Eur J Plant Pathol (2015) 141:159–168
DOI 10.1007/s10658-014-0534-2

Sexual reproduction contributes to genotypic variation in the population of *Puccinia graminis* in Tajikistan

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Accepted: 9 September 2014 / Published online: 20 September 2014

Abstract Stem rust, caused by *Puccinia graminis*, is a potential threat to wheat production in Central Asia. To investigate if sexual reproduction is important for the epidemiology of the disease, the population biology of the fungus was studied. Samples of *P. graminis* were collected from six wheat fields and from wild oats within two of the wheat fields during the growing season of 2010. The population structure of *P. graminis* was investigated by evaluating a total of 121 single uredinia collected from wheat and wild oats, using nine polymorphic simple sequence repeat (SSR) markers. The results presented in this study indicate that there is a selection process by the grass host, in particular wheat, that favours certain clones, which in turn affects the population structure of *P. graminis* in Tajikistan. The genotypic variation was large, both within and between the wheat fields and three populations were in linkage equilibrium, indicating that sexual reproduction within the *P. graminis* population takes place. This leads to the conclusion that the presence of *Berberis* spp. in Tajikistan has an important role in the population dynamics of *P. graminis* within the country, even if the fungus must reproduce primarily in a clonal manner during most of the year. Results also confirm that the two *formae speciales, P. graminis* f. sp. *tritici* and *P. graminis* f. sp. *avenae,* are genetically different even if they were collected in the same field.

Keywords *Avena fatua* · *Triticum aestivum* · *microsatellites* · *alternate host* · Berberis *spp* · Puccinia graminis *f. sp.* tritici · Puccinia graminis *f. sp.* avenae

Electronic supplementary material The online version of this article (doi:10.1007/s10658-014-0534-2) contains supplementary material, which is available to authorized users.

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Introduction

Tajikistan is a mountainous country in Central Asia, with large differences in elevation, ranging from 300 masl (metres above sea level) in the south-western parts to close to 8,000 masl in the eastern parts of the country. Wheat (*Triticum aestivum*) is one of the most important food crops within the country and is cultivated both by small-scale farmers in the mountains and on large collective farms in the lowlands (Rahmatov et al. 2010). Tajikistan is divided into 11 agro-ecological zones, depending on the elevation and climate of the particular areas (Donish 1982). In the mountain areas, most wheat is grown under rain fed conditions, whereas it is usually irrigated in the lowlands. The wheat-growing seasons overlap between the different agro-

ecological zones of the country. In the lowlands, facultative and winter wheat is planted between October and December, and harvested in June–July. In the mountainous areas, spring wheat is planted in March–April and harvested in August–September. Stem rust susceptible wheat crops are thus present in the country from October until September the following year, leaving only approximately a month where no hosts are in the fields.

Stem rust is a serious disease of cereal crops in large areas of the world (Leonard and Szabo 2005). It is caused by the fungus *Puccinia graminis* (Pers.), a heteroecious rust which needs the alternate host barberry (*Berberis* spp.) to complete its sexual life cycle. It is known to be a diverse pathogen (Gäumann 1959; Abbasi et al. 2005; Berlin et al. 2013) and *P. graminis* was early divided into different *formae speciales* (f. sp.) (Eriksson and Henning 1896). The subdivision into different f. sp. reflects the fungus' parasitic adaptation to a certain genus of grass hosts. In Tajikistan, observations of both the wheat type (*P. graminis* f. sp. *tritici*) and the oat type (*P. graminis* f. sp. *avenae*) have been made. Until now, stem rust was usually found in the mountainous areas and was not considered as a major problem in wheat (Pett et al. 2005).

