

Sexual recombination within the “Kranich” race of the yellow rust fungus *Puccinia striiformis* f.sp. *tritici* on *Berberis vulgaris*

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Abstract

An isolate of the “Kranich” race of the basidiomycete *Puccinia striiformis* f.sp. *tritici* (*Pst*), which causes yellow (stripe) rust on wheat, was selfed on *Berberis vulgaris*, the alternate (sexual) host for several rust fungi infecting cereals and grasses. Since 2011, the “Kranich” race has been detected in several European countries and it has contributed to the replacement of the pre-existing European *Pst* population. A sexual origin of the “Kranich” race has been suggested due to the high capacity of teliospore production and the genetic similarity to the populations in the centre of diversity of *Pst* in the near-Himalayan region. For the completion of the sexual life cycle, *B. vulgaris* was inoculated with basidiospores derived from germinating teliospores. Pycnia appeared on the adaxial side of the leaves 8 days after inoculation (dai) and subsequent fertilization resulted in the development of aecia on the abaxial side of the leaves from 15 dai. Inoculation of wheat seedlings with aeciospores from bulked aecia resulted in 124 progeny isolates. Twenty-three of these were randomly selected and genotyped using 19 simple sequence repeat (SSR) markers, which confirmed the parental origin of the progeny isolates. Eight heterozygous markers in the parental isolate revealed segregation in the progenies resulting in 18 novel multilocus genotypes, confirming recombination following sexual reproduction. This study demonstrated a high sexual capacity of the “Kranich” race, which highlights the risks associated with sexual reproduction under suitable conditions for rust development.

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

Puccinia striiformis f.sp. *tritici* (*Pst*), which causes yellow (stripe) rust on wheat, is a macrocyclic rust fungus, which requires an alternate (sexual) host to complete its life cycle (Jin et al. 2010). The sexual life cycle of *Pst* has been a mystery until the discovery of *Berberis* species as the alternate host (Jin et al. 2010). *Mahonia aquifolium* (Oregon grape), belonging to the *Berberidaceae* family, has also been reported to serve as an alternate host for *Pst* under experimental conditions (Wang and Chen 2013). Presently, over thirty barberry species have proven susceptible to *Pst* under experimental or natural conditions (Wang et al. 2016; Zhao et al. 2013; Zhao et al. 2016). Sexual reproduction is considered advantageous regarding the generation of novel and adaptive genetic combinations potentially carrying virulence to the deployed resistance genes (Roelfs and Groth 1980). However, the sexual capacity, measured as the ability to produce teliospores, may vary among *Pst* races of different origin. Particularly, *Pst* races from recombinant populations produced more teliospores compared to races from clonal populations (Ali et al. 2010). Here, we demonstrated that an isolate of the “Kranich” race was able to undergo sexual reproduction resulting in genetically diverse progeny isolates after a selfing on the alternate host, *Berberis vulgaris*. The “Kranich” race was detected in Sweden in 2011 along with two other races of exotic origin, i.e., “Warrior” and aggressive “Triticale2006”, which contributed to the replacement of the pre-existing European *Pst* population at that time and to important rust epidemics on previously resistant wheat cultivars (Ali et al.

2017; Hovmøller et al. 2016). Profuse production of teliospores on seedlings and adult plants suggested that the “Kranich” race had a recent sexual origin, in contrast to prevalent races within the NW European clonal population (Hovmøller et al. 2002; Hovmøller et al. 2016). Similarly, the “Warrior” race was reported to produce abundant teliospores and reproduce sexually under experimental conditions (Rodriguez-Algaba et al. 2014). In addition, comparison to genotypes of reference isolates from six continents suggested that the “Kranich” race originated from a sexually recombining population in the center of diversity of *Pst* in the near-Himalayan region, where *Berberis* species are ubiquitous (Ali et al. 2014; Hovmøller et al. 2016).

