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Characterization of resistance to *Synchytrium endobioticum* in cultivated potato accessions from the collection of Vavilov Institute of Plant Industry

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With 7 figures and 1 table

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Abstract

The causal agent of potato wart (*Synchytrium endobioticum*) is an obligate parasitic chytrid fungus. It is included as a quarantine pathogen in 55 countries, with losses in susceptible cultivars reaching 50–100%. The aim of our study was to characterize the resistance to *S. endobioticum* pathotype 1 in cultivated potatoes from a well-characterized subset of the Vavilov Institute of Plant Industry collection and to determine whether this resistance is associated with cultivated potato species taxonomy, with ploidy, with geographic distance or with a molecular marker Nl25-1400 proposed for molecular screening for resistance to pathotype 1 of *S. endobioticum*. Within the diversity of 52 landrace genotypes, our work shows a lack of such predictive associations with wart resistance. High intraspecific variation of wart diseases resistance allows the selection of extremely resistant and susceptible genotypes available for future genetic and breeding studies.

Key words: marker-assisted selection — potato — potato wart — resistance — *Synchytrium endobioticum* — taxonomy

Synchytrium endobioticum (Schilb.) Perc. is an obligate parasitic chytrid fungus causing potato wart disease. Crop losses with susceptible varieties can reach 50–100% (Hampson 1993, Melnik 1998). The fungus produces a thick-walled winter sporangium, 25–75 μm in diameter, and contains 200–300 diploid resting spores (pro-sori) (Tarasova 1978, OEPP/EPPO 2004). In the spring, sporangia germinate to release motile zoospores that can infect tubers, sprouts, stolons and leaves. In infected cells, sporangia develop during the summer and give rise to new zoospore infections. The infected plant cells swell, divide and form a wart (Laidlaw 1985, EPPO/CABI 1997).

At the end of the nineteenth century, potato wart disease spread from its original range in the Andean region of South America to parts of North America and Europe (Hampson and Proudfoot 1974, Hampson 1993, EPPO/CABI 1997; OEPP/EPPO 2004). More than 40 pathotypes of the fungus exist, but the most widely distributed is pathotype 1 (D1) (Hampson 1993, Baayen et al. 2006). Many varieties have been successfully bred for resistance to *S. endobioticum* pathotype 1 since the beginning of 20th century (Ross 1958a,b, Schick and Hopfe 1962). However, since the 1940s, new pathotypes have been reported which are more difficult to control (Baayen et al. 2006).

Synchytrium endobioticum is included in the list of the quarantine pathogens in 55 countries (Anonymous 1987) and the subject of quarantine worldwide because of persistent resting spores and lack of effective chemical controls. In the Russian Federation, the first localities of disease were registered in the Leningrad region in 1940 with a total area of infection of 1.4 ha (Galanova 1964). Most infections occurred in private plots, where susceptible potato cultivars were grown (Anonymous 2001, 2003, 2006). In 2006 in the Leningrad region, potato wart was registered in nine areas, totalling 8.75 ha (Anonymous 2006). Russian quarantine legislation demands the cultivation of potato only in localities registered against wart, and only with resistant varieties (Tarasova 1978, Anonymous 1988). Resistance to wart is part of the obligatory requirement for the inclusion of new potato varieties in the State Register of Breeding Achievements.

Lack of effective chemical controls stimulates the need to breed-resistant varieties. The development of resistance to wart disease in commercial varieties was reported since the beginning of 20th century. However, the sources of introgression of resistant gene(s) into potato varieties are unclear. As *S. endobioticum* is considered to have co-evolved with potatoes in Andean South America (Hampson 1993, EPPO/CABI 1997), diverse sources of resistant potato species may be found there. Indeed, sources of wart disease resistance were reported mostly for Andean potatoes in both wild species (*S. acaule* Bitter, *S. chacoense* Bitter, *S. commersonii* Dunal, *S. vernei* Bitter and Wittm.) and cultivated species (*Solanum phureja* Juz. and Bukasov, *S. tuberosum* L. subsp. *andigenum* (Juz. and Bukasov) Hawkes) (Hampson 1993, Anisimov et al. 2009).