Eleven wild barberry species have been reported from Tajikistan and Central Asia. The species *B. heterobotrys*, *B. heteropoda*, *B. iliensis*, *B. integerrima*, *B. kaschgarica*, *B. multispinosa*, *B. nummularia*, and *B. stolonifera* were reported by Davlatov and Baikova (2011), whereas *B. oblonga*, *B. vulgaris* and *B. turcomanica* were reported by Ul'anishchev (1978). Of these 11, *B. heteropoda* and *B. nummularia* Bunge (Anonymous 2014), *B. integerrima* (Gäumann 1959), *B. oblonga*, *B. vulgaris* and *B. turcomanica* (Ul'anishchev 1978) are known to be hosts for *P. graminis.* Recent barberry surveillance and monitoring showed that high levels of aecial infection on barberry leaves were present across the country (Rahmatov unpublished). The presence of the aecial host, *Berberis* spp., increases the genotypic diversity within the pathogen (Berlin et al. 2012; Jin 2011).

Since the emergence of a new group of stem rust races virulent to *Sr*31 in Eastern Africa (Singh et al. 2011), the threat of the disease has re-emerged. To study the importance of the presence of barberry on the stem rust population in Tajikistan, simple-sequence repeat (SSR) markers (Szabo 2007; Jin et al. 2009; Zhong et al. 2009; Berlin et al. 2013) were used to investigate the genetic diversity within and between *P. graminis* f. sp. *tritici* from small fields in the mountainous zones and large fields in the plains, as well as samples collected from wild oats (*P. graminis* f. sp. *avenae*) within two of these wheat fields. Since our observations indicate the presence of continuous cropping of wheat from October until September within the country, it was hypothesized that the populations would primarily survive as clones within the area. A previous study of the Swedish populations (Berlin et al. 2012) indicates that the two *formae speciales* have no genetic exchange and we also wanted to test for differentiation between the two *formae speciales* in another population.

Materials and methods

Samples of stem rust-infected wheat and wild oat tillers were collected during the wheat-growing season of 2010 at six locations within Tajikistan (Table 1, Fig. 1). At each location, at least 10 collection sites were identified within each field, randomly distributed in a zig-zag pattern with at least 10 m between each site. At each collection site 5–8 infected stems were collected. DNA from two or three well-defined uredeinia from each collection site was extracted separately. Samples from each collection point were air dried and kept separate in paper bags. At the time of collection, disease severity assessments were made based on the modified Cobb scale (Peterson et al. 1948) and the host response to infection was assessed according to Roelfs et al. (1992). All samples from a field were treated as one population. Samples collected from wheat were considered to be *P. graminis* f. sp. *tritici* and samples collected from wild oats *P. graminis* f. sp. *avenae.*

The DNA extractions were carried out with the OmniPrep kit (GenoTech, St. Louis) according to instructions for fungal tissues with minor modifications as described by Berlin et al. (2013). The samples were analysed using nine polymorphic SSR (simple-sequence repeat) markers (Table 2). Amplification of the SSR markers was carried out as a multiplex reaction according to Berlin et al. (2013). The samples were analysed by capillary electrophoresis (ABI 3730XL DNA Analyzer) and the lengths of the fragments were determined with GeneMarker (Softgenetics).

To confirm the species identity of the samples, the Internal Transcribed Spacer (ITS) region was sequenced. For each sample, a 50 μl PCR reaction was used, including each of the following in the final concentrations: 2 ng/μl DNA template, 0.02 mM dNTP, 0.2 μM each of

Table 1 Summary statistics about the rust populations sampled in Tajikistan

Population	Host	Cultivar	Location	Elevation[a]	Pg score[b]	Day of collection[c]
Wheat 1	Wheat	Navruz	Fayzabod, Mehrobod village	1081	80S	26.05.10
Wheat 2	Wheat	Starshina	Isfara city, Chilgazi farm	818	60S	25.05.10
Wheat 3	Wheat	Soroca	Isfara city, Chilgazi farm	818	40MS	25.05.10
Wheat 4	Wheat	Almaly	Garm, Belgi village	1585	30MR	30.07.10
Wheat 5	Wheat	Navruz	Garm, Askalon village	2014	30MR	30.07.10
Wheat 6	Wheat	Surkhak	Shurabad, Khayr Koron village	1990	20MR	04.08.10
Wild oats 7	Wild oats	–	Garm, Belgi village	1585	40MS	30.07.10
Wild oats 8	Wild oats	–	Shurabad, Khayr Koron village	1990	40S	04.08.10

