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An Easy Assay Technique of Detection and Identification of *Phytophthora Infestans* (Mont.) De Bary From Seed Tubers Before Planting for Healthy Production of Potato

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Received: 01.08.2016 Received in Revised: 24.11.2016 Accepted: 25.11.2016

Abstract

The timely detection and appropriate identification of causal agents associated with disease of crop plants or seeds are considered to be the most important issue in formulating the management strategies for plant diseases. This is particularly important for plant diseases of a fungal nature, where disease-free planting materials is the only effective way to restrict the disease. Beside this, morphological discrimination requires special skill and the expertise of taxonomists or specialists and also time consuming. To simplify the detection, end-point polymerase chain reaction (PCR) assays were developed. Consensus sequences obtained from multiple alignments of target genes, RAPD based methodology was used to design the SCAR maker for rapid detection of *P. infestans* (amplified product 524bp). BLASTn was also used for *in silico* specificity. No cross reactivity was observed when primers were checked against other Phytophthora spp. The described primer sets allowed accurate identification and detection of *P. infestans*. All tests have multiple applications including screening of healthy planting materials, breeding programs and disease diagnosis.

Key words: *P. infestans*, potato seed tubers, direct PCR, diagnosis

Introduction

Potato (*Solanum tuberosum* L.), is one of the world's major non-grain food crops (Haverkort 1990; Scott *et al*., 2000), and optimally thrives in both warm and cool climates. Potato is the world's third-largest food crop after Rice, wheat, and maize (Li, 1985; Haas *et al*., 2009; Chakraborty *et al*., 2010;Hussain, 2016) and is cultivated in several countries worldwide, including China, India, Russia, and Pakistan (Hijmans 2003; Hassanpanah *et al*., 2009; Arab *et al*., 2012). This crop is consumed as a vegetable in Indian subcontinent, and serves as the major food grain dual-purpose crop. India is the second largest producer of potato in the world and providing more nutritious food more quickly, on less land and in harsher climates than any other crop. Its ease of cultivation and high energy content has made it a valuable cash crop for millions of farmers in the developing countries. Potato is an important crop ideally suited to meet the growing food demand associated with population growth in the poor and developing countries of the world especially in the tropics and particularly African nations and South Asia. Its capability to produce high value food in a short duration and amenability to fit into cropping systems makes it a preferred choice to be grown in a variety of environments (Hussain, 2016a). The Indo-Gangetic plains (IGP) is the main potato growing region accounting for almost 85% of the 1.8 million hectares under the crop in India where it is grown as an irrigated crop during the winter season and stored at cold storage during summer season, therefore it is very important to detect the pathogen before and after harvesting of potato seed tubers. Late Blight of potato caused by *Phytophthora infestans* is one of is the most devastating diseases of potatoes not only in India but throughout world were potato is cultivated (Hussain and Singh, 2016). This epidemic had totally destroyed potato crops in the 1840s which led to mass starvation in Europe. Losses up to 85% have been reported if crop (susceptible cultivar) remains unprotected. Disease appears every year in epiphytotic forms in hills as well as in plains (Hussain and Singh, 2016). This disease is mostly

spread through infected potato tuber seeds. Since seed is the carrier of the genetic potential for higher crop production, improved varieties of seed have been produced by modern selection and breeding techniques to help in increasing the yield per unit area and in turn to boost agricultural production leading to green revolution.

Technological advances in molecular detection method sallow fast and accurate detection and quantification of plant pathogens and these are now being applied to practical problems. Polymerase chain reaction (PCR) techniques offer advantages over traditional methods of detection and diagnosis. The practice of diagnosing plant pathogens using PCR has previously been described (Henson and French, 1993; Lévesque, 2001; McCartney *et al*., 2003; Atkins and Clark, 2004, Hussain *et al.,* 2013; 2014a;2014b;2016b). Plant diseases can be controlled most effectively if diagnostics is introduced at an early stage of disease development. Information resulting from molecular diagnostics could be used to make more rational decisions about the choice and use of proper agrochemicals at optimal application times. The information resulting from such experiments could be used to monitor the level of exposure of the crop to pathogen inoculum and to improve disease control by allowing more rational decisions to be made about the choice and the use of fungicides and resistant cultivars. With all these approaches, implementation of appropriate disease management measures requires timely detection and reliable identification of the pathogen and its races.