Cultivated potatoes represent a tremendously diverse gene pool growing under different climatic conditions from Venezuela to south-central Chile. They are easily hybridized to advanced potato cultivars, making them useful as breeding materials, and highlight the importance of a scientifically accurate, stable and predictive taxonomy. Cultivated potato taxonomy has been highly controversial, estimates ranging from 17 species (Bukasov 1978), to seven species and four subspecies Hawkes (1990), to the four species *S.* × *curtilobum* Juz. and Bukasov, *S.* × *juzepczukii* Bukasov, *S. ajanhuiri* and Juz. and Bukasov *S. tuberosum* L. (Spooner et al. 2010, Ovchinnikova

et al. 2011). We use here the seven species terminology of Hawkes (1990) to maintain continuity with previous literature on wart, but relate this to the taxonomy of Spooner et al. (2007) and Ovchinnikova et al. (2011) in our conclusions.

The relationship between disease resistance and taxonomic species, series, clade, ploidy and geographic distance includes examples of both associations and non-associations (Spooner et al. 2009). A review of 10 738 disease and pest evaluations, derived from the literature and geneBank records, of 32 pests and diseases in five classes of organisms (bacteria, fungi, insects, nematodes and viruses) found that resistances are reliably predicted by host taxonomy and climatic variables for only Colorado potato beetle [*Leptinotarsa decemlineata* (Say)] and one pathogen (Potato M Carlavirus), but not for other diseases.

New molecular techniques based on the use of molecular markers closely linked with a resistance gene [or with quantitative trait loci (QTL)] have been used to predict the resistant phenotypes in potato breeding (Gebhardt and Valkonen 2001). This approach requires knowledge about location of the gene(s) conferring resistance to a pathogen and about sequence variation present in candidate gene loci.

Studies of genetic control of resistance to wart disease initially suggested three dominant genes (Black 1935), or one dominant and two complementary genes (Lunden 1950) effective against pathotype 1 (D1). Later studies showed that a single gene *Sen1* (its dominant allele) determines the resistance to pathotype 1 (D1) of *S. endobioticum* (Langerfeld 1984, Lellbach and Effmert 1990). Hehl et al. (1999) reported the genetic mapping of a gene *Sen1* on potato chromosome XI. Later, Gebhardt et al. (2006) reported linkage of marker Nl25 to gene *Sen1* (XI). Brugmans et al. (2006) reported the second independent resistance gene *Sen1-4* against *S. endobioticum* pathotype 1 on potato chromosome IV. Recently, Ballvora et al. (2011) confirmed mapping *Sen1-XI* gene on the same genomic region as Hehl et al. (1999) on chromosome XI. Molecular markers linked with a *Sen1* gene have the potential to assist the selection of wart disease–resistant genotypes.

The goals of our research were (i) to determine the virulence and aggressiveness of *S. endobioticum* populations from different regions Belarus, Moscow and Ukraine; (ii) to characterize intraspecific and interspecific wart resistance variability in a subset of cultivated species accessions and to examine the association of wart disease resistance with taxonomy, ploidy and geographic distance; (iii) to select genotypes that are resistant to *S. endobioticum* pathotype 1; and (iv) to test the ability of the Nl25 marker to identify useful genotypes for wart resistance breeding.

Materials and Methods

Plant materials: We screened 52 cultivated potato species accessions from the experimental subset of the VIR collection, recently well characterized for ploidy, morphology, taxonomy, and molecular marker diversity (Gavrilenko et al. 2010). They included 11 accessions (classification of Hawkes 1990) of *S. phureja*, 15 accessions of *S. stenotomum* Juz. and Bukasov, 13 accessions of *S. tuberosum* subsp. *andigenum* and 13 accessions of *S. tuberosum* subsp. *tuberosum* (Table 1). According to the latest taxonomic treatments (Spooner et al. 2010, Ovchinnikova et al. 2011), all 52 accessions belong to the same species, *S. tuberosum*, which incorporates the diploid and tetraploid cytotypes used here. Each accession was represented by one genotype.

Inoculum of *Synchytrium endobioticum*: For inoculation, we used a population of *S. endobioticum* that was determined on 19 potato differentials to be pathotype 1. For resistance tests, we used samples of three populations *S. endobioticum* from the Moscow Region of Russia, Belarus and Ukraine. These samples were propagated on susceptible potato cultivars 'Liza' and 'Lorkh' by inoculation of potato sprouts in compost, keeping winter zoosporangia of *S. endobioticum*. For inoculum preparations, mature warts were cut into slices and dried at room temperature. The dried warts were pounded by a rubber pestle and screened through mesh. For inoculation of 1 kg of soil, 10–12 g of a sporangia powder was used. The optimum amount of inoculum (30–40 viable zoosporangia in 1 g of soil) was corrected by the determination of sporangia vitality (Saltykova and Tarasova 1982, Dmitraschuk and Romanyuk 1999).