[a] Meters above sea level

[b] Number indicates the disease assessment according to the Cobb scale (Peterson et al. 1948) and the letter indicates host response according to Roelfs et al. (1992)

[c] dd.mm.yy

primers forward TS1rustF10d and reverse StdLSUR2a (Barnes and Szabo 2007), 2.75 mM $MgCl_2$. 0.05 μ/μl of DreamTaq (Fermentas) DNA polymerase and DreamTaq buffer according to the manufacturer's recommendation. The PCR cycling conditions were: initial denaturation at 94 °C for 5 min; 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s; followed by a final extension for 7 min at 72 °C. Successfully amplified samples were purified with Agencourt AMPure PCR Purification kit (Backman Coulter Inc.) according to the manufacturer's recommendations. All purified PCR products were sequenced from both ends by capillary electrophoresis with the same primers that were used for the PCR reactions.

Genetic data analysis

The gene diversity was reported as the number of alleles at each locus. To evaluate the usefulness of the primers (Table 2), the observed heterozygosity (H_o) was calculated for each SSR marker in clone-corrected wheat and wild oat data sets as well as the total clone-corrected data set using GenAlEx6.4 (Peakall and Smouse 2006). H_o is the proportion of samples observed to be heterozygous at the locus. The inbreeding coefficient (F_{IS}) was calculated using the equation F_{IS}=(mean H_o–mean H_e)/ mean H_e, where H_e is the expected heterozygosity.

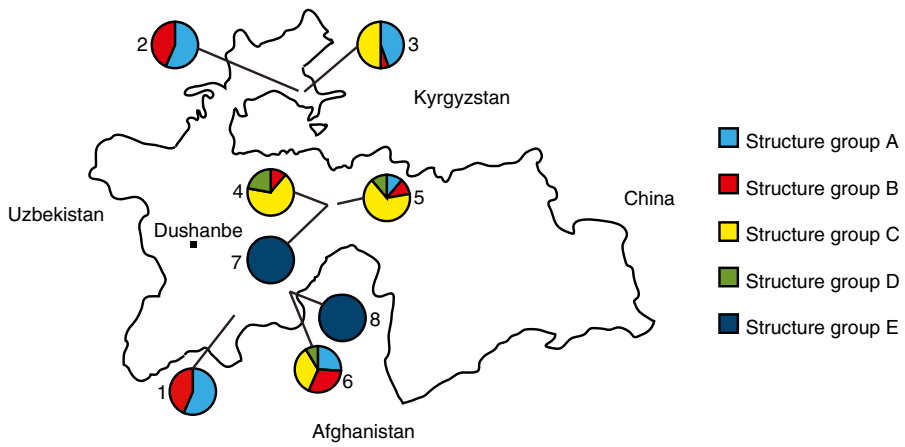

Fig. 1 Map of Tajikistan with each collection site indicated by a number: 1 Fayzabod, Mehrobod village; 2 and 3, Isfara city, Chilgazi farm; 4, Garm, Belgi village; 5 and 7 Garm, Askalon village; 6 and 8, Shurabad, Khayr Koron village. The pie-graphs indicate the groups identified by Structure represented in each population based on the analysis of 9 microsatellite markers

Table 2 Characteristics of the simple sequence repeat (SSR) markers[a] used to analyse populations of *Puccinia graminis* in Tajikistan

	Wheat			Wild oats			Total		
	N	H_o	F_{IS}	N	H_o	F_{IS}	N	H_o	F_{IS}
PgtSSR21[b]	4	0.291	0.543	2	0.917	−0.846	4	0.403	0.435
Pgestrssr021[c]	5	0.964	−0.517	2	0.000	1.000	6	0.791	−0.258
Pgestrssr024[b]	4	0.259	0.601	5	0.417	0.355	9	0.288	0.618
Pgestssr109[d]	2	0.852	−0.706	2	0.083	−0.043	4	0.712	−0.122
Pgestssr255[b]	4	0.889	−0.529	5	0.750	−0.271	9	0.864	−0.223
Pgestssr279[d]	4	0.145	0.440	3	1.000	−0.653	6	0.277	0.393
Pgestssr280[b]	7	0.340	0.454	5	0.667	0.059	8	0.403	0.390
Pgestssr368[d]	3	0.855	−0.445	2	0.667	−0.371	5	0.821	−0.159
PgtCAA53[e]	6	0.963	−0.551	2	0.000	1.000	7	0.788	−0.115

