

Conservation and utilization of Central Asian *Spinacia turkestanica* to improve downy mildew resistance in cultivated spinach

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Abstract

Downy mildew is a plant disease that has devastating effects on spinach cultivation worldwide. Here, we compared resistance against 16 races of downy mildew between cultivated spinach (*Spinacia oleracea*) and its wild relative *S. turkestanica* and studied the distribution of resistance among 75 wild populations from Central Asia. On average, resistance allele frequencies were about four times higher in *S. turkestanica* as compared to cultivated spinach. Resistances against each of the downy mildew races were observed in all regions of the study area. In particular, the region Jizzakh in Uzbekistan showed high levels of downy mildew resistance. In general, resistance allele frequencies for different downy mildew races showed a positive correlation among the *S. turkestanica* populations, which was most pronounced for the older races. Populations were found to harbour lower resistance levels for newer races of downy mildew. Our study results show that *S. turkestanica* harbours wide downy mildew resistance in the study area in Central Asia and it was argued that elevated resistance levels are likely due to selection pressure by the pathogen. Through a resampling study it was shown that all resistance alleles for the studied downy mildew races can be captured with more than 95% probability by sampling 30–50 plants from 5–6 *S. turkestanica* populations from the study area. Recent efforts to collect *S. turkestanica* from this part of its natural distribution area ensures the presence of ample genetic diversity for current research and breeding purposes.

INTRODUCTION

Downy mildew caused by obligate oomycete microbes is a devastating disease responsible for high production losses in a wide variety of agricultural and horticultural crops (Tör et al. 2023). Downy mildew in cultivated spinach (*Spinacia oleracea* L.) is caused by the pathogen *Peronospora effusa* (Greville) Rabenhorst, formerly referred to as *Peronospora farinosa* f.sp. *spinaciae* (Kandel et al. 2019). Spinach downy mildew is by far the most important disease threatening spinach cultivation in production areas all around the world, and new races of the pathogen are emerging at an accelerating pace (Correll et al. 2011). These developments have led to the establishment of the International Working Group on *Peronospora* (IWGP), in which spinach researchers and breeders cooperate to officially denominate newly emerging races that are considered relevant to the industry. To date, 20 races of *P. effusa* have been denominated by the IWGP (ISF 2026).

Due to the ongoing arms race between host plants and pathogens, introgression of new resistances against downy mildew in crop varieties has become core business in spinach breeding (Ribera et al. 2020). Thereby, the genetic resources collections of genebanks serve as important sources for these traits of interest. Until recently, genetic resources collections of spinach mainly consisted of cultivated accessions (Van Treuren et al. 2012). Because finding new resistances against downy mildew in the cultivated gene pool has become increasingly difficult, the interest of breeders has shifted towards the wild relatives of spinach.

S. turkestanica Iljin and *S. tetrandra* Steven ex M. Bieb are of particular interest in spinach breeding because both wild species are interfertile with *S. oleracea*. *S. turkestanica* and *S. tetrandra* share several characteristics with *S. oleracea* as all three species are wind-pollinated, dioecious diploids with the same chromosome number (Ribera et al. 2020). Phylogenetic studies have shown a closer relationship of *S. oleracea* to *S. turkestanica* than to *S. tetrandra*, revealing *S. turkestanica* as the most likely predecessor of cultivated spinach (Fujito et al. 2015; Ribera et al. 2021; She et al. 2022; Xu et al. 2015, 2017). The natural distribution area of *S. tetrandra* is located in the Middle East and in the Trans Caucasus. *S. turkestanica* can be found in Central and South Asia, close to the presumed domestication area of spinach (Morelock and Correll 2008; Ribera et al. 2021). Collecting expeditions in the distribution areas of the species have provided better access to their genetic resources for the research and breeding community (Van Treuren et al. 2020). In January 2026 genebanks worldwide harbored 93 accessions of *S. turkestanica* and 66 of *S. tetrandra* (Genesys 2026).

Management of crop wild relatives under *ex situ* conditions can be a burden to genebank curators because often specific expertise is required for their maintenance. Many wild species display seed dormancy that has to be broken for plant propagation (Finch-Savage and Leubner-Metzger 2006). Also for *S. tetrandra* and *S. turkestanica* specific protocols were developed to enable the regeneration of accessions (Van Treuren et al. 2020). Despite the dormancy-breaking treatments, seeds of *S. turkestanica* and *S. tetrandra* typically show asynchronous germination, which complicates screening tests for downy mildew resistance on seedlings. Here we use hybrid progenies from crosses with *S. oleracea* cultivar 'Viroflay' to overcome these problems. 'Viroflay' is part of the host differential set for spinach downy mildew and is susceptible to each of the races Pe:1 to Pe:20 denominated by the IWGP (ISF 2026). Considering that resistance alleles for spinach downy mildew typically show dominant inheritance (Feng et al. 2018a; Olaoye et al. 2024) and that resistance alleles are absent in 'Viroflay', the presence of resistance in the wild parent can be detected through the screening of hybrid progenies.

Characterisation and evaluation of genetic resources lies at the basis of proper genetic resources management and utilization (Engels and Visser 2003). Despite their increasing importance in genetic resources conservation and plant breeding, diversity data of crop wild relatives are often scarce for the species of interest. A phenotypic and molecular study on *S. turkestanica* from Central Asia showed that the diversity in the investigated region is widespread and is mainly distributed within rather than between populations (Van Treuren et al. 2025). Here we investigate the importance of *S. turkestanica* as a source of downy mildew resistances through the screening of accessions of *S. oleracea* and *S. turkestanica* for a wide spectrum of pathogen races. In addition, we studied the spatial distribution of downy mildew resistance in *S. turkestanica* from Central Asia and used the data for a resampling study to obtain inferences about efficient collecting procedures and collection development.

MATERIAL AND METHODS

Study materials

Materials from the Centre for Genetic Resources, the Netherlands (www.cgn.wur.nl) were used for the present study, which included 404 accessions of *S. oleracea* L. and 75 of *S. turkestanica* Iljin. An overview of the study materials with their main passport data and links to the CGN webpages with accession

information is provided for *S. oleracea* in Suppl. Table 1 and for *S. turkestanica* in Suppl. Table 2. The majority of *S. turkestanica* accessions originated from a collecting expedition organized by CGN in Tajikistan and Uzbekistan in 2008 (Van Treuren et al. 2020). Prior to this expedition, the competent national authorities on access and benefit sharing of Tajikistan and Uzbekistan were contacted to fulfil requirements regarding Prior Informed Consent (PIC) and Mutually Agreed Terms (MAT). A Memorandum of Understanding (MOU) was signed with each of these authorities, adopting the Standard Material Transfer Agreement (SMTA) of the International Treaty for Plant Genetic Resources for Food and Agriculture as the basis for germplasm distribution (IT-PGRFA 2025). The *S. turkestanica* accessions from Turkmenistan were obtained in 2014 from the Iowa State University (Ames, USA) and originated from a collecting expedition in 2000 (GRIN-Global 2024). The geographic origin locations of the *S. turkestanica* accessions with GPS data are presented in Suppl. Figure 1.

