Evaluation of Wild Potato Species for Resistance to Late Blight

W. Pérez[1], A. Salas[1], R. Raymundo[1], Z. Huamán[1,2], R. Nelson[1,3], and M. Bonierbale[1]

Late blight, caused by *Phytophthora infestans*, is a major disease of potato. This study assessed 51 tuber-bearing *Solanum* species for resistance to late blight in a series of greenhouse experiments. A total of 80 out of 133 accessions presented quantitative resistance patterns. Eighteen species from Central and South America presented both qualitative and quantitative resistance responses, whereas accessions of four Mexican and two South American species presented only qualitative resistance to the isolate used. Between one and 90 genotypes in 39 different species have been selected for verification of resistance under field conditions following evaluation of true-seed–grown and tuber-grown plants. The aim of this broad survey is to identify and secure sources of complementary components of stable resistance to late blight for use in breeding programs. The results confirm the value of conserving rare populations of potato genetic resources and provide the first evidence of resistance to late blight in at least seven species endemic to the South American center of origin of potatoes. Implications of the variability detected among species and accessions are discussed in terms of conservation and breeding objectives.

During the past three years, CIP has intensified efforts to evaluate the in-trust germplasm collection of potato for important traits such as resistance to late blight, bacterial wilt, and important viruses. Late blight (LB), caused by *Phytophthora infestans*, is the most devastating disease of potato worldwide. Wild tuber-bearing *Solanum* species from Mexico, especially *S. demissum,* have previously been used in the majority of breeding programs as sources of resistance to late blight. Ross (1986) estimated that *S. demissum* germplasm has been incorporated into more than 50% of the world's potato varieties. However, since it has become evident that new races of *P. infestans* can readily overcome qualitative resistance based on major (R) genes that are incompatible with specific virulence genes in the pathogen, breeders have placed greater emphasis on quantitative, 'horizontal', or non-specific resistance. This type of resistance is expected to be more stable when exposed to the variable and dynamic pathogen population. It was first reported to occur along with specific resistance in certain Mexican wild species including *S. demissum* and *S. stoloniferum* (Toxopeus, 1964). Schober (1981) and Tazelaar (1981) were the first to report horizontal resistance in *Solanum* accessions from outside of Mexico. South American genotypes of the cultivated species *S. tuberosum* subsp *andigena* and *S. phureja* have apparently contributed to the levels of quantitative resistance

[1] CIP, Lima, Peru.
[2] Current address: ProBioAndes, Lima, Peru.
[3] Current address: MacKnight Foundation, Ithaca, NY, USA.

obtained in a number of important varieties such as Monserrate and Atzimba (Niederhauser, 1989). Despite the availability of some varieties that possess stable types of resistance, there is still a need for higher levels of race non-specific resistance, particularly in early varieties.

Wild tuber-bearing *Solanum* species contain numerous sources of resistance (Hanneman and Bamberg, 1986), including potentially useful resistance to *P. infestans* (Hawkes, 1950; Ochoa, 1954, 1981; Van Soest, 1983, 1984; Colon and Budding, 1988; Colon et al., 1995), but only a few have been subject to detailed study or been used in breeding programs. This study presents the results of preliminary evaluation of *Solanum* germplasm held in trust by CIP with a view to promoting the more systematic use of wild species in potato improvement programs to broaden the genetic base of resistance to late blight.

Materials and Methods

A series of 11 experiments was carried out at CIP's highland station at Huancayo (3280 m; 12° 07' S latitude), Peru. A total of 133 *Solanum* accessions (six of which were duplicated, for a total of 139 population samples) in 51 species of 13 taxonomic series of tuber-bearing species were evaluated for resistance to late blight (Table 1).

Priority for screening was given to species never before evaluated and those originating in climatic zones conducive to late blight. Between one and 10 (usually three) accessions, each originating from a different collection site, were evaluated for each species, using about 48 (range 23–56) plants per accession. A sample of 100 seeds was taken at random from each of the selected accessions, which had been maintained in the genebank by full sib mating among 10–30 plants from an original or previously renovated population. Seeds were germinated in trays and the seedlings transplanted to 10 cm pots, which were placed in trays on the greenhouse floor. Each tray contained twelve pots with one plant each, and for each accession, four trays were randomly distributed along the greenhouse sides to avoid any bias due to differentials within the greenhouse environment.

Evaluation of the accessions was carried out in two phases. The first phase (5 true seed experiments) used plants grown from true seed accessions; and in the second phase (6 tuber experiments), plants were grown from tubers of individual plants that had been selected for resistance during the first phase. Guidelines for the different experiments were similar, with the main differences being the time of testing (the total experimental period covered several months), the type of planting material used, and the number of genotypes (plants) included per accession.

For each experiment, 13 standards for quantitative resistance ((SQR); clonal varieties or breeding lines with known levels of blight resistance) were included. Four trays, each representing a replication of the 13 standards were randomly distributed on both sides of the greenhouse. Complete sets of genotypes sampled from a number of accessions were tested within a given experiment. Data from replication of the 13 standards was intended to give information about variation within experiments (due to differences in the environment during a given test) and among experiments (due to changes in experimental conditions through time).

Whole plants of the accessions to be tested and the standards were spray-inoculated with LB at a concentration of 3000 sporangia per ml with the local isolate PCO002, which is virulent on potato differentials carrying R-genes 1, 2, 3, 4, 6, 7, (8), 10, and 11. (The differential reaction of this isolate with R8 requires confirmation.) Inoculation was performed before flowering, at 30–50 days after transplanting seedlings to the pots or planting of the tubers. Special efforts were made to keep environmental conditions in the greenhouse stable throughout the series

Table 1. Solanum accessions evaluated for resistance to late blight, Lima, Peru, 1999-2000.