[a] *Abbreviations*: *N* number of alleles: H_o observed heterozygosity: F_{IS} fixation index F_{IS}=(mean H_e–mean H_o)/ mean H_e

[b] Berlin et al. 2013

[c] Szabo 2007

[d] Zhong et al. 2009

[e] Jin et al. 2009

Identification of genotypes

Multilocus genotypes were obtained by combining data from the nine SSR loci for each sample. Samples from each location and host were grouped into separate populations, giving six populations collected from wheat and two populations collected from wild oats. For each field population, the genotypic diversity was reported as the number of unique genotypes divided by the total number of samples within the population (G/N). Prior to performing the following analyses, clone correction was performed by eliminating duplicate genotypes within each field population, *forma specialis* or wheat cultivar. The inbreeding coefficient (F_{IS}) was calculated using the computer program Fstat (Goudet 1995) according to Weir and Cockerham (1984), which adjusts the F_{IS} for varying sample sizes. To test for association among different loci, the Index of Association (I_A) was calculated for each population separately, using the software Multilocus 1.3b (Agapow and Burt 2001) with 1,000 iterations.

To evaluate differences between the *formae speciales*, between wheat field populations and between wheat cultivars, analyses of molecular variance (AMOVA) were performed using Genalex6.4 with 9,999 iterations on the corresponding clone-corrected data set. Using the same program, the corrected pairwise and overall *theta* (estimation of Wright's F_{ST}) was calculated based on the heterozygosity between individuals within each field population, and the significance of each value was calculated by 9,999 permutations. To visualise the genetic differences between the samples, a principal component analysis (PCA) based on the calculated genetic differences was performed in Genalex6.4.

To infer the common ancestry between genotyped individuals, the Bayesian cluster computer program Structure (Pritchard et al. 2000) was used to investigate the number of genotypic clusters within both the total data set and by field clone-corrected data sets of samples. The assumptions set for the analysis were that the total population was in Hardy-Weinberg equilibrium (panmixia) and that all loci are independent from each other. The range of presumed number of genotypic clusters (*K*) was set to 1–15 and the program was set to a burn-in period of 100 000 iterations followed by 100 000 iterations. The program calculates the Log likelihood estimate for each value of *K*, and the highest likelihood value indicates the most likely number of genotypic clusters. Three independent simulations were performed to test the consistency of the result. Based on the result, each individual was assigned to one of the genotypic clusters (called the Structure group). If a sample was shared between two clusters (i.e. a hybrid), the sample was assigned to the dominant cluster. The distributions of different structure groups in each of the field populations were visualized with pie charts (Fig. 1).

Species identity and genetic relationships

All sequences were checked manually and assembled using SeqMan™II 5.07 (1989–2003 DNASTAR Inc.) and consensus sequences were generated. The identity of the species was decided based on the sample's maximum identity to the sequences available in GenBank through a BLASTn search (Altschul et al. 1997). The sequences were aligned using CLUSTAL W (Thompson et al. 1994) in the program MEGA 5 (Tamura et al. 2011).

Results

The level of disease and host reaction ranged from 20MR (moderate resistant) to 80S (susceptible), and no clear pattern was seen between the early sampling in the lowlands compared to the later sampling in the mountainous regions (Table 1).