S. oleracea x S. turkestanica progenies

Hybrid progenies between *S. oleracea* and *S. turkestanica* were developed using cultivar 'Viroflay' as female parent. The sequenced line "VIR-39-237-04-39-2-3-1-M-4", or a regeneration thereof, was provided by Nunhems Netherlands B.V. and was used as female parent in all crosses.

Considering the dominant inheritance of downy mildew resistance alleles and their absence in 'Viroflay', the probability of detecting downy mildew resistance in hybrid progenies depends on the frequency of resistance alleles in the *S. turkestanica* accessions, the number of male plants used for the hybrid crosses and the progeny size used for disease resistance testing. This probability was estimated through simulation in order to optimize the experimental design. For the simulations a single locus with a susceptibility allele (p) and a resistance allele (q) was considered. Furthermore, the simulation programme assumed random selection of paternal plants for hybrid production and equal contribution of male plants to the progenies. The programme started with simulated values of p and q in the *S. turkestanica* accession, followed by the random sampling of paternal plants for hybrid production and calculating p and q among the selected males. Taking these allele frequencies into account, random offspring were generated and it was determined whether at least one plant carrying the resistance allele was present in the progeny. Simulated values were 0.01, 0.05, 0.10, 0.20, 0.30 and 0.40 for the frequency of the resistance allele, 10, 20, 30, 40 and 50 for the number of paternal plants and 10, 20, 30, 40 and 50 for progeny size. Each parameter combination was tested by 10,000 simulations and the frequency of the tests revealing at least one plant carrying a resistance allele was determined. Simulations were carried out by a tailor-made program written in Turbo Pascal (v. 7.3.5).

Hybrid progenies for each of the 75 *S. turkestanica* accessions were developed in-house in 2017–2019 by a consortium of nine breeding companies. At least 30 male plants per *S. turkestanica* accession were used for progeny development.

Resistance screening

Disease resistance tests were performed in-house by the participating breeding companies. Screening tests were performed in duplo by different companies using at least 30 seedlings in most cases. Host differential sets consisting of plants, e.g. cultivars or breeding lines, with known susceptible and resistant reactions to a given pathogenic race were included in the tests. Resistance screening basically followed the procedures described by Correll et al. (2003). Briefly, seedlings are examined when the susceptible control of the host differential set shows infection. Cotyledons and first true leaves are used to score individual seedlings as 'susceptible', 'partially resistant' or 'resistant'. *S. oleracea* accessions were tested for resistance against downy mildew races Pe:1 to Pe:8 and Pe:10 to Pe:16 in the period 2004–2018. The *S. oleracea* x *S. turkestanica* progenies were tested for the same races during 2020 and 2021. As Pe:17 was officially denominated shortly before to the start of the experiments with *S. turkestanica*, this race was added to the screening of the hybrid progenies.

Data analysis

To facilitate data analysis, results of the screening tests were transformed to a single 'resistance score', ranging from zero to one for each combination of accession and downy mildew race. Firstly, the classes 'susceptible', 'partially resistant' and 'resistant' were assigned the values 0, 0.5 and 1, respectively. Secondly, the average value for the tested seedlings was calculated. Thirdly, the values of the two duplo tests were averaged to obtain the resistance score, unless the difference was more than 0.5. In the latter case the test results were considered ambiguous and disregarded for further analysis. The frequency of ambiguous test results was 1.1% for *S. oleracea* and 1.7% for the *S. oleracea* x *S. turkestanica* progenies. Resistance scores of the study materials can be accessed through CGN's downloadable data website (<https://cgn.websites.wur.nl/Website/downloads/DownloadCnr16.htm>).

Resistance scores were used to estimate the frequency of resistance alleles in accessions. As *S. oleracea* is an allogamous species, Hardy-Weinberg equilibrium was assumed for all accessions of cultivated spinach so that $p^2 + 2pq + q^2 = 1$ (Hartl and Clark 2006). The frequency of the dominant resistance allele (q) was then calculated as $1 - \sqrt{1 - \text{resistance score}}$. As all hybrid offspring received the susceptibility allele (p) from the 'Viroflay' female parent, resistance scores determined for hybrid progenies were considered estimators of the frequency of resistance alleles in the paternal *S. turkestanica* accessions. For each accession the fraction of loci with resistance ($q > 0$) was considered as the degree of polymorphism. In addition, the average resistance allele frequency per accession was determined. Average values of these parameters were used to compare the potential for downy mildew resistance between groups of accessions. The average resistance allele frequency per accession was also used to visualize the relationship between resistance level and geographic origin for the accessions collected in 2008 in Uzbekistan and Tajikistan. For this purpose a geographic map of the study area was constructed in which the origin locations are colour-marked depending on the resistance level of the sample. The map was generated through a Python 3.14.5 script (www.python.org) using Claude Sonnet 4.6 (www.anthropic.com) with the libraries 'NumPy' (Harris et al. 2020), 'Matplotlib' (Hunter 2007), 'SciPy' (Virtanen et al. 2020) and 'Pillow (PIL)' (Clark et al. 2025). Country borders were drawn from Natural Earth (2026) and elevation data downloaded from Terrain Tiles (2026) that were, in turn, collected by Farr et al. (2007).

Scatter plots were made to examine the relationship between the population resistance allele frequencies for different downy mildew races and a linear trendline was fitted to the datapoints. The corresponding correlation coefficient (r) was tested for significant deviation from $r = 0$, using a t-distribution with n-

2 degrees of freedom. The test statistic was calculated as $t = r\sqrt{(n-2)/\sqrt{(1-r^2)}}$ where n is the sample size (Somoray 2023). Similarly, the relationship between resistance allele frequency and altitude of the origin location was investigated.

Resampling study

A software programme was developed to estimate the probability of capturing resistance alleles from *S. turkestanica* through different sampling strategies. For these analyses the estimated resistance allele frequencies (q) of the screening programme were used, assuming that the distributions of resistances in the genebank accessions resemble those in the origin populations. Simulations were carried out with the data from the 66 *S. turkestanica* accessions collected during the expedition in Tajikistan and Uzbekistan in 2008. Data imputation was carried out for 17 ambiguous test results by using the average resistance allele frequency among the other hybrid progenies. The study was carried out by random resampling from a varying number of different populations (1–10) using varying sample sizes per population (10, 20, 30, 40, 50) and the estimated values of q derived from the screening programme. Each parameter combination was analysed by 10,000 simulations that were carried out using a tailor-made program written in Turbo Pascal (v. 7.3.5).

RESULTS

Optimizing experimental design

As could be expected, our simulations showed that the probability of detecting resistance in hybrid progenies increases with a higher frequency of the resistance allele in the accession, a higher number of males used for hybrid production and a larger progeny size used for resistance testing (Table 1). This probability is more than 80% in case the resistance allele frequency in the accession is at least 0.2, even when the number of males for hybrid production and the tested progeny is as low as ten. But when a resistance allele is rare, e.g. occurring with a frequency of 0.01 in the accession, using 50 males and a progeny size of 50 would result in a detection probability of only 33.5%. To carry out an efficient and workable screening protocol it was decided to use at least 30 males for hybrid production and at least 30 seedlings for progeny testing. This experimental design provided a minimum detection probability of 71.4% for alleles with a frequency of 0.05, 91.6% for alleles with a frequency of 0.1 and (nearly) 100% for alleles with a frequency of at least 0.2 in the paternal accession.

