

**Two indigenous *Berberis* species from Spain were confirmed functional alternate hosts of the yellow rust fungus *Puccinia striiformis* f.sp. *tritici***

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**Abstract**

*Puccinia striiformis* f.sp. *tritici* (*Pst*), which causes yellow (or stripe) rust on wheat, is a macrocyclic and heteroecious fungus. In this study, we investigated whether *Berberis vulgaris* subsp. *seroi* and *B. vulgaris* subsp. *australis*, which are indigenous in Spain, may serve as alternate hosts for *Pst*. Wheat leaves bearing telia of an isolate of *Pst* were harvested and used to inoculate plants of both barberry subspecies. Pycnia were observed on the adaxial side of the leaves from 10 days after inoculation (dai). Following successful fertilisation, aecia were observed on the abaxial side of the leaves from 16 dai. At 27 dai, barberry leaves bearing aecia were detached and used to inoculate susceptible wheat seedlings of cultivar ‘Morocco’. Uredinia were observed on wheat seedlings from 12 days after aeciospore exposure. Eighty-three single lesions were recovered from individual wheat leaves, of which 43 were genotyped using 19 *Pst* simple sequence repeat

markers (SSR). In total, 19 multilocus genotypes (MLGs) were identified among the 43 progeny isolates. The SSR genotyping confirmed that all 43 isolates were derived from the parental isolate. Seven heterozygous SSR markers showed segregation among the progenies, whereas none of the 12 homozygous markers resulted in segregation. These results demonstrated that *B. vulgaris* subsp. *seroi* and *australis* can serve as alternate hosts for the yellow rust fungus, which may result in novel virulence combinations that can have a detrimental impact on wheat production. Although *Pst* has not been detected on these barberry species in nature, this study highlights the importance of rust surveillance in barberry areas where suitable conditions for completion of the sexual life cycle may be present.

Keywords. Alternate host, barberry, segregation, sexual reproduction, stripe rust, wheat

Heteroecious cereal rust fungi within the genus *Puccinia* require botanically distant primary and alternate hosts to complete the life cycle involving both asexual and sexual reproduction (Jin et al. 2010; Stubbs 1985a). Sexual reproduction on the alternate host possesses advantages of the generation of novel genotypes (and races), which may have detrimental effects on the durability of rust resistance in wheat cultivars (Roelfs and Groth 1980; Zhao et al. 2016). *Puccinia striiformis* f.sp. *tritici* (*Pst*), which causes yellow (or stripe) rust on wheat, is considered a serious threat to a stable wheat supply (Beddow et al. 2015; Singh et al. 2016). The search for the alternate host of *Pst* date back to the nineteen and twenty centuries, where teliospores were used to inoculate plant species of the *Berberidacea* family. However, all attempts were unsuccessful (Eriksson & Henning, 1894, Tranzschel, 1934, Straib, 1937 cited in Stubbs 1985b). The reasons behind the failure in finding the alternate host of *Pst* at that time may be manifold, e.g., failure in establishing suitable conditions for infection, the use of *Pst* races with low sexual capacity

