

## Screening of Barley Genotypes for Resistance to *Pyrenophora teres* f. *teres* under Controlled Inoculation Conditions

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Net blotch, caused by *Pyrenophora teres* Drechsler, is a major constraint on barley production. We assembled a panel of 276 spring barley genotypes from 18 countries and evaluated resistance to net form net blotch (*P. teres* f. *teres*) using lesion scoring after controlled artificial inoculation of detached leaf segments. Inoculum identity was verified by species-specific PCR, confirming the presence of *P. teres* f. *teres* (378 bp) and the absence of *P. teres* f. *maculata*. To track inoculum performance and normalise lesion scores, three reference barley genotypes ('Pax', 'P22-SP', 'Polo') were included in every inoculation run. Across 26 inoculation time points, lesion severity in reference barley genotypes remained within a narrow, biologically consistent range, indicating stable yet discriminatory infection pressure. Two independent datasets of 276 barley genotypes showed similar distributions of relative lesion score (paired non-parametric test: no shift in central tendency). At the genotype level, categorical agreement between datasets was high: 62.0% exact matches, 37.7% one-step differences and 0.4%  $\geq 2$ -step differences. Rank concordance was moderate (Spearman  $\rho = 0.619$ ; Kendall  $\tau_b = 0.584$ ; both  $p < 0.001$ ), and chance-corrected agreement was fair (Cohen's  $\kappa \approx 0.36–0.40$ ), driven mainly by Susceptible  $\leftrightarrow$  Intermediate reclassifications. Thirty-four genotypes were consistently classified as resistant or highly resistant in both datasets; three ('Romans', 'SK-3212', 'Svit') showed particularly low within-genotype variability across replicates, indicating stable expression of resistance. These results demonstrate the robustness and reproducibility of the phenotyping pipeline and identify promising germplasm for resistance breeding against net form net blotch.

Key words: breeding, detached leaf assay, *Hordeum vulgare*, net form net blotch, plant response

Net form net blotch, caused by *Pyrenophora teres* f. *teres*, is widely recognised as one of the most economically important foliar diseases of barley worldwide. Although a single consolidated global loss estimate is not available, multiple studies consistently report that yield reductions in susceptible cultivars under conducive epidemic conditions may reach up to 40% (Mathre 1982; Robinson & Jalli 1997), and regional economic assessments indicate

substantial national-level impacts (Zia *et al.* 2025). In addition to quantitative yield losses, net form net blotch negatively affects grain quality traits relevant to malting and feed production (Robinson & Jalli 1997).

Overwintering inoculum on crop residues leads to recurrence at the affected site in subsequent seasons (Abebe 2021; Backes *et al.* 2021). The pathogen's life cycle allows several disease cycles within

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a single growing season, during which both sexual and asexual spores may contribute to spread to adjacent fields, while infected seeds may facilitate long-distance dispersal to new locations (Backes *et al.* 2021; Tomić *et al.* 2024). The incidence of net blotch is characterised by high inter-annual variability, driven by fluctuating precipitation and temperature patterns during critical crop growth stages (Vahamidis *et al.* 2020), as well as by cultivation practices and crop management, particularly residue retention under reduced tillage systems (Jordan & Allen 1984). Although cereal cropping systems with reduced soil disturbance, such as no-till or reduced tillage, may offer agronomic benefits, they also favour the persistence of infected crop residues on the soil surface, thereby increasing inoculum potential and promoting earlier and more severe net blotch development (Jordan & Allen 1984). Barley is a major, but not the only, host of *P. teres*, indicating that crop rotation may be an insufficient precaution. Taken together, the deployment of resistant genotypes is an important measure in affected locations, along with cultural, chemical, and biological disease management (Abebe 2021).

*P. teres* Drechsler (anamorph *Drechslera teres* (Sacc.) Shoem) is currently treated as a single species comprising two infraspecific forms, *P. teres* f. *teres* (causal agent of net form net blotch) and *P. teres* f. *maculata* (spot form). These forms differ in symptom expression and represent genetically distinct lineages (Bakonyi & Justesen 2007; Ellwood *et al.* 2012; Hassett *et al.* 2024). Resistance to net form net blotch in barley is genetically complex. While major-effect loci have been identified in specific biparental populations (e.g., a chromosome 6H QTL explaining a large proportion of seedling resistance variance; Friesen *et al.* 2006), broader germplasm surveys and association studies consistently reveal multiple loci of small to moderate effect distributed across the genome (Vatter *et al.* 2017; Adhikari *et al.* 2019; Rozanova *et al.* 2019; D'Souza *et al.* 2025). Moreover, resistance expression is often isolate-specific and growth-stage-dependent, reflecting the interaction between host quantitative trait loci and pathogen virulence determinants (Clare *et al.* 2019; Backes *et al.* 2021). This polygenic and context-dependent architecture complicates the identification of stable resistance sources and necessitates

rigorous, reproducible phenotyping strategies.

Phenotyping net form net blotch resistance presents additional challenges. Pathogen populations are genetically diverse and may differ in virulence spectra across regions (Bouajila *et al.* 2011; Akhavan *et al.* 2017), and both greenhouse and field evaluations are influenced by isolate composition, environmental heterogeneity, and growth stage effects (Jönsson *et al.* 1998; Dinglasan *et al.* 2019). Given the economic importance of net form net blotch, the quantitative nature of resistance, and the documented variability in resistance expression across environments and pathogen populations, there is a clear need to evaluate not only resistance levels but also the consistency and reproducibility of phenotypic classifications. The present study, therefore, aims to (i) assess resistance of a diverse panel of spring barley genotypes to *P. teres* f. *teres* under controlled artificial inoculation, (ii) compare resistance classifications across two independent phenotyping datasets generated under comparable conditions, and (iii) identify genotypes exhibiting stable and robust resistance suitable for breeding applications.

## MATERIAL AND METHODS

### Plant Material

The primary dataset comprised 276 spring barley (*Hordeum vulgare* L.) genotypes selected from the *ex-situ* collection at the Gene Bank of the Slovak Republic within the National Agricultural and Food Centre. The selection represented broad geographic diversity, comprising genotypes from 18 countries, predominantly in Central and Eastern Europe. The highest number of accessions originated from the Czech Republic (84), followed by Slovakia (65), the former Soviet Union (34), Austria (26), and Poland (23). The evaluated material encompassed various biological types of genetic resources, including: (i) registered cultivars (n=156): bred varieties developed through formal breeding programmes; (ii) synthetic populations and breeding lines (n=104): intermediate material used for selection and trait improvement; (iii) landraces (n=12): traditionally cultivated, locally adapted varieties passed down through generations; and (iv) unspecified types (n=4): entries lacking available classification (Table 1).

Seeds of barley genotypes were sown in horticultural substrate (Klasmann KTS1). Plants were grown for 6–8 days in a growth chamber under constant light (light intensity of 200  $\mu\text{mol}/\text{m}^2/\text{s}$ ) at 20°C until they reached growth stage GS 11–12 according to the Zadoks growth scale (first leaf unfolded). For the detached leaf assay, adapted from Afanasenko *et al.* (1995), a 30 mm-long leaf segment was cut from the subapical part of the first true leaf. For each genotype, ten segments, each from a different plant, were placed on filter paper moistened with distilled water containing 0.004% benzimidazole in plastic trays.

Three barley reference genotypes, selected based on preliminary screening and prior experimental experience, served as internal standards for score normalisation: ‘Pax’, a cultivar from Slovakia; ‘P22-SP’, a genotype from a synthetic population in Slovakia; and ‘Polo’, a cultivar from Poland. These genotypes were included in each artificial inoculation run to monitor infection dynamics and ensure stable inoculum pressure. Their inclusion in each plastic tray enabled reliable calibration and correction of lesion scores, ensuring comparability across independent evaluations.

#### *Pyrenophora teres* – Isolates and their PCR Verification

A local population of *Pyrenophora teres* f. *teres* was collected from barley leaves naturally infected with net form net blotch, growing in experimental fields at the Department of Plant Production in Piešťany, Slovakia (GPS: 48.585308, 17.811417) within the National Agricultural and Food Centre. Isolates were grown on PDA agar at 20°C under a 16-hour photoperiod (200  $\mu\text{mol}/\text{m}^2/\text{s}$ ) in a growth chamber for storage and vegetative propagation. The virulence of the mixed inoculum was not quantified using a differential set; instead, its biological performance was assessed by lesion development on three reference barley genotypes included in each inoculation run.