Series and species		2n	EBN[1]	Country	No.	s	m	r	O	Proportion	No.
CIP No.	Collector's No.			of origin	tested[2]					resistant genotypes (m+r+0) /No.	selected[3]
ACAULIA											
S. albicans (alb)											
CIP 761452	OCH 12089	72	4	PERU	48	38	10	0	0	0.21	0
CIP 762120	OCH 14789	72	4	PERU	48	47	1	0	0	0.02	0
CIP 762584	OCHS 16028	72	4	PERU	48	48	0	0	0	0.00	0
CIRCAEIFOLIA											
S. circaeifolium (crc)											
CIP 761344	OCHS 11909	24	1	BOLIVIA	51	42	4	5	0	0.18	0
S. circaeifolium var. capsicibaccatum (cap)											
CIP 761030	**OCHS 11915**	**24**	**1**	**BOLIVIA**	**56**	**1**	**4**	**27**	**24**	**0.98**	**42**
CIP 761030	**OCHS 11915**	**24**	**1**	**BOLIVIA**	**48**	**0**	**0**	**0**	**48**	**1.00**	**0**
CIP 762303	OCHS 15489	24	1	BOLIVIA	48	11	31	2	4	0.77	1
COMMERSONIANA											
S. commersonii (cmm)											
CIP 761087	FB 4010	24	1	URUGUAY	48	42	4	2	0	0.13	3
CIP 762454	URY 4	24	1	URUGUAY	48	19	20	7	2	0.60	19
CIP 762475	URY 31	24	1	URUGUAY	48	5	9	17	17	0.90	23
CONICIBACCATA											
S. chomatophilum (chm)											
CIP 761587	OCH 13341	24	2	PERU	48	47	1	0	0	0.02	0
CIP 762568	OCHS 12553	24	2	PERU	48	35	12	1	0	0.27	0
CIP 762611	OCHS 16072	24	2	PERU	48	46	0	1	1	0.04	0
S. colombianum (col)											
CIP 762793	**SCL 5050**	**48**	**2**	**ECUADOR**	**48**	**10**	**36**	**2**	**0**	**0.79**	**0**
CIP 762793	**SCL 5050**	**48**	**2**	**ECUADOR**	**48**	**43**	**5**	**0**	**0**	**0.10**	**0**
S. flahaultii (flh)											
CIP 761877	OCH 14105	48		COLOMBIA	48	11	31	4	2	0.77	0
CIP 761878	OCH 14106	48		COLOMBIA	47	24	20	2	1	0.49	4
S. irosinum (irs)											
CIP 761252	OCH 11640	24	2	PERU	48	40	2	6	0	0.17	0
CIP 762257	OCHS 15210	24	2	PERU	48	18	2	28	0	0.63	0
S. paucijugum (pcj)											
CIP 762800	SCL 5084	48		ECUADOR	42	26	14	2	0	0.38	0
CIP 762803	SCL 5096b	48		ECUADOR	39	7	14	8	0	0.76	0
CIP 762816	SCLG 5151	48		ECUADOR	37	21	16	0	0	0.43	0
S. urubambae (uru)											
CIP 761044	OCH 13781	24	2	PERU	48	0	0	5	43	1.00	4
CIP 762368	OCHS 15654	24	2	PERU	48	18	20	10	0	0.63	0
S. violaceimarmoratum (vio)											
CIP 760563	VSOA 7	24	2	BOLIVIA	48	1	15	22	10	0.98	0
DEMISSA											
S. demissum (dms)											
CIP 761893	OCH 14154	72	4	MEXICO	44	0	1	26	17	1.00	38
CIP 761050	OCH 14218	72	4	MEXICO	48	0	13	35	0	1.00	28
S. hougasii (hou)											
CIP 761902	OCH 14172	72	4	MEXICO	48	0	0	12	36	1.00	17
CIP 761899	OCH 14168	72	4	MEXICO	48	0	0	11	37	1.00	26

Table 1. (continued)