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and the use of non-susceptible plant species. Recently, Jin et al. (2010) discovered that aeciospores from *B. chinensis* and *B. koreana* could infect *Poa pratensis* (Kentucky bluegrass) and identified that *P. pseudostriiformis* (syn. *P. striiformis* f.sp. *poae*), as the rust specie involved. Further experiments confirmed that teliospores of *Pst* were able to infect *B. chinensis*, *B. koreana*, *B. holstii*, and *B. vulgaris* and that aeciospores from *B. chinensis* infected wheat seedlings. Thus, the yellow rust fungus was confirmed heteroecious and macrocyclic represented by five spore stages, *i.e.*, uredia, telia, basidia, pycnia and aecia. About 500 barberry species have been described within the genus *Berberis* (Kim et al. 2004). To date, approximately 50 *Berberis* spp. have been confirmed susceptible to *Pst* under natural or experimental conditions (Li et al. 2020; Mehmood et al. 2020). Additionally, *Mahonia aquifolium* (Oregon grape) has also been identified as alternate host for *Pst* under experimental conditions (Wang and Chen 2013). Similarly, natural aecial infections collected in Western China from *B. aggregata*, *B. brachypoda*, *B. polyantha*, *B. shensiana* and *B. soulieana* have been reported as *Pst*, but only at low frequencies (Wang et al. 2016; Zhao et al. 2013). This may suggest that proper conditions for sexual reproduction may exist in certain parts of Asia, where barberry is ubiquitous and frequently growing close to wheat crops. However, the role of *Berberis* species as alternate host of *Pst* in other parts of the world is still unclear. In Europe, *B. vulgaris* (also known as European or common barberry) has historically been used as a hedge shrub to avoid livestock from damaging cereal crops and for medicinal and ornamental purposes since it was introduced from Asia in the Middle Ages (Barnes et al. 2020; Hermansen 1968; Roelfs 1982). Since the seventeenth century, eradication and legislation campaigns were established to remove barberry species and particularly common

barberry, aiming to reduce the novel inoculum of *P. graminis* (the stem rust fungus infecting cereal and grasses) generated after sexual reproduction (Hermansen 1968; Stakman 1923). Currently, common barberry is widely distributed throughout Europe (iNaturalist 2021) due to the abolishment of eradication programs and legislation restricting the planting of *B. vulgaris*. Recently, Rodriguez-Algaba et al. (2020); (2014) reported that *Pst* isolates of exotic origin can undergo sexual reproduction under experimental conditions using *B. vulgaris* plants collected in Denmark and Sweden from natural areas. Although *Pst* has not yet been found to reproduce sexually in Europe under natural conditions, the high prevalence of common barberry could initiate the infection by *Pst* races of sexual origin (characterized by high teliospore production) and that have been widely detected across Europe since 2011 (Ali et al. 2010; Ali et al. 2017; Hovmøller et al. 2016). Besides *B. vulgaris*, indigenous barberry species, e.g., *B. catartica* in Croatia (Kosalec et al. 2009), *B. cretica* in Greece, *B. aetnensis* in Italy (Ahrendt 1961), and *B. vulgaris* subsp. *seroi* (syn. *B. garciae*) and *B. vulgaris* subsp. *australis* (syn. *B. hispanica*) in Spain (López González 1986) are present in Europe. In this study, we investigated whether *B. vulgaris* subsp. *seroi* and *B. vulgaris* subsp. *australis* may serve as alternate hosts for *Pst*. Better knowledge of the role of indigenous barberry species in rust epidemiology in Europe is crucial as these could potentially contribute to increase genetic diversity in rust populations and thereby pose a threat to European wheat production.

Seeds of *B. vulgaris* subsp. *seroi* and *australis* were collected from Huesca and Albacete provinces in Spain, respectively, where these barberry subspecies grow naturally.

*Berberis vulgaris* subsp. *seroi* is found at altitudes from 470 to 2000 m, occasionally growing close to cereal crops. *Berberis vulgaris* subsp. *australis* grows at altitudes from 1000 to 2500 m, but it is infrequently found in close proximity to cereal crops (López González 1986). Species identification of barberry plants were confirmed based on specific morphological characters, e.g., colour of one year old stems, size of racemes, colour of matured fruits, type and morphology of leaves (López González 1986). After harvesting, seeds were dried at room temperature. Ten seeds of each *B. vulgaris* subspecies were placed in petri dishes containing a peat-based substrate and stratified at 5°C for approximately three months to promote seed germination. After germination, seedlings were transferred to 10 cm square pots containing an organic peat-based substrate with slow release of plant nutrients and grown in rust quarantine spore-proof greenhouse cabins. Subsequently, barberry plants were grown for approximately one year until specific morphological characters used for species identification were clearly visible.

