Screening for field resistance to Powdery Mildew *(Eyrsiphe polygoni* D.C.) in the JI Pisum Collection

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Introduction

Powdery mildew is a serious disease in peas grown in areas with dry warm days and nights cool enough for dew formation. The infection causes a reduction in plant productivity, delays the drying out of plant tissues and can cause discoloration and off-flavors to the seed (1). Resistance was first reported by Harland in 1948 in material originating from Huancabamba in Northern Peru growing at an altitude of 1948m (2). This resistance (erZ) has proved highly durable to date and Cousin reports in 1997 that the resistance had held up in France from when it was first introduced in1965 (3). Notes of resistant accessions in the John Innes Pisum collection made in the early 1980'S are still resistant today. The use of the resistance by breeders in the many regions of the world (including Australia, Canada and the US) where the disease is a problem has been steady since the 1970'S. In Europe this has accelerated as part of the drive for higher agronomic sustainability and implementation of changes in EU pesticide legislation (Council Directive 91/414 EEC) which is reducing the availability of fungicide treatments. Two further loci conferring resistance have been reported, *er2* (4) and *Er3* (5). While a number of research groups are focused on identifying, isolating and sequencing these loci, establishing the distribution of resistance in broader germplasm is still of interest in helping to target future screening for naturally occurring allelic variation.

Field resistant accessions in the JIC collection have been grown periodically as part of regeneration programs and opportunistically scored for mildew resistance/susceptibility. Resistance scores collected over a period of 26 years may include false positives where the resistant scores of some accessions may have been due to the lack of symptoms through disease avoidance such as early maturity. This study was undertaken to assess the current status of all accessions previously scored a showing field resistance in a single growing season and to explore the distribution and origins of that material.

Materials and Methods

Field resistance to powdery mildew (PMR) had previously been scored in 66 *Pisum sativum* accessions during the period 1982 to 2008. Five seeds of each accession were sown against wire with a susceptible control (JI 502: Rondo) sown at the start of the row and every 11^{th} line position along the row. The trial was grown on experimental plots adjacent to the JIC. Peas have been continuously grown in this vicinity since the early 1970'S and infection each year is; therefore, effectively guaranteed.

Plants were scored at mid-podding at the start of June two weeks after mildew was first noted. This was well into the flowering and early podding period and white patches of mycelium were clearly visible on susceptible lines over the entire plant including pods. A second scoring was conducted in mid July when plants were senescing.

Resistant lines were cross-referenced to the output of a new structure analysis output based on a genotype dataset of 45 RBIP retro-element markers scored across the JI Pisum Collection (6). The recent availability of this output marks the start of a new era of more predictive management and selection of germplasm utilising the entire collection and contrast to earlier approaches where such detailed resolution was only feasible on subsets of lines which led to the development of core collections to represent the wider diversity.

Results and Discussion

Of the 66 lines, 60 lines were found to exhibit strong resistance with no visible spores over leaves, stipule, stems or pods. The second scores, conducted blind later in the growing season, confirmed the earlier scores. At this stage the resistant material was clearly visible as the only living green material in any of the pea trials. Six lines previously scored as resistant were found to be susceptible. It is not clear whether the resistance in these accessions had been overcome but the evidence of the remaining material suggests this is not the case. What is more likely is the fact that the previous resistant scores may have been false due to the material maturing before the infection could take hold or the infection being particularly light that year. A table of resistant accessions is presented in Table 1.

Table 1. Accessions scored as field resistant to powdery mildew. Combined order = Structure analysis order, CTY = country of origin, Group= Structure subgroup membership. * denotes line with reduced stipules (st). NA = = not assigned.