Table 1

Probability of sampling at least one plant carrying a resistance allele in screening tests of *S. oleracea* x *S. turkestanica* hybrid progenies for simulated resistance allele frequencies in the *S. turkestanica* population (q), different numbers of males for hybrid production and different progeny sizes for resistance testing. Values equal or greater than 0.95 are shaded in grey colour.

q = 0.01		Males for hybrid production					q = 0.20		Males for hybrid production				
		10	20	30	40	50			10	20	30	40	50
Tested progeny	10	0.081	0.090	0.087	0.089	0.098	Tested progeny	10	0.833	0.857	0.870	0.878	0.876
	20	0.124	0.146	0.157	0.170	0.172		20	0.938	0.971	0.978	0.981	0.983
	30	0.144	0.197	0.212	0.226	0.236		30	0.969	0.990	0.995	0.995	0.996
	40	0.164	0.230	0.255	0.267	0.289		40	0.978	0.997	0.998	0.999	0.999
	50	0.169	0.258	0.293	0.313	0.335		50	0.982	0.998	1.000	0.999	1.000
q = 0.05		Males for hybrid production					q = 0.30		Males for hybrid production				
		10	20	30	40	50			10	20	30	40	50
Tested progeny	10	0.345	0.368	0.382	0.383	0.386	Tested progeny	10	0.940	0.958	0.959	0.966	0.963
	20	0.487	0.560	0.592	0.589	0.606		20	0.988	0.996	0.998	0.998	0.998
	30	0.548	0.660	0.714	0.723	0.732		30	0.996	0.999	1.000	1.000	1.000
	40	0.593	0.727	0.772	0.798	0.817		40	0.998	1.000	1.000	1.000	1.000
	50	0.614	0.766	0.825	0.850	0.867		50	0.998	1.000	1.000	1.000	1.000
q = 0.10		Males for hybrid production					q = 0.40		Males for hybrid production				
		10	20	30	40	50			10	20	30	40	50
Tested progeny	10	0.561	0.602	0.629	0.627	0.637	Tested progeny	10	0.981	0.988	0.990	0.992	0.993
	20	0.738	0.813	0.838	0.847	0.849		20	0.998	1.000	1.000	1.000	1.000
	30	0.807	0.893	0.916	0.927	0.938		30	1.000	1.000	1.000	1.000	1.000
	40	0.839	0.928	0.957	0.965	0.965		40	1.000	1.000	1.000	1.000	1.000
	50	0.860	0.953	0.978	0.979	0.985		50	1.000	1.000	1.000	1.000	1.000

Downy mildew resistance in *S. oleracea*

The data set for *S. oleracea* comprised 404 accessions, including 140 cultivars, 122 landraces and 142 accessions for which no population type was registered (Suppl. Table 1). The number of accessions screened per downy mildew race ranged from 131 to 140 for cultivars and from 105 to 122 for landraces. For each of the races Pe:1 - Pe:8 and Pe:10 - Pe:16 resistance was observed in cultivars as well as in landraces (Suppl. Figure 2), although it could be rare in *S. oleracea*, such as for Pe:13. In general, the average resistance allele frequency was higher in cultivars as compared to landraces, and was most pronounced for Pe:1 and Pe:2 (Suppl. Figure 2, top chart). No systematic differences were observed between these population types regarding the proportion of accessions with resistant plants (Suppl. Figure 2, bottom chart). For this parameter, resistant plants were more common in landraces than in cultivars for the races Pe:2, Pe:3, Pe:10 and Pe:11. The combined results for the tested Pe races showed that, compared to cultivars, resistance against downy mildew is not rare among landraces but the frequency of resistance is about five times as low in individual accessions (Table 2a).

Table 2
Average degree of polymorphism and average resistance allele frequency for accessions of (a) *S. oleracea* (15 Pe races) and (b) *S. turkestanica* (16 Pe races).

Accession origin	Number accessions	Polymorphism	Allele frequency
(a) <i>S. oleracea</i>	404	0.426	0.051
Population type			
Cultivars	140	0.475	0.104
Landraces	122	0.377	0.021
Origin region			
Europe West	113	0.486	0.115
Europe South	25	0.339	0.014
Asia West	104	0.366	0.020
Asia South	47	0.467	0.029
Asia East	53	0.431	0.027
(b) <i>S. turkestanica</i>	75	0.870	0.199
Origin region			
TJK-Rep. Sub.	6	0.894	0.161
TJK-Kathlon	24	0.859	0.149
UZB-Surxondaryo	3	0.808	0.119
UZB-Qashqadaryo	4	0.859	0.147
UZB-Jizzakh	22	0.942	0.323
UZB-Tashkent	4	0.779	0.113
Turkmenistan	5	0.766	0.143

The average degree of polymorphism (0.486) and the average resistance allele frequency (0.115) were relatively high for West European accessions due to the fact that this group mainly consists of cultivars (Table 2a). South European accessions, that mainly comprised landraces collected in Macedonia and Greece, showed rather low values for polymorphism (0.339) and resistance allele frequency (0.014). Cultivated materials from Asia largely consisted of landraces collected in West Asia (Turkey, Armenia and Azerbaijan), South Asia (Iran, Afghanistan, Pakistan and India) and East Asia (China, South Korea and Japan). Among the Asian origin regions, the highest value for polymorphism (0.467) and resistance allele frequency (0.029) were observed for the accessions from South Asia, which is the presumed area where spinach was domesticated.

Comparison of resistance with *S. turkestanica*

Resistance screening of the 75 hybrid progenies between cultivated spinach and *S. turkestanica* for 16 downy mildew races resulted in 1180 data points and 20 ambiguous test results. Resistance to downy mildew was observed in all hybrid progenies. For each of the investigated downy mildew races, the hybrid progenies typically showed higher average resistance allele frequencies compared to cultivated spinach (Fig. 1). Only for the older races Pe:1 and Pe:2 cultivated spinach showed relatively high frequencies of accessions with high resistance allele frequency. For all examined downy mildew races the proportion of accessions with resistant plants was considerably higher in *S. turkestanica* than in cultivated spinach (Fig. 2). Resistance against Pe:13, which was almost absent in cultivated spinach, was observed in all hybrid progenies, underlining the importance of *S. turkestanica* as a source of downy mildew resistance for spinach breeding. Also for Pe:2, Pe:4 and Pe:7 resistant plants were found in all hybrid progenies. For all Pe races combined the average degree of polymorphism (0.870) was about two times as high and the average resistance allele frequency (0.199) about four times as high in *S. turkestanica* as compared to *S. oleracea* (Table 2).

Distribution of resistance in *S. turkestanica*

In general, resistance alleles were detected in all origin countries and in all national regions of the examined *S. turkestanica* accessions for each of the investigated downy mildew races (Suppl. Figure 3). However, allele frequencies could differ markedly within and between regions. In particular, populations from the region Jizzakh in Uzbekistan appeared relatively rich sources of downy mildew resistance. The average resistance allele frequency in populations from this area (0.323) was at least twice as high compared to the other investigated origin regions (Table 2b). Within the region Jizzakh high resistance levels were observed particularly for accessions collected south and east of the city of Jizzakh (Fig. 3). Accession CGN25010 showed the highest average resistance level of 0.520.

