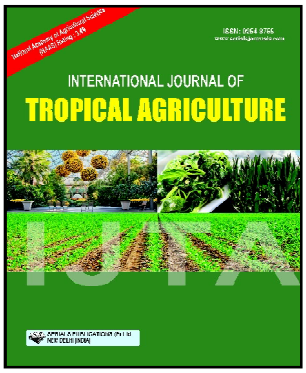

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Potato pre-breeding: Identification of late blight resistant wild *Solanum* species by challenge inoculation and protoplast fusion

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Abstract: Potato is third most important food crop in the world after rice and wheat in terms of human consumption. Potato suffers from various biotic and abiotic stresses. Late blight caused by the oomycete Phytophthora infestans (Mont.) de Bery is the most disease of potato worldwide. Wild Solanum species are reservoir of resistance source for various biotic and abiotic stresses including late blight. However, it is hard to transfer the desirable traits from wild Solanum into the cultivated species via conventional breeding, due to sexual incompatibility. Potato breeding using conventional methods has limitations because the genetic base of cultivated potato is narrow. Therefore, this study signifies the importance of wild species late blight resistance and their use in protoplast fusion for development of potato somatic hybrids, as pre-breeding genetic material for breeding uses. It is of great significance to explore these pre-breeding alternatives to complement the traditional way, so as to fully use the wild species for potato improvement.

1. INTRODUCTION

Potato is the staple food in the developed countries, whereas considered as a vegetable crop in the developing countries. Potato is originally a native of Peru, South America and introduced in India in late 16th to early 17th century. The cultivated commercial potato (*Solanum tuberosum* L.) is a tetraploid ($2n = 4x = 48$) crop with basic chromosome number $x = 12$. Wild species represent ploidy ranging from diploid ($2n = 2x = 24$) to hexaploid ($2n = 2x = 72$) level (Chakrabarti *et al.*, 2017). Potato crop production is affected by various biotic and abiotic stresses, such as bacterial, fungal, viral diseases and adverse environment. Of them, late blight caused by the oomycete fungus, *Phytophthora infestans* is the most devastating disease. This pathogen may causes losses up to 85% in the hills and 60–70% in the plains of India depending upon the disease incidence. The

qualitative resistance to late blight is based on a series of 11 single dominant *R* genes, designed *R1* to *R11* (Shaw, 1991). Unfortunately, all the present-day Indian potato cultivars have very narrow genetic base those rely upon few parents having mainly the major R genes providing vertical resistance to late blight, which are broken down with the appearance of complex races of *P. infestans* (Tiwari *et al.*, 2017). Henceforth, race non-specific or horizontal or durable resistance to potato late blight is now being preferred for varietal development in India (Tiwari *et al.*, 2018). Importantly, wild *Solanum* species are an important source of resistance for various biotic and abiotic stresses. The genetic improvement of cultivated species is a key to successful potato production to withstand these stresses. Nevertheless, it is hard to transfer the desirable traits from wild *Solanum* into the cultivated species *via* conventional breeding, due to sexual incompatibility. Potato breeding using conventional methods has limitations because the genetic base of cultivated potato is narrow. Therefore, it is of great significance to explore other breeding alternatives to complement the traditional way, so as to fully use the wild species for crop genetic improvement. Protoplast fusion is one of the many breeding tools available to create new genomic combination and enables to broaden the genetic base in a single step by allowing transfer of multi genes including nuclear and cytoplasmic organelles genes. Somatic hybridization is very useful to overcome sexual incompatibility barriers and facilitates the development of tetraploid genotypes using pre-selected diploid/dihaploid parents (Tiwari *et al.*, 2018). This technique provides pre-breeding resources with increased genetic variability and desirable traits for introgression into cultivated background.