In recent years, the increasing use of molecular methods in fungal diagnostics has emerged as a possible answer to the problems associated with existing phenotypic identification systems. As a results, in the last two decades, molecular tools have had a major impact on the identification of plant pathogens. Furthermore, early diagnosis may help to restrict disease spread. Because infected seeds can carry the pathogen and spread the disease (Michail *et al.*, 1999), a molecular assay for tuber seeds is important to recognize infected tubers and prevent spread to new areas where the disease does not occurs/spread. This study consisted of newly developed group-specific PCR assays for the genus *Phytophthora* and a new species-specific PCR assay for Late blight of potato and can be very easily carried out at Krishi Vigayan Kendra at economical levels.

Materials and Methods

Plant material and extraction of DNA from tuber tissues

Hundred samples of different potato varieties were collected from different agro-climatic potato growing regions of the Western U.P and neighbouring area (Table 1). Reference pure culture of *P. infestans* were artificially infected potato tuber affected kept in artificial Biological incubators at ±18˚C (Fig. 1). While before DNA extraction, area wise samples were pooled into single (10 tubers) samples. Pure genomic DNA from *P. infestans* as well as other *Phytophthora* species and other potato fungal pathogens mycelium (100 mg) (cultured maintained in lab.) was extracted by using Qiagen Plant DNA Miniprep Kit (according to manufacturer protocol). Total genomic DNA was extracted from the host tissues (100 mg) (potato tubers sampled and collected during potato season, 2013-2014, before planting) using modified protocol as described in previous study of Hussain *et al.* (2014). RNAs treatment was performed by adding 2 µl of RNAse (10 mg/ml) to 1.5ml Eppendorf tube containing 100 µl of extracted DNA and then incubated for 3 hours at 37°C in a water bath. The concentration of DNA was determined by UV visible spectrophotometer (Nano drop, Thermofisher).

Table 1. Different Potato varieties samples collected from farmers' fields before planting (Season 2013-2014)

S.No.	Potato variety	Location	Symptoms
1	Kufri Bahar	Modipuram, meerut	Invisible to naked eyes
2	Kufri Bahar	Partapur Bypass	Invisible to naked eyes
3	Kufri Baadshah	Daurala,Meerut	Invisible to naked eyes
4	Kufri Phukraj	Pabli village	Invisible to naked eyes
5	Kufri Bahar	Haathras	Invisible to naked eyes
6	Kufri Bahar	Kannauj	Invisible to naked eyes
7	Kufri Anand	Haridwar	Invisible to naked eyes
8	Kufri Bahar	Sambhal	Invisible to naked eyes
9	Kufri Bahar	Aligarh	Invisible to naked eyes
10	Kufri Sadabahar	Babugarh, Hapur	Invisible to naked eyes

Table 2. List of Fungi used to screen the PCR (Primers) for amplification specific to *P. infestans*

Isolate	Host	Source	Mating Type
P. colocasiae	Taro	IISR, Calicut	A1
P. cactorum	Apple, Strawberry	IISR, Calicut	A1
P. palmivora	Coconut	IISR, Calicut	A1
P. capsici	Black Pepper	IISR, Calicut	A1
Fusarium spp.	Potato	CPRIC, Modipuram	A1
Rhizoctonia solani AG-3	Potato	CPRIC, Modipuram	-
A. solani	Potato	CPRIC, Modipuram	-

Figure 1. Artificially infected potato tubers kept in BOD incubator at ±18˚C, dark.