The sexual life cycle of *Pst* was completed according to Rodríguez-Algaba et al. (2014), with slight modifications. Yellow rust infected wheat leaves bearing uredinia and telia were harvested from an experimental field nursery previously inoculated with *Pst* isolate DK219\_19, originally collected in Denmark (2019) from wheat cultivar 'Ambition' and characterised by a high production of teliospores on seedlings and adult plants. The wheat leaves were kept dried at 5°C until further use. Telia germination was initiated by soaking the dried leaves bearing telia in distilled water at room temperature (22-25°C) for two days, followed by incubation on 2.5% water agar containing 50 mg/l chloramphenicol

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4 120 at 12°C for two days in darkness. Microscopic observations confirmed that basidiospores  
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6 121 were produced after two days of incubation. Inverted agar plates containing germinating  
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8 122 teliospores were placed on top of open-ended transparent plastic cylinders each  
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10 123 containing barberry plants of a single barberry subspecies and incubated at 12°C for 4  
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12 124 days in darkness, 100% relative humidity (RH). Pycnia were observed on the adaxial leaf  
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14 125 surface from 10 days after inoculation (dai) (Fig. 1a and 1e). Infected plants were regularly  
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16 126 sprayed with mist water to facilitate fertilization of pycnia. Aecia were observed on the  
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18 127 abaxial leaf surface from 16 dai (Fig. 1b and 1f). The recovery of aeciospores was carried  
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20 128 out by exposing susceptible wheat seedlings of cultivar 'Morocco' below barberry leaves  
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22 129 bearing multiple aecial clusters at 27dai. The wheat seedlings were incubated at 10°C for  
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24 130 24h in darkness, 100% RH, and transferred to spore-proof cabins under the conditions  
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26 131 described in Rodriguez-Algaba et al. (2014). Subsequently, new wheat seedlings were  
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28 132 placed below the infected barberry leaves and incubated as described above. Uredinia  
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30 133 and telia were observed on wheat seedlings from 12 and 28 days after aeciospore  
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32 134 exposure, respectively (Fig. 1c and 1g; 1d and 1h). Variability in telia production was  
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34 135 observed among the progeny isolates (data not shown). From 14 to 22 days after  
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36 136 aeciospore exposure, 83 clearly separated single lesions bearing uredia (59 derived from  
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38 137 *B. vulgaris* subsp. *seroi* and 24 from *B. vulgaris* subsp. *australis*, respectively) were  
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40 138 collected from wheat leaves. A total of 43 single lesions (19 derived from *B. vulgaris*  
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42 139 subsp. *seroi* and 24 from *B. vulgaris* subsp. *australis*) were selected for genotypic analysis  
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44 140 using 19 *Pst* simple sequence repeat (SSR) markers according to Rodriguez-Algaba et  
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46 141 al. (2014) (Table 1). Telia representing the parental isolate was included as control in the  
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48 142 SSR marker analysis. New alleles were not observed among the progeny isolates, in  
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4 143 comparison to the parental isolate, confirming that all progeny isolates were indeed  
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6 144 descendants of the parental isolate. The parental isolate was homozygous at 12 loci and  
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8 145 heterozygous at 7 loci, which revealed segregation among the progenies resulting in 19  
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10 146 novel multilocus genotypes (MLGs) (Table 1, Fig. 2). The identification of the MLGs was  
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12 147 carried out using the Poppr genetic analysis software for sexual populations (Kamvar et  
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14 148 al. 2014). Progeny isolate DK219\_19\_F1 (MLG number 8) shared MLG with the parental  
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16 149 isolate. Other progeny isolates also shared MLG with one another, suggesting that some  
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18 150 progeny isolates originated from the same fertilisation event at the pycnial stage and thus  
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20 151 from spores produced within a single aecial cup (a.k.a. aecium) (Table 1, Fig. 2)  
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22 152 (Rodriguez-Algaba et al. 2017). Particularly, progenies derived from *B. vulgaris* subsp.  
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24 153 *australis* showed fewer MLGs compared to *B. vulgaris* subsp. *seroi*, which may be  
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26 154 explained by the relatively few aecia present on the leaves of the former being used for  
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28 155 aeciospore recovery on wheat seedlings.  
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36 157 This study demonstrated that *B. vulgaris* subsp. *seroi* and *B. vulgaris* subsp. *australis* are  
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38 158 susceptible to *Pst* and may serve as alternate hosts for this important wheat rust  
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40 159 pathogen. Although there are no indications of sexual recombination in the contemporary  
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42 160 European *Pst* population (Hovmøller et al. 2016), the results stress the importance of rust  
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44 161 surveillance in areas where barberry and wheat coexist and appropriate environmental  
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46 162 and physiological conditions for completion of the sexual cycle of *Pst* are present. Further  
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48 163 studies aiming to investigate the role of alternate hosts (barberry species), primary host  
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50 164 (wheat) and auxiliary hosts (wild cereal and grasses) would help to better understand the  
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52 165 epidemiology of *Pst* in Europe. In conclusion, sexual reproduction occurring on  
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indigenous *Berberis* subspecies, such as the ones present in Spain, could result in novel and unique virulence combinations (Rodriguez-Algaba et al. 2020; Zhao et al. 2016) that may have negative consequences for wheat production in Europe and beyond.