A significant negative correlation was found between resistance allele frequency and altitude of the origin location for Pe:4 ($r = -0.245$, $t = 1.998$, $p < 0.05$), Pe:5 ($r = -0.217$, $t = 1.794$, $p < 0.05$), Pe:15 ($r = -0.229$, $t = 1.908$, $p < 0.05$), Pe:16 ($r = -0.222$, $t = 1.854$, $p < 0.05$) and Pe: 17 ($r = -0.275$, $t = 2.323$, $p < 0.05$). However, the relatively low altitude of the collecting sites in Jizzakh might confound these correlations and for all races combined ($r = -0.179$, $t = 1.482$) the relationship was non-significant (Suppl. Table 3).

Population resistance allele frequencies were positively correlated between different downy mildew races in many cases (Table 3). Exceptions were Pe:8 and to a lesser extent Pe:10. Pe:8 showed significant ($p < 0.05$) correlations only with Pe:10 and Pe:13. For Pe:10 the relationship with other races was more diverse. In particular Pe:1 to Pe:7 showed highly significant correlations for all combinations of these older downy mildew races. A significant trend was observed of lower average resistance allele frequencies for newer races of the pathogen (Fig. 4, Spearman's $\rho = -0.57$, $p = 0.021$, two-tailed test).

Table 3
Correlations (above diagonal) and corresponding Student t values (below diagonal) between the resistance allele frequencies for different downy mildew races (Pe) based on the 75 study populations of *S. turkestanica*.

Pe:1	Pe:1	Pe:2	Pe:3	Pe:4	Pe:5	Pe:6	Pe:7	Pe:8	Pe:10	Pe:11	Pe:12	Pe:13	Pe:14	Pe:15	Pe:16	Pe:17
		0.777	0.702	0.646	0.704	0.590	0.626	0.042	0.177	0.667	0.636	0.434	0.356	0.625	0.458	0.4
Pe:2	10.468		0.732	0.666	0.725	0.698	0.669	0.001	0.282	0.609	0.525	0.530	0.267	0.787	0.397	0.4
Pe:3	8.302	9.124		0.687	0.635	0.507	0.614	-0.028	0.294	0.584	0.551	0.271	0.216	0.593	0.190	0.3
Pe:4	7.038	7.477	7.854		0.607	0.530	0.541	-0.135	0.267	0.510	0.558	0.228	0.395	0.498	0.296	0.2
Pe:5	8.356	8.926	6.922	6.397		0.736	0.706	0.133	0.366	0.727	0.590	0.493	0.314	0.616	0.508	0.5
Pe:6	6.071	8.162	4.883	5.113	9.042		0.568	0.075	0.330	0.588	0.523	0.571	0.313	0.574	0.541	0.4
Pe:7	6.671	7.522	6.467	5.269	8.283	5.653		0.009	0.240	0.529	0.398	0.317	0.250	0.494	0.301	0.4
Pe:8	0.355	0.012	0.237	1.123	1.123	0.624	0.074		0.232	0.004	-0.153	0.216	-0.059	-0.124	-0.020	0.1
Pe:10	1.523	2.513	2.624	2.316	3.336	2.922	2.067	2.013		0.215	0.165	0.354	0.237	0.324	0.114	0.3
Pe:11	7.553	6.507	6.059	4.930	8.910	6.034	5.183	0.038	1.870		0.652	0.568	0.507	0.560	0.483	0.4
Pe:12	6.749	5.084	5.411	5.428	5.979	4.953	3.496	1.260	1.379	7.043		0.456	0.337	0.435	0.277	0.4
Pe:13	4.087	5.341	2.388	1.958	4.804	5.825	2.795	1.861	3.238	5.850	4.221		0.410	0.474	0.418	0.3
Pe:14	3.236	2.371	1.876	3.601	2.805	2.753	2.164	0.499	2.081	4.996	2.952	3.845		0.327	0.403	0.2
Pe:15	6.790	10.888	6.244	4.801	6.636	5.872	4.754	1.057	2.928	5.735	3.980	4.597	2.953		0.389	0.4
Pe:16	4.377	3.697	1.644	2.596	5.007	5.377	2.641	0.169	0.981	4.677	2.382	3.930	3.758	3.605		0.5
Pe:17	3.863	3.891	3.620	2.332	5.295	4.757	3.692	1.000	3.210	4.454	3.602	3.200	2.652	3.827	5.207	
	p < 0.001		p < 0.01		p < 0.05		ns									

Table 4

Simulation results of the resampling study using varying numbers of the 66 *S. turkestanica* populations from Tajikistan and Uzbekistan and varying numbers of sampled plants per population. (A) Fraction of the total number of resistance alleles captured for the 16 investigated downy mildew races, (B) Probability of capturing all resistance alleles of the 16 investigated downy mildew races. Values equal or greater than 0.95 are shaded in grey colour.

(A) Fraction of the total number of resistance alleles captured											
		Number of populations									
		1	2	3	4	5	6	7	8	9	10
Sample size	10	0.779	0.924	0.966	0.984	0.991	0.996	0.998	0.999	0.999	0.999
	20	0.838	0.950	0.980	0.991	0.996	0.998	0.999	0.999	1.000	1.000
	30	0.861	0.958	0.985	0.993	0.997	0.999	0.999	1.000	1.000	1.000
	40	0.869	0.964	0.987	0.994	0.998	0.999	0.999	1.000	1.000	1.000
	50	0.876	0.966	0.988	0.995	0.998	0.999	0.999	1.000	1.000	1.000
(B) Probability of capturing all resistance alleles											
		Number of populations									
		1	2	3	4	5	6	7	8	9	10
Sample size	10	0.087	0.360	0.607	0.771	0.877	0.930	0.962	0.980	0.990	0.995
	20	0.132	0.474	0.723	0.869	0.932	0.971	0.986	0.993	0.998	0.999
	30	0.159	0.532	0.777	0.903	0.953	0.980	0.992	0.996	0.998	0.999
	40	0.175	0.565	0.810	0.917	0.963	0.984	0.993	0.996	0.998	0.999
	50	0.183	0.588	0.818	0.925	0.967	0.986	0.993	0.998	0.999	0.999

Resampling study

Simulations revealed that on average 77.9% of the total number of resistance alleles can be captured when 10 plants from a single randomly chosen population are sampled (Table 4A). This percentage rises rapidly with higher sample sizes per population and larger numbers of sampled populations. More than 95% of the alleles can be captured when at least 20 plants are sampled from two populations or when at least 10 plants are sampled from at least three populations. When looking at individual downy mildew races, sampling at least 20 plants from five populations would be sufficient to pick up the resistance allele with at least 95% probability (Suppl. Table 4). To have a more than 95% probability of capturing all resistance alleles, sampling 30–50 plants from 5–6 populations would be needed (Table 4B). The results of the resampling study are in line with the finding that the distribution of downy mildew resistance alleles is widespread rather than locally rare.