Here, the sexual life cycle was completed by using a modified version of the experimental system developed by Rodriguez-Algaba et al. (2014). In brief, the flag and flag -1 leaves of adult plants of near-isogenic wheat line Avocet/Yr8 were inoculated with urediniospores of isolate DK02d/12 (“Kranich” race), followed by harvesting of wheat leaves containing telia after approximately 8 weeks. Leaf segments harbouring telia were dried in a desiccator and maintained at 5°C until further use. Dried teliospores were activated in distilled water at 7°C for 3 days. Subsequently, leaf segments were placed on 2.5% water agar containing 50 mg/l chloramphenicol and incubated at 13°C. Germination was monitored daily and when profuse production of basidiospores was observed (approximately 2 days after incubation), agar plates containing germinating teliospores were suspended onto *B. vulgaris* plants for 4 days at 13°C, 100% relative humidity (RH). Pycnia appeared on the adaxial side of barberry leaves 8 days after inoculation (dai) (Fig. 1a). Fertilization of pycnia was facilitated by daily spraying with mist water to allow aerial movement of pycniospores. Subsequently, aecia appeared on the abaxial side of the leaves from 15 dai (Fig. 1b). Aeciospores were recovered 25 dai by inoculating wheat seedlings of four generally susceptible wheat cultivars, *i.e.*, Avocet S, Anja, Cartago, and Morocco, where the second leaf was fixed on a plastic stand beneath barberry leaves bearing aecia. The stand was covered and incubated at 10°C for 24h, 100% RH to facilitate the release of aeciospores onto wheat seedlings. First uredinia appeared 9 dai and from 13 to 15 dai, 124 distinctly separated lesions were sampled from individual wheat leaves. Twenty-three progeny isolates were randomly selected and genotyped using 19 simple sequence repeat (SSR) markers as described in Rodriguez-Algaba et al. (2014). Novel alleles were not observed in the progenies confirming the parental origin of all the progeny isolates. Furthermore, no mixed genotypes were detected among the progeny isolates demonstrating an efficient procedure for recovery of genetically pure progeny isolates. The SSR markers used in the current study have successfully been applied in previous genetic studies resolving segregation patterns of *Pst* after a selfing on the alternate host (Rodriguez-Algaba et al. 2017; Rodriguez-Algaba et al. 2014). Eight of the 19 SSR markers were heterozygous in the parental isolate, which revealed segregation within the progenies resulting in 18 novel multilocus genotypes (MLGs), confirming recombination following sexual reproduction (Table 1). Five MLGs (MLG2, MLG7, MLG9, MLG10 and MLG14) contained progeny isolates displaying the same SSR genetic profile indicating that these progenies were derived from the same aecium (a.k.a. aecial cup).

Although this study demonstrated a high sexual capacity of the “Kranich” race, this does not necessarily imply a risk of sexual reproduction in areas where this race has been detected, *i.e.*, Sweden, Denmark, Norway, Finland, Poland, France, and UK (www.wheatrust.org). *Pst* has so far not been reported on *Berberis* species in Europe (A. F. Justesen et al. unpublished; Berlin et al. 2012). There may be various reasons for the absence of the sexual life cycle of *Pst* in Europe. First, teliospores have very short or no dormancy, which may lead to germination after maturation under optimal environmental conditions (Wang and Chen 2015; Wright and Lennard 1978). After teliospore germination, successful infection by basidiospores occurs primarily on new barberry leaves at specific regimes of temperature and humidity (Leonard and Szabo 2005; Zhao et al. 2016). Additionally, the coexistence of the alternate host in close

proximity to the primary cereal host is required since basidiospores cannot travel long distances due to sensitivity to ultraviolet light and dry weather conditions (Zhao et al. 2016). A synchrony between both the primary and the alternate host with respect to host susceptibility is also essential for a successful completion of the sexual life cycle. In summary, due to the increased sexual capacity of prevalent races detected in Europe, this may increase the risk that *Berberis* species may become part of the epidemiology of *Pst* and generate new virulence combinations, as reported in Western China (Wang et al. 2016). The findings presented here emphasize the need to improve our understanding regarding the capacity of *Pst* races of different origin to undergo sexual reproduction and continue searching in agroecosystems where suitable conditions for sexual reproduction may occur, which may have detrimental effects on wheat production.

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Compliance with ethical standards

The authors declare that their research is in compliance with the ethical responsibilities and standards of the European Journal of Plant Pathology.

Conflict of interest

The authors declare that they have no conflict of interest.