Series and species		2n	EBN[1]	Country of origin	No. tested[2]	s	m	r	O	Proportion resistant genotypes (m+r+0) /No.	No. selected[3]
CIP No.	Collector's No.										
S. iopetalum (iop)											
CIP 761928	OCH 14208	72	4	MEXICO	48	0	0	0	48	1.00	23
CIP 761935	OCH 14221	72	4	MEXICO	48	0	0	0	48	1.00	46
CIP 761930	OCH 14212	72	4	MEXICO	48	11	21	16	0	0.77	24
LIGNICAULIA											
S. lignicaule (lgl)											
CIP 761210	OCH 11315	24	1	PERU	48	29	17	2	0	0.40	0
CIP 761236	OCH 11617	24	1	PERU	47	12	27	8	0	0.74	0
CIP 761652	OCH 13585	24	1	PERU	48	21	24	3	0	0.56	0
LONGIPEDICELLATA											
S. fendlerii (fen)											
CIP 761921	OCH 14199	48	2	MEXICO	48	9	2	6	31	0.81	34
CIP 761923	OCH 14202	48	2	MEXICO	45	45	0	0	0	0.00	0
CIP 761926	OCH 14205	48	2	MEXICO	48	48	0	0	0	0.00	0
S. stoloniferum (sto)											
CIP 761884	OCH 14135	48	2	MEXICO	44	3	13	11	17	0.93	3
CIP 761062	OCH 14145	48	2	MEXICO	48	0	0	0	48	1.00	30
MEGISTACROLOBA											
S. dolichocremastrum (dcm)											
CIP 761043	OCH 12071	24	1	PERU	48	48	0	0	0	0.00	0
CIP 761439	OCH 12074	24	1	PERU	47	47	0	0	0	0.00	0
CIP 761470	**OCH 13013**	**24**	**1**	**PERU**	**48**	**45**	**1**	**2**	**0**	**0.06**	**1**
CIP 761470	**OCH 13013**	**24**	**1**	**PERU**	**48**	**48**	**0**	**0**	**0**	**0.00**	**0**
S. megistacrolobum subsp. toralapanum (mga)											
CIP 760535	HAM 200	24	2	BOLIVIA	43	1	0	21	21	0.98	16
CIP 761109	OCH 7609	24	2	PERU	48	27	15	6	0	0.44	0
CIP 761403	OCH 12032	24	2	BOLIVIA	48	47	1	0	0	0.02	8
S. megistacrolobum subsp. toralapanum (tor)											
CIP 760459	HHA 6616	24	2	BOLIVIA	48	1	5	16	26	0.98	1
CIP 761369	OCH 11964	24	2	BOLIVIA	27	25	1	1	0	0.07	0
CIP 761055	OCHS 11914	24	2	BOLIVIA	32	10	16	6	0	0.69	0
S. raphanifolium (rap)											
CIP 761113	OCH 7613	24	2	PERU	48	1	26	17	4	0.98	15
CIP 761640	OCH 13572	24	2	PERU	48	3	29	14	2	0.94	24
CIP 761671	OCH 13610	24	2	PERU	48	5	29	9	5	0.90	17
S. sogarandinum (sgr)											
CIP 761586	OCH 13336	24	2	PERU	48	1	10	10	27	0.98	16
CIP 762410	OCHS 15723	24	2	PERU	47	42	4	0	1	0.11	2
PINNATISECTA											
S. cardiophyllum (cph)											
CIP 762561	OCH 14157	24	1	MEXICO	29	0	0	0	29	1.00	12
PIURANA											
S. cantense (cnt)											
CIP 762241	OCHS 15159	24	2	PERU	48	0	22	21	5	1.00	8
S. chiquidenum (chq)											
CIP 761588	OCH 13345	24	2	PERU	48	37	11	0	0	0.23	12
CIP 761870	OCH 13963	24	2	PERU	48	33	15	0	0	0.31	11
CIP 762573	OCHS 12566	24	2	PERU	48	35	11	2	0	0.27	14

Table 1. (continued)

Series and species		2n	EBN[1]	Country of origin	No. tested[2]	s	m	r	O	Proportion resistant genotypes (m+r+0) /No.	No. selected[3]
CIP No.	Collector's No.										
S. humectophilum (hmp)											
CIP 761052	OCH 11753	24	1	PERU	53	50	2	1	0	0.06	2
S. hypacrarthrum (hcr)											
CIP 761259	OCH 11692	24	1	PERU	48	15	11	2	0	0.46	0
CIP 761204	OCHS 11308	24	1	PERU	23	23	0	0	0	0.00	0
CIP 762104	OCHS 14715	24	1	PERU	48	41	6	1	0	0.15	0
S. paucissectum (psc)											
CIP 761247	OCH 11634	24	2	PERU	44	41	2	1	0	0.07	0
CIP 762124	OCHS 14816	24	2	PERU	47	8	26	0	13	0.83	0
S. piurae (pur)											
CIP 761868	OCH 13959	24	2	PERU	48	0	0	2	46	1.00	20
CIP 761072	OCHS 11615	24	2	PERU	48	0	0	0	48	1.00	4
POLIADENIA											
S. polyadenium (pld)											
CIP 761911	OCH 14187	24		MEXICO	48	0	0	0	48	1.00	12
TUBEROSA											
S. alandiae (aln)											
CIP 760469	HHA 6657	24		BOLIVIA	48	28	20	0	0	0.42	18
CIP 760473	HHA 6665	24		BOLIVIA	48	48	0	0	0	0.00	0
CIP 761394	OCH 12013	24		BOLIVIA	48	48	0	0	0	0.00	0
S. ambosinum (amb)											
CIP 761842	OCH 13852	24	2	PERU	48	48	0	0	0	0.00	4
CIP 761194	OCHS 11298	24	2	PERU	48	47	1	0	0	0.02	0
CIP 761053	OCHS 11865	24	2	PERU	48	48	0	0	0	0.00	0
S. brevicaule (brc)											
CIP 760461	HHA 6619	24	2	BOLIVIA	48	48	0	0	0	0.00	0
CIP 760479	HHA 6690	24	2	BOLIVIA	48	47	1	0	0	0.02	0
CIP 761074	OCH 11934	24	2	BOLIVIA	48	48	0	0	0	0.00	0
S. bukasovii (buk)											
CIP 761120	OCH 7717	24	2	PERU	48	30	17	1	0	0.38	7
CIP 761517	OCH 13166	24	2	PERU	48	20	23	5	0	0.58	1
CIP 761664	OCH 13602	24	2	PERU	48	47	1	0	0	0.02	0
CIP 761720	OCH 13679a	24	2	PERU	48	35	10	3	0	0.27	3
CIP 761805	OCH 13796	24	2	PERU	48	47	0	1	0	0.02	0
CIP 761806	OCH 13798	24	2	PERU	47	26	17	4	0	0.45	0
CIP 761847	OCH 13858	24	2	PERU	48	33	13	2	0	0.31	2
CIP 762434	OCH 15822	24	2	PERU	30	30	0	0	0	0.00	0
CIP 761152	OCHS 10114	24	2	PERU	48	47	1	0	0	0.02	0
CIP 761301	OCHS 11851	24	2	PERU	43	10	26	7	0	0.77	0
S. cajamarquense (cjm)											
CIP 762616	OCHS 16118	24	1	PERU	48	1	10	30	7	0.98	18
CIP 762619	OCHS 16121	24	1	PERU	48	2	14	30	2	0.96	11
S. candolleanum (cnd)											
CIP 761041	OCHS 11913	24	2	BOLIVIA	48	48	0	0	0	0.00	0
CIP 762168	OCHS 14959	24	2	BOLIVIA	48	48	0	0	0	0.00	3
CIP 762185	OCHS 15011	24	2	BOLIVIA	48	48	0	0	0	0.00	8