Primer specificity and sensitivity

The nucleotide sequence search program located in the 'Entrez' browser provided by the National Center for Biotechnology Information (NCBI) (Bethesda, MD) was used to retrieve and recheck the sequences of different *Phytophthora* species. Nucleotide sequences of all the GenBank isolates were aligned using the program CLUSTALX2 Larkin *et al*., (2007) and were re-examined for the conserved regions. Primers were Re-synthesize from Imperial Life science, Gurgaon, India). The specificity of each primer was confirmed *in silico* by screening the primer sequences with BLASTn (Altschul e*t al*., 1990).

PCR amplification

PCR assays were carried out in 25µl reaction mixtures containing 25 µl 2.5X Green Taq Buffer with 1.5mM $MgCl_2$, 2.5µl dNTPs master mix, 0.2U/µl Taq DNA polymerase (Fermentas) and 2µl (10pmol) of each Forward (Pinth2F-GGGGGTCTTACTTGGCGGCG) and Reverse primer (Pinth2R-CAAACCGGTCGCCAACTCGC), 2µl genomic DNA template (50ng/µl)and volume make up with milli Q water. PCR amplification was carried out in Eppendorf thermal cycler (Eppendorf, Germany).Thermal cycling parameters were initial denaturation at 94°C for 2 min followed by 30 cycles consisting of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1min. A final extension at 72°C for 10 min followed. Negative controls (no template DNA) were used in every experiment to test for the presence of contamination in reagents.

A volume of 20µl of amplified PCR product was electrophoresed in a 1.5% agarose gel containing ethidium bromide (0.25 mg/ml) in 1X TAE buffer, and amplicon sizes were estimated using 100bp ladders (Fermentas). The PCR amplicons were visualized using UV gel documentation system (BioVis, U.K).

Results and Discussion

It is a well-known fact that infected or contaminated seed is a primary source of inoculums for a large number of destructive diseases of important food, fodder and fiber crops (Neergaard, 1977). Besides affecting the crop yields, the seed-borne pathogens affect the nutritive quality and value of the seeds, leading to trade barriers. In some cases infected seeds are the only source of initial inoculums in the field. Late Blight is the most important pathogen of potato and tomato worldwide.

Identification of the causal agent and prevalence of a disease is very essential for adequate and timely management of disease, which in turns depends on proper accurate diagnosis and early detection of the pathogen before sowing into the fields. If not followed these methods, not only potato foliage is destroyed but by the time potato tubers can also become

infected (Carrier). Often, many countries import plant germplasm to diversify the genetic base of crop to improve yields and raise the levels of disease resistance and other economic and agronomic characteristics. But due to indiscriminate international exchange of germplasm, areas hitherto free of certain pathogens now have new population. So, in the present investigation the species-specific SCAR marker was used and validated during crop season 2013-2014, for early detection of *P. infestans* presence. Thus, this marker proved an efficient marker for species-specific discrimination which would be useful in developing a rapid and sensitive diagnostic PCR based assay for early detection and timely management of *P. infestans,* before and heavy loss to farmers as well as potato growers and can lead to epidemic situation.

Sampling of potato tubers was done before crop planting, 2013-14 season (*i.e.* four months of storage), sequence characterized amplified (SCAR) marker as expected amplified an amplicon of 524 bp fragment from isolates of all gDNA of *P. infestans* (cultures preserved and maintained in Pathology lab., Fig. 2). Although *P. infestans* populations may contain sexual compatible types and isolates with different metalaxyl sensitivities, they all were detectable. SCAR marker also amplified an amplicon of 524bp fragment from both *P. infestans* mating type A1 and A2.No cross-reactivity was observed with any non-target Phytophthora species as well other fungal pathogen of potato (Fig. 3). Judelson and Tooley (2000) reported an improved detection limit of 10 fg DNA with several new primer sets designed in repeated DNA families of *P. infestans* but they also cross-reacted with *P. mirabilis*, *P. phaseoli*, *P. hibernalis*, *P. ilicis*, *P. quinine*, *P. katsurae*, and *P. capsisci*. Similarly, the PCR assay described by Trout *et al.* (1997) for Internal Transcribed Spacer (ITS) regions cross-reacted with *P. mirabilis* and *P. cactorum* but was not tested with *P. phaseoli* and no sensitivity limits of detection were reported. With the Pinth 2-f and Pinth 2-r primer set, 10 fg of *P. infestans* DNA was detectable (Fig. 5) in PCR.