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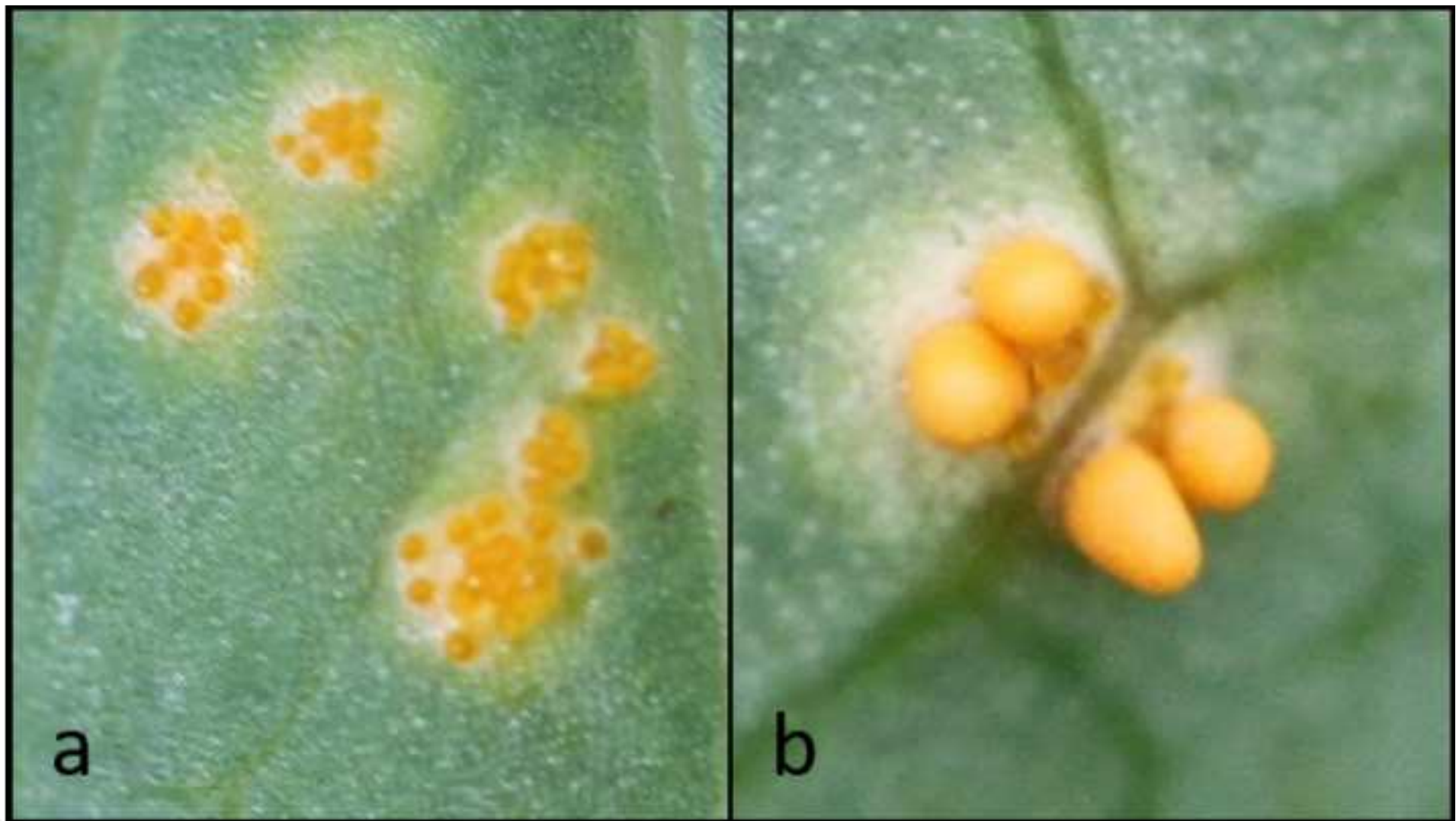
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Legends

Fig. 1. Pycnia and aecia of *Puccinia striiformis* f.sp. *tritici* developed on common barberry (*Berberis vulgaris*). **1a:** Clusters of pycnia on the adaxial side of the leaf observed at 8 days after inoculation (dai), **1b:** A cluster of closed aecial cups on the abaxial side of the leaf at 15 dai.