Combined order	JI Acc. No.	Name	CTY	Group
NA	1050	WIS-7104	USA	NA
NA	2480	CGN 3352	PER	NA
NA	1064	X13463	PER	NA
26	2216	POWDERY MILDEW RESISTANT		1.1
51	73	WBH 1238		1.1
268	1391	WBH 2138-wp	USA	1.2
411	2217	POWDERY MILDEW RESISTANT		1.3
544	26	STIPULA-IMMINUATA-stim	SWD	1.4
634	229	P.SATIVUM-MALAYA	UTK	1.4
683	1749	OFI	USA	1.4
710	2302	B76-197 (STRATAGEM)	FRN	1.4
766	105	P.SATIVUM-AFGHANISTAN	AFG	1.5
835	2301	MEXIQUE 4	MEX	1.5
897	1559	MEXIQUE 4	MEX	1.5
947	1401	MANOA SUGAR		1.6
1015	1180	MARX G-TYPE	USA	1.6
1068	1243	NEW LINE E.PERF./PMR	USA	1.7
1163	1239	DSP/PMR	USA	1.7
1327	1048	WIS-7102	USA	1.7
1335	1069	WILTY-wil		1.7
1501	1205	MISOG-2:tl	USA	2.1
1503	1203	MISOG-2:af	USA	2.1
1514	1207	MISOG-2:af,tl	USA	2.1
1542	1769	MELTON-af	UTK	2.1
1555	1568	CM-014F		2.1
1560	1204	MISOG-2:st	USA	2.1
1575	1241	NEW ERA/PMR	USA	2.1
1610	1199	MISOG-1:af,tl	USA	2.1
1627	1567	EDISON		2.1
1639	1201	MISOG-1:af,st,tl	USA	2.1

Combined order	JI Acc. No.	Name	CTY	Group
1698	1562	B277-457-1		2.1
1740	1237	SPRITE/PMR	USA	2.1
1745	1213	ERYLIS	FRN	2.1
1760	1424	VR74-1492-1	USA	2.1
1766	2072	VIP		2.1
1789	1194	MISOG-1:CONVENTIONAL	USA	2.1
1876	1049	WIS-7103	USA	2.1
1910	1047	WIS-7101	USA	2.1
1958	1766	BARTON-af,st	UTK	2.1
2011	1566	ALMOTA		2.1
2017	1171	P.SATIVUM-INDIA	IND	2.1
2129	1412	MARLIN	USA	2.2
2174	1817	HJA 51902-af	FIN	2.2
2318	143	B268-394-3		2.2
2357	1780	TWIGGY-af	USA	2.2
2375	1128	P.SATIVUM-INDIA	IND	2.2
2443	1816	HJA 51893-af	FIN	2.2
2457	210	LUCKNOW BONIYA	UTK	2.2
2753	1214	TRIANON	FRN	3.1
2756	1951	P.SATIVUM-CHINA	CHN	3.1
2793	1557	P.SATIVUM-AFGHANISTAN	AFG	3.3
2799	92	P.SATIVUM-AFGHANISTAN	AFG	3.3
2802	96	P.SATIVUM-AFGHANISTAN	AFG	3.3
2809	101	P.SATIVUM-AFGHANISTAN	AFG	3.3
2812	95	P.SATIVUM-AFGHANISTAN	AFG	3.3
2813	102	P.SATIVUM-AFGHANISTAN	AFG	3.3
2814	100	P.SATIVUM-AFGHANISTAN	AFG	3.3
2856	1388	FENOMEN		3.4
3027	2019	P.SATIVUM-LADAKH	IND	3.7

and JI 2480) were not screened as part of the structure analysis and so could not be assigned to a group. This is unfortunate as JI 1050 and JI 1064 can both be traced back to the original resistant material from Peru first reported by Harland some 51 years ago (2,4). Resistant lines were found to be spread throughout the three major groups identified in the structure analysis (Fig. 1). The 12 accessions in group 1, which constitutes predominantly landraces, are relatively evenly spaced.