Detection of P. infestans in infected host tissues

Template genomic DNA was extracted from nearby node and internodes adjoining areas using the method of Hussain et al., (2014). No PCR products were amplified from uninfected tubers, or the healthy control, but there was a single band (of 524 bp) amplified from artificially and naturally infected tubers. In the tuber assay, *P. infestans* was detected in dark, sunken lesions as well as healthy tuber tissue. No amplification was observed from samples collected from survey samples instead of samples collected from Hathras, Sambhal and Hapur area were found to be infected with *P .infestans* inoculum (Fig.4).

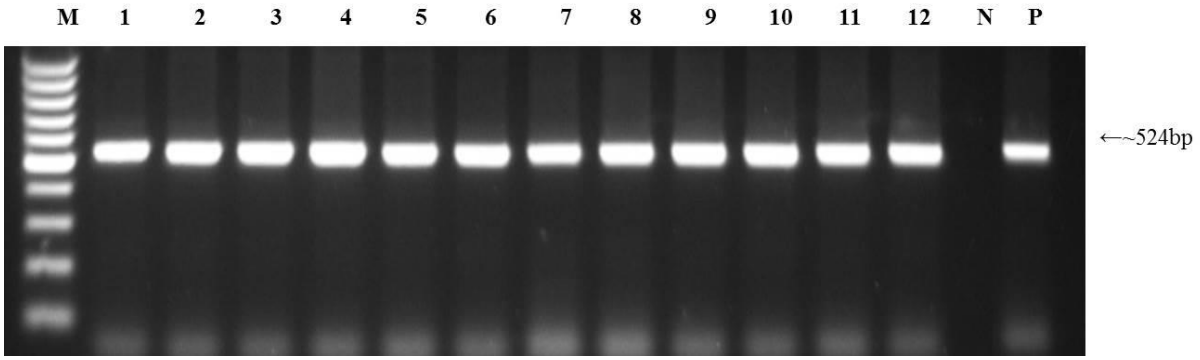

Figure 2. PCR amplification from genomic DNA extracted from crushed *P. infestans* mycelium. Lane 1 to 5 = Shimla isolates, 6 to 12= U.P. isolates, N= Negative control, P= Positive control, M= 100bp DNA ladder (Fermentas)

DNA-based identification

Identification of *P. infestans* with the molecular assay was verified by sequencing (data not shown). All isolates examined presented high homology (>95%) to previously described (Hussain *et al*., 2015, under press). DNA-based detection and identification methods described here can be used to confirm the morphological identification facilitate detection of genus *Phytophthora* and *P. infestans* and be applied during pathogen control activities as well as epidemiological studies of pathogen. The assays is practical, rapid and low-cost, and efficient for the identification and discrimination of genus Phytophthora and *P. infestans*.

Hussain *et al*., (2015) developed SCAR marker that could be used for the identification of *P. infestans* unique nucleotide sequence, proved earlier to be species-specific (Hussain *et al*.,2014c), as a monitoring tool (Fig. 4 and 5). The modified

PCR method detected the fungus in host tissues and was sensitive enough to reveal variations in the amount of DNA above threshold levels, according to band intensity in assay gels. Our molecular findings on disease progression in host plants are consistent with previous literature observations. This PCR method is considered to be the most sensitive diagnostic technique, and extremely low amounts of inoculum of the target pathogen in the sample analysed could have resulted in a positive amplification.