The nine simple-sequence repeat (SSR) markers were polymorphic, with 4–10 alleles for each marker, and a total of 58 alleles was found: 39 within the wheat populations and 28 within the wild oat populations (Table 2). Only nine of the 58 alleles were found in samples collected from both wheat and wild oats (2 for PgtSSR21, 1 for Pgestssr021, 1 for Pgestssr279, 4 for Pgestssr280 and 1 for PgtCAA53). The observed heterozygosity (H_o) ranged between 0.277 and 0.864 for the total clone-corrected data set, 0.145 and 0.964 for the samples collected from wheat and 0.000 and 1.000 for the samples collected from oats. The F_{IS} values also differed between the data sets (Table 2), but no clear pattern could be seen. None of the loci identified by these primers was close to Hardy-Weinberg equilibrium, i.e., F_{IS} values were clearly deviating from zero, with the exception of Pgestssr280 in the samples collected from wild oats. The overall percentage of missing values was low, and could only be considered high for the loci Pgestssr280 for the samples collected from wheat and Pgestssr279 for the samples collected from wild oats.

By comparing the ITS region sequences to GenBank submissions, 107 of the 155 collected samples were identified as *Puccinia graminis*. Of the remaining sequences some were identified as *Puccinia coronata* or their identity could not be determined by the ITS region (Supplementary Table 1). Fourteen samples, not confirmed as *P. graminis*, were included in the study since they produced similar SSR patterns to known *P. graminis* samples, although amplification of their ITS region was not successful. Thus, for the population study, a total of 121 single uredinia were included (Table 3) originating from six wheat fields and two collections from wild oats from within two of the wheat fields. In total 69 unique genotypes were found. The genotypic diversity (reported as G/N) of the collected material ranged between 0.45 and 0.83 for the populations collected from wheat and 0.80 and 1.00 for the populations collected from wild oats (Table 3). The number of observed alleles within the populations ranged between 25 and 32 for the samples collected from wheat whereas the two populations collected from wild oats had 28 and 15 alleles (Table 3). The inbreeding coefficients, F_{IS}, were negative for all wheat field populations (Table 3), indicating outbreeding. For the collections made from wild oats, F_{IS} was close to zero for one population, indicating equilibrium, and negative for the other population. The Index of Association (I_A) ranged between −0.231 and 2.986 and was statistically significant for all but two populations collected from wheat and one collected from wild oats (Table 3), which indicates that only three populations were in linkage equilibrium.

The analysis of molecular variance (AMOVA) showed that 59 % of the variation ($p<0.0001$) was present within the *formae speciales* and 41 % was due to the differences between the *formae speciales* (Table 4). AMOVAs were also carried out to test if the location and cultivar affected the result, and they explained 12 % ($p<0.0001$) and 7 % ($p<0.004$), respectively, of the genetic differences between the locations and cultivars. The pairwise *theta* (estimation of Wright's F_{ST}) ranged between 0.000 and 0.512 and was significant for 17 of the 28 pairwise comparisons (Table 5). Low and non-significant pairwise *theta* indicates no genetic difference between the populations, and was found only between populations collected from the same host (Table 5).

The first axis of the principal component analysis (PCA) explained 38.9 % and the second axis 25.5 % of the pairwise genetic differences between the samples (Fig. 2). In general, the PCA shows that there are genetic differences between *P. graminis* collected from wheat compared to the samples collected from wild oats within the same field. In the Bayesian cluster analysis performed in Structure, the highest Log likelihood value was obtained for a *K*-value of 5, i.e., the samples clustered into five groups (output for clone-corrected data set in supplementary Figure 1). For wheat, four groups

Table 3 Population genetic data for the field populations based on clone-corrected data

Population	N	G/N	N_a	H_o (s.e.)	H_e (s.e.)	F_{IS}	I_A	*p*
Wheat 1	23	0.83	25	0.599 (0.155)	0.484 (0.052)	−0.200	−0.184	0.966
Wheat 2	22	0.77	26	0.594 (0.147)	0.497 (0.034)	−0.097	−0.164	0.825
Wheat 3	22	0.45	27	0.669 (0.125)	0.565 (0.037)	−0.110	2.108	<0.001
Wheat 4	9	0.78	29	0.614 (0.116)	0.495 (0.080)	−0.067	2.827	<0.001
Wheat 5	9	0.78	26	0.635 (0.109)	0.502 (0.066)	−0.191	2.986	<0.001
Wheat 6	23	0.61	32	0.589 (0.110)	0.549 (0.060)	−0.021	1.681	<0.001
Wild oats 7	8	1.00	28	0.500 (1.128)	0.458 (0.073)	0.061	1.113	0.016
Wild oats 8	5	0.80	15	0.500 (0.161)	0.299 (0.080)	−0.588	−0.231	1.000