Table 1. (continued)

Series and species		2n	EBN[1]	Country of origin	No. tested[2]	s	m	r	O	Proportion resistant genotypes (m+r+0) /No.	No. selected[3]
CIP No.	Collector's No.										
S. coelestipetalum (cop)											
CIP 761660	OCH 13596	24	2	PERU	48	39	6	3	0	0.19	7
CIP 761728	OCH 13686	24	2	PERU	48	48	0	0	0	0.00	0
CIP 762005	OCH 14351	24	2	PERU	48	47	0	1	0	0.02	0
S. huancabambense (hcb)											
CIP 761238	**OCH 11619**	**24**	**2**	**PERU**	**48**	**31**	**14**	**3**	**0**	**0.35**	**6**
CIP 761238	**OCH 11619**	**24**	**2**	**PERU**	**48**	**41**	**4**	**3**	**0**	**0.15**	**0**
CIP 761239	OCH 11626	24	2	PERU	48	26	10	12	0	0.46	6
CIP 762123	OCHS 14815	24	2	PERU	48	45	2	1	0	0.06	3
S. huarochiriense (hro)											
CIP 761215	OCH 11325	24	2	PERU	34	33	1	0	0	0.03	0
CIP 761224	**OCH 11335**	**24**	**2**	**PERU**	**48**	**44**	**4**	**0**	**0**	**0.08**	**5**
CIP 761224	**OCH 11335**	**24**	**2**	**PERU**	**48**	**46**	**2**	**0**	**0**	**0.04**	**0**
CIP 761265	OCH 11699	24	2	PERU	48	48	0	0	0	0.00	0
S. leptophyes (lph)											
CIP 761766	OCH 13730	24	2	PERU	48	48	0	0	0	0.00	0
CIP 760805	VSAL 126	24	2	BOLIVIA	46	41	5	0	0	0.11	1
CIP 760895	VSH 248	24	2	BOLIVIA	46	38	8	0	0	0.17	3
S. marinasense (mrn)											
CIP 761669	OCH 13608	24	2	PERU	51	42	9	0	0	0.18	0
CIP 761774	OCH 13737	24	2	PERU	47	41	5	1	0	0.13	0
CIP 761812	OCH 13809	24	2	PERU	48	27	20	1	0	0.44	0
S. medians var. autumnale (aut)											
CIP 761198	OCHS 11302	24	2	PERU	48	6	33	8	1	0.88	0
CIP 762629	OCHS 12573	24	2	PERU	48	39	9	0	0	0.19	0
CIP 762256	OCHS 15205	24	2	PERU	48	42	5	1	0	0.13	2
S. microdontum (mcd)											
CIP 762314	OCHS 15534	24	2	BOLIVIA	48	37	6	5	0	0.23	15
CIP 760531	HAM 176	24	2	BOLIVIA	48	45	1	2	0	0.06	6
CIP 760534	HAM 187	24	2	BOLIVIA	48	16	17	15	0	0.67	38
S. mochiquense (mcq)											
CIP 761037	OCHS 14870	24	1	PERU	48	11	25	12	0	0.77	0
S. multiinterruptum (mtp)											
CIP 761260	OCH 11693	24	2	PERU	48	26	18	2	2	0.46	3
CIP 761422	OCHS 12055	24	2	PERU	48	2	9	24	13	0.96	14
CIP 762405	OCHS 15716	24	2	PERU	48	19	27	2	0	0.60	0
S. oplocense (opl)											
CIP 761352	OCH 11927	24	2	BOLIVIA	45	11	26	8	0	0.76	8
CIP 761362	**OCH 11947**	**48**	**4**	**BOLIVIA**	**44**	**44**	**0**	**0**	**0**	**0.00**	**0**
CIP 761362	**OCH 11947**	**48**	**4**	**BOLIVIA**	**48**	**48**	**0**	**0**	**0**	**0.00**	**0**
S. orophilum (orp)											
CIP 761440	OCH 12077	24	2	PERU	48	0	17	28	3	1.00	1
CIP 761443	OCH 12080	24	2	PERU	48	1	37	10	0	0.98	2
CIP 761475	OCH 13020	24	2	PERU	48	2	22	24	0	0.96	
S. sparsipilum (spl)											
CIP 760475	HHA 6669a	24	2	BOLIVIA	48	46	2	0	0	0.04	8

Table 1. (continued)