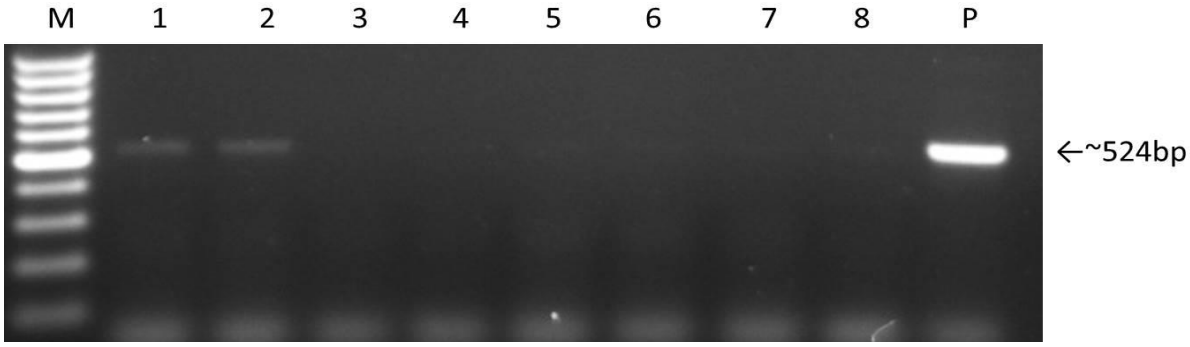

Figure 3. Cross-reactivity test with other *Phytophthora* spp. and other fungal pathogen of Potato. Lane 1= *P. infestans* A1 type, 2= *P. infestans*A2 type, 3= P. *palmivora*, 4= P. *capsici*, 5= P. *cactorum*, 6= *R. solani* AG-3, 7= *Fusarium* spp., 8= *A. solani*, P= positive control, M= 100bp DNA ladder (Fermentas)

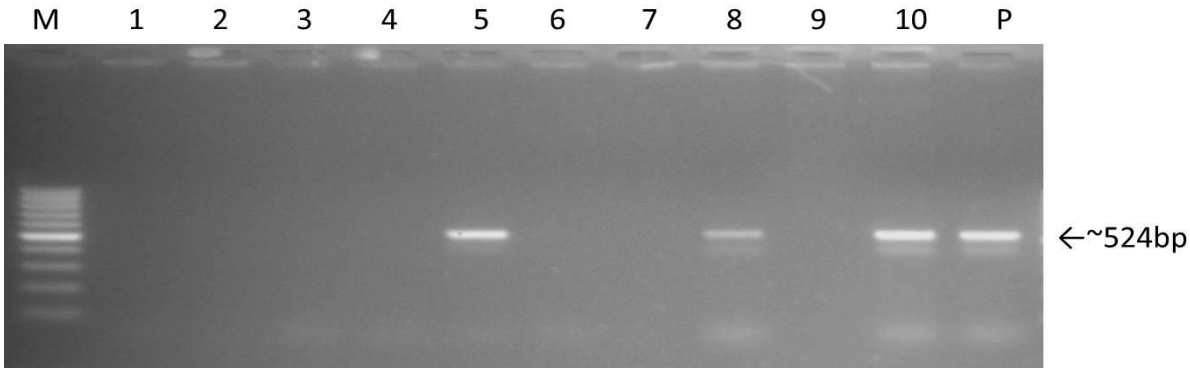

Figure 4. PCR amplification from suspected samples of potato seed tubers with SCAR Pinth 2-F/Pinth 2-R marker. Lane 1 to 5 suspected tuber samples