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## References

- Ahrendt, L. W. A. 1961. *Berberis* and *Mahonia*. A taxonomic revision. J. Linn. Soc. (Bot.) 57.
- Ali, S., Leconte, M., Walker, A. S., Enjalbert, J., and de Vallavieille-Pope, C. 2010. Reduction in the sex ability of worldwide clonal populations of *Puccinia striiformis* f.sp. *tritici*. Fungal Genetics and Biology 47:828-838.
- Ali, S., Rodriguez-Algaba, J., Thach, T., Sørensen, C. K., Hansen, J. G., Lassen, P., Nazari, K., Hodson, D. P., Justesen, A. F., and Hovmøller, M. S. 2017. Yellow Rust Epidemics Worldwide Were Caused by Pathogen Races from Divergent Genetic Lineages. Frontiers in Plant Science 8.
- Barnes, G., Saunders, D. G. O., and Williamson, T. 2020. Banishing barberry: The history of *Berberis vulgaris* prevalence and wheat stem rust incidence across Britain. Plant Pathology 69:1193-1202.
- Beddow, J. M., Pardey, P. G., Chai, Y., Hurley, T. M., Kriticos, D. J., Braun, H. J., Park, R. F., Cuddy, W. S., and Yonow, T. 2015. Research investment implications of shifts in the global geography of wheat stripe rust. Nat Plants 1:15132.
- Hermansen, J. E. 1968. Studies on the Spread and Survival of Cereal Rust and Mildew Diseases in Denmark The Royal Veterinary and Agricultural University, Copenhagen.
- Hovmøller, M. S., Walter, S., Bayles, R. A., Hubbard, A., Flath, K., Sommerfeldt, N., Leconte, M., Czembor, P., Rodriguez-Algaba, J., Thach, T., Hansen, J. G., Lassen, P., Justesen, A. F., Ali, S., and de Vallavieille-Pope, C. 2016. Replacement of the European wheat yellow rust population by new races from the centre of diversity in the near-Himalayan region. Plant Pathology 65:402-411.
- iNaturalist. 2021. Available from <https://www.inaturalist.org>. Accessed 03.02.2021.
- Jin, Y., Szabo, L. J., and Carson, M. 2010. Century-old mystery of *Puccinia striiformis* life history solved with the identification of *Berberis* as an alternate host. Phytopathology 100:432-435.
- Kamvar, Z. N., Tabima, J. F., and Grunwald, N. J. 2014. Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ 2:e281.
- Kim, Y. D., Kim, S. H., and Landrum, L. R. 2004. Taxonomic and phytogeographic implications from ITS phylogeny in *Berberis* (Berberidaceae). J Plant Res 117:175-182.