To confirm the identity of the fungal isolates used in inoculation experiments, we performed a PCR-based assay to differentiate *P. teres* f. *teres* (Ptt) from *P. teres* f. *maculata* (Ptm), following the protocol of Williams *et al.* (2001). Genomic DNA was extracted from mycelial cultures using the DNeasy Plant Mini

Kit (Qiagen, Germany). The PCR reaction included primer pairs specific to Ptt (PTT-F/PTT-R) and Ptm (PTM-F/PTM-R), yielding amplicons of 378 bp for Ptt and 411 bp for Ptm, respectively (Williams *et al.* 2001). PCR amplifications were carried out in a total volume of 15  $\mu\text{L}$  containing: 1.5  $\mu\text{L}$  of 10 $\times$ PCR buffer, 1.5 mM  $\text{MgCl}_2$ , 0.5  $\mu\text{M}$  of each primer (PTT-F, PTT-R, PTM-F, PTM-R), 0.2 mM dNTPs, 0.8 U of Taq DNA polymerase (Invitrogen), 2  $\mu\text{L}$  of template DNA (30 ng), and nuclease-free water to volume. Thermal cycling was performed using a touchdown protocol: initial denaturation at 94°C for 1 min; 10 cycles of 94°C for 30 s, annealing starting at 65°C (decreasing by 1°C per cycle) for 30 s, and extension at 72°C for 1 min; followed by 24 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min; with a final extension at 72°C for 5 min. PCR products were separated on a 1.4% agarose gel stained with ethidium bromide and visualised under UV light.

#### *Artificial Inoculation*

To prepare inoculum for the detached leaf assay, *P. teres* f. *teres* isolates were transferred to Czapek-Dox agar and incubated for 5 days. Conidiation was then induced by UV-C radiation for 24–48 hours, followed by 3–4 days under a 16-hour photoperiod at 20°C. A conidial suspension was prepared by flooding the culture in a Petri dish with distilled water supplemented with Tween 20 to a final concentration of 0.01%, then passing the suspension through a 0.355 mm mesh sieve. For inoculum, suspensions from 2–3 Petri dishes with different isolates were pooled, and the final concentration was adjusted to  $2.5\text{--}6 \times 10^3$  conidia per mL. An 8  $\mu\text{L}$  droplet of the inoculum, containing approximately 20–50 conidia, was applied to each leaf segment, while a slight mechanical injury was induced on the leaf surface with the pipette tip. Leaf segments were arranged in trays with sufficient spacing to prevent contact between adjacent segments and interference among inoculation droplets. In each tray, 15 tested genotypes, along with three reference barley genotypes, were inoculated, and control segments (excised from reference genotypes) were either treated with 0.01% Tween 20 in distilled water (mock treatment,  $n=6$ ) or left untreated (absolute control,  $n=6$ ). To ensure maximal humidity, trays with leaf

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List of the 276 barley (*Hordeum vulgare* L.) genotypes evaluated in this study, including their origin, biological type of genetic resource, and name. Genotypes were categorised into four groups based on their genetic background: 1 – Registered cultivars, 2 – Synthetic populations and breeding lines, 3 – Landraces, and 4 – Unspecified types. The numbers preceding genotype names refer to their unique Sample IDs used throughout the phenotypic analyses in blind tests.

| Origin | Genetic background | Sample ID + name of genotype                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|--------|--------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| CZE    | 1                  | 69. 'Heris', 76. 'Rubin', 84. 'Ametyst', 92. 'Michalovický', 112. 'Proskovcův hanacký', 113. 'Selekcny hanacký', 119. 'Akcent', 131. 'Hanacký exportný', 132. 'Hanacký jubilejny', 133. 'Hanacký Kargyn', 134. 'Hanacký Moravan', 135. 'Heran', 149. 'Kredit 210', 150. 'Ladik', 151. 'Malvaz', 161. 'Safir', 167. 'Hana', 168. 'Jantar', 172. 'Merkur', 173. 'Opal', 175. 'Atlas', 178. 'Denar', 179. 'Diabas', 180. 'Dukat', 184. 'Favorit', 192. 'Topas', 197. 'Amulet', 199. 'Olbram', 239. 'Bohatyr', 240. 'Detenický Kargyn', 241. 'Dobrovický Starocesky', 242. 'Dregeruv Imperial', 243. 'Export Ratborsky', 244. 'Janovický', 245. 'Jarohnevický', 246. 'Jindřichovický K 64', 250. 'Kvasický', 251. 'Moravský nepolehavy IGB', 253. 'Novodvorský Hanacký', 254. 'Olesenský', 255. 'Pisarecký', 256. 'Semčický hospodarský', 257. 'Semčický pivovar', 258. 'Stupický Hanacký', 259. 'Stupický plnozrnný' |
|        | 2                  | 71. 'KM-245-16', 105. 'DB-132', 125. 'BR-2385', 130. 'HE-5038', 136. 'KM-1112', 140. 'M-3/2', 141. 'M-3/9', 143. 'N-331 84', 159. 'SG-S-159', 160. 'SG-S-167-A', 164. 'HE-6037', 165. 'HE-6621', 171. 'KM-1375', 183. 'F 24575', 190. 'SG-S-185', 193. 'V 1625/79', 198. 'SG-S-167', 201. 'KM-1635', 206. 'K 26822', 207. 'KM 178509/77', 208. 'KM-BR-G 1114', 211. 'ST 17113', 228. 'HE-1440', 229. 'KM 123', 230. 'KM 1378', 231. 'BR 5914', 232. 'BR 6464', 233. 'BR 6481', 234. 'HE 7928', 262. 'HE 9540', 263. 'HE 9859', 264. 'HE 9901', 265. 'HE 9439'                                                                                                                                                                                                                                                                                                                                                     |
|        | 3                  | 247. 'Krajova St. Hrozenkov', 248. 'Krajova z Orlove', 249. 'Krukanický horský', 252. 'Nolc Dregeruv velerany'                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|        | 4                  | 4. 'RTG Valtický', 94. 'KM-S-2'                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| SVK    | 1                  | 1. 'Sladko', 3. 'Nitriansky exportný', 5. 'Slovenský dunajský trh', 6. 'Slovenský jemný', 7. 'Slovenský kvalitný', 8. 'Viglasský polojemný', 9. 'Buciánsky Kneifel', 10. 'Donum', 11. 'Garant', 12. 'Horal', 13. 'Orbit', 14. 'Pax', 15. 'Poprad', 16. 'Svit', 17. 'Terrasol Pivovarský', 18. 'Vladan', 19. 'Zlatan', 31. 'Slovenský RTG 802', 64. 'Kumran', 65. 'Valis', 66. 'Exalis', 67. 'SK Levitus'                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|        | 2                  | 20. 'P3', 21. 'P4', 22. 'P40', 23. 'P41', 24. 'P51', 25. 'P59', 26. 'P61', 27. 'SK 13-9-91', 28. 'TR-SK-3767-3877', 29. 'SK-2664', 30. 'SK-2736', 32. 'SK-3212', 33. 'SK-4062', 34. 'P 19-SP', 35. 'P 22-SP', 36. 'P 23-SP', 37. 'P 7-PY', 38. 'P 9-PY', 39. 'PL-6291', 40. 'SK-4294', 45. 'PY-29', 46. 'PY-30', 47. 'PY-31', 48. 'PY-32', 49. 'PY-36', 50. 'PY-37', 51. 'TR-4031', 52. 'TR-4276', 53. 'TR-2125', 54. 'TR-2159', 55. 'TR-4452', 56. 'TR-4520', 57. 'SK 4101', 58. 'SK 4653', 59. 'SK 4770', 60. 'SK 4101-592', 61. 'SK 4908', 62. 'SK 6109', 63. 'SOH 517'                                                                                                                                                                                                                                                                                                                                        |
|        | 3                  | 41. 'Tatry 1995-245', 42. 'Tatry 1995-198', 43. 'Tatry 1995-196', 44. 'Tatry 1995-246'                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| SUN    | 1                  | 72. 'Dnepropetrovskij', 74. 'Ogul', 75. 'Temp', 77. 'Viking', 78. 'Jenisej', 80. 'Tismin', 81. 'Veras', 82. 'Verchacskij', 83. 'Agul 2', 88. 'Beloruskij 18', 95. 'Kaskad', 96. 'Kedr', 99. 'Odesskij 125', 103. 'Berezinskij', 106. 'Dzugajaj', 108. 'Pirogovskij', 109. 'Romans', 110. 'Zazerskij 85', 115. 'Viner', 117. 'Zazerskij', 126. 'Boratinskij', 127. 'Duzy', 129. 'Goland', 138. 'Linga', 147. 'Odisej', 148. 'Olimpiec', 169. 'Judiz'                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|        | 2                  | 97. 'Nutans 752', 98. 'Nutans 778', 144. 'Nutans 765', 145. 'Nutans 92', 146. 'Nutans-0353/133', 209. 'Nutans 244', 210. 'Nutans 3'                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| AUT    | 1                  | 114. 'Valenta', 116. 'Viva', 118. 'Admira', 120. 'Amalia', 122. 'Australische Fruhe', 152. 'Erika', 153. 'Esta', 154. 'Extra', 162. 'Tina', 181. 'Effekta', 182. 'Elke', 194. 'Verbesserte Mehlaures. Pumper', 212. 'Elisa', 213. 'Ohara', 214. 'Panorama', 215. 'Prosa', 216. 'Santiago', 217. 'Secura', 273. 'Avilla'                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
|        | 2                  | 111. 'P-433-86', 157. 'SE-245/92', 158. 'SE-289/90', 187. 'SE-307/93', 188. 'SE-312/94', 189. 'SE-313/94'                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|        | 3                  | 196. 'Westendorfi Tirol'                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| POL    | 1                  | 100. 'Antalek', 121. 'Ars', 137. 'Klimek', 139. 'Lot', 155. 'Polo', 156. 'Rodos', 163. 'Edgar', 200. 'Boss', 218. 'Atol', 219. 'Bies', 220. 'Bryl', 222. 'Gwarek', 223. 'Orlik', 224. 'Poldek', 225. 'Rastik', 226. 'Rataj', 227. 'Stratus', 272. 'Antek', 274. 'Johan'                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
|        | 2                  | 68. 'STH 1448', 123. 'BKH-89 1055', 124. 'BKH-988', 128. 'GOH 1527'                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| HUN    | 1                  | 79. 'Kompolti', 142. 'Matyo', 166. 'Nora', 221. 'GK Isis'                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|        | 2                  | 107. 'GKSC-7214', 170. 'K 81/31'                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|        | 4                  | 2. 'Hatvani'                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| UKR    | 1                  | 73. 'Slavutic', 93. 'Odesskij 135', 202. 'Mironovskij 86', 203. 'Spomin', 204. 'Podolyan', 205. 'Odesskij 151', 275. 'Nudum 0155'                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| AFG    | 1                  | 174. 'Andak', 176. 'Batman Ghulghula'                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|        | 2                  | 195. 'Wechenanai'                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|        | 3                  | 185. 'Roa', 186. 'Rugut', 191. 'Takana Kala'                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| BGR    | 1                  | 104. 'Beroc'                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|        | 2                  | 85. 'BGRC 24132', 86. 'BGRC 24202', 87. 'BV 65 Orzovica', 102. 'BGRC 24129'                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| SYR    | 1                  | 70. 'Icarda 110154', 261. 'Nutans Syrie'                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|        | 2                  | 89. 'Icarda 108546', 90. 'Icarda 110145', 91. 'Icarda 111030'                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
| TUR    | 1                  | 260. 'Anatolien'                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
|        | 2                  | 235. '3063', 236. '3857', 237. '3875', 238. '4513'                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| CAN    | 1                  | 269. 'Peregrine', 176. 'CDC Candle'                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| DEU    | 1                  | 267. 'Dometzkoer Paradies', 271. 'Despina'                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| ITA    | 4                  | 270. 'Orfeo'                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| MNG    | 2                  | 266. 'MK 1257'                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| NOR    | 1                  | 101. 'Arve'                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| ROU    | 1                  | 177. 'Cluj 230'                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| USA    | 1                  | 268. 'Bear'                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |

CZE = Czech Republic; SVK = Slovak Republic; SUN = former Soviet Union; AUT = Austria; POL = Poland; HUN = Hungary; UKR = Ukraine; BGR = Bulgaria; SYR = Syria; TUR = Türkiye; CAN = Canada; DEU = Germany; ITA = Italy; MNG = Mongolia; NOR = Norway; ROU = Romania; USA = United States of America.

segments were covered with glass after inoculation and incubated in the dark for 24 hours. Thereafter, they were exposed to constant light, and the temperature was maintained between 16–20°C. Lesion symptoms were assessed five days after inoculation.

#### Plant Response Evaluation

As in Afanasenko *et al.* (2007), we evaluated the lesion category. However, we aimed to compare the degree of resistance among genotypes more precisely, based on lesion size. Accordingly, we used slightly longer segments (30 mm) and added more lesion categories, as follows: 0 = no lesion; 1 = pinpoint brown lesion, no chlorosis; 2 = lesion extending longitudinally up to 5 mm, no chlorosis; 3 = brown lesion extending both longitudinally and transversely up to 6 mm, no or slight chlorosis; 4 = net-forming brown lesion extending up to 40% of the segment surface, surrounded by chlorosis; 5 = net-forming brown lesion with chlorosis occupying 40–49% of the segment surface. Our categories 6 to 9 correspond to lesion type 4 in the scale of Afanasenko *et al.* (2007) and thus represent a highly susceptible reaction, differing in the area occupied by the necrotic lesion: 6 = lesion covering 50–59% of the segment surface; 7 = lesion covering 60–79% of the segment surface; 8 = lesion covering 80–99%, completely chlorotic segment (no remaining green tissue); 9 = whole leaf segment covered by a brown necrotic lesion.

To compare the analysed genotypes with respect to temporal variations in inoculum aggressiveness, the Relative Lesion Score (RLS) was calculated by normalising this mean against the reference factor:

$$RLS = \frac{LC}{Rf}$$

Where (LC) represents the lesion category value for a particular segment, and a reference factor (Rf) was set as the grand mean lesion category value across three reference barley genotypes (each n=6) in the respective infection run.

The calculation of *Rf* value was:

$$Rf = \frac{\sum_{j=1}^3 \sum_{k=1}^6 LC}{18},$$

Where LC represents the value of lesion category

(above-mentioned 1–9 scale) of the  $k^{th}$  replication, and  $j$  (1–3) refers to the three reference barley genotypes.

#### Statistical Evaluation

To assess the stability of artificial inoculation across 26 independent time points, the lesion category of three reference barley genotypes was evaluated on a 1–9 scale (see above). Statistical analyses and visualisations were performed using Python (v3.12.7). For comparative evaluation of 276 barley genotypes, two independently generated datasets of relative lesion scores (RLS) were used for all downstream analyses. Score distributions were visualised using violin plots combined with box plots to depict variability across phenotypic response categories. To quantify between-dataset concordance on the ordinal scale, relative lesion scores were classified into five ordered categories (Table 2). The thresholds used for this classification were established specifically for the present study and were not directly adopted from previous publications.

Concordance was evaluated using (i) Spearman's rank correlation ( $\rho$ ) and Kendall's  $\tau$ -b to assess rank association; (ii) Cohen's kappa to estimate agreement beyond chance, computed as unweighted  $\kappa$  and as linearly and quadratically weighted  $\kappa$ , appropriate for ordered categories; and (iii) simple agreement summaries based on the absolute category difference per genotype (exact match, off-by-one, off-by-two-or-more), together with a confusion matrix. P-values were obtained for the rank correlations; kappa statistics were reported with the chosen weighting scheme. Principal component analysis (PCA) was performed on relative lesion scores from 10 biological replicates per genotype to evaluate the

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Classification criteria for phenotypic response of barley genotypes to *P. teres* f. *teres* based on relative lesion scores

| Phenotypic response | Relative lesion score (RLS) |
|---------------------|-----------------------------|
| Highly susceptible  | $RLS \geq 1.32$             |
| Susceptible         | $0.98 \leq RLS < 1.32$      |
| Intermediate        | $0.84 \leq RLS < 0.98$      |
| Resistant           | $0.72 \leq RLS < 0.84$      |
| Highly resistant    | $RLS < 0.72$                |

multivariate structure and grouping patterns based on phenotypic response categories. PCA was conducted on centred and standardised variables (correlation matrix). Data from both datasets were analysed separately, and the first two principal components were visualised using biplots in PAST v4.17 (Hammer *et al.* 2001). A subset of 34 genotypes consistently classified as resistant or highly resistant in both datasets was further analysed. For these genotypes, all 20 relative lesion score values (10 replicates from each dataset) were merged and visualised as box plots.

## RESULTS AND DISCUSSION

### *PCR Verification of Fungal Isolates*

Prior to inoculation trials, the identity of fungal isolates was verified using species-specific PCR primers. All tested cultures produced a specific 378 bp amplicon and showed no signal at 411 bp, providing evidence that the inoculum mixture consisted exclusively of *P. teres* f. *teres*, with no detection of *f. maculata* (Figure 1). This was consistent with our expectations, as all isolates were derived from barley leaves showing symptoms of net form net blotch. Three isolates (PT4470, PT4570, PT4630) were selected for further use to better approximate field conditions, where multiple pathogen strains typically co-occur.