Series and species		2n	EBN[1]	Country	No.	s	m	r	O	Proportion	No.
CIP No.	Collector's No.			of origin	tested[2]					resistant genotypes (m+r+0) /No.	selected[3]
S. velardei (vlr)											
CIP 761730	OCH 13688	24		PERU	48	20	23	5	0	0.58	2
CIP 762027	OCH 14387			PERU	48	23	25	0	0	0.52	0
CIP 762028	OCH 14387a	24		PERU	48	32	15	1	0	0.33	0
S. wittmackii (wtm)											
CIP 761566	OCH 13267	24	1	PERU	48	12	32	3	1	0.75	0
CIP 761205	OCHS 11309	24	1	PERU	48	26	21	1	0	0.46	0
CIP 762077	OCHS 14626	24	1	PERU	48	47	1	0	0	0.02	0
YUNGASENSA											
S. berthaultii (ber)											
CIP 761390	OCH 12008	24	2	BOLIVIA	44	23	15	2	4	0.48	15
S. chacoense (chc)											
CIP 761399	OCH 12026	24	2	BOLIVIA	44	27	10	7	0	0.39	5
CIP 762270	OCH 15266b	24	2	PARAGUAY	48	26	16	5	1	0.46	3
CIP 762274	OCH 15271	24	2	PARAGUAY	48	41	7	0	0	0.15	0
S. tarijense (tar)											
CIP 761007	OCH 12001	24	2	BOLIVIA	48	9	35	4	0	0.81	10
CIP 762334	OCHS 15596	24	2	BOLIVIA	48	47	1	0	0	0.02	3
				Total	6306	3532	1316	685	743		**801**

Note: Accessions noted in bold were evaluated in duplicate.

s = susceptible; m = moderately susceptible; r = resistant; 0 = qualitatively resistant.

[1] EBN = endosperm balance number.

[2] No. tested = Number of plants tested in true seed experiments.

[3] No. selected = Number of genotypes selected following tuber experiments.

of experiments. After inoculation, conditions were maintained conducive to late blight by the delivery of cool mist through an overhead sprinkler system operating on a thermostat. Shade cloth was used to help keep temperatures in the range of 15–20° C.

The proportion of total leaf area with symptoms was recorded for each individual plant at two-day intervals beginning on the third or fourth day after inoculation, for a total of three or four readings per experiment. A contact fungicide was applied to stop the disease when the susceptible controls reached 60% foliar infection.

Relative area under the disease progressive curve (rAUDPC) was calculated for each individual plant from the ratings of foliar infection (Fry, 1978). Analyses of variance were conducted on the rAUDPC values of the standards (SQRs) for each greenhouse experiment, and the estimated least squares means were used to find the SQRs ranking within each experiment. Spearman correlation coefficients calculated on these rankings were used to compare pairwise performance of standards between greenhouse experiments. Discriminant analyses for the greenhouse experiments were conducted to appreciate natural grouping of the SQRs into relative resistance categories, using data from the true seed and tuber phases.

Individuals with infection levels of 40% or lower by the third reading and AUDPC values lower than or equal to the moderately resistant controls (SQRs) within each experiment were selected for evaluation from tuber-grown plants in the second phase of the study. These tuber experiments followed the same guidelines as the

true seed experiments, except for the use of one to three tuber-grown clones of the putatively resistant genotypes. For each accession in the first (true seed) and second (tuber) phase, the response pattern to environment was studied through a frequency distribution of their ADUPC values.

Individuals of each accession, within a particular experiment, that became infected with the pathogen were assigned one of three disease reaction levels (resistance ratings): susceptible (s), moderately resistant (m) and resistant (r); based on the comparison of their rAUDPC values with those of the SQRs in each category of resistance. An additional rating, 0 (qualitative resistance, not represented in the standards) was used for individuals that showed either no visible disease reaction or necrotic flecks assumed to be indicative of race-specific resistance. The proportions of total resistant, quantitatively resistant, and qualitatively resistant individuals were calculated as:

$$\frac{m + r + 0}{n}, \quad \frac{m + r}{n}, \quad \text{and} \quad \frac{0}{n}, \quad \text{respectively,}$$

where *n* indicates the number of individuals inoculated in the True Seed experiments.

In order to assess the representative nature of the greenhouse assay, performance of the standards (SQRs) in greenhouse experiments was compared, through Kendall's correlation coefficient, with their reaction to late blight in five field trials at Comas, Peru. The field trials were performed in randomized complete block designs using 4 replications of 5-plant experimental units, their full analysis will be presented elsewhere.

Results

Initial symptoms of infection were observed three days after inoculation, and susceptible individuals reached 100% foliar infection by day 8. Spearman's correlation coefficients for pairwise comparison of the rAUDPC rankings of the controls (SQRs) among the 11 greenhouse experiments ranged from 0.25 ($P > 0.05$) to 0.71 ($P < 0.05$) for true-seed experiments; from 0.40 ($P > 0.05$) to 0.86 ($P < 0.05$) for tuber experiments; and from 0.21 ($P > 0.05$) to 0.87 ($P < 0.05$) between true-seed and tuber experiments. Despite moderate variations in rankings of the SQRs, expressed as a low correlation coefficient, discriminant analysis on the greenhouse results identified three consistent response categories: susceptible (7 clones), moderately resistant (5 clones) and resistant (1 clone) across all greenhouse experiments. AUDPC values corresponding to each of these groups in each experiment were used to align the responses of the test accessions within the categories s, m, and r.