Kosalec, I., Gregurek, B., Kremer, D., Zovko, M., Sankovic, K., and Karlovic, K. 2009. Croatian barberry (*Berberis croatica* Horvat): a new source of berberine—analysis and antimicrobial activity. *World J Microbiol Biotechnol* 25:145–150.

Li, S., Chen, W., Ma, X., Tian, X., Liu, Y., Huang, L., Kang, Z., and Zhao, J. 2020. Identification of eight *Berberis* species from the Yunnan-Guizhou plateau as aecial hosts for *Puccinia striiformis* f.sp. *tritici*, the wheat stripe rust pathogen. *Journal of Integrative Agriculture* 19(0):2–8.

López González, G. 1986. *Berberidaceae*. Pages 402-405 in: *Flora Iberica*, vol. 1. G. López González, ed. Real Jardín Botánico, CSIC, Madrid, Spain.

Mehmood, S., Sajid, M., Zhao, J., Huang, L., and Kang, Z. 2020. Alternate Hosts of *Puccinia striiformis* f. sp. *tritici* and Their Role. *Pathogens* 9.

Rodriguez-Algaba, J., Hovmøller, M. S., and Justesen, A. F. 2020. Sexual recombination within the “Kranich” race of the yellow rust fungus *Puccinia striiformis* f.sp. *tritici* on *Berberis vulgaris*. *European Journal of Plant Pathology* 156:1169–1173.

Rodriguez-Algaba, J., Walter, S., Sorensen, C. K., Hovmoller, M. S., and Justesen, A. F. 2014. Sexual structures and recombination of the wheat rust fungus *Puccinia striiformis* on *Berberis vulgaris*. *Fungal Genetics and Biology* 70:77-85.

Rodriguez-Algaba, J., Sørensen, C. K., Labouriau, R., Justesen, A. F., and Hovmøller, M. S. 2017. Genetic diversity within and among aecia of the wheat rust fungus *Puccinia striiformis* on the alternate host *Berberis vulgaris*. *Fungal biology* 12:541-549.

Roelfs, A. P. 1982. Effects of barberry eradication on stem rust in the United States. *Plant Dis.* 66:177–181.

Roelfs, A. P., and Groth, V. J. 1980. A comparison of virulence phenotypes in wheat stem rust populations reproducing sexually and asexually. *Phytopathology* 70:855–862.

Singh, R. P., Singh, P. K., Rutkoski, J., Hodson, D. P., He, X., Jorgensen, L. N., Hovmoller, M. S., and Huerta-Espino, J. 2016. Disease Impact on Wheat Yield Potential and Prospects of Genetic Control. *Annu Rev Phytopathol* 54:303-322.

Stakman, E. C. 1923. Barberry eradication prevents black rust in Western Europe. *United States Department of Agriculture*:1–15.

Stubbs, R. W. 1985a. *The Cereal Rusts*. A.P. Roelfs & W.R. Bushnell, Academic Press, Orlando, FL.

Stubbs, R. W. 1985b. *Stripe Rust*. Pages 77-115 in: *The Cereal Rusts Vol. II: Diseases, Distribution, Epidemiology, and Control* A.P. Roelfs & W.R. Bushnell, Academic Press, Orlando, FL.

Wang, M. N., and Chen, X. M. 2013. First report of Oregon grape (*Mahonia aquifolium*) as an alternate host for the white stripe rust pathogen (*Puccinia striiformis* f. sp. *tritici*) under artificial inoculation *Plant Disease* 97:839.

Wang, Z., Zhao, J., Chen, X., Peng, Y., Ji, J., Zhao, S., Lv, Y., Huang, L., and Kang, Z. 2016. Virulence Variations of *Puccinia striiformis* f. sp. *tritici* Isolates Collected from *Berberis* spp. in China. *Plant Disease* 100:131-138.

Zhao, J., Wang, M., Chen, X., and Kang, Z. 2016. Role of Alternate Hosts in Epidemiology and Pathogen Variation of Cereal Rusts. *Annual Review of Phytopathology* 54:207-228.