### *Temporal Stability of Inoculum and P. teres f. teres Infection Dynamics*

To assess the temporal consistency of the inoculum, lesion category was evaluated across 26 inoculation time points for three internal reference barley genotypes: ‘Pax’, ‘P22-SP’, and ‘Polo’ (Figure 2). The overall mean lesion category across all genotypes and time points was  $3.58 \pm 0.93$  (mean  $\pm$  SD) on the 1–9 lesion category scale, indicating that the inoculum mixture consistently induced moderate, discriminatory disease symptoms throughout the experimental period. Although minor temporal variation was observed, most notably at inoculation time point 2, where the highest mean value (4.67) was recorded for genotype ‘Pax’, the majority of time points fell within a relatively narrow interval of 3.1 to 4.0. No systematic increase or decline in

lesion severity was detected across the 26 inoculation events.

In addition to monitoring inoculum performance, the reference genotypes provided insight into inherent differences in disease response. Tuohy *et al.* (2006) showed that isolates exhibited similar virulence (either inducing symptoms or not), whereas the cultivars varied in their response to individual isolates. When assessing barley genotype responses, it is important to recognise that crops are often exposed to pathogen populations comprising multiple co-occurring strains under field conditions. The use of a mixed inoculum is particularly relevant given documented pathogenic variability within *P. teres* f. *teres* populations. Studies across diverse geographic regions have demonstrated substantial differences in virulence spectra among isolates (Bouajila *et al.* 2011; Akhavan *et al.* 2017; Poudel *et al.* 2019). Consequently, resistance expression may vary depending on the specific isolate or isolate composition used for phenotyping. By combining multiple isolates from the local population, we aimed to minimise isolate-specific bias and approximate the heterogeneous infection pressure encountered under field conditions. The time course of the reference genotype responses (Figure 2) shows expected fluctuations rather than an undesired gradual attenuation of aggressiveness, indicating sufficient stability of infection pressure throughout the phenotyping experiment. Because all 276 genotypes could not be evaluated within a single inoculation run, variation in inoculum performance between runs could potentially affect the comparative assessment of cultivar responses. To prevent bias in the evaluation of tested genotypes, we introduced the relative lesion score. Similarly, Jørgensen *et al.* (2000) related the assessment of barley response in field trials to a control cultivar with a known level of resistance. The inclusion of internal reference genotypes in each inoculation run also aligns with recommendations for standardised resistance evaluation in barley – *P. teres* pathosystems. The development of international differential sets for *P. teres* (Afanasenkov *et al.* 2009) underscores the necessity of stable reference cultivars to ensure comparability across experiments and laboratories. In our study, the consistent ranking of ‘Pax’, ‘P22-SP’, and ‘Polo’ across 26 inoculation

events further supports the robustness of the phenotyping system.

Mock-treated and untreated reference leaf segments were examined to rule out senescence as a contributor to lesion development during the post-inoculation incubation period. Although a slight loss of chlorophyll was observed after 5 days in both control variants, the colour remained uniform, with no distinct chlorotic or necrotic lesions, spots, or irregular pigmentation. Consequently, the lesion score remained zero, regardless of normalisation.

#### *Phenotypic Response of 276 Barley Genotypes to Artificial Inoculation*

No genotype was fully immune to pathogen infection, as at least a pinpoint lesion (type 1) was observed in all inoculated genotypes, whereas mock-treated controls remained symptomless.

To assess the consistency and reproducibility of phenotypic scoring, relative lesion scores for 276 barley genotypes were compared across two independent datasets (Dataset\_1 and Dataset\_2), each collected in different time periods under comparable conditions using the same isolate mixture (PT4470, PT4570, PT4630). Dataset\_1 had a mean relative lesion score of  $0.937 \pm 0.119$ , ranging from 0.32 to

1.20, while Dataset\_2 showed a comparable mean of  $0.945 \pm 0.108$ , ranging from 0.48 to 1.41. Although both datasets deviated from normality, the Wilcoxon signed-rank test ( $p=0.30$ ) indicated no significant shift in central tendency between them.

As shown in Figure 3A, the overall distribution of relative lesion scores was highly similar between datasets, with comparable medians and ranges. However, slight differences were observed in the classification of phenotypic response categories. In Dataset\_1, genotypes were grouped into four phenotypic categories, whereas Dataset\_2 included five categories, with two genotypes classified as highly susceptible. Additionally, Dataset\_1 showed a broader spread among highly resistant genotypes, while Dataset\_2 contained a larger number of genotypes classified as resistant. Despite these subtle differences, the phenotypic pattern remained largely consistent, as supported by Spearman correlation analysis ( $\rho=0.684$ ,  $p<0.001$ ; Figure 3B), indicating a statistically significant positive association between continuous relative lesion scores in both datasets. This moderate but significant correlation between datasets is consistent with previous reports indicating that disease expression in the barley – *P. teres* pathosystem may vary over time and in rela-

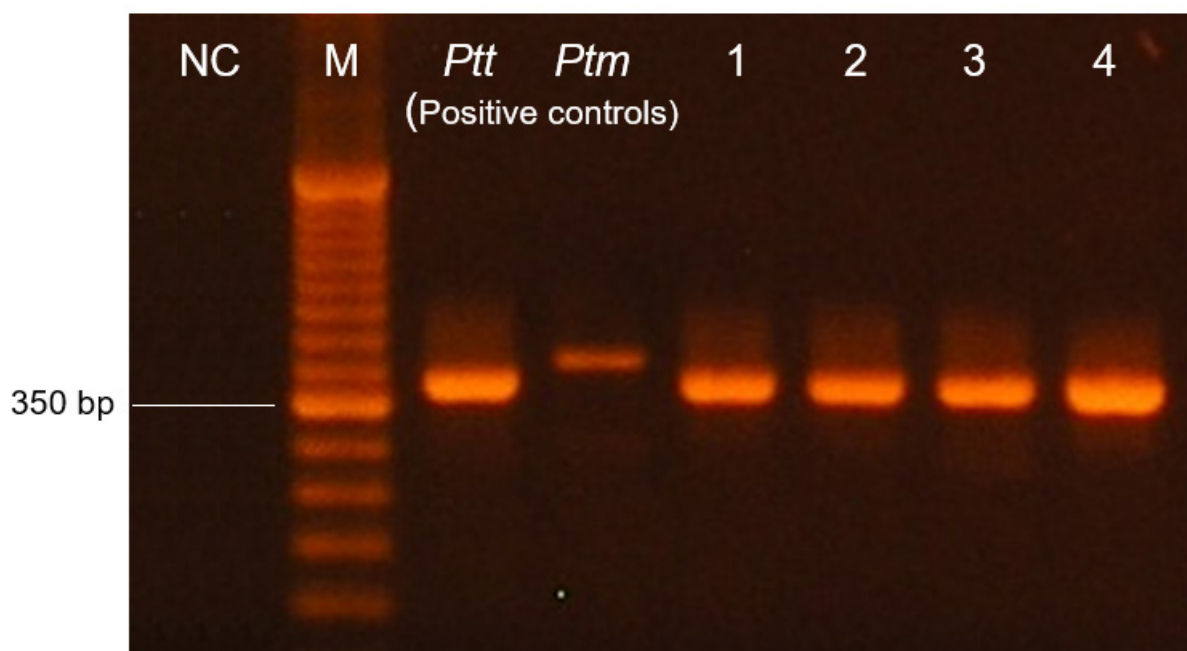


Figure 1. Agarose gel illustrating the results of the diagnostic PCR with primer sets Ptt (*P. teres* f. *teres*-specific, expected ~378 bp) and Ptm (*P. teres* f. *maculata*-specific, expected ~411 bp). NC (negative control); M (50 bp DNA ladder); positive controls for Ptt and Ptm; lanes 1–4, tested isolates (PT4380, PT4470, PT4570, and PT4630).

tion to pathogen population dynamics and environmental conditions (Poudel *et al.* 2019; Vahamidis *et al.* 2020). Even under controlled conditions, minor shifts in infection dynamics may influence the ex-

pression of quantitative resistance without altering overall genotype ranking.

