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DETECTION OF PHYTOPLASMA FROM DISEASED POTATO SAMPLE

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Abstract

The stunted and deformed potato (*Solanum tuberosum*) samples were received from Potato Research Station, Sahiwal at Crop Diseases Research Program, National Agriculture Research Centre, Islamabad for diognosis of the disease. The symptoms of little leaf, stunted growth and deshaped tubers were observed in the sample which was assumed as infected by Phytoplasma. Polymerase Chain Reaction (PCR) technique was selected for identification of the pathogen. An amplified fragment of 550 base pair was obtained which confirmed the presence of phytoplasmal pathogen in infected tubers and plant.

Introduction

Phytoplasma, previously known mycoplasma like organism (MLO), are causative agent of many plant diseases including potato. Different symptoms like yellowing, phyllody, witches broom and dwarfing are due to MLO in phloem tissue (Doi *et al*., 1967). Among different phytoplasmal diseases of potato, three possible groups i.e., Aster Yellow, Witches broom and Stolbur are more common (Salazar, 1996). These diseases can significantly reduce the yield like purple top roll, marginal flavescence and witches broom causing yield reduction ranging from 50–75%, 50–95% and 15–65 % respectively in India (Nagaich *et al*., 1982). In Pakistan, Mycoplasma-like symptoms in potato were first time observed in 1985 by Turkenstein in Swat, Gilgit and Mansehra. Later it was observed in early 90s (Khalid and Mughal–unpublished) in the district of Sahiwal. Similar symptoms were also recorded by Shafiq *et al*., (1995) in autum crop in the Punjab. They were of two types a) infected plants have long and thin stem, swollen nodes, lush green foliage with slight effect of late blight and having numerous tubers with deep eyes b) Plants showing stunted growth with purple discoloration of leaves with numerous small size tubers. The incidence of first type (a) was higher in Sahiwal (0-80%), while that of second type (b) was higher in Okara (0-95%). Recently in May, 2000 potato plants showing identical symptoms were also noticed in Mansehra area (unpublished). Although no systematic studies have been carried out on phytoplasma diseases of potato in Pakistan but type of symptoms observed in potato suggests that witches broom and purple top are present in Pakistan (Fauqia & Ahmad, 2001). The current study was undertaken when potato samples with similar type of symptoms like swollen nodes, stunted growth with little leaves and deshaped tubers were received from Potato Research Station, Sahiwal, Punjab.

Materials and Methods

Phytoplasmal DNA extraction: An infected potato tuber sample of about 0.1 gram was grounded in liquid nitrogen and hot buffer of 0.5 ml Cetyl trimethyl ammonium bromide (CTAB) was added. The sample was incubated at 65°C for 30 minutes and equal volume of chloroform and isoamyl alcohol (24:1) was added. The suspension was centrifuged at 4000 rpm for 10 minutes. The upper layer was removed and two third volume of cold isopropanol and 1/10 volume of 3M sodium acetate was added to the solution and left over night at -20°C. Then mixture was spun at 10,000 rpm for 30 minutes and supernatant was poured off. The pellet was dried in vacuum desiccators and resuspended in 0.5ul TE buffer and transferred to eppendorf tube (Daire *et al*., 1997).

DNA amplification: Phytoplasmal DNA was amplified by the Polymerase Chain Reaction (PCR) using the universal primer pairs derived from highly conserved regions of the 16SrRNA gene of Oenothera (O) MLO (Lim and Sears, 1989).

Sequence of the primers

Forward primer
5'-ACGAAAGCGTGGGGAGCAAA-3'

Reverse primer
5'-GAAGTCGAGTTGCAGACTTC-3'

Polymerase chain reaction (PCR): Extracted DNA of potato tuber with typical symptoms of phytoplasma was taken. The 50ul of reaction mixture contained 3ul of extracted DNA, 1ul of each primer, 8ul of dNTPs, 0.5ul of Taq polymerse, 5ul of 10x PCR buffer, 3ul of 25Mm $MgCl_2$ and 29.5ul of (double distilled deionized) H_2O were prepared. The Gene Amp PCR system 2400 (Perkin Elmer) was used. During first cycle denaturation was carried out at 94°C for 5 minutes, Annealing at 50°C for 1 minute and Extension was conducted at 72°C for 3 minutes. After that 35 cycles were conducted at 94°C denaturation temperature for 1 minute, 52°C Annealing for 1 minute and 72°C Extension for 1 minute. Post PCR Extension was carried out at 72°C for 10 minutes and finally material was stored at 4°C.

Gel electrophoresis: Electrophoresis of the amplified PCR product was carried on a 0.8% horizontal agarose gel in Tris borate EDTA (TBE) buffer at 510 volt and current of 50 mA for one and a half hours. The gel was visualized under UV Tran illuminator after staining with ethidium bromide (EtBr).

Results and Discussion

The potato sample showing little leaf, stunting and tuber deformation symptoms (Fig. 1) which were different to those of potato purple top symptoms previously described (Nasir *et al*., 2007). The similar type of symptoms like long and thin stem, swollen nodes having numerous tubers with the incidence of (0-80%) had also been observed in Sahiwal (Shafiq *et al*., 1985). That observation was only on the basis of symptoms but in current study the disease has been identified by molecular method like PCR and product of 550 base pair was obtained (Fig. 2). The same sample of potato was further processed at Rothamsted Resaearch Station to find out the group and subgroup of the disease by RFLP, Sequencing. The accession number FJ178388 was given (Fahmeed *et al*., 2008) and their results further confirmed our findings of the presence of phytoplasma in potato sample.

Fig. 1. De shaped potato tubers infected with Phytoplasma.

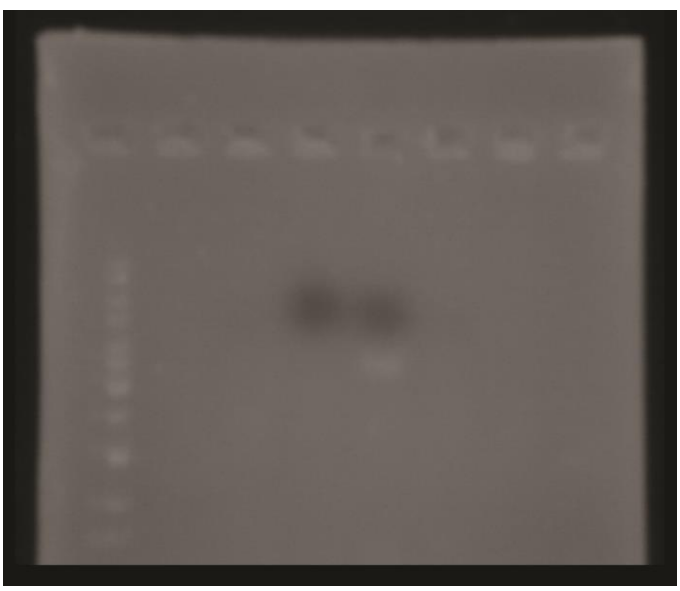

Fig. 2. PCR Ampliefied 16S rRNA with primer.

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