2. MATERIALS AND METHODS

2.1. *In vitro* regeneration of wild *Solanum* species

True potato seed (TPS) of wild species were sterilized and plants were regenerated through tissue culture. List of the wild species used in seed sterilisation and *in vitro* regeneration is given in Table 1. All the glass wares, distilled water and filter papers used in seed sterilisation were sterilised as per the standard practices in the autoclave (Mediquep, Medicare equipment Co., Baroda, India) for 21 minutes at 121 °C. TPS of different accessions of different wild potato species were washed under the tap water for 20-30 min. These TPS were transferred in a sterile beaker and rinsed with sterile Milli-Q water (Millipore Elix®3, Millipore, France). These seeds were sterilised with 5% sodium hypochlorite for 20-30 min followed by rinsing with sterile Milli-Q water (3-4 times). Then TPS were again surface-disinfested with 0.1% mercuric chloride ($HgCl_2$) (0.1 g/100 ml water) for 4-5 min followed by thorough rinsing (four times) with sterile Milli-Q water under aseptic conditions. The TPS were then dried over sterile filter paper and cultured for *in vitro* germination. The seeds were allowed to germinate in magenta GA-7 (Sigma-Aldrich) boxes containing 50 ml of Murashige and Skoog (MS) medium pH 5.8, without organic compounds, glycine and plant growth regulators (PGRs). pH was adjusted with pH meter (pH tutor, Eutech Instruments, Singapore). The balance used for weighing all chemicals was Mettler Toledo (Mettler Toledo, Switzerland). The composition of MS medium is given in Table 3.2. All the work of sterilization was performed under laminar air flow (Klenz Flo™, Klenzaids Contamination Control PVT. LTD., Valsad, India).

2.2. Late blight resistance test

In vitro grown plants were tested twice for late blight resistance by challenge inoculation of *P. infestans* under the controlled conditions as detailed procedures described by Tiwari *et al.* (2015). The *in vitro* plants were grown (in triplicates) in earthen pots (20 × 25 cm^2) containing a sterile mixture of soil/FYM-based compost (1: 1, v/v) under the glass house conditions during the summer season in Shimla (31.10 °N, 77.17 °E, 2200 m above mean

sea level), Himachal Pradesh under an average of 14 h day length, with a mean temperature of 20 °C in day and 15 °C in night. Eight weeks-old plants (fully grown three pots of each accession) were shifted to the controlled chamber (18±2 °C temperature and 80–90 % relative humidity). The *P. infestans* isolate HP09/40 (A2 mating type and races 1.2.3.4.5.6.7.8.9.10.11) was mass-cultured on tuber slices of a highly susceptible potato *cv.* Kufri Chandramukhi. Temperature shock of 4 °C was given to release the zoosporngia and then zoosporangia were washed from the tuber surface to dislodge them and diluted to 5×10^4 (sporangia/ml). The sporangial suspension was incubated at 12 °C for 60-90 min for releasing of zoospores. Zoospore suspension was sprayed on plants having concentration 5×10^4 (sporangia/ml) by using hand sprayer. Throughout the screening period, relative humidity in the controlled chamber was maintained to 80–90 % using mist generation system (Rajdeep Agrotech, New Delhi, India) and temperature was ranged between 18±2 °C by automatically operated air conditioner system.

At different time interval after inoculation with *P. infestans* spores the percentage of leaves showing late blight lesions was recorded. During the late blight progression, percent foliar necrosis was recorded five times i.e. at 3, 5, 7, 9 and 10 days after inoculation (DAI). The late blight resistance score was determined by visual observation (three replicates) at 10 DAI. The area under disease progress curve (AUDPC) was calculated as described by Camphell and Madden (1990). Accessions were categorized based on the AUDPC value: HR (< 50), R (50–100), MR (100–150) and S (> 150) (HR = highly resistant, R = resistant, MR = moderately resistant and S = susceptible). The AUDPC was calculated for the clones showing different late blight resistance levels as described earlier by Singh and Birhman (1994). Based on the resistance levels, species were selected as donor parent for protoplast fusion.