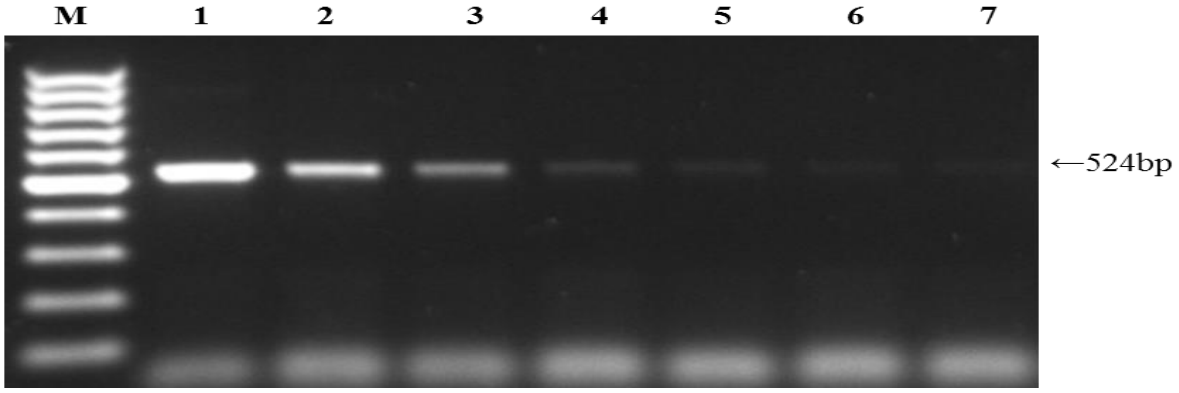

Figure 5. Determination of sensitivities of Pinth 2-f/Pinth2-r SCAR marker with different dilutions of genomic DNA of *P. infestans*. Lane 1=10 ng, 2=1 ng, 3=1000 pg, 4=100 pg, 5=50 pg, 6=10 pg, 7=1 pg, M=100 bp DNA ladder (Fermentas)

It is well known that about 90% of all the food crops grown are propagated by seed. Seeds are both vehicles and victims of disease. The significance of transmission of plant diseases through seeds was realized long ago. Seed health testing is important for research and development purpose. In previous studies (Samra *et al.*, 1963; Fathi, 1966) reported that the pathogen can cause diseases outbreak. Although infected seeds do not show discernible external symptoms and cannot be identified visually, *P. infestans* can be cultured

from infected seeds by plating them on a specific Rye agar medium (Caten and Jinks, 1968).

Conclusions

Our results demonstrate the potential use of a molecular assay, proved earlier to be species-specific (Zeller *et al.*, 2000; Saleh *et al.*, 2003; Hussain, *et al.,* 2005; Hussain *et al.,* 2013), to test healthy, deliberately infested and naturally infected potato tubers (Fig. 5). This method is an important initial step towards the goal of efficient healthy seed testing programmes as well as helps in quarantine management. Several additional validation steps are still required before the molecular assay presented here can be introduced as a tool to be used for regulatory purposes. Developing a molecular seed health assay is even more relevant given that *P. infestans* can survive for several months in plant seeds, while during cold storage (due to longer survival at low temperatures). The pathogen can also persist on potato tuber stubble for 12 to 15 months (Sabet *et al.*, 1970). The ability of the pathogen to become established and survive in stored potato tuber seeds (even from apparently healthy parental plants) stresses the urgent need to develop new ways to monitor seed health and control disease spread. There is, therefore, an urgent need for developing sensitive, reliable and quick tests for detection of seed-transmitted pathogen which may be present at a low percentage and also the hosts may be symptomless carriers. The tests will be quite helpful at quarantine centres as well as field trials. Appropriate control procedures can only be applied effectively if the pathogen is correctly identified and distribution in an area or crop is known.

Precise identification and diagnosis of plant pathogens during early stages of infection can help a lots in better management of the diseases. Molecular data, combined with classical characterization of fungi in the field, provide new aspects about fungal functions and interactions within terrestrial communities and results of analyses will be available in a few hours. Thus, farmers can get early information about the disease pathogen, which can contribute to good decision making about a strategy of disease management and it will help to operate an early warning system or to select growing seasons or areas for special crops where infection is unlikely. Furthermore, research frontiers will have more detailed and in depth studies of host-pathogen interactions, disease resistance, pathogen population structure. To summarize, results from this study demonstrate the usefulness of the PCR-based molecular detection of *P. infestans* in potato production and will helped in preventing or reducing the crop losses.

