



Late blight resistance status in wild potato species against Indian population of *Phytophthora infestans*

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ABSTRACT

Late blight caused by oomycete pathogen *Phytophthora infestans* is the most destructive disease affecting potato crop world-wide with losses up to 90% in India. Resistant varieties offers safe and economical mean for management of the disease. Wild species of potato are the reservoirs of resistance against many insect pest and diseases including late blight. In the present study, 539 clones of 91 potato accessions belonging to 18 wild species maintained at CPRI, Shimla were evaluated for presence of durable resistance against late blight. The clones SS 1764-19 (*S. alandiae*), SS 1763-09 and SS 1763-25 (*S. albicans*), SS 1769-04, SS 1769-08 and SS 1770-14 (*S. arnezii*), SS 1784-07 (*S. berthaultii*), SS 1794-07 (*S. brevicaulis*), SS 0551-02, SS 0680-06, SS 1671-01 and SS 1671-03 (*S. chacoense*), SS 1835, SS 1846-05, SS 1847-09, SS 1850-0, SS 1850-01 and SS 1850-04 (*S. demissum*), SS 1926-09, SS 1926-10, SS 1926-11 and SS 1926-13 (*S. microdontum*), SS 2615-01, SS 2616-01, SS 2616-02, SS 2655-01, SS 2656-02, SS 2658-01, SS 2658-02 and SS 2658-03 (*S. pinnatisectum*), SS 1664-02 and SS 1724-40 (*S. sparsipilum*), SS 2038-04 and SS 2048-0 (*S. tuberosum* ssp. *andigena*) and SS 2082-0 (*S. vernei*) were found to be most promising having high late blight resistance under laboratory and field testing. Although some difficulties exist in direct utilization of these clones due to ploidy and EBN differences, but these can be overcome through both short and long-term breeding strategies viz., ploidy and EBN manipulation, bridging species, embryo rescue, somatic hybridization and molecular techniques.

Key words: *Solanum tuberosum*, *Phytophthora infestans*, wild species, EBN.

INTRODUCTION

Late blight caused by oomycete pathogen *Phytophthora infestans* is the most destructive disease affecting potato crop and incurs huge expense world-wide in crop losses and control measures (Havekort *et al.*, 4). In India, late blight appears in most of the potato growing regions in varying degree causing losses up to 90% depending upon the variety and control measures adopted (Singh *et al.*, 12). The disease is usually managed by applying fungicides but it increases the cost of production and poses environmental hazards. Resistant varieties offer safe and economical mean for controlling the disease. Early potato breeding for *Phytophthora infestans* resistance was based on major gene resistance derived mainly from the Mexican hexaploid species *Solanum demissum* but it proved to be unstable. Attempts have been made to incorporate quantitative resistance but little success is achieved (Bormann *et al.*, 2) due to strong linkage between foliage resistance and late foliage maturity (Visker *et al.*, 13). The pathogen *Phytophthora infestans* has proved to be notoriously rapid in evolving complex races and

matching virulence types and adapting to changing environmental conditions. High genetic uniformity among the varieties released during last century has further made potato cultivation vulnerable to losses from diseases, insect pests *etc.* (Provan *et al.*, 7). Wild species of potato have been reported to possess resistance against many insect pests and diseases including late blight which confers broad spectrum resistance and also broaden the genetic base of the future varieties. Late blight resistance genes have been mapped in many wild species like *S. pinnatisectum*, *S. berthaultii*, *S. microdontum* *etc.* With recent advances in the gene transfer technology, it has become possible to efficiently transfer only the gene of interest either through cisgenesis or transgenesis (Havekort *et al.*, 4).

Central Potato Research Institute, Shimla serves as national repository for collection and conservation of potato germplasm in India from all over the world. Every year new accessions are being added to this collection from different sources. Evaluation of these wild species for resistance to different biotic and abiotic stresses affecting potato crop has been a continuous activity (Bhardwaj *et al.*, 1; Luthra *et al.*, 6). Many wild species maintained in different parts of the world have been evaluated separately

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for resistance to late blight (Douches *et al.*, 3) but only a part of the accessions found resistant in one study can be confirmed in other studies owing to differences in the variability of the pathogen, evaluation methodology used or segregation of character expression between different accessions or species. Thus, it becomes essential to evaluate the existing germplasm against the local pathogen population present in the respective countries. The present study was undertaken to evaluate the existing wild species potato germplasm for presence of durable resistance against late blight populations in India.