Abbreviations: *N* number of genotyped samples: *G/N* number of genotypes divided by number of genotyped samples: N_a number of alleles found within each field population: F_{IS} inbreeding coefficient according to Weir and Cockerham (1984): I_A Index of Association and its *p*-value based on 1,000 iterations

(Structure groups A, B, C and D) were found and for wild oat samples one group was found (Structure group E). The geographical distribution of the groups is shown in Fig. 1.

The differences between the two *formae speciales* were confirmed by the neighbour-joining tree (Saitou and Nei 1987) based on the ITS sequences (Supplementary Figure 2), but no differences between the locations could be detected based on the ITS region.

Discussion

In Tajikistan, wheat is present in the fields almost throughout the year and it could be anticipated that *P. graminis* primarily reproduces clonally. Population genetic analyses showed that some genotypes are more successful during clonal reproduction, which is indicated by negative values of the inbreeding coefficient F_{IS} together with a significant I_A. However, population genetic analyses indicate variation in how *P. graminis* is reproducing. For populations Wheat 1 and 2, a G/N ratio close to 1, along with a non-significant index of association (I_A), is consistent with sexual reproduction. For the other wheat populations, which include those collected late in the season, the data supports conflicting conclusions. A relatively large G/N ratio indicates that a large number of different genotypes have infected the field (which could be a result of sexual reproduction), whereas the excess of heterozygosity is more a sign of clonal reproduction. The significant I_A in these fields could also be indicative of clonal reproduction, or could

Table 4 Analyses of molecular variance (AMOVA) between *Puccinia graminis* f. sp. *tritici* and *P. graminis* f. sp. *avenae* among wheat field populations and among wheat cultivars

Source	Df[a]	SS	MS	Est. Var.	%	*P*-value
Between *formae speciales*	1	144.67	144.67	7.10	59 %	0.0001
Within *formae speciales*	65	316.68	4.87	4.87	41 %	
Total	66	461.36		11.97	100 %	
Among wheat field populations	5	56.30	11.26	0.580	12 %	0.0001
Within wheat field populations	68	293.64	4.32	4.318	88 %	
Total	73	349.95		4.898	100 %	
Among wheat cultivars	4	37.05	9.26	0.34	7 %	0.004
Within wheat cultivars	68	310.08	4.56	4.56	93 %	
Total	72	347.14		4.90	100 %	

Abbreviations: *Df* degrees of freedom: *SS* sum of squares: *MS* mean squares: *Est. Var.* estimated variance

[a] Clone correction was done separately for each of the three AMOVAs

Table 5 Pairwise *theta* (estimates of Wright's F_{ST}) below the diagonal based on 9,999 permutations. Level of significance is shown above the diagonal. Bold figures are not significant. Population corresponds to the populations in Tables 1 and 3

	Wheat 1[a]	Wheat 2[b]	Wheat 3[b]	Wheat 4[c]	Wheat 5[d]	Wheat 6[e]	Wild oats 7[c]	Wild oats 8[e]
Wheat 1[a]		0.356	0.068	***	**	*	***	***
Wheat 2[b]	**0.000**		0.111	***	**	0.073	***	***
Wheat 3[b]	**0.042**	**0.029**		0.056	0.293	0.337	***	***
Wheat 4[c]	0.185	0.154	**0.057**		0.399	0.063	***	***
Wheat 5[d]	0.143	0.120	**0.011**	**0.000**		0.322	***	***
Wheat 6[e]	0.055	**0.030**	**0.005**	**0.045**	**0.007**		***	***
Wild oats 7[c]	0.460	0.437	0.401	0.438	0.442	0.412		0.086
Wild oats 8[e]	0.512	0.487	0.457	0.489	0.503	0.458	**0.074**	