Zhao, J., Wang, L., Wang, Z., Chen, X., Zhang, H., Yao, J., Zhan, G., Chen, W., Huang, L., and Kang, Z. 2013. Identification of eighteen *Berberis* species as alternate hosts of *Puccinia striiformis* f. sp. *tritici* and virulence variation in the pathogen isolates from natural infection of barberry plants in China. *Phytopathology* 103:927-934.

**Legend**

**Fig. 1.** Pycnia and aecia developed on *Berberis vulgaris* subspp. *seroi* (top) and *australis* (bottom) and uredinia and telia observed on wheat seedlings, **a,e**: Pycnia observed on the adaxial side of the leaf at 10 days after inoculation (dai), **b,f**: Aecia observed on the abaxial side of the leaf at 20 dai, **c,g**: Uredinia observed on wheat seedlings of cultivar 'Morocco' at 14 days after aeciospore recovery, **d,h**: Telia observed on wheat seedlings of cultivar 'Morocco' at 28 days after aeciospore recovery.

**Fig. 2.** Minimum distance spanning tree of the parental isolate (in yellow) and the 19 multilocus genotypes (MLG) detected among the 43 progeny isolates. Sizes of nodes are proportional to the number of progeny isolates detected on each MLG, which are indicated by different colours.

**Table 1.** Sizes of a1 and a2 alleles given in base pairs at twelve homozygous and seven heterozygous (in bold) simple sequence repeat (SSR) loci in the parental isolate (DK219\_19) and 43 progeny isolates of *Puccinia striiformis* f.sp. *tritici*. The number of segregated SSR loci and the barberry subspecies from which the progeny isolates were originated are also indicated.

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Isolate(s)	Barberry subspecie	No. of segregated SSR loci	a1	a2
DK219_19 (Parental isolate)	-	-	199	199
DK219_19_F1	seroi	0	199	199
DK219_19_F2, F9	seroi	4	199	199
DK219_19_F3	seroi	3	199	199
DK219_19_F4	seroi	4	199	199
DK219_19_F5	seroi	3	199	199
DK219_19_F6	seroi	4	199	199
DK219_19_F7	seroi	3	199	199
DK219_19_F8	seroi	3	199	199
DK219_19_F10	seroi	1	199	199
DK219_19_F11	seroi	2	199	199
DK219_19_F12, F18, F20	seroi	3	199	199
DK219_19_F13	seroi	5	199	199
DK219_19_F14	seroi	1	199	199
DK219_19_F16	seroi	3	199	199
DK219_19_F17	seroi	2	199	199
DK219_19_F19	seroi	3	199	199
DK219_19_F51, F52, F53, F54, F55, F56, F57, F58, F59, F60, F61, F67, F68, F70	australis	1	199	199
DK219_19_F74, F75	australis	4	199	199
DK219_19_F76, F77, F78, F79, F80, F81, F82, F83	australis	3	199	199