Figure 1 illustrates the outcome of the correlation analyses conducted to compare the average performance of the SQRs in the 11 greenhouse experiments with their average performance in the five field experiments in Peru. The median rankings for true-seed, tuber, and field experiments were obtained for each control and Kendall correlation coefficients were calculated among these three sets. Kendall's coefficient ranged from 0.47 ($P < 0.05$, between true-seed and field experiments), to 0.62 ($P < 0.05$, between tuber and field experiments), to 0.70 ($P < 0.05$, between True-Seed and Tuber experiments.)

Resistance patterns observed in tests of true seed-derived accessions

The majority of seed-derived accessions showed variation in their levels of resistance, as would be expected from their heterogeneous constitution. However, because individual plants were used to represent genotypes within accessions, some experimental error is confounded in the measures of variability (see Discussion). Of the 139 series of plants tested,

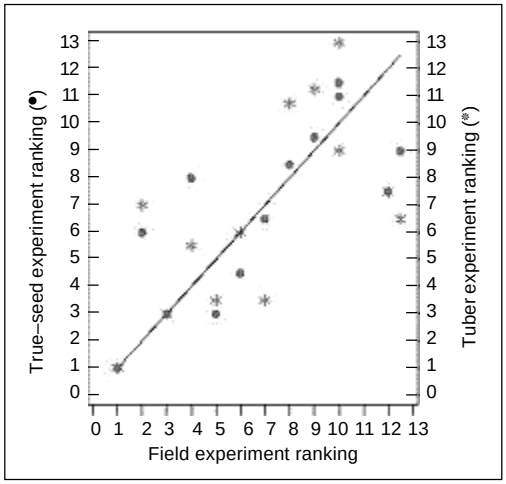

Figure 1. Ranked performance of 13 standards for quantitative resistance to late blight evaluated in each of the two sets of greenhouse experiments used to evaluate germplasm accessions, versus their ranking in a set of field experiments in Comas, Peru. (* = true-seed experiments; • = tuber experiments). The solid line indicates the hypothetical case of equal rankings of the standards in the greenhouse and field experiments.

110 (> 80%) showed mixed responses (more than one response category) and 29 showed clear, single category responses (22 totally susceptible and 7 totally resistant). When the data from all the accessions are considered together, the majority of individuals (3532 plants out of a total of 6306 tested) fell into the most susceptible (s) class. In addition, the frequency distributions of most accessions were skewed toward the more susceptible categories (s and m) (Table 1). However, 16 species each contained at least one accession that showed moderate resistance (m) and resistance (r), including accessions of *S. cajamarquense*, *S. orophilum*, *S. raphanifolium, S. cantense* and *S. violaceimarmoratum* (Table 1). Additional promising accessions showing more resistant than susceptible individuals were encountered among those of: *S. circaeifolium*, *S. commersonii*, *S. demissum* and *S. hougasii, S. iopetalum, S. irosinium, S. megistacrolobum, S. multiinterruptum, S. sogorandinum,* and *S. stoloniferum.*

Distribution of resistance types: r vs. 0

Data for the true seed-grown plants shows that 26 of the 51 species evaluated each contained some individuals that were not visibly infected (rated 0*)* with the *P. infestans* isolate PCO002, despite its complex virulence nature. Although we have not tested these individuals against other isolates and histopathological observations were not made, we expect this type of reaction to reflect qualitative resistance. This interpretation, as opposed to the possibility of escapes from inoculation, is strengthened by the distribution of individuals with this rating in the greenhouses, which could not be attributed to obvious experimental conditions, and the low coefficients of variability (< 30%) for resistance data taken as the standards in most experiments.

In addition to its expected occurrence in species from Mexico, qualitative resistance (rating 0) was observed in 19 accessions native to South America. All individuals of one accession of each of the South American species *S. piurae* and *S. circaeifolium*, and of five accessions of four Mexican species - *S. cardiophyllum*, *S. polyadenium, S. stoloniferum* and *S. iopetalum* showed qualitative resistance (0). The latter species was represented by two accessions that appeared uniformly incompatible with our isolate (48/48 individuals rated 0), and one accession that expressed quantitative variation*.* Both qualitative and quantitative types of resistance were observed within and among at least 18 Mexican and South American accessions. Figure 2 presents an overview of the distribution of the resistance types observed in the tested accessions in terms of percent of individuals that showed quantitative and qualitative disease responses.

In all, 1868 individuals from 93 accessions in 45 species classified as m, r, or 0 in the experiments with true seed-grown plants, were selected for re-evaluation using plants derived from tuber-grown plants.