Populations from the same filed are marked with the same letter: [a] Fayzabod, Mehrobod village, [b] Isfara city, Chilgazi farm, [c] Garm, Belgi village, [d] Garm, Askalon village, [e] Shurabad, Khayr Koron village

be the result of selection processes, which favour certain genotypes over others. This selection of clones is seen more in wheat, probably because of the uniformity of wheat genotypes in the fields while the wild oats represent a natural, variable population.

The pairwise *theta* (Table 5) shows that there are small genetic differences within populations collected in May/June (Populations Wheat 1–3) and within the populations collected later in the season (Populations Wheat 4–6). These results are reflected in the repartition of the Structure groups (Fig. 1). The field populations collected early in the season had a limited number of groups whereas the field populations collected later included more groups. Populations one and two seem to have the same ancestors, although the geographical distance between the two populations is large. In contrast, the two geographically closely collected populations two and three do not share the same groups, or proportion of the groups, present within the area. This may be due to the difference in susceptibility of the wheat cultivars, and this leads to selection on the grass host that favour certain genotypes. More structure groups are represented in the populations collected later in the season. This could be related to sexual

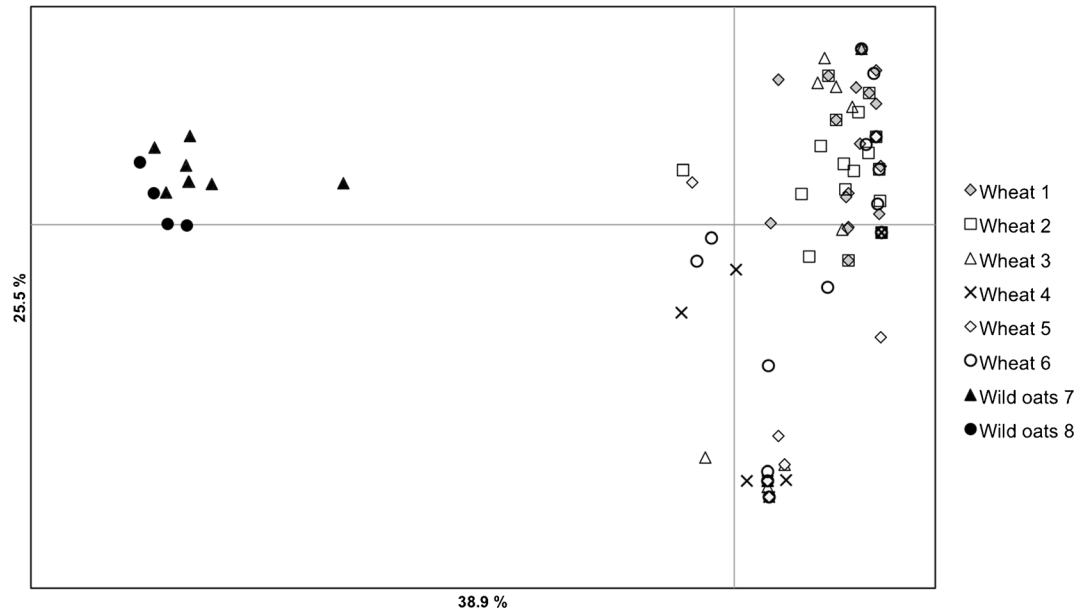

Fig. 2 Principal coordinate analysis (PCA) based on simple sequence repeat data of *Puccinia graminis* and grouped by host plant. The first axis explains 38.9 % and the second 25.5 % of the differences between the genotypes of the samples