To stimulate the germination of potato sprouts, variable times and temperatures were used from 7 days at +23–25°C, to 5 days at +3–5°C and to 5 days at +23–25°C. Tubers with sprouts up to 1 mm long were used for inoculation. Coarse sand was placed to a 3- to 5-cm layer at the base of cardboard boxes (40 × 30 × 20 cm) for drainage, upon which were slightly pressed into the sand 20 tubers of cultivars 'Liza' or 'Lorkh'. Tubers were moistened with water and covered with humid compost. Humidity of the compost was maintained at 70–80% and temperature at 16–18°C throughout the experiment. Fresh warts containing fast-germinated summer zoosporangia were obtained 2–2.5 months after inoculation. Eighty boxes were used every year for 3 years of evaluations to propagate the inoculum of *S. endobioticum*.

Pathotype determination: To determine the pathotype composition in samples of three geographic populations, 19 potato differentials that were present in the Vavilov Institute of Plant Industry (VIR) collection ('Alma', 'Antares', 'Apollo', 'Barbara', 'Bozhedar', 'Cardula', 'Fontana', 'Giewont', 'Lorkh', 'Lugovskoi', 'Lvovskiibelyi', 'Nezabudka', 'Ora' ('Mira'), 'Polesskii rosovyi', 'Prolisok', 'Resurs', 'Spadshchina', 'Temp' and 'Volovetskii') were used. Varieties from this set were used by several researchers for the determination of *S. endobioticum* pathotype composition on the territory of the former Union of Soviet Socialist Republics (USSR) (Saltykova 1988, Malakhanova and Melnik 1998) and Germany (Maris 1961), and the Netherlands (Baayen et al. 2006). A virulence test was conducted by the method recommended by the European and Mediterranean organization of plant protection with some modifications (OEPP/EPPO 2004). Tubers were placed on pallets with moist sterile sand two cm thick. Inoculation of sprouts on the tubers was carried out with 1 g pieces of fresh warts, white to light brown in colour, containing large numbers of summer sporangia, obtained from the susceptible varieties 'Liza' and 'Lorkh'. One test was conducted with ten potato tubers of each accession. Before inoculation, sprouts 1–2 mm in length were ringed on tubers with plastic sticky tape, sealed with silicone. According to the method of the European and Mediterranean Plant Protection Organization (OEPP/EPPO, 2004, http://www.eppo.org/), the rings were prepared with warm petroleum jelly or with petroleum jelly and paraffin. Pieces of fresh wart tissue were placed inside the rings with water. Inoculated tubers were placed inside the moist chamber and were placed inside a plastic box. After 24-h incubation at 16–18°C, the wart tissue was removed and new warts were put on the sprouts. Forty-eight hours after inoculation, the sprouts were moistened with distilled water. The evaluation of the reaction types was made after 25 days, using the five-scale method of OEPP/EPPO (2004).

Ten tubers of each differential and 10 tubers of susceptible varieties in three replications were used for inoculation. After 25 days, the results of inoculation were scored by a five-score scale recommended by OEPP/EPPO (2004): (i) extremely resistant: early defence necrosis; no visible sorus formation; (ii) resistant: late defence necrosis; sorus formation partially visible, sori immature or necrotic before maturity; (iii) weakly resistant: very late defence necrosis, single ripe sori or sorus fields developed, but completely surrounded by necrosis, defence reactions dominant, but not always faster than sorus or sorus field maturation, scattered infections, up to five non-necrotic sori, clear necrosis in other zones of the same tuber piece, high degree of attack of the control cultivar (essential! – as mentioned in protocol), the present

Table 1: Resistance to pathotype 1 of *Synchytrium endobioticum* of 52 genotypes of cultivated potato species