DISCUSSION

Downy mildew resistance

Genetic erosion of the cultivated gene pool has reduced the genetic diversity available for present and future crop breeding (Van de Wouw et al. 2010). Due to the possibility to transfer traits between interfertile species by conventional crossing, crop wild relatives have become an indispensable source of genetic diversity for crop improvement (Hajjar and Hodgkin 2007). Particularly for resistances to biotic and abiotic stresses, crop wild relatives are considered important suppliers, and hence play an essential role in developing a more sustainable agriculture and in ensuring food security (Mammadov et al. 2018). The genetic resources of plant species that occur *in situ* are usually difficult to access by users, and in general crop wild relatives are poorly represented in *ex situ* collections (Castañeda-Álvarez et al. 2016; Khoury et al. 2010). This was true for the crop wild relatives of spinach, but recent collecting expeditions have greatly improved their access in *ex situ* collections (Van Treuren et al. 2020). Here we evaluated the importance of *S. turkestanica* as a source of downy mildew resistance for spinach breeding and investigated how the spatial distribution of downy mildew resistance in *S. turkestanica* in Central Asia may impact efficient collection management.

The genetic basis of downy mildew resistance in spinach is not yet fully understood. Major qualitative dominant genes are considered to control the resistance and at least six Resistance to *Peronospora farinosa* (RPF) loci have been reported to be involved in resistance against the Pe races denominated to date (Bhattarai et al. 2022; Correll et al. 2011; Feng et al. 2018a; Olaoye et al. 2024). RPF1, RPF2 and RPF3 were mapped to a 1.5 Mb region on chromosome 3, each controlling resistance against multiple denominated Pe races. A Genotyping by Sequencing (GBS) study resulted in several markers linked to RPF1, RPF2, and RPF3 and supported the close linkage of these resistance loci (Feng et al. 2018a). A Genome Wide Association Study (GWAS) using whole genome resequencing data identified various significantly associated SNP markers with spinach downy mildew resistance, and Quantitative Trait Loci (QTL) alleles were detected at the chromosome 3 region harboring RPF loci (Bhattarai et al. 2022). Partial resistance against spinach downy mildew has been reported but the genetic basis thereof has not yet been elucidated (Correll et al. 2011). Data analysis in the present study was facilitated by converting data on resistance and partial resistance into a combined resistance score per accession and by hypothesizing a single resistance allele per

investigated Pe race. When partial resistance was disregarded the fraction of *S. turkestanica* accessions that harbor resistance was 60% on average, ranging from 13% for Pe10 to 92% for Pe2 (Suppl. Figure 4). Thus, also absolute resistance alone showed a wide distribution among the investigated *S. turkestanica* accessions.

Downy mildew resistance was detected in *S. oleracea* for all of the investigated Pe races, albeit that its presence was typically less than ten percent and rather rare in some cases. Only for Pe:1 and Pe:2 high resistance levels were observed for *S. oleracea*, which was most pronounced in cultivars as compared to landraces. This finding is most likely related to the fact that the start of downy mildew resistance breeding in the late 1950s focused on the introgression of resistance to Pe:1 and Pe:2 into cultivars (Ribera et al. 2020). As a result, cultivars that were introduced since then can be expected to be resistant to Pe:1 and Pe:2. Pe:3 was denominated by the IWGP in 1976, Pe:4 to Pe:7 in the nineties and Pe:8 to Pe:17 between 2004 and 2018 (Ribera et al. 2020). Because the CGN collection does not contain many cultivars from these more recent periods, a lower occurrence of resistance can be expected for the newer races.

Downy mildew resistance was clearly more common in *S. turkestanica* than in *S. oleracea*, indicating their usefulness for spinach resistance breeding. Resistance levels in *S. turkestanica* were correlated between different Pe races, which may be related to the tight linkage of the underlying resistance loci (Feng et al. 2018a), or because certain resistance loci may confer resistance against different Pe races (Feng et al. 2018b). Exceptions were observed for Pe:8 and to a lesser extent for Pe:10 that showed no or limited correlation with the other Pe races. For these two Pe races resistance reactions of lines from the differential set are associated with known RPF loci (ISF 2026; Ribera et al. 2020). Compared to the other Pe races a lower level of differentiation among the examined *S. turkestanica* populations was observed for Pe:8 and Pe:10 (Suppl. Figure 3). It remains to be determined whether the lack of correlation with other Pe races is due to experimental or genetical causes.

In none of the *S. turkestanica* populations fixation of the resistance allele was observed for any of the investigated Pe races. Polymorphism maintenance for resistance genes is presumed to be due to a balance between the benefits of plant defense and the fitness costs of disease resistance (Brown 2003; Brown and Rant 2013). It has also been argued that costs of resistance or virulence in gene-for-gene interactions are no prerequisite for polymorphism maintenance under natural conditions as dispersal scale was found to have a large impact on genetic variation for resistance in metapopulations (De Meaux and Mitchell-Olds 2003; Thrall and Burdon 2002). *S. turkestanica* was typically found in cultivated non-irrigated steppes with a loess soil along agricultural fields (Van Treuren et al. 2020). Therefore, the exchange of *S. turkestanica* seeds between existing populations or suitable habitats may be common through agricultural activities. In addition, the spiny pericarp of *S. turkestanica* fruits can be considered to promote seed dispersal by animals (Van Treuren et al. 2020). Because *S. turkestanica* is a wind-pollinated outbreeder, gene flow between populations through pollen transfer may also be common. The dispersal and reproduction characteristics of *S. turkestanica* may underlie the high level of polymorphism observed for downy mildew resistance in the majority of the examined populations.

For each of the investigated Pe races, resistant plants could be found throughout the study area in Central Asia, rather than being confined to specific geographic regions. Interestingly, populations from the region Jizzakh in Uzbekistan showed twofold higher average resistance levels than populations from other sampled regions. Especially populations collected eastward and southward of the city of Jizzakh showed high average resistance levels, suggesting a high disease pressure in this area (Fig. 3). *Peronospora* typically thrives during cool, moist nights and dry, warm mornings but the precise determinants for optimal *Peronospora* infection, growth, sporulation and spread are complex (Choudhury et al. 2016). The Jizzakh region, like most regions in Uzbekistan has a dry, hot summer, with highest precipitation in winter and spring. However, conditions differ markedly within the country. The sampled regions south of Samarkand (Fig. 3) experience much higher temperatures in summer, and receive less precipitation, resulting in a short spinach growth season and less favorable conditions for *Peronospora* (Anonymous 2020). The regions north of Samarkand (and north of the Zarafshan and Turkestan mountain ranges) have a more temperate climate and experience more rainfall (Anonymous 2020). The foothills of the Turkestan range have a complex orogenic structure and feature a range of microclimates with significant spatial contrasts in temperature, precipitation and humidity (Shermanov 2025). It is likely that regional differences in climatic conditions exist, resulting in an ecological “sweet spot” for downy mildew in wild spinach around the city of Jizzakh and causing selection for elevated resistance levels against downy mildew in this region. The observation that accessions collected near Tashkent do not show the same, high resistance levels as accessions around Jizzakh, in spite of generally similar climatic conditions, supports the idea that high disease pressure around Jizzakh is not explained by climate alone. Disease resistance levels have previously been correlated with climatic effects and geographical variables (Ahrens et al. 2019; Keepers et al. 2024; Stam et al. 2017), but is typically thought to result from the interplay of different variables. In the light of the growth (and dispersal) cycles of both host and pathogen, such is not unexpected for spinach downy mildew (Choudhury et al. 2016).