Principal component analysis (PCA) was conducted independently for each dataset (Figure 3 C)

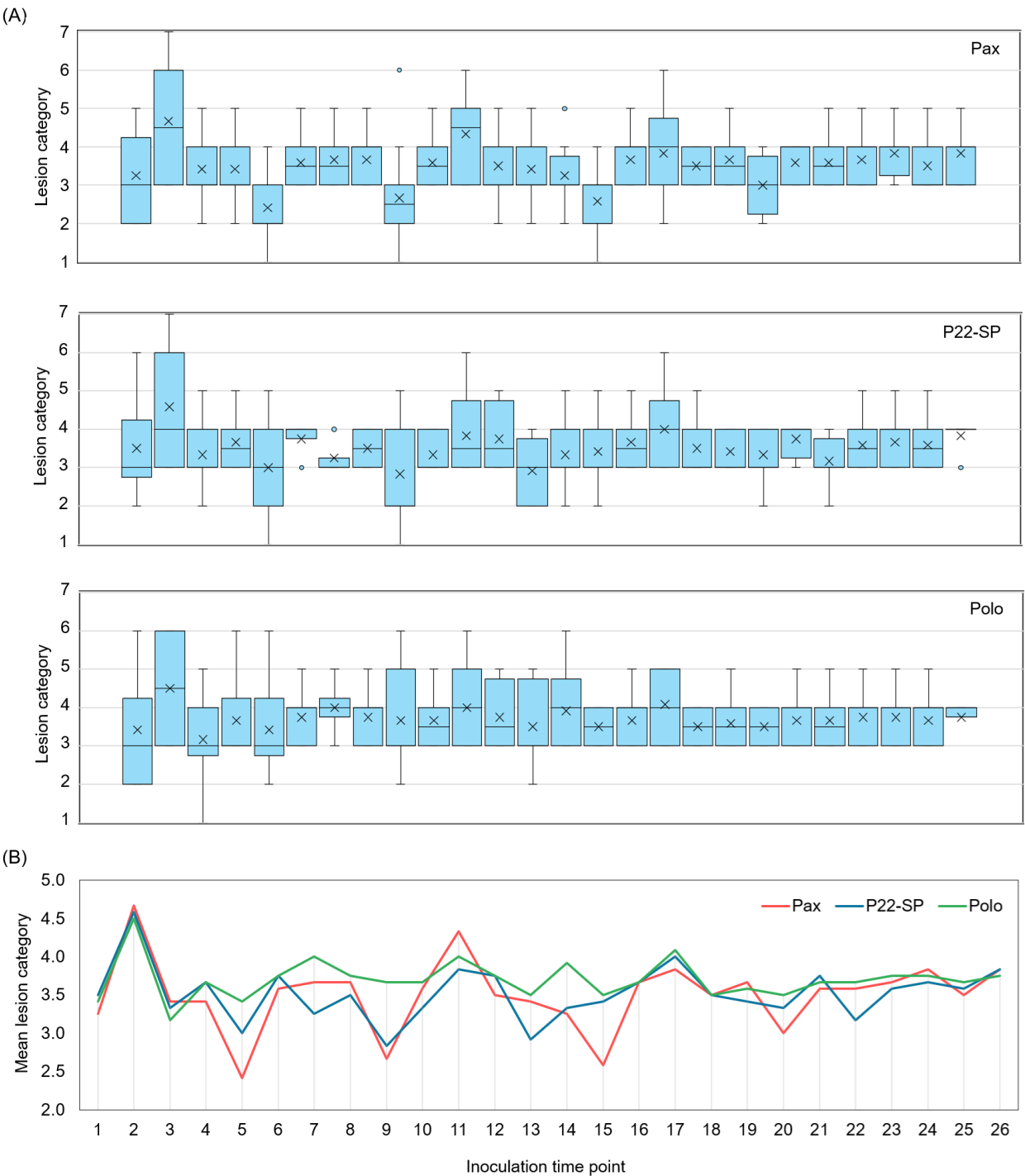


Figure 2. Temporal variability of plant responses to the inoculation with *P. teres* f. *teres*. (A) Box plots displaying the distribution of lesion category values recorded across 26 inoculation time points for reference genotypes: ‘Pax’, ‘P22-SP’, and ‘Polo’. Each box plot represents one inoculation event (1–26) in chronological sequence of experiments. The lesion categories were scored using the 1–9 scale (1 = pinpoint lesion, 9 = necrotic lesion covering the whole leaf segment, i.e. proportional to susceptibility). (B) Line plot illustrating the temporal progression of mean lesion category for each genotype across the 26 inoculation events, highlighting their dynamic phenotypic response throughout the experiment.

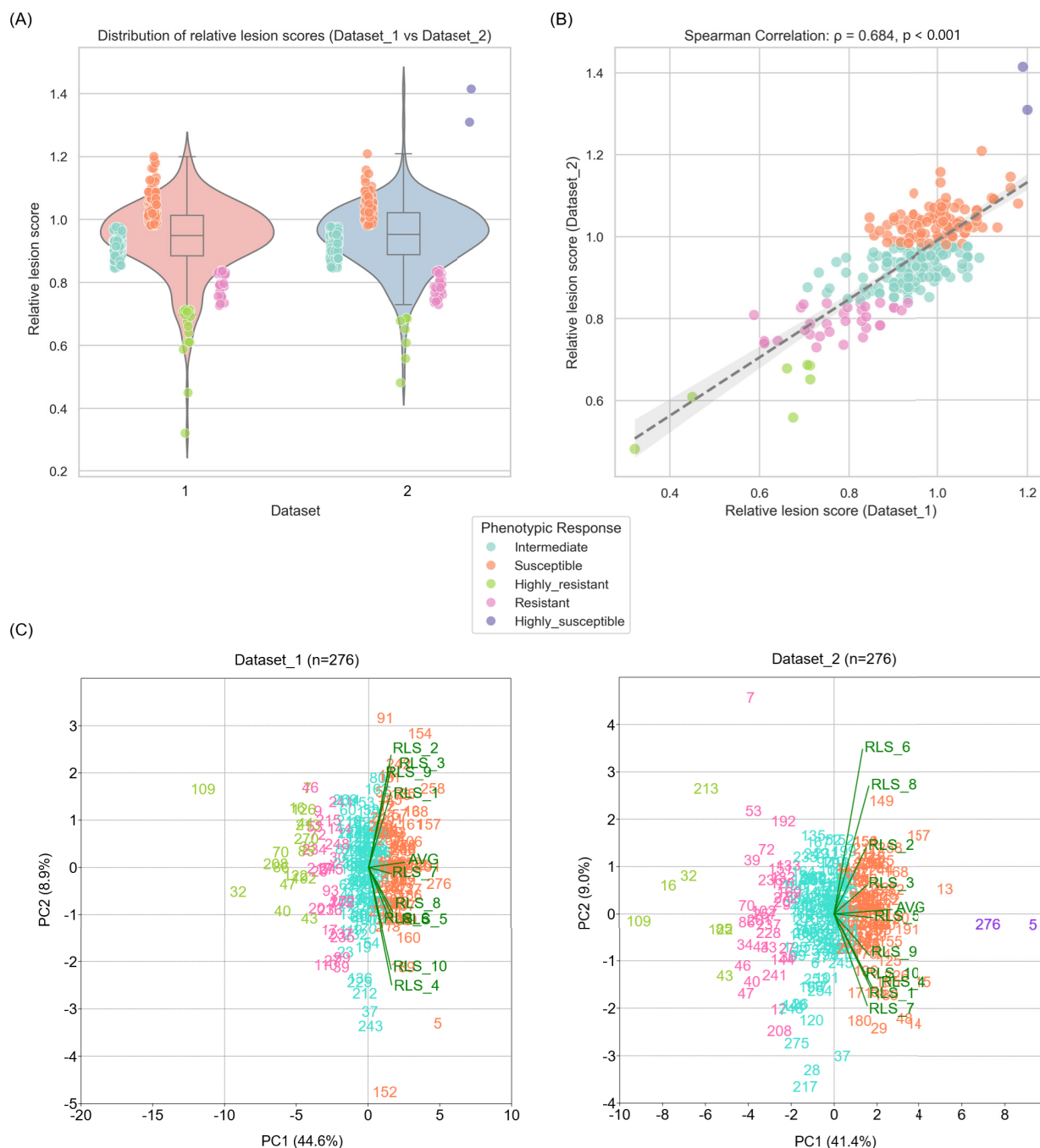


Figure 3. Comparison of relative lesion scores of 276 barley genotypes between two independent phenotyping datasets. Lesion categories were originally assessed on a 1–9 scale and normalised relative to the grand mean lesion category value of three reference barley genotypes within each dataset to minimise temporal effects during extended evaluation periods. (A) Violin and box plots illustrating the distribution of mean relative lesion scores (Dataset\_1 and Dataset\_2). Each point represents an individual genotype, color-coded by its phenotypic response category. (B) Scatter plot depicting the correlation of relative lesion scores between the two datasets, with colour-coded points according to phenotypic responses based on Dataset\_2. Spearman correlation coefficient ( $\rho$ ) and p-value are displayed. (C) Principal component analysis (PCA) biplots based on genotype  $\times$  replicate relative lesion score matrices for Dataset\_1 (left) and Dataset\_2 (right). Numbers represent sample IDs of individual genotypes ( $n = 276$  per dataset) and are colour-coded by phenotypic response category. Green lines indicate variable loadings corresponding to individual replicate relative lesion scores (RLS\_1–RLS\_10) and the average relative lesion score (AVG).