No	VIR catalogue number[1]	New VIR introduction number[2]	Species[3]	Types of reactions in different replications	Resistance group[4]	Presence of Nl25 allele 1200 bp	Presence of Nl25 allele 1400 bp
1	8271	0144780	phu	2; 1.8; 1.6	2	–	–
2	22210	0144802	phu	2; 2	2	+	–
3	22221	0144804	phu	1; 1	1	+	–
4	31439 GLKS (k-1817)	0144810	phu	1; 1	1	+	–
5	9836	9836	phu	2; 1.8; 1.4	2	+	–
6	16530	0144793	phu	2; 2	2	–	–
7	11291	0144787	phu	1; 1	1	–	–
8	9333	0144781	phu	1; 1	1	+	–
9	9393	0144783	phu	1; 1; 1	1	+	–
10	12789	0144788	phu	5; 3.5	5	+	–
11	15845	0144790	phu	3.4; 3.7; 3; 2.1	4	+	–
12	8991	0144762	stn	1; 1; 1	1	+	–
13	8865	0144750	stn	2; 1.8; 2; 1.8	2	+	–
14	8935	0144759	stn	2; 1.6; 2	2	+	–
15	8892	0144756	stn	1; 1; 1	1	–	–
16	8863	0144749	stn	2.2; 2.6; 2.1; 2.2	3	+	–
17	8880	0144754	stn	1; 1; 1	1	+	–
18	8929	0144758	stn	1; 1; 1	1	+	–
19	9039	0144763	stn	1; 1; 1	1	+	–
20	16911	0144825-a	stn	1; 2	2	–	–
21	16911-a	0144825	stn	2; 4.5	5	–	–
22	9889	0144816	stn	3; 3; 2.7	3	+	–
23	9889-a	0144816-a	stn	1; 1	1	+	–
24	7126	0144833	stn	2; 1.8; 1.6	2	+	–
25	10194	0144829	stn	1; 1; 1	1	+	–
26	11053	0144830	stn	2; 2	2	–	–
27	1697	0144703	adg	1; 1.6; 2	2	–	–
28	1763	0144713	adg	2; 1.4	2	+	–
29	1796	0144719	adg	1; 1; 1	1	+	–
30	1793	0144718	adg	3; 3; 3; 3	3	+	–
31	1775	0144716	adg	2; 5; 5; 3.5; 4.6; 4.8	5	+	–
32	1741	0144708	adg	2; 3.6; 3.7; 3.8	4	+	+
33	4617	0144728	adg	1; 1; 1	1	–	–
34	4634	0144729	adg	1; 1; 1; 1	1	+	–
35	8931	0144734	adg	2; 1.8; 2; 2	2	+	–
36	9002	0144735	adg	1; 1; 1	1	+	–
37	9571	0144736	adg	2; 1.7; 1.4; 2	3	+	+
38	12892	0144737	adg	4; 3.14	4	–	–
39	3231	0144725	adg	1; 2; 1.8; 1.1; 1.2	3	+	–
40	7530	0144891	tub	1; 1; 1	1	+	–
41	7535	0144892	tub	2; 1.6; 2	2	+	–
42	7568	0144896	tub	1; 1; 1	1	+	+
43	7580	0144899	tub	2; 1.6; 1.8	2	+	+
44	7583	0144900	tub	2; 2	2	+	–
45	1673	0144911	tub	1; 1	1	+	–
46	3456	0144885	tub	1; 1	1	+	+
47	3484	0144886	tub	1; 1.4; 1; 1; 1.3	2	–	–
48	1816	1816	tub	1; 1	1	+	–
49	2148	0144912	tub	2; 2; 2	2	+	–
50	2083	0144909	tub	1; 1; 1; 1	1	–	–
51	7586	0144901	tub	1; 1; 1; 1	1	+	–
52	24602	0144906	tub	4; 4	4		

[1]VIR catalogue number, '-a' – means seedling.
[2]Each accession (VIR catalogue number) in this study was represented by one genotype which received a new VIR 'introduction number'.
[3]Species codes: adg = *Solanum tuberosum* subsp. *andigenum*; phu = *S. phureja*; stn = *S. stenotomum*; tub = *S. tuberosum* subsp. *tuberosum*.
[4]Resistance phenotype codes: 1 = extremely resistant; 2 = resistant; 3 = weakly resistant; 4 = slightly susceptible; 5 = extremely susceptible.

class 3 includes the old class 4 of Hille (1965); (iv) slightly susceptible: scattered infections, sori or sorus fields non-necrotic, few in number, late necrosis can be present on other infection sites on the sprout, the sprout can be slightly malformed (thickened); (v) extremely susceptible: dense infection fields, numerous ripe non-necrosed sori and sorus fields, fields with dense non-necrotic infection sites, predominant tumour formation.

Average means for each experiment were counted, and the group of resistance was determined on the basis of reaction type of the majority of tubers. If the results of replications were different, the group of resistance was determined on the basis of maximum score in any replication.