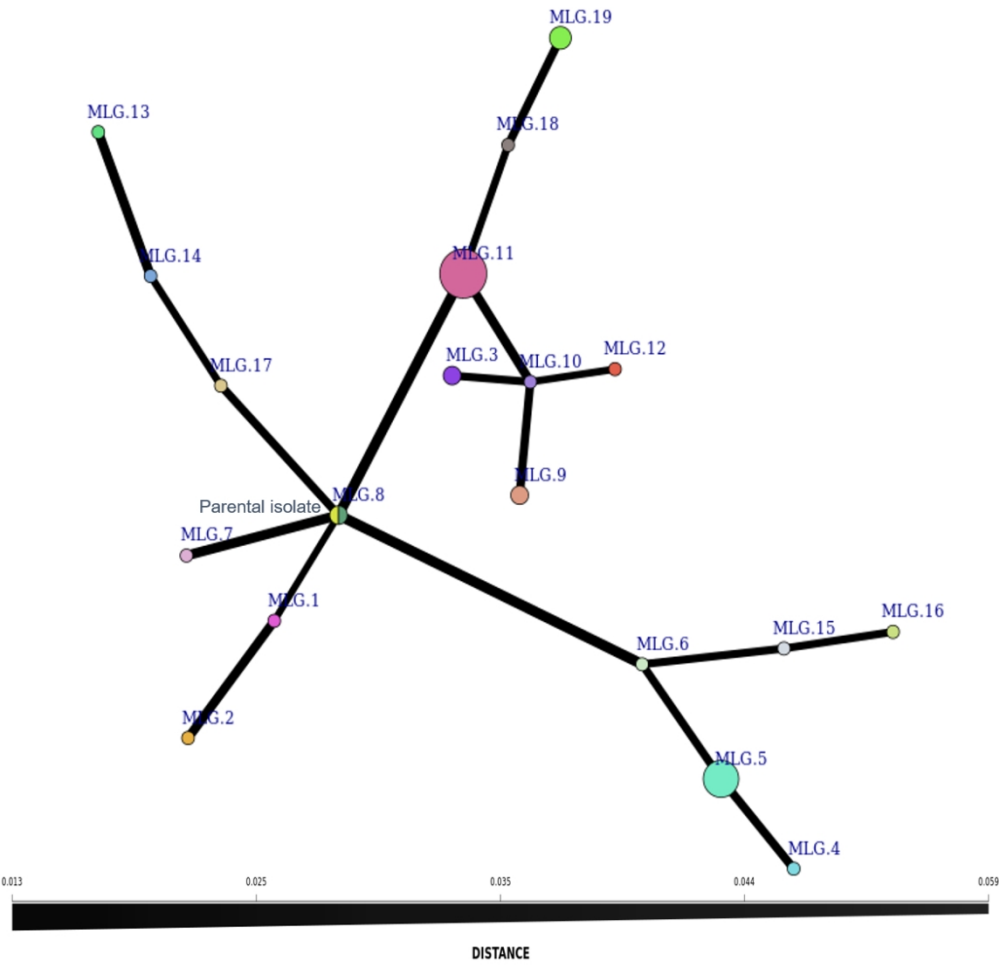
	RJO24		RJN12		RJN8		RJN13		RJN3		RJN11		RJO27		RJN6		RJO21		RJN10		RJO18		WU6		RJO20	
	a1	a2	a1	a2	a1	a2	a1	a2	a1	a2	a1	a2	a1	a2	a1	a2	a1	a2	a1	a2	a1	a2	a1	a2	a1	a2
6	284	293	196	196	307	316	147	147	336	336	176	180	242	242	318	318	170	170	221	224	331	331	210	210	284	287
7	284	293	196	196	307	316	147	147	336	336	176	180	242	242	318	318	170	170	221	224	331	331	210	210	284	287
8	284	293	196	196	316	316	147	147	336	336	180	180	242	242	318	318	170	170	221	221	331	331	210	210	284	287
9	284	284	196	196	307	316	147	147	336	336	176	180	242	242	318	318	170	170	224	224	331	331	210	210	284	287
10	293	293	196	196	316	316	147	147	336	336	176	180	242	242	318	318	170	170	221	224	331	331	210	210	284	284
11	284	284	196	196	307	307	147	147	336	336	176	180	242	242	318	318	170	170	221	221	331	331	210	210	284	287
12	284	284	196	196	316	316	147	147	336	336	180	180	242	242	318	318	170	170	221	224	331	331	210	210	287	287
13	284	293	196	196	307	316	147	147	336	336	180	180	242	242	318	318	170	170	221	224	331	331	210	210	287	287
14	284	284	196	196	316	316	147	147	336	336	176	180	242	242	318	318	170	170	221	224	331	331	210	210	287	287
15	284	293	196	196	307	316	147	147	336	336	176	180	242	242	318	318	170	170	224	224	331	331	210	210	284	287
16	284	284	196	196	307	316	147	147	336	336	176	180	242	242	318	318	170	170	221	224	331	331	210	210	284	284
17	284	284	196	196	307	307	147	147	336	336	176	176	242	242	318	318	170	170	221	224	331	331	210	210	284	287
18	284	293	196	196	307	316	147	147	336	336	176	176	242	242	318	318	170	170	221	221	331	331	210	210	287	287
19	284	293	196	196	307	316	147	147	336	336	176	180	242	242	318	318	170	170	221	224	331	331	210	210	284	287
20	284	284	196	196	307	316	147	147	336	336	176	180	242	242	318	318	170	170	224	224	331	331	210	210	284	287
21	284	293	196	196	307	316	147	147	336	336	176	180	242	242	318	318	170	170	221	221	331	331	210	210	284	287
22	284	293	196	196	307	316	147	147	336	336	176	180	242	242	318	318	170	170	224	224	331	331	210	210	284	287
23	284	293	196	196	307	316	147	147	336	336	176	180	242	242	318	318	170	170	221	221	331	331	210	210	284	287
24	293	293	196	196	316	316	147	147	336	336	176	180	242	242	318	318	170	170	221	224	331	331	210	210	284	284
25	284	293	196	196	307	316	147	147	336	336	176	180	242	242	318	318	170	170	221	221	331	331	210	210	284	287
26	284	293	196	196	307	316	147	147	336	336	176	180	242	242	318	318	170	170	221	221	331	331	210	210	287	287
27	284	293	196	196	307	316	147	147	336	336	176	180	242	242	318	318	170	170	224	224	331	331	210	210	287	287