2.3. Protoplast fusion

Late blight resistant wild diploid species (donor parent) and known androgenic dihaploid C-13 (recipient parent) were used for protoplast fusion (Chandel *et al.*, 2015). The source plants were maintained and multiplied through tissue culture in MS medium (pH 5.8) in boxes magenta GA-7. Three-week-old *in vitro*-grown plants raised from single nodal cuttings were used to isolate mesophyll protoplasts under sterile conditions. Cultures were grown at 20 °C under a 16-h photoperiod (light intensity 50-60 µmol m^{-2} s^{-1}). Protoplasts were isolated from 3-week-old plants of both the diploid and dihaploid plants.

3. RESULTS AND DISCUSSION

The late blight, caused by oomycete *Phytophthora infestans* is the most devastating disease worldwide and greatest threat to potato cultivation. Late blight disease is responsible for the 10-15% reduction in the global production of potato tubers (Smyda *et al.*, 2013). The fungicides are frequently used to control late blight that has a negative effect on the economic feasibility of the crop and on the environment. Therefore, development and application of cultivars with a high level of resistance to potato late blight is highly desirable. The most effective and environmental friendly way of controlling late blight is to incorporate natural and durable resistance source from wild/semi- or cultivated species into commercial potato cultivars. Therefore, durable resistance is now being favoured world over. Genetic improvement through conventional hybridization is difficult and time consuming because of sexual incompatibilities and reproductive isolation caused by difference in ploidy number and endosperm balance number between wild and cultivated potato species. Hence, to circumvent this problem, protoplast fusion is one of the very suitable methods to introgress valuable resistant gene source from wild species into the cultivated potato gene pool.

Total of 22 accessions of 18 wild potatoes (*Solanum*) species were regenerated *in vitro* from TPS of hundreds of accessions for the study (Table 1). The *in vitro* plants were maintained *in vitro* on MS medium under controlled growth chamber 18-20 °C under a 16-h photoperiod (light intensity 50-60 µmol m^{-2} s^{-1}). Twenty-two accessions of 18 wild species were tested for late blight resistance by challenge inoculation of *P. infestans* under controlled conditions (Table 1 and Fig. 1). Of which, 15 accessions of 12 species namely *S. berthaultii*, *S. cardiophyllum, S. hougsii, S. huancabambense, S. iopetalum, S. jamesii, S. lesteri, S. microdontum, S. polyadenium, S. polytrichon, S. stoloniferum* and *S. trifidum* were found highly resistant (HR) to

Table 1
List of wild *Solanum* species used in the study for regeneration of plants from TPS and late blight resistance testing by challenge inoculation of *P. infestans* under controlled conditions

S. No.	*Solanum species*	*Accession number*	*Late blight infection (AUDPC)*		*Class*
			I	*II*	
1.	*S .berthaultii*	PI 265857	0	4	HR
2.	*S. cardiophyllum*	CGN 22387	25	20	HR
3.	*S. cardiophyllum*	PI 283062	0	0	HR
4.	*S. cardiophyllum*	PI 341233	0	0	HR
5.	*S. hougsii*	PI 161727	20	0	HR
6.	*S .huancabambense*	CGN 18306	10	0	HR
7.	*S. iopetalum*	PI 230459	0	2	HR
8.	*S. jamesii*	PI 498407	0	0	HR
9.	*S. lesteri*	CGN 24429	0	0	HR
10.	*S. microdontum*	PI 218224	10	0	HR
11.	*S. polyadenium*	CGN 17747	0	0	HR
12.	*S. polyadenium*	PI 230480	0	0	HR
13.	*S. polytrichon*	CGN 22362	10	0	HR
14.	*S. stoloniferum*	PI 225661	0	0	HR
15.	*S. trifidum*	CGN 22722	20	0	HR
16.	*S. vernei*	PI 320330	125	160	S
17.	*S. acaule*	CGN 17938	175	175	S
18.	*S. acaule*	PI 210029	120	145	MR
19.	*S. stenophyllidium*	CGN 17603	105	115	MR
20.	*S. chacoense*	PI 197760	100	125	MR
21.	*S. medians*	PI 283081	140	155	MR
22.	*S. michoacanum*	CGN 22371	140	160	MR
23.	Kufri Jyoti		180	190	S
24.	Kufri Girdhari		25	30	HR

[a] SN # 1-18: wild species; 19-20: control varieties

[b] Categorization of plants for resistance/susceptible is based on the area under disease progress curve (AUDPC) value: HR (< 50), R (50–100), MR (100–150), and S (>150) (HR=highly resistant, R=resistant, MR= moderately resistant and S=susceptible).