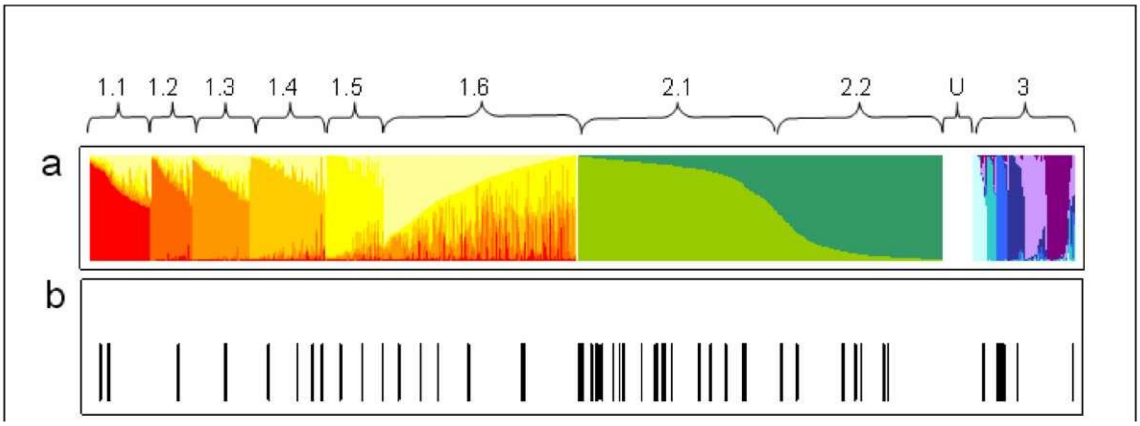
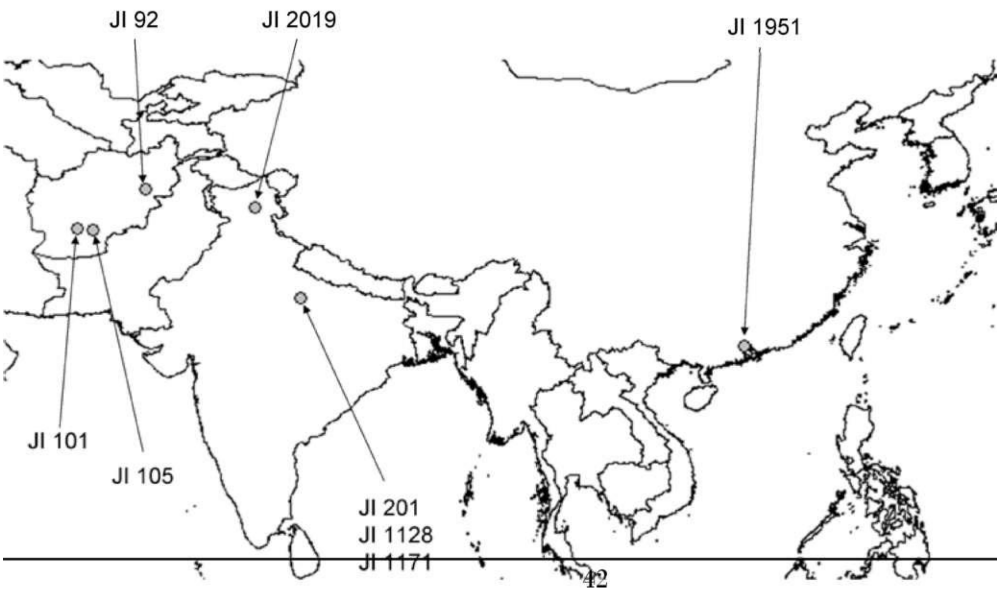


Figure 1. a. Output of structure analysis of 45 RPIB markers on 3029 accessions of the JIC Pisum Collection based on K=3, b. Bars indicate the position of PMR accessions listed in Table 1.

The distribution of lines in group 2, which is predominantly composed of cultivars, is more concentrated in subgroup 2.1. This is largely due to the 7 near isogenic lines (MISOG1, MISOG2 series)(7), occurring in close order. The presence of resistant accessions in group 3 is of considerable interest. Group three has the strongest sub-group structure and all the accessions of wild species *(P. fulvum, P. elatius)* along with an array of *P. sativum,* including cultivated forms which show a higher degree of relatedness to these forms than the majority of *P. sativum* (6). Among this material are accessions from a range of countries including France, China and India and, most notably, a group of seven from Afghanistan. Collection site data are available for 8 of these from Afghanistan to China which are presented in Figure 2.

Figure 2. Collection sites of 8 accession showing field resistance to powdery mildew.



The geographic range over which these resistant lines from group 3 are spread is extremely large. This is the first such report of resistance to powdery mildew being reported in material from this region and raises some intriguing questions. The high altitude associated with the secondary region of diversity (Asiatic highland including the Hind Kusch) as the collection sites of two of these accessions (JI 92 and JI 2019 above 2000m) bears close parallels with the initial source of resistant germplasm in Peru. Both are associated with high altitude which would provide the warn days and cool nights favoured by the disease. There is no knowledge as to the genetic basis of the PMR observed in this material or how many resistance genes might be present in each accession. A detailed comparison will only become possible through lengthy genetic studies or comparisons of sequences from each of the resistance genes once they become known. Until that time it is impossible to establish whether the resistance found in the new world material was present in peas taken over to the Americas by early pioneers and is; therefore, derived from these old world sources outlined or whether the resistance is novel and emerged independently due to high selection pressure of the disease. Either way, the nature and genetic basis for the resistance reported for the first time here in this old world germplasm will clearly be of interest in future studies. All the germplasm cited above is available on request.

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