Table 1. Sizes of alleles (a1: allele 1, a2: allele 2 given in base pairs) at 11 homozygous and 8 heterozygous simple sequence repeat (SSR) loci in parental isolate (DK02d/12) and in 23 segregating progeny isolates of *Puccinia striiformis* f.sp. *tritici*. Genetic segregation of progeny isolates is presented based on the number of segregated markers and the corresponding multilocus genotype (MLG).



MLG ID	Isolate(s)	No. of segregated SSR loci	RJO4		RJO24		RJN12		RJN8		RJN13		RJN3		RJN11	
			a1	a2	a1	a2	a1	a2	a1	a2	a1	a2	a1	a2	a1	a2
-	DK02d/12	-	199	199	284	293	196	196	307	307	147	147	336	340	176	180
1	69a	1	199	199	284	293	196	196	307	307	147	147	336	340	176	180
2	35a, 37a	3	199	199	284	293	196	196	307	307	147	147	336	336	176	180
3	100a	3	199	199	284	293	196	196	307	307	147	147	340	340	176	180
4	1a	4	199	199	293	293	196	196	307	307	147	147	340	340	176	180
5	61a	4	199	199	284	284	196	196	307	307	147	147	336	340	176	180
6	77a	4	199	199	284	293	196	196	307	307	147	147	336	340	176	176
7	91a ,92a	4	199	199	284	284	196	196	307	307	147	147	340	340	176	180
8	114a	4	199	199	284	293	196	196	307	307	147	147	336	340	176	180
9	8a, 10a	5	199	199	293	293	196	196	307	307	147	147	340	340	176	180
10	30a, 34a	5	199	199	284	284	196	196	307	307	147	147	340	340	176	180
11	55a	5	199	199	284	284	196	196	307	307	147	147	336	340	176	180
12	81a	5	199	199	293	293	196	196	307	307	147	147	336	340	176	176
13	2a	6	199	199	293	293	196	196	307	307	147	147	340	340	176	180
14	47a, 51a	6	199	199	284	284	196	196	307	307	147	147	336	336	180	180
15	94a	6	199	199	284	284	196	196	307	307	147	147	336	336	180	180
16	15a	7	199	199	284	284	196	196	307	307	147	147	336	336	176	176
17	41a	7	199	199	284	284	196	196	307	307	147	147	336	336	180	180
18	74a	7	199	199	293	293	196	196	307	307	147	147	336	336	176	180

RJO27		RJN6		RJO21		RJN10		RJO18		WU6		RJO20		RJN2		RJN4		RJN9		RJN5	
a1	a2	a1	a2	a1	a2	a1	a2	a1	a2	a1	a2	a1	a2	a1	a2	a1	a2	a1	a2	a1	a2
232	242	315	318	170	170	221	224	331	331	210	210	287	287	183	183	253	255	332	332	222	226
242	242	315	318	170	170	221	224	331	331	210	210	287	287	183	183	253	255	332	332	222	226
242	242	315	318	170	170	221	224	331	331	210	210	287	287	183	183	255	255	332	332	222	226
242	242	315	318	170	170	221	224	331	331	210	210	287	287	183	183	253	255	332	332	222	222
242	242	315	318	170	170	221	224	331	331	210	210	287	287	183	183	253	255	332	332	222	222
242	242	318	318	170	170	224	224	331	331	210	210	287	287	183	183	253	255	332	332	222	226
242	242	315	318	170	170	224	224	331	331	210	210	287	287	183	183	253	255	332	332	222	222
242	242	318	318	170	170	221	224	331	331	210	210	287	287	183	183	253	255	332	332	222	226
242	242	315	315	170	170	221	224	331	331	210	210	287	287	183	183	255	255	332	332	222	226
242	242	318	318	170	170	221	221	331	331	210	210	287	287	183	183	253	255	332	332	222	226
242	242	315	315	170	170	221	221	331	331	210	210	287	287	183	183	255	255	332	332	222	226
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242	242	315	315	170	170	224	224	331	331	210	210	287	287	183	183	253	255	332	332	222	222
242	242	315	318	170	170	221	221	331	331	210	210	287	287	183	183	253	253	332	332	222	222
242	242	315	315	170	170	224	224	331	331	210	210	287	287	183	183	255	255	332	332	226	226

WU12

[illegible]