to further examine phenotypic relationships among genotypes and the consistency of their classification across datasets. The first two principal components (PC 1 + PC 2) explained 53.5% and 50.4% of the total variance in Dataset\_1 and Dataset\_2, respectively. In both datasets, PC 1 showed uniformly positive loadings across all replicate scores and the average relative lesion score (AVG), indicating that this compo-

nent primarily represents the overall resistance–susceptibility gradient. Genotypes positioned towards higher PC 1 values consistently exhibited higher relative lesion scores (greater susceptibility), whereas those with negative PC 1 values showed uniformly lower relative lesion scores across replicates (greater resistance). The strong alignment of AVG with PC 1 confirmed that the mean relative lesion score

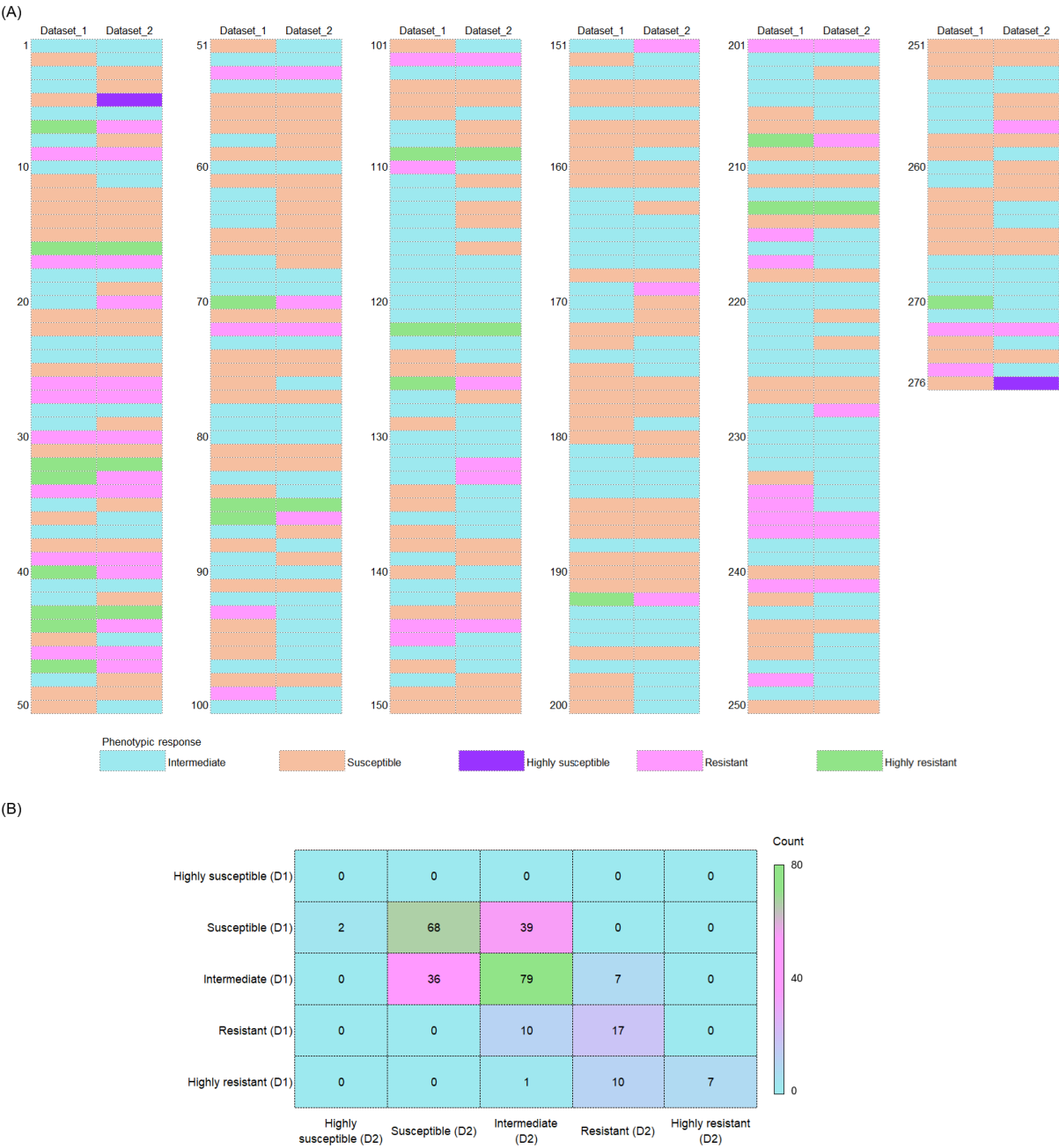


Figure 4. Cross-dataset consistency of phenotypic categories for 276 barley genotypes. (A) Tile map showing, for each genotype (rows; ordered by Sample ID), the phenotypic class assigned in two independent experiments (columns: Dataset\_1 and Dataset\_2). Colours denote five ordinal categories: highly susceptible, susceptible, intermediate, resistant, highly resistant. (B) Confusion matrix summarising counts of category assignments in Dataset\_1 (rows) versus Dataset\_2 (columns); the colour bar indicates the number of genotypes per cell. D1 – Dataset\_1, D2 – Dataset\_2.

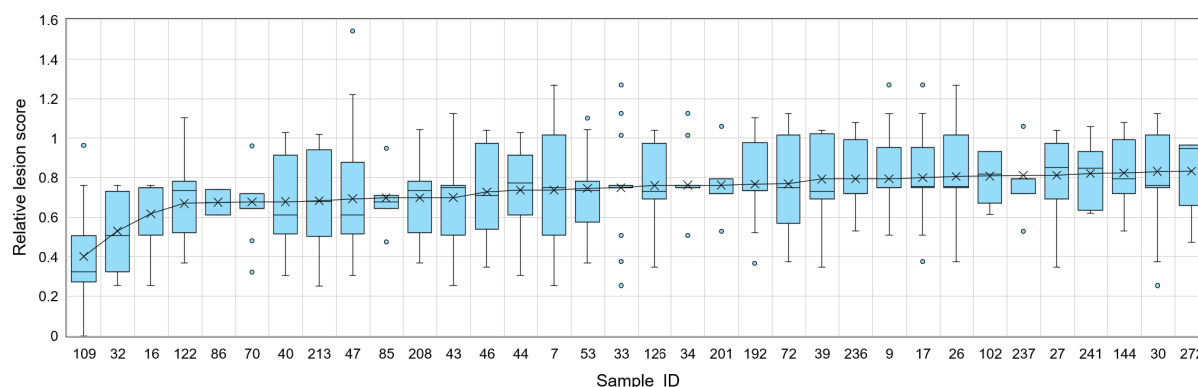


Figure 5. Box plots showing the distribution of relative lesion scores for 34 selected barley genotypes classified as resistant or highly resistant in both datasets. Sample IDs are sorted from left to right based on increasing mean scores, highlighting the variability in resistance performance among consistently resistant genotypes.

adequately captures overall resistance intensity. In contrast, PC 2 displayed a mixed pattern of positive and negative loadings across replicate scores in both datasets, while the AVG contributed minimally to this component, indicating that PC 2 reflected variation in the stability of genotype response across replicates rather than absolute resistance intensity. Genotypes located near the horizontal axis ( $PC\ 2 \approx 0$ ) exhibited relatively uniform responses across all inoculation runs, whereas those with more extreme PC 2 values showed greater phenotypic fluctuation. The continuous distribution of genotypes along the PC 1 axis, without discrete clustering boundaries, corroborates the quantitative nature of resistance to net form net blotch. This pattern is consistent with genetic studies demonstrating that resistance to net form net blotch is generally quantitative and often involves multiple loci of small to moderate effect, although major-effect loci have also been identified in some biparental populations (Friesen *et al.* 2006; Vatter *et al.* 2017; Wonneberger *et al.* 2017; Adhikari *et al.* 2019). Therefore, the absence of discrete phenotypic clusters in our dataset supports the polygenic architecture of resistance in diverse barley germplasm. The presence of a secondary stability-related axis (PC 2) confirms that genotypes with comparable mean resistance levels may differ in the consistency of their response across independent inoculation events. Similar patterns of stable versus fluctuating resistance expression have been reported in QTL mapping studies, where some loci show effects across developmental stages, whereas others are stage-dependent (Wonneberger *et al.* 2017; D' Souza *et al.* 2025). The secondary PCA axis ob-

served here may therefore reflect genotype-specific sensitivity to subtle experimental variation rather than fundamental differences in resistance intensity.