Aggressiveness of *S. endobioticum* geographic populations: In order to choose a more aggressive population for resistance evaluation, comparative studies of *S. endobioticum* populations from the Moscow, Belarus and Ukraine regions were conducted. The number of infected plants after inoculation of five susceptible varieties ('Lorkh', 'Liza', 'Polesskii rosovyi', 'Tulunskii' and 'Alma') was studied. Inoculation

with fresh warts of 10 tubers of each cultivar was carried out in three replications.

Method of resistance evaluation: For resistance evaluation, we used the method of inoculation of tuber sprouts with fresh warts, containing summer sporangia (Fig. 1). The procedure of inoculation and scale for resistance evaluation were the same as described above for pathotype determination. Results of resistance evaluation are considered authentic if disease developed on not less than on 75% of tubers of the susceptible cultivars 'Lorkh' or 'Liza'. The number of tubers per replication and the number of replications depended on the availability of tuber material (Table 1), but when possible we used ten tubers of each accession per replication. We conducted two independent replications for 19 potato accessions, three for 20 accessions, four for 10 accessions, five for two accessions and six replications for one accession. Average means for each replication were counted. If the results of replications were different, the group of resistance was determined on the basis of the maximum score in any replication.

Assessment of the diagnostic value of marker Nl25: We assessed the same subset of 52 landrace genotypes with the SCAR-marker Nl25, which is linked to a gene *Sen1* (XI) conferring resistance to pathotype 1 of *S. endobioticum* and which amplified a 1400-bp fragment in the wart-resistant diploid breeding clone and its progeny (Gebhardt et al. 2006). Young leaves from each accession were harvested from the field-grown plants and frozen in liquid nitrogen. Total DNA was isolated from frozen leaf material using the CTAB method (Murray and Thompson 1980) with small modifications. PCR was performed according to Bormann et al. (2004).

GIS mapping the accessions: We mapped the distribution of cultivated species potato accessions and their resistance phenotypes with MAPINFO, version 9.5.

Results

Virulence and aggressiveness of *S. endobioticum* populations

By using the set of 19 potato differentials, it was possible to determine the pathotypes distributed on the territory of the former USSR: 1 (D1), 11 (M1), 13 (R2), 16 (S1), 18 (I), 20, 21, 22, and also pathotypes, additionally recorded in Germany 2 (G1), 4 (P1), 5 (K1), 6 (O1), 7 (S1), 8 (F1), 9 (R1), 10 (E1), 18 (T1) and Czech Republic 15 (P2), 16 (N1), 17 (M2). The Moscow, Ukrainian and Belorussian populations of *S. endobioticum* were virulent to susceptible potato cultivars ('Lorkh', 'Polesskii rosovyi' and 'Alma') and avirulent to all other cultivars, allowing us to determine that all pathotypes are D1.

The number of infected tubers of susceptible cultivars 'Liza', 'Lorkh', 'Tulunskii' and 'Polesskii rosovyi' used for inoculation was similar (93–100%) to all three populations (see Materials and Methods). Pathogen populations differed on aggressivity only on 'Alma'. It showed the least number of infected tubers when populations from Belarus (44% infected tubes) and Ukraine (62% infected tubes) were used. At the same time, 79% tubers of this cultivar were infected by the Moscow population (Fig. 2). On the basis of these results, we propose that the Moscow population would be more aggressive on other potato accessions, and we used the Moscow population of *S. endobioticum* (pathotype D1) for evaluations of resistance and as susceptible controls – cultivars 'Liza' and 'Lorkh'.

Characterization of cultivated potato subset for resistance to *Synchytrium endobioticum*

The results of evaluation of cultivated potato species for resistance/susceptibility to potato wart are presented in Table 1 and in Figs 1, 3 and 4. Most of the evaluated potato accessions have the same types of reactions to *S. endobioticum* in all replications (Table 1). In few cases, we have contradictions between evaluations in different replications. The group of resistance for accessions with different reaction types was determined on the basis of the maximum score in any replication. For example, *S. tuberosum* subsp. *andigenum* (0144725) was scored in five replications as 1; 1.1; 1.2; 1.8 and 3, and thus, it was characterized as weakly resistant (resistance group 3). Accession 0144716 was scored in six replications as 4.6; 4.8; 5; 5; 5 and in one as 2, and thus, it was characterized as extremely susceptible (resistance group 5).