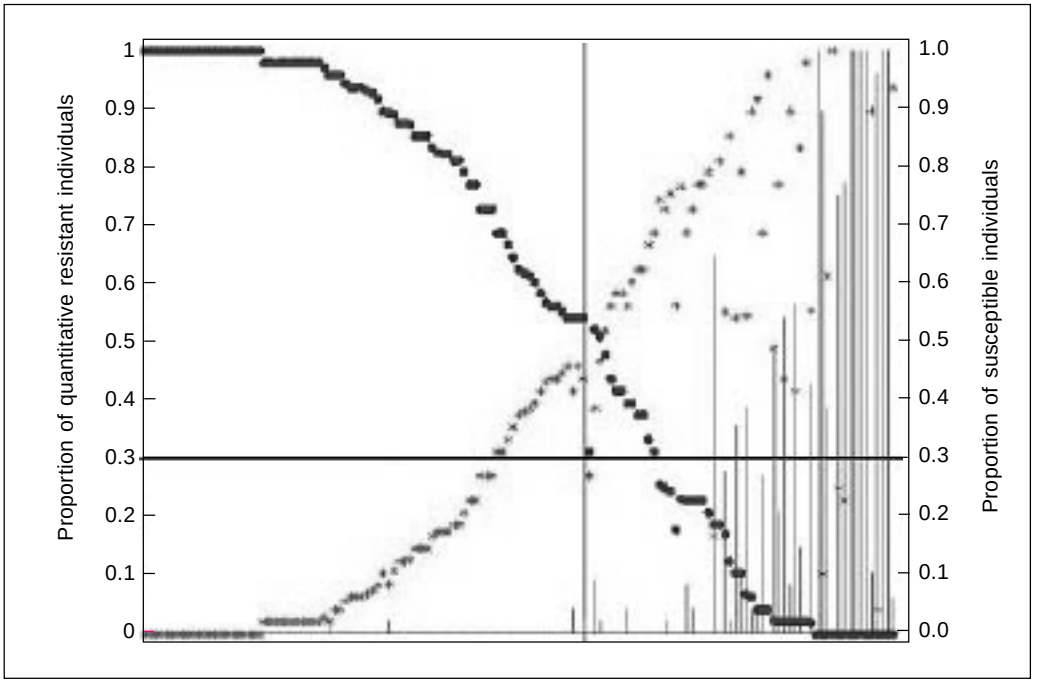

Figure 2. Proportions of individuals in each of 139 germplasm samples tested in true-seed experiments falling into quantitative resistance (m + r), susceptible (s) and qualitative resistance (0) categories. (* = quantitatively resistant; • = susceptible; | = qualitatively resistant).

Evaluation of tuber-grown plants of individuals selected from resistant accessions

Genotypes of *S. piurae, S. iopetalum, S. hougasii, S. fendlerii, S. cardiophyllum*, and *S. circaeifolium* accessions collected in Mexico, Bolivia, and Peru showed incompatible responses (48/48 individuals rated 0) in both the true seed- and the tuber-derived inoculations, indicating the likely presence of R genes with which PCO002 is incompatible. Genotypes of *S. albicans, S. alandiae, S. ambosinum, S. circaeifolium, S. chiquidenum, S. cajamarquense, S. commersonii, S. coelestipetalum, S. microdontum, S. huancabambense, S. megistacrolobum, S. toralapanum, S. multiinterruptum, S. sogorandinum* and *S. stoloniferum* collected in Peru, Bolivia, Colombia, Uruguay, Paraguay, and Mexico showed moderate and variable response to the disease when evaluated from tubers, confirming the likely presence of quantitative resistance. Eight hundred and one promising genotypes in 35 species of 11 taxonomic series were selected by comparison of the results obtained from the inoculation of plants grown from true seed and from tubers. These selections are currently being propagated for resistance evaluation at the genotypic level (Table 1).

Discussion

Limitations to the greenhouse assay

The greenhouse assay was a convenient way to evaluate large samples of germplasm under applied disease pressure. However, susceptible individuals reached 100% foliar infection only eight days after inoculation. In contrast, it takes 3–4 weeks for moderately resistant or susceptible clones to reach 80–100% infection in the field. The rapid greenhouse epidemics may not provide the best conditions to

differentiate realistically between genotypes. This population-based evaluation has therefore permitted a broad overview of the resistance levels in the germplasm, but validation in field trials is still required. Replicated trials under controlled conditions would also permit resistance components to be measured, elucidating factors that may vary independently among different sources of resistance.

Variability in resistance types within and among accessions

Disease ratings of 0 were observed among 26 species from South America and Mexico. It is likely that the 0 rating reflects the presence of major resistance (R) genes that are not matched by the virulence genes of the test isolate used, masking quantitative resistance that might be detectable with compatible isolates. Screening with a range of isolates would confirm whether the individuals with 0 ratings carry one of the known R genes (e.g., R5 or R9) with which our isolate was not compatible, or whether their resistance was due to other factors.

Elsewhere, genetic mapping in an interspecific hybrid population has revealed the presence of both qualitative and quantitative resistance (Ewing et al., 2000), and permitted description of three apparently novel R genes from *S. berthaultii* (Sanchez et al., 2000; W.E. Fry and G. Sanchez, Cornell University, personal communication). Given this precedent, it is quite likely that more R genes than those reported in *S. demissum* and *S. berthaultii* are present in *Solanum* germplasm. Selected genotypes will therefore be analyzed further using a set of differential isolates with known interaction patterns with the currently recognized R genes.

Implications of intraspecific variation for late blight resistance

Better knowledge of patterns of variability for resistance to late blight among different accessions of the same species can help to develop appropriate strategies for the conservation and use of genetic resources. The differences between accessions of the same species observed in this study might be explained by: (1) genetic differences among source populations; (2) sampling error in selecting seeds for testing accessions, (3) genetic drift associated with the conservation procedure, or (4) experimental error due to lack of replication. In the first case, the observed variation might stem from different pressures acting on the common gene pool, such as the influence of different habitats. For example, *S. sogarandinum*, was represented by two accessions collected from localities on the eastern (OCH 13336) and western (OCHS 15723) slopes of the Andes. When inoculated in the same greenhouse experiment, the accession collected at the more easterly site (OCH 13336), exhibited higher levels of resistance; this might reflect adaptation to a more humid environment and higher blight pressure*. S. fendlerii* also presented strikingly different resistance patterns between accessions collected in different locations, reinforcing the need to take account of potential variability among isolated populations when devising sampling strategies that seek to represent widely distributed species.