Acknowledgements

This study was made possible through the financial support and fellowship to the first author from the ICAR-New Delhi-Phytofura Project.

References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. 1990. Basic local alignment search tool. *J. Mol. Biol.* 215(3): 403-410.

Arab, H.R, Afshari, H., Daliri, M.S., Laei, G. and Toudar, S.R. 2012. The effect of planting date, depth and density on yield and yield components of potato in Shahrood (Iran). *Journal of Research in Agricultural Science*, **7**: 141-149.

Atkins, S.D. and Clark, I.M. 2004. Fungal molecular diagnostics: a mini review. *J. Appl. Genet*. 45: 3-15.

Chakraborty, S., Chakraborty, N. and Datta, A. 2010. Increased nutritive value of transgenic potato by expressing a non-allergenic seed albumin gene from *Amaranthus hypochondriacus*. Proceedings of the National Academy of Sciences USA, 97: 3724-3729.

Caten, C.E. and Jinks, J.L. 1968. Spontaneous variability of single isolates of *Phytophthora infestans*. I. Cultural Variation. *Canadian Journal of Botany,* 46: 329-348.

Fathi, S.M. 1966. Some Studies on Seed and Seedling Disease of Corn. M.Sc. Thesis, Agriculture, Ain Shams, Egypt.

Haverkort, A J. 1990. Ecology of potato cropping systems in relation to latitude and altitude. *Agricultural Systems*, 32: 251-272.

Haas, B.J, Kamoun, S., Zody, M.C., Jiang, R.H.Y., Handsaker, R.E., Cano, L.M., Grabherr, M., Kodira, C.D, Raffaele, S. and Torto-Alalibo, T. 2009. Genome sequence and analysis of the Irish potato famine pathogen *Phytophthora infestans*. *Nature*, 461: 393-398.

Henson, J.M., and French, R. 1993. The polymerase chain reaction and plant disease diagnostics. *Annu. Rev. Phytopathol.* 31: 81-109.

Hussain, S, Lees, A.K., Duncan, J.M. and Cooke, D.E.L. 2005. Development of a species-specific and sensitive detection assay for *Phytophthora infestans* and its application for monitoring of inoculum in tubers and soil. *Plant Pathol.* 54: 373-382.

Hussain, T., Sharma, S., Singh, B.P., Jeevalatha, A., Sagar, V., Sharma, N.N., Kaushik, S.K., Chakrabarti, S.K. and Anwar, F. 2013.

Detection of latent infection of *Phytophthora infestans* in potato seed tubers.*Potato Journal* 40(2): 142–148.

Hussain, T., Singh, B.P. and Firoz, A. 2014a. A quantitative real time PCR based method for the detection of *P. infestans* causing late blight of potato, in infested soil. Saudi J. Biol. Sci. 21: 380-386.

Hussian,T.,Singh,B.P.andTomar,S.2014b.Comparative study between different DNA extraction methods for detection of Late Blight of potato caused by *Phythophthora infestans*. Trends in Biosciences, 7(14):1707-1711.

Hussain T., Singh, B.P, Kaushik, S.K, Lal, M and Gupta, A.2014c. PCR protocol for quick and combined detection of Early and Late Blights of Potato. National Seminar on Emerging Problems of Potato, 1st and 2nd Nov. 2014, CPRI and IPA, Shimla, H.P.pg.169.

Hussain, T., Singh, B.P. and Anwar, F. 2015. Development of specific marker for PCR diagnostic of Late blight of potato caused by *Phytophthora infestans* using RAPD based SCAR methodology. *Journal of Saudi Society of Agricultural Science* Manuscript ID. JSSAS-D-13-00120R1 Accepted, under press.