MATERIALS AND METHODS

The accessions used in the study were imported from Potato Introduction Station, Sturgeon Bay, USA and Institute of Plant Genetics and Crop Plant Research (GLKS), Gross Luessewitz, Germany in true potato seed (TPS) form for evaluation and utilization under Indian conditions. The TPS belonging to different accessions were sown in plastic trays and later transplanted in the pots for converting into tuber form. The tubers obtained from each seedling represent a different clone and these clones were used in the present study. A total of 539 clones of 91 potato accessions belonging to 18 wild species were evaluated for late blight resistance following laboratory screening through detached leaf methodology and field screening under natural epiphytotic condition during 2014 & 2015.

The detached leaf tests were done in 2014 (June to August) and 2015 (July to September) by challenge inoculation of *Phytophthora infestans* under environment-controlled conditions ($18 \pm 2^\circ\text{C}$ temperature and $>90\%$ relative humidity). The *P. infestans* isolate HP10/42 (A2 mating type and races 1.2.3.4.5.6.7.8.9.10.11) was used as inoculum and was multiplied through a tuber-slice method on a R-gene free susceptible potato cultivar, Kufri Chandermukhi. The zoospore concentration was adjusted to a level of 6×10^4 zoospores/ml using a hemocytometer. The artificial inoculation of this complex race was done in a minimum of six leaflets of top 4-5th leaf from 2-4 plants/clone obtained from the six weeks old crop grown under glass house. Based on lesion area, clones were grouped into highly resistant (lesion area $<1.0 \text{ cm}^2$), resistant (lesion area 1.1 to 2.5 cm^2), moderately resistant (lesion area $2.51-6.0 \text{ cm}^2$) and susceptible (lesion area $>6.0 \text{ cm}^2$) categories. The field evaluations were done during the summer season (April-September) in Shimla (31.10°N , 77.17°E , 2200 m (above mean sea level), Himachal Pradesh, India and the mean temperature and relative humidity during the main cropping season

(July to September) ranged from $14.71-24.21^\circ\text{C}$ and $75-93\%$, respectively with a total rainfall of 1855mm. The data on disease severity were recorded at weekly intervals and used to calculate Area Under Disease Progress Curve (AUDPC) following Shaner and Finney (9). Clones were classified as highly resistant (AUDPC < 50), resistant (AUDPC 50-150), moderately resistant (AUDPC 151-250), susceptible (AUDPC 251-500) and highly susceptible (AUDPC > 500) categories based on the AUDPC values. The laboratory as well as field observations were recorded in three replications for two years and parameters like mean, range, analysis of variance and different interaction effects were estimated following standard statistical procedures.

RESULTS AND DISCUSSION

Out of 539 clones belonging to 18 *Solanum* species screened against late blight, more than 25% belonged to highly resistant or resistant group indicating high proportion of resistant genotypes among the wild species (Table 1). Maximum proportion of highly resistant clones was found in *S. pinnatisectum* (69.2%) followed by *S. chacoense* (23.4%), *S. microdontum* (12.5%) and *S. demissum* (10.3%). Maximum proportion of resistant clones was found in *S. vernei* (54.5%) followed by *S. demissum* (41.4%), *S. cardiophyllum* (33.3%), *S. sparsipilum* (30.8%), *S. berthaultii* (25.9%), *S. arnezii* (25%) and *S. microdontum* (25%). *S. haancabambense* and *S. gourlayii* had only moderately resistant or susceptible clones while *S. cardiophyllum* had only resistant and moderately resistant clones. Thus, clones from the wild species *S. pinnatisectum*, *S. chacoense*, *S. microdontum*, *S. demissum*, *S. cardiophyllum*, *S. sparsipilum*, *S. berthaultii* and *S. arnezii* can be used as donors for late blight resistance genes. The analysis of variance of the late blight resistance of the accessions scored through laboratory testing revealed significant variances among clones which indicated presence of sufficient variability in the experimental material under study (Table 2). The variance due to species was also significant indicating presence of variability among different species for late blight resistance. The variance due to accessions was significant which was expected as each accession represents an individual genotype.

The accessions PI-498089 and PI-568913 (*S. alandiae*), PI-498201 (*S. albicans*), PI-545880 and PI-545958 (*S. arnezii*), PI-473331 and PI-595507 (*S. berthaultii*), PI-473378 (*S. brevicaulis*), PI-GLKS-95 (*S. cardiophyllum*), PI-230495, PI-189217, PI-217451, PI-133073 and PI-320285 (*S. chacoense*), PI-GLKS-269, PI-GLKS-306, PI-160208, PI-161169, PI-161366, PI-161719 and PI-175423 (*S. demissum*),

Table 1. Status of late blight resistance in different *Solanum* species under field conditions.