reproduction, but selection on the *P. graminis* populations by the grass host may prevent Hardy-Weinberg equilibrium. The wheat cultivars probably carry some resistance and barberry is more common in mountainous areas (Davlatov and Baikova 2011). Differences between the early and late collections might not only be due to the time differences, but also elevation and type of field. The early collections were made in the lowlands, in large fields on large farms, whereas the later collections were made in small-scale farmer's fields in the mountainous regions. To disentangle these differences, more samples at different time points would be necessary. Two of the three populations collected early in the season did not have a significant value of I_A, indicating random mating. This is consistent with the presence of *Berberis* spp., which will not only enable sexual reproduction, but will also ensure the survival of a local population within an area (Roelfs 1982; Berlin et al. 2013). The possibilities for sexual recombination do not alter the conclusion that long-distance dispersal of urediniospores (Kolmer 2005) is important for the development of populations. Within a sexual population that receives new individuals through rare immigration events, the genetic diversity will increase when the new alleles are spread within the population (Wingen et al. 2013).

The hypothesised genetic differentiation between *P. graminis* f. sp. *tritici* and *P. graminis* f. sp. *avenae* in Tajikistan (Zambino and Szabo 1993; Berlin et al. 2012), was confirmed (Fig. 2, Tables 4 and 5). No samples collected from wild oats had similar genotypes to samples collected from wheat (Fig. 2), and the low and non-significant pairwise *theta* values (Table 5) between the populations collected from wheat show that these wheat populations are part of the same large population. The *theta* values indicate that the oat populations also form a single population, and that the oat population is different from the one on wheat (Table 5). This clear division between the two types may be surprising since both *formae speciales* were collected within the same field, earlier studies have reported that a *forma specialis* may infect the "wrong" host and crosses have been made between the two f. sp. under controlled conditions (Johnson 1949). Since the genetic data and the presence of barberry ensure sexual reproduction, it is clear that while the two populations can meet on the alternate host, no genetic exchange takes place.

The primers were originally developed for *P. graminis* f. sp. *tritici* and were also shown to be useful on *P. graminis* f. sp. *avenae* (Berlin et al. 2013). Although these SSR markers were tested on samples collected in North America and Scandinavia, they are also informative for *P. graminis* f. sp. *tritici* and *P. gramnis* f. sp. *avenae* from Tajikistan in Central Asia, confirming that they are useful for many different stem rust populations. In this study, we found that some samples identified as *P. coronata* by using the ITS region sequence also produced fragments with some of the markers (data not shown).

Since *P. graminis* has a mixed reproduction system in Tajikistan with both sexual and clonal reproduction, it fits well with the definition of a pathogen with a high evolutionary potential (McDonald and Linde 2002). Sexual recombination limits the importance of resistance genes within the host crop since recombination will enable the fungus to broaden its genetic diversity and gene-for-gene resistances may potentially break down within a short time. Sexual recombination may result in a large number of genotypes with low fitness (Lehtinen et al. 2009). Whether an aggressive strain will succeed depends on access to a suitable host, the host's resistance genes, the level of disease within the field at the time of infection, and the possibility to survive in the clonal uredinial stage. In the presence of barberry, it could be difficult for this to happen since the alternate host provides for a possibility of sexual recombination. However, presence of barberry may enable early disease onset with a large number of genotypes and this could increase the initial inoculum levels as well as the number of races.

Analysis of the populations of *P. graminis* in Tajikistan with molecular markers shows that sexual reproduction of the pathogen and selection during the clonal reproduction phase has likely shaped the population structure of the pathogen. In addition, the *formae speciales* on wild oats and wheat are clearly different from each other, even when collected within the same field. A better understanding of the biology of *P. graminis* is an important tool in the management of stem rust. The presence of barberry and the resulting sexual reproduction increases the evolutionary potential of *P. graminis* (McDonald and Linde 2002), with the increased probability of the emergence of new virulent races on wheat (Jin 2011). This can cause high yield losses in an area where many small-scale farmers do not have access to fungicides. Removal of the *Berberis* species that serve as the alternate host will limit the yield and quality losses due to this disease and at the same time decrease the genotypic variation within the pathogen.

Acknowledgments The authors wish to thank Bernt Pett for assistance with sample collection, Annika Djurle for valuable comment on the manuscript and the Swedish University for Agricultural Sciences (SLU) for funding this research.

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