A review study on natural plant-pathogen associations showed that variation in disease resistance is a widespread phenomenon, observed across all spatial scales (Laine et al. 2011). However, whether the resistance structure of plant populations is shaped by selective or non-adaptive forces remained unclear. Previously, 18 of the populations from Uzbekistan and Tajikistan investigated in the present study were analyzed for SNP and phenotypic variation. The SNP variation was positively correlated with phenotypic variation and geographic distance (Van Treuren et al. 2025). For this set of populations, genetic distances calculated for downy mildew resistance allele frequencies (Hartl and Clark 2006) were uncorrelated with genetic distances based on SNP variation ($r = -0.047$, $t = 0.577$) and phenotypic variation ($r = -0.033$, $t = 0.408$) and with geographic distance ($r = 0.042$, $t = 0.522$). As the SNP and phenotypic data can be considered to represent largely neutral variation, the lack of correlation with the resistance data may point towards the influence of selection in the distribution of resistance alleles. The expedition materials from Uzbekistan and Tajikistan were collected in 2008, in the year that Pe:11 was denominated, and prior to the introduction of Pe:12 to Pe:17 (Ribera et al. 2020). For the older races Pe:1 to Pe:10 relatively high levels of resistance were observed (Fig. 4), which may have resulted from natural selection by pathogen pressure.

The *S. turkestanica* accessions collected in Central Asia undoubtedly represent a valuable resource to improve downy mildew resistance in cultivated spinach. For example, CGN25259 has been successfully used in a breeding program resulting in spinach plants resistant to the pathogenic races Pe 1–9, 11–15 and 17 (De Visser 2022). Similarly, an introgressed sequence from CGN25007 was used to enhance downy mildew resistance for Pe races 1–18 in spinach (Lokossou and de Lange 2024).

Conservation aspects

S. turkestanica is native to Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan and Uzbekistan in Central Asia and to Afghanistan, Iran and Pakistan in South Asia (Hassler 2018). A substantial part of this distribution area remains unexplored as organizing collecting expeditions with the national authorities of these countries is difficult, with the exception of Tajikistan and Uzbekistan. Populations of *S. turkestanica* in Tajikistan and Uzbekistan were typically found in the margins of agricultural fields. Such populations are at risk due to human activities when management practices change from low-input farming to an irrigation system and the application of chemical fertilizers and herbicides. The absence of local conservation programs underlines the importance of collecting expeditions to conserve the genetic diversity of *S. turkestanica ex situ* (Van Treuren et al. 2020).

Knowledge about the distribution of genetic variation among populations is key to sound sampling procedures and efficient collection development (Engels and Visser 2003). Such knowledge is generally lacking for wild species that are underrepresented in *ex situ* collections. As a general guideline for diploid outbreeders, a sample size of 30 individuals has been proposed to capture at least one copy of 95% of the alleles that occur in a population at frequencies greater than 0.05 (Marshall and Brown 1975). It has also been argued that under realistic population structure the sampling of 25–30 individuals per population from few but widely-spaced populations forms an optimal strategy (Hoban and Schlarbaum 2014). These recommendations are in line with our findings for *S. turkestanica*. Research on SNP and phenotypic markers showed that variation of *S. turkestanica* in the study area is mainly distributed within rather than between populations and that sampling 30–50 plants from each of 5–6 geographically widespread populations is sufficient to capture more than 98% of the observed SNP alleles and more than 99% of the observed phenotypic diversity (Van Treuren et al. 2025). Here we showed that this sampling strategy in the study area would capture all resistance alleles of the studied downy mildew races with more than 95% probability. In case of rare resistances against yet undetermined races recollecting from wild populations may be needed in the future when frequencies have increased as a result of selection.

Adaptive traits that can be expected to occur only in specific habitats, such as tolerance to drought or salinity, will have a low probability to be picked up through moderate untargeted sampling. In case of interest in such characters extensive sampling may then be needed to find the needle in the haystack. In case of such specific interests, targeted collecting using available habitat information or focusing on marginal instead of core populations constitutes a more efficient strategy.

Conclusions

Downy mildew resistance represents the most important horticulture trait of spinach. As new pathogenic races continue to emerge, the availability of novel resistances is essential in spinach breeding. Compared to cultivated spinach, its wild predecessor *Spinacia turkestanica* constitutes a much richer source of downy mildew resistances. Downy mildew resistance is widespread in the Central Asian distribution area of *S. turkestanica*. All resistance alleles of the 16 studied pathogenic races can be captured with more than 95% probability by sampling 30–50 plants from 5–6 populations from this area. Limited sampling of *S. turkestanica* from its Central Asian distribution area ensures the availability of ample genetic diversity in *ex situ* collections for research and breeding.

Declarations

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

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Author Contribution

R.v.T. was responsible for the study conception and design, software programming, data curation and first draft of the manuscript. Both authors contributed to the data analysis and the reviewing and editing of the manuscript. Both authors read and approved the final manuscript.