<i>Solanum</i> sp.	Chr No.	EBN	Total clone (s) tested	Late blight status (%)*			
				HR	R	MR	S
<i>S. alandiae</i>	24	2	42	2.4 (1)	16.7 (7)	81.0 (34)	-
<i>S. albicans</i>	72	4	39	5.1 (2)	17.9 (7)	66.7 (26)	10.3 (4)
<i>S. arnezii</i>	24	2	32	9.4 (3)	25.0 (8)	50.0 (16)	15.6 (5)
<i>S. avilesii</i>	24	2	15	-	6.7 (1)	26.7 (4)	66.7 (10)
<i>S. berthaultii</i>	24	2	27	3.7 (1)	25.9 (7)	63.0 (17)	7.4 (2)
<i>S. brevicaule</i>	24	2	10	10.0 (1)	-	70.0 (7)	20.0 (2)
<i>S. cardiophyllum</i>	24	1	15	-	33.3 (5)	66.7 (10)	-
<i>S. chacoense</i>	24	2	17	23.5 (4)	29.4 (5)	41.2 (7)	5.9 (1)
<i>S. demissum</i>	72	4	58	10.3 (6)	41.4 (24)	46.6 (27)	1.7 (1)
<i>S. gandavillasii</i>	24	2	14	-	-	28.6 (4)	71.4 (10)
<i>S. gourlayii</i>	48	4	14	-	7.1 (1)	28.6 (4)	64.3 (9)
<i>S. haancabambense</i>	24	2	14	-	-	85.7 (12)	14.3 (2)
<i>S. microdontum</i>	24	2	32	12.5 (4)	25.0 (8)	46.9 (15)	15.6 (5)
<i>S. pinnatisectum</i>	24	1	13	69.2 (9)	15.4 (2)	7.7 (1)	7.7 (1)
<i>S. sparsipilum</i>	24	2	39	5.1 (2)	30.8 (12)	46.2 (18)	17.9 (7)
<i>S. spegazzinii</i>	24	2	57	-	24.6 (14)	47.4 (27)	28.1 (16)
<i>S. tuberosum</i> ssp. <i>andigena</i>	48	4	90	3.3 (3)	8.9 (8)	50.0 (45)	37.8 (34)
<i>S. vernei</i>	24	2	11	9.1 (1)	54.5 (6)	36.4 (4)	-
Kufri Jyoti (check)	48	4	1	-	-	-	S
Total			539	37	115	278	109

Late blight status (%)*: HR = Highly resistant; R = Resistant; MR = Moderately resistance; S = Susceptible

*Figures in parentheses indicate number of clones.

PI-595509 and PI-218224 (*S. microdontum*), PI-275236, PI-347766, PI-275231, PI-230489, PI-275231 and PI-184774 (*S. pinnatisectum*), PI-113531, PI-DA 019 and PI-CGN-17838 (*S. sparsipilum*), PI-CGN-17839 and PI-442686 (*S. spegazzinii*), PI-186178, PI-243361 and PI-243404 (*S. tuberosum* ssp. *andigena*) and PI-473306 (*S. vernei*) were found to be highly resistant to late blight under laboratory testing.

The response of late blight reaction was almost similar under field and lab evaluations though variable response was observed in few accessions. The lesion expansion rate decrease with decrease in inoculum load and, therefore, the expression of virulence in detached leaf test depend on the inoculum concentration (Sharma and Singh, 10). Though a standardized inoculum load was used under lab evaluations, but the same may vary under field conditions. Similarly, the environmental conditions i.e. temperature and humidity also vary in the field vis-à-vis controlled chambers which together with inoculum load explain aberration in results obtained under both testing methods. The accessions