Hussain, T. and Singh, B.P. 2016. Molecular diagnosis of Killer pathogen of Potato: *Phytophthora infestans* and its, management. *Current Trends in Plant Diagnostics and management practices*. Edited by Pradeep Kumar, V.K Gupta, A.K. Tiwari and Madhu Kamle. Springer International Publishing Switzerland, pp 2.

Hussain, T. 2016. Detection and quantification of *Phytophthora infestans* from host tissue and infested soil.Ph.D.Thesis. Dept. of Life Science,Uttarakhand Technical University, Dehradun, U.K.p1.

Hussain, T.2016a.Potatoes: Ensuring food for the future.*Advances in Plant and Agricultural Research,* 3(6):00117.

Hussain, T.2016b.Diagnostic of most important pathogen on potato through PCR techniques: A systematic Review.*Trends in Biosciences.9(4):203-212.*

Hijmans, R.J. 2003. The effect of climate change on global potato production. *American Journal of Potato Research*, 80: 271-280.

Hassanpanah, D., Hosienzadeh, A.A. and Allahyari, N. 2009. Evaluation of planting date effects on yield and yield components of *Savalan* and *Agria* cultivars in Ardabil region. *Journal of Food, Agriculture & Environment*, 7: 525-528.

Judelson, H.S. and Tooley, P.W. 2000. Enhanced PCR methods for detecting and quantifying *Phytophthora infestans* in plants. *Phytopathology,* 90: 1112-1119.

Lévesque, A.C. 2001. Molecular methods for detection of plant pathogens e what is the future? *Can. J. Plant Pathol*. 24: 333-336.

Li, P.H. 1985. Potato Physiology.*Academic Press Inc. (USA)*, 1-602.

Larkin, M.A, Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J. and Higgins, D.G. 2007. Clustal W and Clustal X version 2.0. Bioinformatics, 23: 2947-2948.

Michail, S.H., Abou-Elseoud, M.S. and Nour Eldin Mona, S. 1999. Seed health testing of corn for *Cephalo sporiummaydis*. *Acta Phytopathologicaet Entomologica Hungarica*. 34: 35-42.

McCartney, H.A., Foster, S.J., Fraaije, B.A. and Ward, E. 2003. Molecular diagnostics for fungal plant pathogens. *Pest Manage. Sci.* 59: 129-142.

Neergaard, P. 1977. Seed-borne viruses. Chapter 3. In: Seed Pathology, vol I. Macmillan, London/Madras, 839 pp.

Saleh, A.A., Zeller, K.A., Ismael, A.S.M., Fahmy, Z.M., El Assiuty, E.M. and Leslie, J.F. 2003. Amplified fragment length polymorphism diversity in *Cephalo sporiummaydis* from Egypt. *Phytopathology,* 93: 853-859.

Samra, A.S., Sabet, K.A. and Hingorani, M.K. 1963. Late wilt disease of maize caused by *Cephalosporium maydis. Phytopathology* 53: 402-406.

Scott, G.J., Rosegrant, M.W. and Ringler, C. 2000. Global projections for root and tuber crops to the year 2020. *Food Policy*, 25: 561-597.

Sabet, K.A., Zaher, A.M., Samra, A.S. and Mansour, I.M. 1970. Pathogenic behaviour of *Cephalosporium maydis* and *C. acremonium*.*Annals of Applied Biology*, 66: 257-263.

Trout, C.L, Ristaino, J.B., Madritch, M. and Wangsomboondee, T. 1997. Rapid detection of *Phytophthora infestans* in late blight infected potato and tomato using PCR. *Plant Disease* 81: 1042-1048.

Zeller, K.A., Jurgenson, J.E., El-Assiuty, E.M. and Leslie, J.F. 2000. Isozyme and amplified fragment length polymorphisms from *Cephalo sporiummaydis* in Egypt. *Phytoparasitica* 28: 121-130.