To visualise genotype-level consistency across both experiments, we summarised the phenotypic response category assigned to each accession in Dataset\_1 and Dataset\_2 using a tile map (Figure 4A). Adjacent tiles per genotype reveal concordance or shifts between experiments, providing a transparent visual bridge between the distributional comparisons in Figure 3 and the identification of consistently resistant accessions. Agreement between datasets was generally high: the category matched exactly for 171 genotypes (62.0%), differed by only one adjacent class for 104 genotypes (37.7%), and differed by  $\geq 2$  classes for one genotype (0.4%). The mean absolute category difference was 0.38 (median = 0), indicating that, when discrepancies occurred, they were typically small. Rank-based concordance between the two ordinal series was moderate (Spearman  $\rho=0.619$ , Kendall  $\tau_b=0.584$ , both  $p<0.001$ ). Agreement beyond chance was fair: Cohen's  $\kappa=0.397$  (unweighted),  $\kappa_w$  (linear)=0.357, and  $\kappa_w$  (quadratic)=0.362, consistent with the fact that nearly all disagreements were confined to neighbouring categories.

The confusion matrix (Figure 4B) clearly illustrates this pattern. Most counts were concentrated along the diagonal or in adjacent cells, particularly around the Susceptible-Intermediate boundary, whereas pronounced shifts were rare. This indicates that differences between datasets were generally minor and unlikely to substantially affect the identification of robust resistance donors. The need for

standardised phenotyping frameworks in net blotch research has been emphasised previously, particularly to harmonise resistance classifications across laboratories and environments (Afanasenko *et al.* 2009; ElMor *et al.* 2018). Overall, the direction of reclassification was slightly biased towards higher resistance in Dataset\_2 (58 upgrades vs 46 downgrades; 171 unchanged), but the discrepancies were minor and concentrated around the Susceptible-Intermediate boundary and are unlikely to substantially affect the identification of robust resistance donors. Similar shifts have been observed in net blotch phenotyping studies, where minor fluctuations in infection pressure, microclimatic conditions, or pathogen population structure may influence the expression of quantitative resistance (Poudel *et al.* 2019; Vahamidis *et al.* 2020).

Thirty-four genotypes were consistently classified as resistant or highly resistant across both datasets, representing approximately 12% of the evaluated panel. Only two cultivars were classified as highly susceptible in Dataset\_2, whereas none fell into this category in Dataset\_1. The detached leaf segment assay used in this study is a well-established phenotyping approach that enables precise discrimination among genotypes under controlled infection pressure. Importantly, this method is primarily intended for high-throughput pre-breeding screening rather than definitive confirmation of field-level resistance. Previous comparative studies have reported substantial concordance between detached leaf and whole-plant resistance assessments (Sharma 1984; Deadman & Cooke 1986; Tuohy *et al.* 2006; El-Mor *et al.* 2018), although environmental complexity under field conditions may modulate symptom expression (Vahamidis *et al.* 2020). Some divergence between controlled and field responses is expected, given the greater environmental complexity that influences host-pathogen interactions under field conditions. For instance, Tekauz (1985) reported that symptom expression may differ between experimental systems, reflecting the multifactorial nature of disease development rather than methodological limitations.

The 34 consistently resistant genotypes originated from diverse geographic regions, with a predominance of accessions from Central and Eastern Europe, particularly Slovakia (17 genotypes), followed

by the Czech Republic (4), Bulgaria (3), Austria (2), Poland (1), Syria (1), Türkiye (2), and the former Soviet Union (4). For many of the older cultivars, breeding lines, and gene bank accessions included in this panel, directly comparable published data on resistance to *P. teres* f. *teres* are limited or unavailable, thereby increasing the value of the present screening as a baseline phenotypic characterisation. The presence of resistant genotypes across diverse geographic origins aligns with previous reports demonstrating that quantitative resistance to net blotch is widely distributed among landraces and breeding materials from different regions (Vatter *et al.* 2017; Oğuz *et al.* 2019; Wallwork *et al.* 2024).

The merged relative lesion scores (n=20 per genotype), visualised using box plots to assess internal variability and average performance of each genotype, are shown in Figure 5. Genotypes were ordered by ascending mean relative lesion score, with those with the lowest (i.e. most favourable) average scores on the left side of the plot. The visualisation highlights several genotypes (e.g., 109 – ‘Romans’, SUN; 32 – ‘SK-3212’, SVK; 16 – ‘Svit’, SVK) with consistently low lesion scores, suggesting robust resistance. In contrast, genotypes towards the right side of the plot (e.g., 7 – ‘Slovensky kvalitny’, SVK; 26 – ‘P61’, SVK; 30 – ‘SK-2736’, SVK; or 47 – ‘PY-31’, SVK) showed slightly higher mean scores and wider distributions, indicating moderate variability in resistance expression. The identification of a limited subset of consistently resistant genotypes is consistent with the polygenic architecture of resistance to *P. teres* f. *teres*, where multiple small-effect QTL collectively determine the overall phenotype (Friesen *et al.* 2006; Wonneberger *et al.* 2017; Adhikari *et al.* 2019).

## CONCLUSIONS

This study demonstrates that a controlled, PCR-verified *P. teres* f. *teres* inoculation system, coupled with three reference barley genotypes and score normalisation, provides a robust and reproducible pipeline for phenotyping net blotch resistance in a large, diverse barley panel. Across 26 inoculation time points, infection pressure was biologically consistent yet sufficiently discriminatory.

Two independent datasets showed similar distributions, with predominantly one-step (neighbouring class) differences between categorical assignments; most genotypes retained the same class across experiments. Multivariate summaries, together with the tile-map and confusion-matrix views, corroborated this overall concordance while still resolving meaningful genotypic contrasts. Of the 276 genotypes, we identified 34 that consistently exhibited high levels of resistance to *P. teres* f. *teres* across experiments. Among these, three genotypes ('Romans', 'SK-3212', and 'Svit') combined low lesion scores with narrow within-genotype dispersion, making them strong candidates for pre-breeding and cultivar improvement. These data enabled the assignment of initial resistance ratings against net form net blotch to particular barley accessions in the Genetic Resources Information System of Slovakia (GRISS). Future studies should aim to (i) validate these findings using other parameters of resistance (e.g., the duration of the latent period, conidial sporulation) under controlled conditions; (ii) compare these results with responses under field conditions; (iii) characterise the underlying genetic loci; and (iv) integrate the most promising donors into marker-assisted selection pipelines for the development of improved barley cultivars with enhanced resistance to net blotch.

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## REFERENCES

- Abebe, W. (2021). Barley net blotch disease management: A review. *International Journal of Agriculture and Environmental Research*, 7(9), 69–81. Available at: [https://ijoeear.com/assets/articles\\_menuscripts/file/IJOEAR-SEP-2021-20.pdf](https://ijoeear.com/assets/articles_menuscripts/file/IJOEAR-SEP-2021-20.pdf) [Accessed 16 March 2026].
- Adhikari, A., Steffenson, B., Smith, M., and Dill-Macky, R. (2019). Genome-wide association mapping of seedling net form net blotch resistance in an Ethiopian and Eritrean barley collection. *Crop Science*, 59(4), 1625–1638. DOI:10.2135/cropsci2019.01.0003.
- Afanasenkov, O., Hartleb, H., Guseva, N., Minarikova, V., and Janosheva, M. (1995). A set of differentials to characterize populations of *Pyrenophora teres* Drechs. for international use. *Journal of Phytopathology*, 143(8), 501–507. DOI:10.1111/J.1439-0434.1995.Tb04562.X.
- Afanasenkov, O., Mironenko, N., Filatova, O., Kopahnke, D., Krämer, I., and Ordon, F. (2007). Genetics of host-pathogen interactions in the *Pyrenophora teres* f. *teres* (net form)–barley (*Hordeum vulgare*) pathosystem. *European Journal of Plant Pathology*, 117(3), 267–280. DOI:10.1007/s10658-006-9093-5.
- Afanasenkov, O., Jalli, M., Pinnschmidt, H., Filatova, O., and Platz, G. (2009). Development of an international standard set of barley differential genotypes for *Pyrenophora teres* f. *teres*. *Plant Pathology*, 58(4), 665–676. DOI:10.1111/j.1365-3059.2009.02062.x.
- Akhavan, A., Strelkov, S., Kher, S., Askarian, H., Tucker, J., Legge, W., Tekauz, A., and Turkington, T. (2017). Resistance to *Pyrenophora teres* f. *teres* and *P. teres* f. *maculata* in Canadian barley genotypes. *Crop Science*, 57(1), 151–160. DOI:10.2135/cropsci2016.05.0385.
- Backes, A., Guerriero, G., Barka, E.A., and Jacquard, C. (2021). *Pyrenophora teres*: Taxonomy, morphology, interaction with barley, and mode of control. *Frontiers in Plant Science*, 12, 614951. DOI:10.3389/fpls.2021.614951.
- Bakonyi, J. and Justesen, A. (2007). Genetic relationship of *Pyrenophora graminea*, *P. teres* f. *maculata* and *P. teres* f. *teres* assessed by RAPD analysis. *Journal of Phytopathology*, 155(2), 76–83. DOI:10.1111/j.1439-0434.2007.01192.x.
- Bouajila, A., Zoghalmi, N., Ahmed, M., Baum, M., Ghorbel, A., and Nazari, K. (2011). Comparative virulence of *Pyrenophora teres* f. *teres* from Syria and Tunisia and screening for resistance sources in barley: implications for breeding. *Letters in Applied Microbiology*, 53(5), 489–502. DOI:10.1111/j.1472-765x.2011.03127.x.
- Clare, S., Wyatt, N., Brueggeman, R., and Friesen, T. (2019). Research advances in the *Pyrenophora teres*–barley interaction. *Molecular Plant Pathology*, 21(2), 272–288. DOI:10.1111/mpp.12896.
- Deadman, M. and Cooke, B. (1986). A comparison of detached leaf, greenhouse and field experiments for screening barley cultivars to *drechslera-teres*. *Irish Journal Of Agricultural Research*, 25(1), 63–70. Available at: <http://www.jstor.org/stable/25556135> [Accessed 16 Feb. 2026].
- Dinglasan, E., Hickey, L., Ziemis, L., Fowler, R., Anisimova, A., Baranova, O., Lashina, N., and Afanasenkov, O. (2019). Genetic characterization of resistance to *Pyrenophora teres* f. *teres* in the international barley differential Canadian lake shore. *Frontiers in Plant Science*, 10, 326. DOI:10.3389/fpls.2019.00326.
- D'Souza, N., Dinh, H., Lawrence, J., Palmiero, E., Muria-Gonzalez, M., Gupta, S., Forrest, K., and Ellwood, S. (2025). Exploiting barley landraces to reintroduce lost genetic diversity: Resistance to net form net blotch. *Crop Science*, 65(3), e70088. DOI:10.1002/csc2.70088.
- El-Mor, I., Fowler, R., Platz, G., Sutherland, M., and Martin, A. (2018). An improved detached-leaf assay for phenotyping net blotch of barley caused by *Pyrenophora teres*. *Plant Disease*, 102(4), 760–763. DOI:10.1094/pdis-07-17-0980-re.
- Ellwood, S., Syme, R., Moffat, C., and Oliver, R. (2012). Evolution of three *Pyrenophora* cereal pathogens: Recent divergence, speciation and evolution of non-coding DNA. *Fungal Genetics and Biology*, 49(10), 825–829. DOI:10.1016/j.fgb.2012.07.003.
- Friesen, T., Faris, J., Lai, Z., and Steffenson, B. (2006). Identification and chromosomal location of major genes for resistance to *Pyrenophora teres* in a doubled-haploid barley pop-