PI-568913 (*S. alandiae*), PI-595507 (*S. berthaultii*), PI-GLKS-95 (*S. cardiophyllum*), PI-133073 and PI-320285 (*S. chacoense*), PI-GLKS-269, PI-GLKS-306 and PI-161169 (*S. demissum*), PI-595509 and PI-218224 (*S. microdontum*), PI-113531 (*S. sparsipilum*), PI-CGN-17839 and PI-442686 (*S. spegazzinii*) and PI-243361 (*S. tuberosum* ssp. *andigena*), that were highly resistant in laboratory testing showed less resistance under field conditions while reverse trend was observed in the accessions PI-545958 (*S. arnezii*), PI-473101 (*S. gourlayii*), PI-320314 (*S. microdontum*), PI-275231 (*S. pinnatisectum*), PI-CGN-17838 and PI-CGN-17839 (*S. spegazzinii*) and PI-243390 and PI-243435 (*S. tuberosum* ssp. *andigena*). Such variation among late blight reaction in laboratory and field screening can be due to differences in methodology, testing condition, duration of testing, plant age, canopy structure, pubescence of leaves and spatial and temporal variation in pathogen pressure (Rogozina *et al.*, 8). It can be safely concluded that the accessions resistant under both laboratory and field conditions are of much breeding value.

Table 2. Late blight status of different potato species based on laboratory testing.

Species name	Accessions (P.I. No.) evaluated [#]	Lesion area (cm ²)	Promising clone(s)*
<i>S. alandiae</i>	498089 (21), 568913 (21)	0.73 - 4.23	1764-19
<i>S. albicans</i>	365310 (12), 498201 (27)	0.67 - 7.39	1763-09, 1763-25
<i>S. arnezii</i>	545880 (17), 545958 (15)	0.51 - 7.54	1769-04, 1769-08, 1770-14
<i>S. avilesii</i>	498091 (10), 498093 (5)	1.39 - 6.46	-
<i>S. berthaultii</i>	218215 (10), 265857 (1), 265858 (2), 310926 (1), 473330 (3), 473331 (1), 473339 (1), 473340 (1), 498104 (3), 595507 (4)	0.62 - 7.08	1784-07
<i>S. brevicaula</i>	473378 (7), 545971 (3)	0.53 - 7.05	1794-07
<i>S. cardiophyllum</i>	GLKS-95 (3), 283062 (1), 341233 (11)	0.59 - 5.20	-
<i>S. chacoense</i>	230495 (1), 3297 (1), 189217 (1), 113443 (3), WRF-286 (1), 217451 (4), EC 329480 (1), 133073 (4), 320285 (1)	0.53 - 7.06	0551-02, 0680-06, 1671-01, 1671-03
<i>S. demissum</i>	GLKS-221 (1), GLKS-233 (2), GLKS-269 (6), GLKS-306 (1), 160208 (1), 160212 (1), 160220 (3), 160222 (2), 161149 (1), 161169 (3), 161366 (8), 161719 (7), 161729 (17), 175423 (4), 201853 (1)	0.48 - 6.53	1835, 1846-05, 1847-09, 1850-0, 1850-01, 1850-04
<i>S. gandavillasii</i>	265866 (2), 545862 (12)	3.48 - 8.10	-
<i>S. gourlayii</i>	473101 (14)	1.64 - 7.78	-
<i>S. haancabambense</i>	458400 (14)	2.89 - 7.37	-
<i>S. microdontum</i>	218225 (1), 458358 (3), 473170 (8), 595505 (1), 595509 (12), 320314 (6), 218224 (1)	0.58 - 7.44	1926-09, 1926-10, 1926-11, 1926-13
<i>S. pinnatisectum</i>	190115 (1), 275236 (1), 347766 (3), 275231 (1), 230489 (1), 275231 (3), 184774 (3)	0.53 - 7.46	2615-01, 2616-01, 2616-02, 2655-01, 2656-02, 2658-01, 2658-02, 2658-03
<i>S. sparsipilum</i>	113531 (1), DA 019 (2), 310972 (2), CGN-17838 (34)	0.50 - 7.53	1664-02, 1724-40
<i>S. spegazzinii</i>	205407 (2), CGN-17839 (51), 442686 (4)	0.47 - 7.76	-
<i>S. tuberosum</i> ssp. <i>andigena</i>	161716 (2), 161683 (1), 186178 (7), 225629 (14), 237208 (7), 243361 (15), 243390 (1), 243404 (4), 243406 (8), 243411 (2), 243435 (3), 243437 (5), 460701 (6), 243443 (4), 243448 (2), 296086 (9)	0.80 - 7.77	2038-04, 2048-0
<i>S. vernei</i>	WAC 4085 (1), 473306 (1), 500062 (9)	0.44 - 5.42	2082-0
K. Chandermukhi (check)	-	6.94 - 9.49	-
CD (5%)			
Clone	0.09	Species	0.15
Accession	0.13	Year	NS
Clones × Year	NS	Species × Year	NS
Accession × Year	NS		

*Clones exhibiting high level of late blight resistance under laboratory conditions as well as in field testing.