RJN2		RJN4		RJN9		RJN5		WU12	
a1	a2	a1	a2	a1	a2	a1	a2	a1	a2
181	187	253	255	332	332	226	226	332	332
181	187	253	255	332	332	226	226	332	332
187	187	253	255	332	332	226	226	332	332
181	181	253	255	332	332	226	226	332	332
181	181	253	255	332	332	226	226	332	332
181	187	253	255	332	332	226	226	332	332
181	187	253	255	332	332	226	226	332	332
187	187	253	255	332	332	226	226	332	332
181	187	253	255	332	332	226	226	332	332
181	187	253	255	332	332	226	226	332	332
181	187	253	255	332	332	226	226	332	332
181	187	253	255	332	332	226	226	332	332
181	187	253	255	332	332	226	226	332	332
181	187	253	255	332	332	226	226	332	332
181	187	253	255	332	332	226	226	332	332
181	187	253	255	332	332	226	226	332	332
181	187	253	255	332	332	226	226	332	332
181	187	253	255	332	332	226	226	332	332
181	187	253	255	332	332	226	226	332	332
181	187	253	255	332	332	226	226	332	332
187	187	255	255	332	332	226	226	332	332
181	187	253	253	332	332	226	226	332	332
187	187	253	255	332	332	226	226	332	332
181	187	253	255	332	332	226	226	332	332
181	187	253	255	332	332	226	226	332	332
187	187	255	255	332	332	226	226	332	332
187	187	253	255	332	332	226	226	332	332



Pycnia and aecia developed on *Berberis vulgaris* subspp. *seroi* (top) and *australis* (bottom) and uredinia and telia observed on wheat seedlings, a,e: Pycnia observed on the adaxial side of the leaf at 10 days after inoculation (dai), b,f: Aecia observed on the abaxial side of the leaf at 20 dai, c,g: Uredinia observed on wheat seedlings of cultivar 'Morocco' at 14 days after aeciospore recovery, d,h: Telia observed on wheat seedlings of cultivar 'Morocco' at 28 days after aeciospore recovery.

233x184mm (150 x 150 DPI)



Minimum distance spanning tree of the parental isolate (in yellow) and the 19 multilocus genotypes (MLG) detected among the 43 progeny isolates. Sizes of nodes are proportional to the number of progeny isolates detected on each MLG, which are indicated by different colours.

198x190mm (150 x 150 DPI)