Of the 52 genotypes, 23 were extremely resistant (score 1) and included most accessions of each analysed cultivated species; 17 were resistant (score 2) and likewise were distrib-

Fig. 1: Rings from plastic sticky tape, fixed with silicone to form a hermetic seal on *Solanum stenotomum* (0144750)

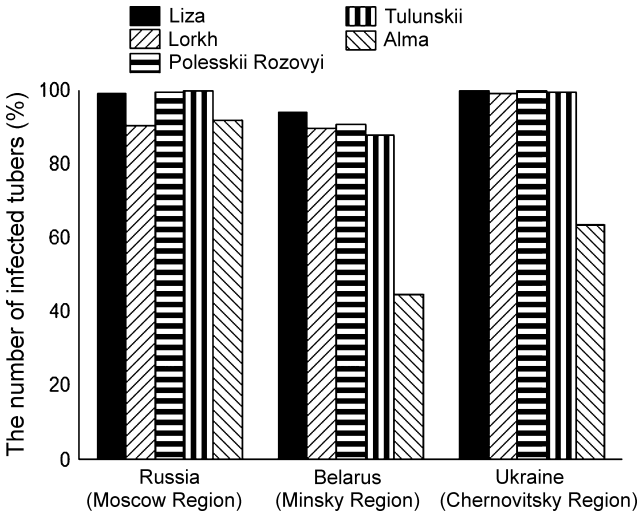

Fig. 2: Percentage of infected tubers after inoculation of five potato cultivars ('Alma', 'Liza', 'Lorkh', 'Polesskii rosovyi' and 'Tulunskii') with fresh warts of pathotype 1 (D1) of *Synchytrium endobioticum*. The three regions are from Russia (Moscow Region), Belarus (Minsky Region) and Ukraine (Chernovetsky Region)

uted among all analysed cultivated species; five were weakly resistant (score 3); four were slightly susceptible (score 4); and three were extremely susceptible (score 5) (Table 1).

Extremely resistant (score 1) and resistant (score 2) accessions were widely distributed among all analysed cultivated species (Fig. 3). Five weakly resistant (score 3) accessions were found among *S. stenotomum* (0144749, 144816) and *S. tuberosum* subsp. *andigenum* (0144718, 014736, 0144725); four were slightly susceptible (score 4), belonging to *S. phureja* (0144790), *S. tuberosum* subsp. *andigenum* (0144708, 0144737), *S. tuberosum* subsp. *tuberosum* (0144906); and three were extremely susceptible (score 5), belonging to *S. phureja* (044788), *S. stenotomum* (0144825) and *S. tuberosum* subsp. *andigenum* (0144716) (Fig. 3, Table 1). Therefore, our study did not find associations of traditional taxonomy with wart resistance.

Ploidy level potentially could influence gene dosage effect, so we analyzed associations of wart resistance with ploidy level. Of the 52 accessions, 26 are diploid (*S. phureja*, *S. stenotomum*) and 26 are tetraploid (*S. tuberosum* subsp. *andigenum* and subsp. *tuberosum*). Of the 26 diploid clones, twelve were assigned to the score 1, nine to score 2, two to score 3, one to score 4 and two to score 5. Of the 26 tetraploid clones, 11 were assigned to the score 1, eight to score 2, three to score 3, three to score 4 and one to score 5 (Fig. 5). Resistant accessions are widely distributed throughout the Andean regions of South America (Fig. 6) and therefore also lacked associations with geography.

As mentioned above, Spooner et al. (2007) and Ovchinnikova et al. (2011) treated the above-mentioned taxa *S. tuberosum*. Following this classification, high intraspecific diversity to wart disease resistance is detected both in diploid and in tetraploid cytotypes of *S. tuberosum* collected in different geographic regions. Our study therefore shows a lack of predictive associations between wart resistance, ploidy and cultivated species geographic distance.