Figure 1: Late blight resistant wild potato (*Solanum*) species

late blight and scored AUDPC between 0.0 to 20.0 compared to susceptible control variety Kufri Jyoti (AUDPC = 180-190) and highly resistant control variety Kufri Girdhari (AUDPC = 25-30). However, other species were found moderately resistant (MR) and susceptible (S), therefore not selected for somatic fusion work. Protoplast fusion work was carried out to regenerate interspecific potato somatic hybrids (Chandel *et al.*, 2015).

Wild species were confirmed twice for late blight resistance by challenge inoculation of *P. infestans* under controlled conditions. A number of experiments have been demonstrated on testing of potato for late blight resistance assay through challenge inoculation by *P. infestans* (Tiwari *et al.*, 2013, 2015). Indeed, this study confirmed the high level of resistance in somatic hybrids between C-13 and *S. cardiophyllum*. In particular, a large number of somatic hybrids have been developed through protoplast fusion for late blight resistance derived from wild species such as *S. etuberosum* (Tiwari *et al.*, 2010), *S. pinnatisectum* (Sarkar *et al.*, 2011) and so on. Consequently, a significant development has been achieved in terms of exploitation of these somatic hybrids for potato improvement by these researchers. Importantly, somatic hybrids between *S. tuberosum* and *S. bulbocastanum* and back-cross progenies were exploited for mapping and cloning of the *RB* gene, the most important late blight resistance gene in potato (Song *et al.*, 2003). According to Szczerbakowa *et al.* (2005), the severely virulent pathogen races used in inoculation of the detached leaves reflects the level of specific resistance conferred by R genes. Susceptibility depends upon the lack of major resistance genes and durable resistance depends on the combined action of many minor genes. Late

blight resistance was successfully transmitted from *S. bulbocastanum* to tetraploid potato by Helgeson *et al.* (1998) and high resistance of the *S. bulbocastanum* was retained by the hexaploid somatic hybrids. According to Zimnoch-Guzowska *et al.* (2003), the interaction of the plasmon and the phenomenon of complementation of nuclear gene may be important for expressing the higher level of leaflet resistance in the fusion progenies in comparison to that present in the resistant fusion parent.

To conclude, durable resistance to late blight has been found in several wild potato species, which needs to be tapped and utilized in breeding of cultivated potato by conventional or biotechnological approaches (Tiwari *et al.*, 2017). The identification of late blight resistant wild species in this study and somatic hybrids developed in later part of this study (Chandel *et al.,* 2015) have great scope for breeding potato for development of varieties to manage this disease. It is of great significance to explore protoplast fusion technique as pre-breeding tool to complement the potato breeding.

REFERENCES

Chakrabarti, S.K., Conghua, X. and Tiwari, J.K. (Eds.) (2017), The Potato Genome. Springer International Publishing AG 2017. DOI https://doi.org/10.1007/978-3-319-66135-3. (Print ISBN 978-3-319-66133-9).

Chandel, P., Tiwari, J.K., Ali, N., Devi, S., Sharma, S.H., Sharma, S.A., Luthra, S.K. and Singh, B.P. (2015), Interspecific potato somatic hybrids between *Solanum tuberosum* and *S. cardiophyllum*, potential sources of late blight resistance breeding. *Plant Cell Tissue and Organ Culture* 123:579–589.

Helgeson, J.P., Pohlman, J.D., Austin, S., Haberlach, G.T., Wielgus, S.M., Ronis, D., Zambolim, L., Tooley, P., McGrath, J.M., James, R.V. and Stevenson, W.R. (1998), Somatic hybrids between *Solanum bulbocastanum* and potato: a new source of resistance to late blight. *Theoretical and Applied Genetics* 96:738–742.