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Data Availability

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Figures

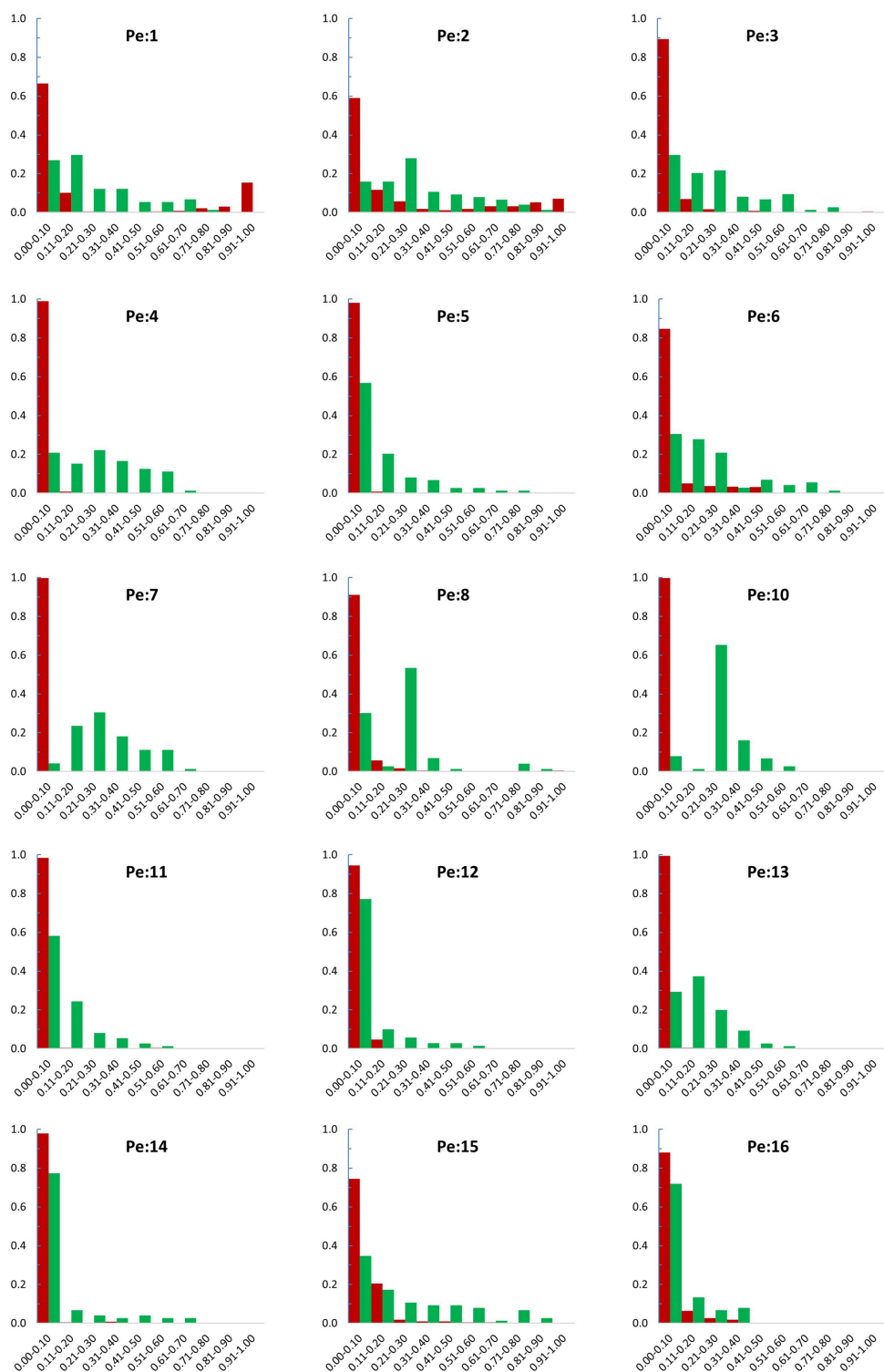


Figure 1

Frequency distribution of accessions per category of resistance allele frequency for each of 15 races of downy mildew (Pe) for *S. oleracea* (red) and *S. turkestanica* (green)

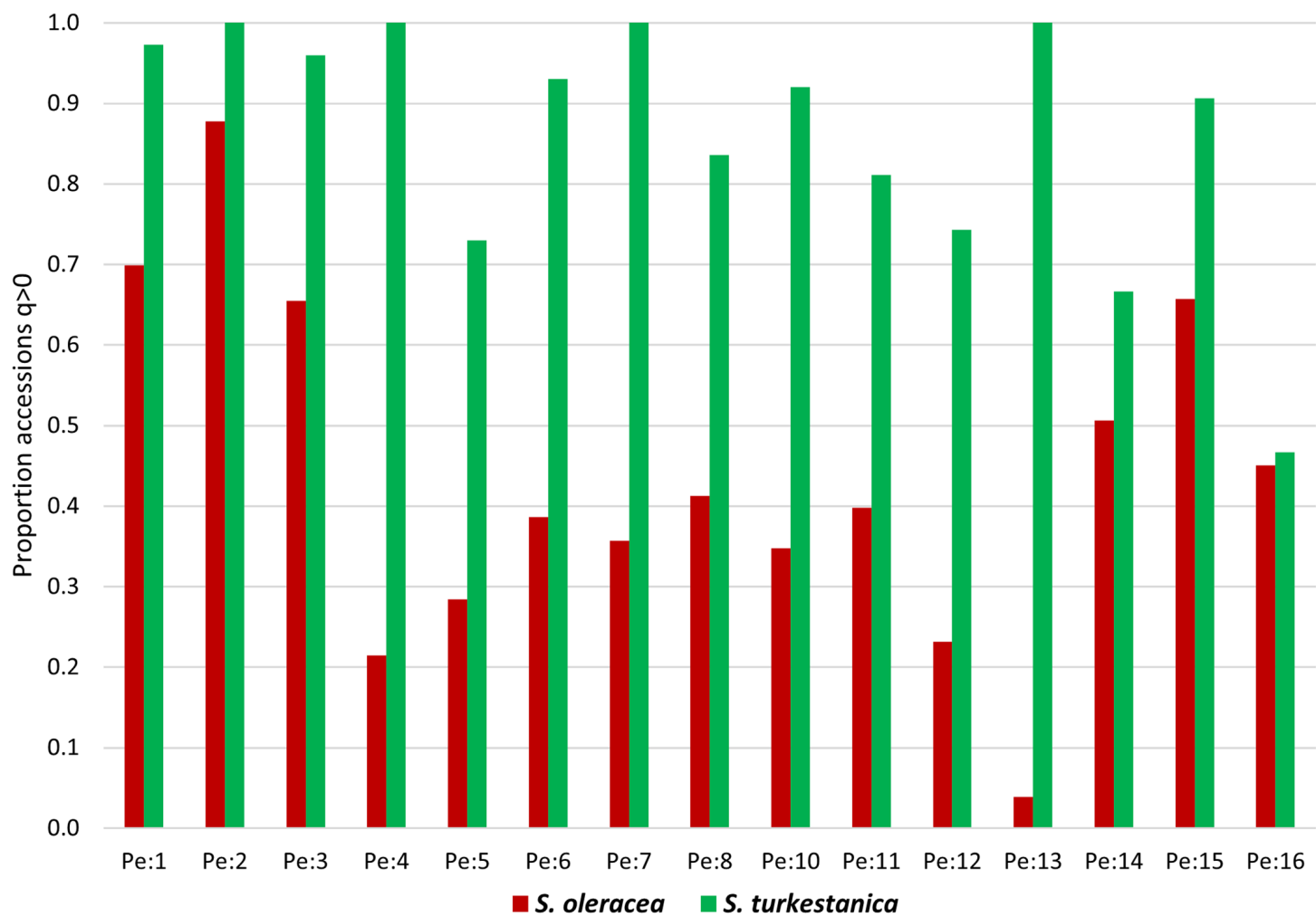


Figure 2

Proportion accessions with resistant plants for each of 15 races of downy mildew (Pe) for *S. oleracea* and *S. turkestanica*