Assessment of the diagnostic value of marker Nl25

All 52 genotypes of the same subset were used to test the effectiveness of the Nl25-1400-bp marker. Thirty-nine accessions (both susceptible and resistant genotypes) all had the same non-diagnostic fragment Nl25-1200 bp (Table 1, Fig. 7). Twelve genotypes had no amplification of fragments obtained

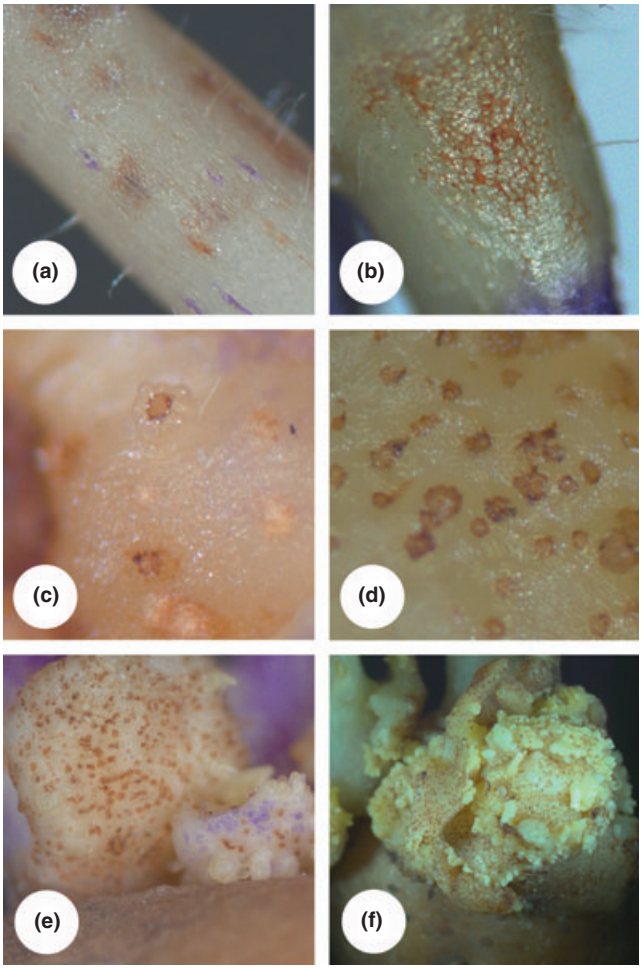

Fig. 3: Examples of resistance phenotypes: (a) Group 1, *Solanum tuberosum* subsp. *andigenum* (0144719); (b) Group 2, *S. phureja* (0144802); (c) Group 3, *S. stenotomum* (0144749); (d) Group 4, *S. tuberosum* subsp. *andigenum* (0144708); (e) Group 5, *S. tuberosum* subsp. *andigenum* (0144716); (f) susceptible control, cultivar 'Lorkh'

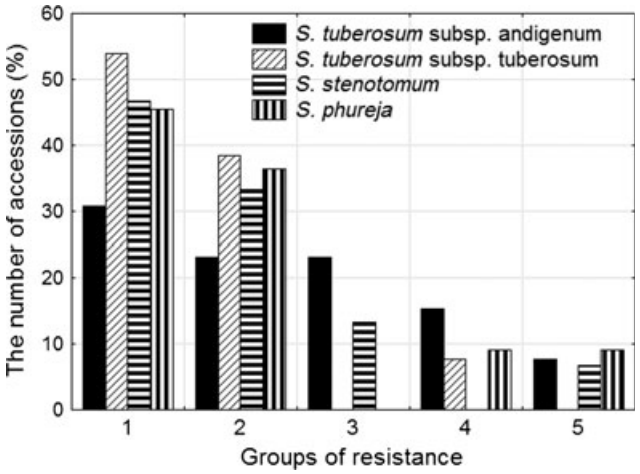

Fig. 4: Histogram representing the percentage of accessions of different potato species within five-score scale groups (following recommendations of OEPP/EPPO, 2004)

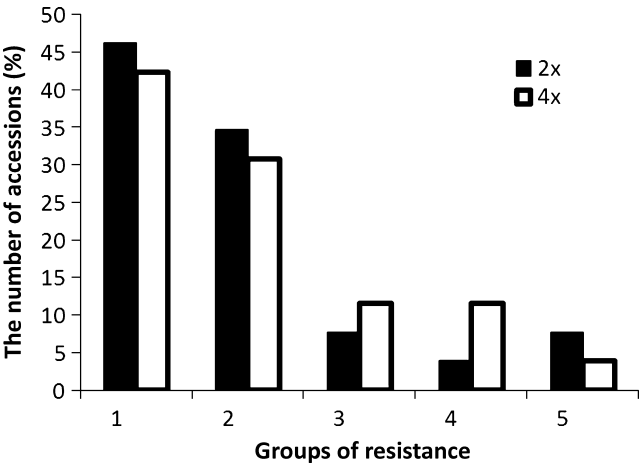

Fig. 5: Comparison of wart resistance within and between ploidy groups of cultivated potato