The process of sampling seeds from variable gene bank accessions may induce errors. There is evidence that sampling errors may have occurred in this study in cases when duplicate samples of seed from the same regeneration cycles were sown as back-ups following slow germination. Two samples of accession OCHS 11915 *(S. circaeifolium*), and two of SCL 5050 (*S. colombianum*) showed variability in resistance levels that was probably due to sampling error. However, duplicate samples from accessions in four other species presented nearly identical patterns of resistance. Although they differed from one another in resistance

pattern, the two pairs of duplicate samples of *S. circaeifolium* accession OCHS 11915 were consistently more resistant than the other two accessions tested from this species. Different sample sizes may be required to represent accessions of which between 200 and 6000 seeds are available in the gene bank, especially if different diversity levels are expected among the species due to breeding habit or other factors affecting the genetic structure of the source populations. Genetic drift also has important implications for germplasm conservation. Renovation procedures in the gene bank may result in unintentional selection for linked features and hence affect patterns of resistance. The sampling procedures used in this study did not provide the opportunity to examine genetic drift.

Finally, the possibility of escapes from infection or variation within the testing environment cannot be ruled out as possible causes of this apparent genetic variability. An alternative testing strategy involving prior establishment of clonal materials would afford more robust evaluations of genotypes, but would significantly reduce the scope or increase the cost of a germplasm survey.

The value of rare species

These experiments provide new information concerning the distribution of levels and types of resistance in 12 of 18 little-known species previously suggested to carry resistance. For example, the suggestion by Van Soest (1984) that two species from Peru, *S. chiquidenum* and *S. multiinterruptum* carry race non-specific resistance has been confirmed by the demonstration of quantitative resistance to our test isolate. A large number of additional genotypes have been identified that showed quantitative resistance in tests conducted on both true seed- and tuber-grown plants (Table 1). Species which have not been evaluated for this form of resistance before and showed high levels of resistance in these experiments include: *S. urubambae, S. violaceimarmoratum, S. cantense, S. cajamarquense, S. orophilum, S. velardei* and *S. wittmackii*. We therefore report the first evidence of resistance to late blight in 7 species endemic to the South American center of origin of potatoes.

Some accessions showed unfamiliar responses to infection with *P. infestans*. For example, *S. colombianum* presented watery lesions on the underside of its leaves; *S. megistacrolobum* developed irregular, streak-like lesions on the upper leaf surface and small lesions on the underside; and *S. mochiquense* presented small water-soaked lesions on both leaf upper and lower surfaces. These unusual reactions suggest different types of host–pathogen interactions, which may indicate resistance mechanisms that could be exploited in genetic improvement programs.

Utilization of new diversity/exotic species

The potential for using these genetic resources in conventional breeding depends on their ability to cross breed with *S. tuberosum*, the inheritance of the resistance they express, and the strength of associations between desirable and undesirable traits that might be encountered in the course of an enhancement program. Ability to cross breed is greater among species with compatible endosperm balance numbers (Johnston and Hanneman, 1980) (see Table 1), with the frequency of unreduced gametes influencing the success of interploidy crosses. Polygenes, which are difficult to maintain intact in breeding, are believed to underlie quantitative resistance. It is thus desirable to identify sources with high heritability, and to ensure robust screening procedures that are suitable for large numbers of hybrid individuals if introgression or upgrading programs are proposed.

The poor tuberization of some accessions led to the loss of some promising genotypes identified in the true seed

experiments. For example, *S. chomatophilum* and certain accessions of *S. albicans, S. ambosinum, S. circaeifolium,* and *S. dolichocremastrum* produced no or only a few short-lived tubers and therefore could not be re-evaluated as tuber-grown plants. If crosses with such donors are accomplished in breeding programs, the genetic association of desirable and undesirable traits in hybrid populations may limit advances in improving resistance and agronomic characteristics. For example, Ochoa (1999) proposes *S. chiquedenum* as useful for its resistance to late blight and earliness, but its moniliform tuberization habit (formation of tubers in chains) is likely to be a detracting horticultural characteristic in breeding programs. On the other hand, it is notable that several species presenting resistance in these evaluations produce large tubers in their wild states (e.g., *S. urubambae, S. violaceimarmoratum, S. cajamarquense, S. coelestispetalum, S. medians, S. microdontum, S. multiinterruptum, S. oplocense,* and *S. orophilum*). This may facilitate their use in breeding.

This population approach to germplasm evaluation provides important information needed to select and optimize strategies for the use of new resistance sources in breeding. For example this effort will orient the selection of new donors of resistance for tests of heritability and complementarity with currently deployed resistance types. The use of multiple genotypes of a given donor species and a carefully chosen set of diverse recurrent parents may help circumvent the poorly understood genetic interactions between wild and cultivated species that often result in fertility problems in F_1 and F_2 generations (Santini et al., 2000). The selection of diverse donor species using taxonomic, ecogeographic and mechanistic criteria should also contribute to building complex resistance types that are not readily overcome by the pathogen.

Tools of modern genetics including molecular diversity surveys, testing hypotheses on the role of candidate genes for resistance and defense, and comparative mapping, will be valuable in helping to develop strategic combinations of resistance sources, and possibly lead to the identification and direct transfer of superior alleles from wild to cultivated genetic backgrounds.

Acknowledgments

This work was supported in part by the Department for International Development (DFID), United Kingdom. We thank E. de la Torre, H. Ponce, G. Marticorena and O. Gaspar for technical assistance in the greenhouse and laboratory, V. Otazu for input on design and execution of the experiments, C. Arellano for statistical consultation, and R. Hijmans, A. Panta, and E. Mihovilovich for helpful comments on the manuscript.

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