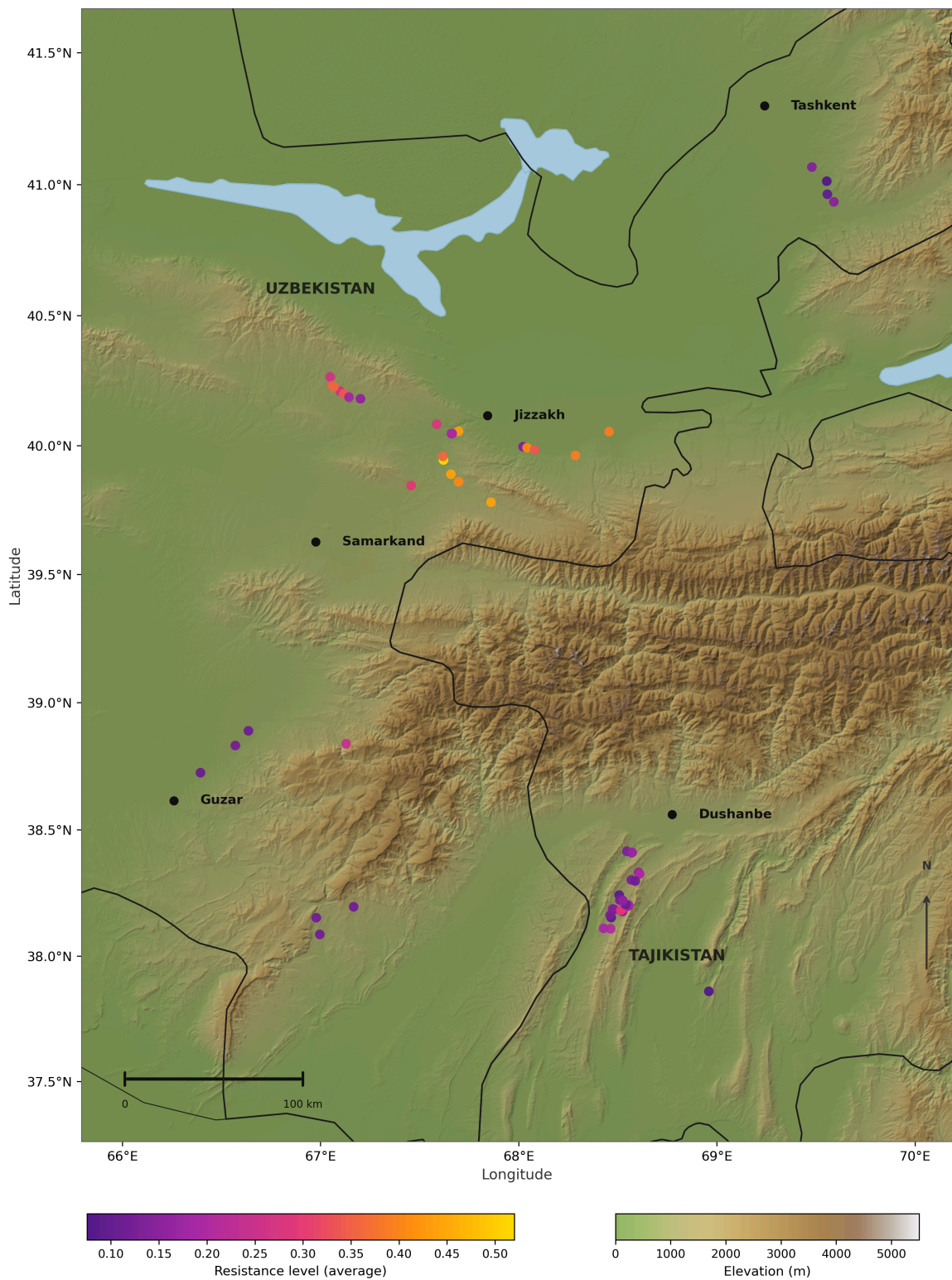


Figure 3

Geographic origin of the *S. turkestanica* samples from Uzbekistan and Tajikistan. Origin locations are denoted by dots, their colour depending on the average resistance level of the sample against the investigated downy mildew races

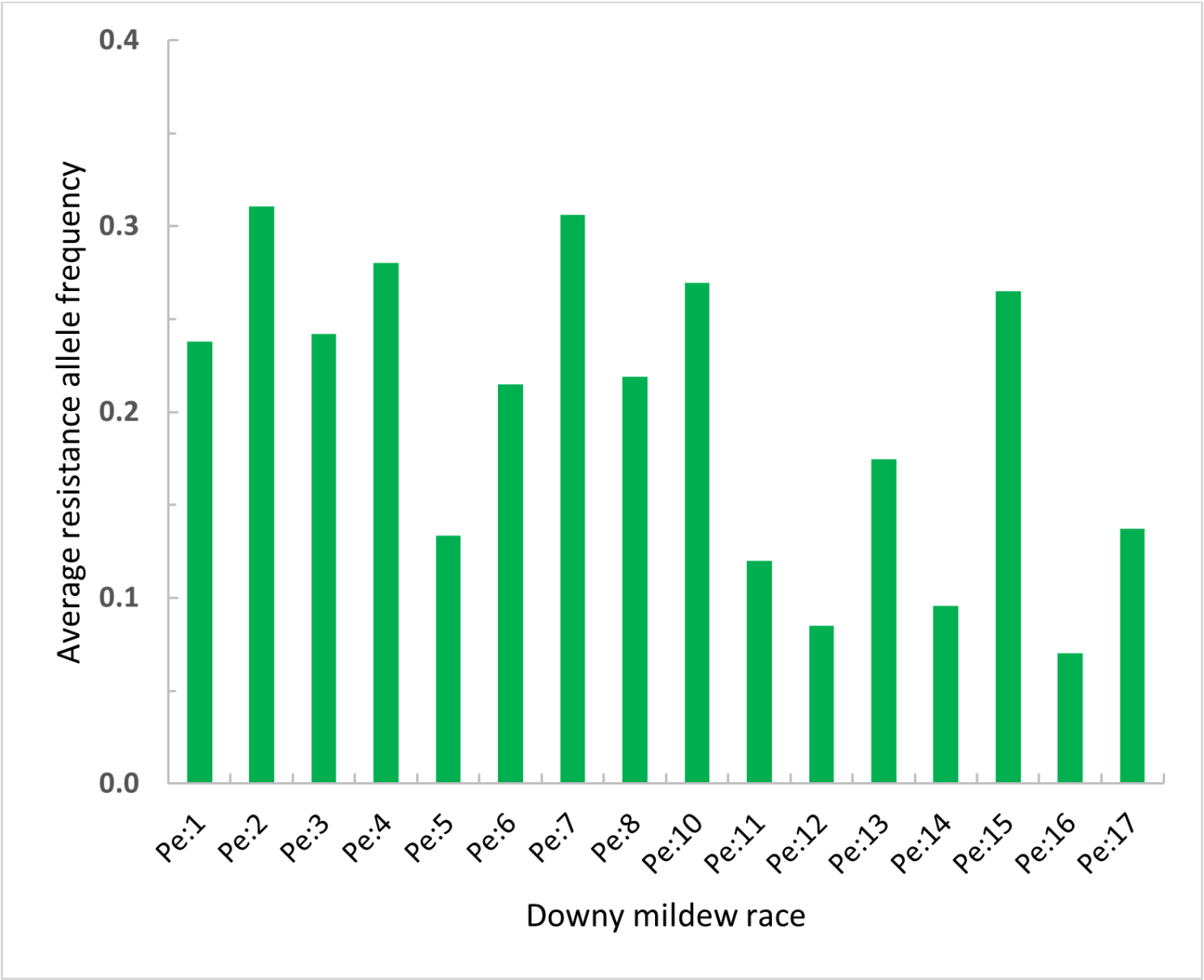


Figure 4
Average resistance allele frequency of the *S. turkestanica* accessions per downy mildew race

Supplementary Files

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- [MSvanTreurenWijnkerSuppl.Fig.1.pdf](#)
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