[#]Values in parenthesis indicate the number of clones evaluated in particular accession (P.I. No.).

The clone SS 1764-19 (*S. alandiae*), SS 1763-09 and SS 1763-25 (*S. albicans*), SS 1769-04, SS 1769-08 and SS 1770-14 (*S. arnezii*), SS 1784-07 (*S. berthaultii*), SS 1794-07 (*S. brevicaule*), SS 0551-02, SS 0680-06, SS 1671-01 and SS 1671-03 (*S. chacoense*), SS 1835, SS 1846-05, SS 1847-09, SS 1850-0, SS 1850-01 and SS 1850-04 (*S. demissum*), SS 1926-09, SS 1926-10, SS 1926-11 and SS 1926-13 (*S. microdontum*), SS 2615-01, SS 2616-01, SS 2616-02, SS 2655-01, SS 2656-02, SS 2658-01, SS 2658-02 and SS 2658-03 (*S. pinnatisectum*), SS 1664-02 and SS 1724-40 (*S. sparsipilum*), SS 2038-04 and SS 2048-0 (*S. tuberosum ssp. andigena*) and SS 2082-0 (*S. vernei*) were found to be most promising having high late blight resistance under both laboratory and field conditions. Presence of high level of late blight resistance among clones of potato wild species *S. arnezii*, *S. berthaultii*, *S. chacoense*, *S. demissum*, *S. microdontum*, *S. pinnatisectum*, *S. sparsipilum*, *S. tuberosum ssp. andigena* and *S. vernei* have also been reported earlier (Hoekstra, 5).

The clones found promising in this study forms excellent material for pre-breeding that can be used later to breed late blight resistant tetraploid germplasm and will also broaden the genetic base of the cultivated potato. Besides, the highly resistant clones from *S. tuberosum ssp. andigena* (SS 2038-04 and SS 2048-0), having endosperm balance number (EBN) and ploidy level same as that of cultivated *S. tuberosum ssp. tuberosum* (2n = 48, 4 EBN), can directly be utilised in crossing with cultivated potato varieties. For transferring late blight resistance from the promising clones of hexaploid species (2n = 6x), viz., *S. albicans* (SS 1763-09 and SS 1763-25) and *S. demissum* (SS 1835, SS 1846-05, SS 1847-09, SS 1850, SS 1850-01 and SS 1850-04) with 4 EBN to *S. tuberosum*, bridge species like *S. chacoense* and *S. phurja* can be utilised. Diploid 2 EBN germplasm generally crosses more readily with cultivated germplasm than diploid 1EBN germplasm due to 2n gamete formation via first division restitution (FDR) and second division restitution (SDR) (Singh *et al.*, 11). By following this breeding strategy, the late blight resistance from the promising clones of diploid species *S. alandiae* (SS 1764-19), *S. arnezii* (SS 1769-04, SS 1769-08 and SS 1770-14), *S. berthaultii* (SS 1784-07), *S. brevicaule* (SS 1794-07), *S. chacoense* (SS 551-02, SS 680-06, SS 1671-01, SS 1671-03), *S. microdontum* (SS 1926-09, SS 1926-10, SS 1926-11 and SS 1926-13), *S. sparsipilum* (SS 1664-02 and SS 1724-40) and *S. vernei* (SS 2082-0) can be transferred to cultivated potatoes.

The promising clones of diploid *S. pinnatisectum* (SS 2615-01, SS 2616-01, SS 2616-02, SS 2655-01, SS 2656-02, SS 2658-01, SS 2658-02 and SS

2658-03) with 1 EBN are difficult to utilize in short term. These can be utilized through long term breeding strategies by adopting various techniques of ploidy and EBN manipulation, utilizing bridging species, embryo rescue and somatic fusion (Zlesak and Thill, 14).

It can be concluded that there is high intra and inter genetic diversity in accessions and species of potato maintained in India. The genetically diverse and most promising clones from different species as identified in this study can be exploited both for short and long-term breeding strategies in terms of deploying different set of genes for different environmental conditions i.e. inoculum load and duration of congenial conditions for development of late blight thus avoiding mono-culture of the host that has resulted in the quick breakdown of resistance in the past globally. Further, these strategies would help to broaden the genetic base of potato gene pool leading to stronger and more durable resistance in the potato cultivars.

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