Sarkar, D., Tiwari, J.K., Sharma, S.H., Poonam, Sharma, S.H., Gopal, J., Singh, B.P., Luthra, S.K., Pandey, S.K. and Pattanayak, D. (2011), Production and characterization of somatic hybrids between *Solanum tuberosum* L. and *S. pinnatisectum* Dun. *Plant Cell Tissue and Organ Culture* 107:427-440

Shaw, D.S. (1991), Genetics. Advances in Plant Pathology. **7**:131–170.

Singh, B.P., Birhman, R.K. (1994), Laboratory estimation of field resistance of potato late blight. *Journal of Phytopathology* 140:71-76.

Smyda, P., Jakuczun, H., Debski, K., Sliwka, J., Thieme, R., Nachtigall, M., Wasilewicz-Flis, I. And Zimnoch-Guzowska, E. (2013), Development of somatic hybrids *Solanum* × *michoacanum* Bitter. (Rydb.) (+) *S. tuberosum* L. and autofused 4*x S.* × *michoacanum* plants as potential sources of late blight resistance for potato breeding. *Plant Cell Report* 32:1231–1241.

Song, J., Bradeen, J.M., Naess, S.K., Raasch, J.A., Wielgus, S.M., Haberlach, G.T., Liu, J., Kuang, H., Austin-Phillips, S., Buell, C.R., Helgeson, J.P. and Jiang, J. (2003), Gene *RB* cloned from *Solanum bulbocastanum* confers broad spectrum resistance to potato late blight. *Proceeding of National Academy of Sciences USA* 100:9128–9133.

Szczerbakowa, A., Boltowicz, D., Lebecka, R., Radomski, P. and Wielgat, B. (2005), Characteristics of the interspecific somatic hybrids *Solanum pinnatisectum* (+) *S. tuberosum* H-8105. *Acta Physiologia Plantarum* 3:265–273.

Tiwari, J.K., Devi, S., Ali, N., Luthra, S.K., Kumar, V., Bhardwaj, V., Singh, R.K., Rawat, S. and Chakrabarti, S.K. (2018) Progress in somatic hybridization research in potato during the past 40 years. *Plant Cell Tissue and Organ Culture* 132:225-238.

Tiwari, J.K., Devi, S., Sharma, S., Chandel, P., Rawat, S. and Singh, B.P. (2015), Allele mining in *Solanum* germplasm: cloning and characterization of RB-homologous gene fragments from late blight resistant wild potato species. *Plant Molecular Biology Reporter* 33:1584–1598.

Tiwari, J.K., Kumar, V., Devi, S., Luthra, S.K., Chakrabarti, S.K., Rawat, S. and Nagesh, M. (2017) Genomics in Management and Genetic Enhancement of Potato Germplasm. In: Chakrabarti SK, Conghua X, Tiwari JK (eds) The Potato Genome. Compendium of Plant Genomes. Springer, Cham. DOI https://doi.org/10.1007/978-3-319-66135-3_8

Tiwari, J.K., Poonam, Kumar, V., Singh, B.P., Sharma, S., Luthra, S.K., Bhardwaj, V. (2013), Evaluation of potato somatic hybrids of dihaploid *S. tuberosum* (+) *S. pinnatisectum* for late blight resistance. *Potato Journal* 40:176-179.

Tiwari, J.K., Poonam, Sarkar, D., Pandey, S.K., Gopal, J. and Kumar, S.R. (2010) Molecular and morphological characterization of somatic hybrids between *Solanum tuberosum* L. and *S. etuberosum* Lindl. *Plant Cell Tissue and Organ Culture* 103:175–187.

Zimnoch-Guzowska, E., Lebecka, R., Kryszczuk, A., Maciejewska, U., Szczerbakowa, A. and Wielgat, B. (2003), Resistance to *Phytophthora infestans* in somatic hybrids of *Solanum nigrum* L. and diploid potato. *Theoretical and Applied Genetics* 107:43–48.