- ulation. *Genome*, 49(7), 855–859. DOI:10.1139/g06-024.
- Hammer, Ø., Harper, D. and Ryan, P. (2001). PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, 4, 1–9. Available at: [http://palaeo-electronica.org/2001\\_1/past/issue1\\_01.htm](http://palaeo-electronica.org/2001_1/past/issue1_01.htm) [Accessed 5 March 2026].
- Hassett, K., Muria-Gonzalez, M., Martin, A., Karakaya, A., Oğuz, A., Bakonyi, J., Knight, N., Prins, R. and Ellwood, S. (2024). Global spread, genetic differentiation, and selection of barley spot form net blotch isolates. *Phytopathology*, 114(7), 1542–1553. DOI:10.1094/phyto-11-23-0442-r.
- Jönsson, R., Bryngelsson, T., Jalli, M., and Gustafsson, M. (1998). Effect of Growth Stage on Resistance to *Drechslera teres* f. *teres* in Barley. *Journal of Phytopathology*, 146(5–6), 261–265. DOI:10.1111/j.1439-0434.1998.tb04688.x.
- Jordan, V. and Allen, E. (1984). Barley net blotch: influence of straw disposal and cultivation methods on inoculum potential, and on incidence and severity of autumn disease. *Plant Pathology*, 33, 547–559. DOI:10.1111/j.1365-3059.1984.tb02879.x.
- Jørgensen, J., Bech, C. and Jensen, J. (2000). Reaction of European spring barley varieties to a population of the net blotch fungus. *Plant Breeding*, 119(1), 43–46. DOI:10.1046/j.1439-0523.2000.00455.x.
- Mathre, D. (1982). *Compendium of Barley Diseases*. American Phytopathology Society, St. Paul, Minnesota, USA. 78 p.
- Oğuz, A., Rahimi, A. and Karakaya, A. (2019). Seedling response of iranian barley landraces to *Pyrenophora teres* f. *teres* and *Pyrenophora teres* f. *maculata*. *Journal of Agricultural Sciences*, 25(1), 86–92. DOI:10.15832/ankutbd.539006.
- Poudel, B., Vaghefi, N., McLean, M., Platz, G., Sutherland, M., and Martin, A. (2019). Genetic structure of a *Pyrenophora teres* f. *teres* population over time in an Australian barley field as revealed by Diversity Arrays Technology markers. *Plant Pathology*, 68(7), 1331–1336. DOI:10.1111/ppa.13035.
- Robinson, J. and Jalli, M. (1997). Grain yield, net blotch and scald of barley in Finnish official variety trials. *Agricultural and Food Science*, 6(5–6), 399–408. DOI:0.23986/afsci.72803.
- Rožanova, I., Lashina, N., Mustafin, Z., Gorobets, S., Efimov, V., Afanasenko, O., and Khlestkina, E. (2019). SNPs associated with barley resistance to isolates of *Pyrenophora teres* f. *teres*. *BMC Genomics*, 20(S3), 292. DOI:10.1186/s12864-019-5623-3.
- Sharma, H. (1984). Assessment of the reaction of some spring barley cultivars to *Pyrenophora teres* using whole plants, detached leaves and toxin bioassay. *Plant Pathology*, 33(3), 371–376. DOI:10.1111/j.1365-3059.1984.tb01332.x.
- Tekauz, A. (1985). A numerical scale to classify reactions of barley to *Pyrenophora teres*. *Canadian Journal of Plant Pathology*, 7(2), 181–183. DOI: 10.1080/07060668509501499
- Tomić, A., Trkulja, V., Matić, S., Trkulja, N., Ilić, R., Scortichini, M., and Milovanović, T. (2024). Net blotch (*Pyrenophora teres* Drechsler): An increasingly significant threat to barley production. *Plant Protection Science*, 60, 1–30. DOI:10.17221/122/2023-pps.
- Tuohy, J., Jalli, M., Cooke, B., and Sullivan, E. (2006). Pathogenic variation in populations of *Drechslera teres* f. *teres* and *D. teres* f. *maculata* and differences in host cultivar responses. *European Journal of Plant Pathology*, 116, 177–185. DOI:10.1007/s10658-006-9001-z.
- Vahamidis, P., Stefopoulou, A., Lagogianni, C., Economou, G., Dercas, N., Kotoulas, V., Kalivas, D., and Tsitsigiannis, D. (2020). *Pyrenophora teres* and *Rhynchosporium secalis* establishment in a Mediterranean malt barley field: assessing spatial, temporal and management effects. *Agriculture*, 10(11), 553. DOI:10.3390/agriculture10110553.
- Vatter, T., Maurer, A., Kopahnke, D., Perović, D., Ordon, F. and Pillen, K. (2017). A nested association mapping population identifies multiple small effect QTL conferring resistance against net blotch (*Pyrenophora teres* f. *teres*) in wild barley. *Plos One*, 12(10), e0186803. DOI:10.1371/journal.pone.0186803.
- Wallwork, H., Vassos, E., Garrard, T., Butt, M., Abood, E., Szajder, B., and Mather, D. (2024). Adult-plant resistance to net form net blotch in barley (*Hordeum vulgare* L.): Durability and genetic control. *Plant Breeding*, 143(4), 587–596. DOI:10.1111/pbr.13191.
- Williams, K., Smyl, C., Lichon, A., Wong, K., and Wallwork, H. (2001). Development and use of an assay based on the polymerase chain reaction that differentiates the pathogens causing spot form and net form of net blotch of barley. *Australasian Plant Pathology*, 30, 37–44. DOI:10.1071/AP00063.
- Wonneberger, R., Ficke, A. and Lillemo, M. (2017). Identification of quantitative trait loci associated with resistance to net form net blotch in a collection of Nordic barley germplasm. *Theoretical and Applied Genetics*, 130(10), 2025–2043. DOI:10.1007/s00122-017-2940-2.
- Zia, R., Popper, Z. and Kildea, S. (2025). Barley Disease screening: A multiplex digital droplet PCR approach for the detection of *Ramularia collo-cygni*, *Rhynchosporium graminicola* and *Pyrenophora teres*. *Plant Pathology*, 74(6), 1752–1762. DOI:10.1111/ppa.14123.

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