Fig. 6: Geographic distribution (collecting sites) of 52 accessions of four cultivated species used in our study. Red dots (OEPP/EPPO 2004 resistance scores), extremely resistant (score 1); blue, resistant (score 2); green, slightly susceptible (score 3); yellow, weakly resistant (score 4); lilac, extremely susceptible (score 5)

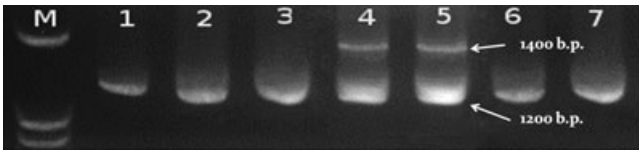

Fig. 7: Results of screening cultivated potato species accessions for the presence of marker Nl25_1400 bp. Lane 1, *Solanum phureja* (0144781); 2, *S. tuberosum* subsp. *andigenum* (0144719); 3, *S. stenotomum* (0144758); 4, *S. tuberosum* subsp. *andigenum* (0144708); 5, *S. tuberosum* subsp. *andigenum* (0144736); 6, *S. tuberosum* subsp. *tuberosum* (0144900); lane 7, *S. tuberosum* subsp. *tuberosum* (0144892). M – the molecular weight marker. Lanes 1,2,3, correspond to score 1 of resistance; lane 4 corresponds to score 4; lane 5 corresponds to score 3, and lanes 6,7 correspond to score 2

with Nl25 marker, although PCR was repeated three to four times.

Only five of the 52 genotypes possessed the fragment Nl25-1400 bp: two accessions of *S. tuberosum* subsp. *andigenum* (0144708 and 0144736) and three of *S. tuberosum* subsp. *tuberosum* (0144896, 0144899 and 0144885) (Table 1, Fig. 7). Four of these five accessions were resistant (scores 1, 2 or 3); however, one accession of *S. tuberosum* subsp. *andigenum* (0144708) had resistance score 4 (Table 1). The majority of resistant genotypes with scores 1 and 2 did not have the marker fragment Nl25-1400 bp.

Discussion

All studied accessions were collected in the Andean region of South America, the origin of potato wart disease. It is reasonable to expect therefore that many of the accessions within each species were extremely resistant or resistant (Fig. 4). The Andean region of South America also is the centre of cultivated potato genetic diversity, where landrace cultivars are highly diverse. Our results show that taxonomic relationships, ploidy and geographic data cannot be reliably used to predict where additional sources of wart resistance will be found. This is similar to findings of a wider diversity panel of wild potato species of resistance to white mould, early blight and potato wart, not associated with species, taxonomic series and geographic distance (Jansky et al. 2006, 2008, Spooner et al. 2009). These authors likewise concluded that it was impractical to use taxonomic and biogeographic factors of potato resistance to fungal pathogens. Selection of wart-resistant genotypes therefore requires broad and laborious disease screening.

It is known that gene *Sen1* completely blocks development and reproductive abilities of pathotype 1 (D1) of *S. endobioticum* (Langerfeld 1984, Lellbach and Effmert 1990, Hehl et al. 1999). Most tested accessions here demonstrated such resistance (extreme resistance). Different reaction types of other genotypes of the subset used in our experiments to the same inoculums in the same conditions suggested that other genes could be involved in host–pathogen interactions, or the influence of different genetic backgrounds on the expression of gene *Sen1*.

Our results also suggest the possible presence of another gene(s) or QTL in cultivated potatoes differing from *Sen1* (XI) which confers resistance to the pathotype 1 of *S. endobioticum,* or recombination between Nl25 marker allele and *Sen1* gene. Thus, our study failed to correlate the absence/presence of the detected fragment Nl25-1400 bp and the resistance to wart disease in a broad genetic background of cultivated potatoes. Further genetic studies are necessary to determine the genetic diversity of resistance in new sources of wart resistance.

In a practical matter for potato breeding, high intraspecific variation in wart disease resistance detected within all of the cultivated potato species we tested allowed us to select extremely resistant and susceptible genotypes at both diploid and tetraploid levels which are available for future genetic and breeding programmes. A crossing programme has been initiated and berries with numerous seeds have been obtained in such crosses. We plan to characterize these families for resistance to wart disease and screen them with different DNA markers including recently developed by Ballvora et al. (2011).

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