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Short Communication

THE POSSIBILITY OF PHYTOPLASMA DISEASE TRANSMISSION THROUGH BITTER GOURD (*Mormodica charantia)* SEEDS

T.M.N.D.TENNAKOON[1], P.M.C.P.NAGASENA and K.D.NANDASENA[1]

[1] *Plant Virus Indexing Center, Gabadawatte, Homagama. Sri Lanka.*

[2] *Department of Botany, Faculty of Applied Science, University of Sri Jayawardenapura, Sri Lanka*

INTRODUCTION

Phytoplasma are associated with diseases in several hundreds of plant species. Infected plants show symptoms such as virescence, phyllody, yellowing, witches' broom and generalized decline. Transmission of phytoplasma from plant to plant occurs preliminary during the feeding activity of vector insects, through vegetative propagation of infected plant materials and graft inoculation (Kirkpatrick, 1992). Apart from these, a few recent studies have highlighted the possible seed transmission of phytoplasma in certain crops species. Khan *et al.*, (2002) showed that phytoplasma witch's broom disease in both seed and seedling progeny of alafalfa plants. The presence of phytoplasma DNA in coconut embryos raises the possibility of seed transmission of Lethal Yellowing disease in Atlantic tall coconut palm species (Cordova *et al.*, 2003).

During last few years phytoplasma in bitter gourd has been spread rapidly and it affected the yield in a significant way. Identifying the reason for this rapid distribution is essential for management of the disease. Therefore, this research was carried out to find out whether there is any possibility to transfer the phytoplasma DNA through bitter gourd seeds.

MATERIALS AMD METHODS

Twenty three bitter gourd vines with phytoplasma symptoms were selected from farmer fields in the Central province of Sri Lanka and seeds

were collected. Mother plants were tested for phytoplasma by using direct and nested PCR techniques (Table 01) and further confirmed by DNA sequencing. Then, 248 seeds were collected from infected vines and 183 seeds were allowed to germinate under sterile conditions. Sixty five seeds were in abnormal shape so they were not allowed to germinate. Total genomic DNA was extracted by using phenol extraction method (Zambo, 2011) from the seedlings which was 5- 10 days old and 13 PCR amplicons which represent each mother plant were sent for DNA sequencing for further confirmation. Although there were 17 mother plants, seedlings did not obtained from 4 mother plants.

Table 1. Primers used for the detection of phytoplasma infection seed and plant samples for PCR.

	primer	Sequence(5'- 3')	Amplified region	Expected amplicon size	References
Direct PCR	P1	ACGAAAGCGTGGGG AGCAAA	16SrDNA	557bp	Ahrens *et al* (1992)
	P2	GAAGTCGAGTTGCA GACTTC	16SrDNA		
Nested 01	P1	AAGAGTTTGATCCT GGCTCAGGATT	16SrDNA	1784 bp	Jomantiene*et al* (1998)
	P7	CGTCCTTCATCGGCT CTT	16SrDNA		
Nested 02	R16F2n	GAAACGACTGCTAA GACTGG	16SrDNA	1229 bp	Jomantiene *et al* (1998)
	R16R2	TGACGGGCGGTGTG TACAAACCCCG	16SrDNA		

RESULTS AND DISCUSSION

Mother plants were selected with symptoms of little leaf disease including dwarf, thickened and puckered leaves and shortened internodes. These are the typical symptoms of phytoplama in bitter gourd vines. For this research, 23 vines which showed the symptoms at the latter stage were selected and fruits were harvested. Seventeen mother plants out of 23 gave positive results for direct PCR and nested PCR. All the primers used for the study were universal primers and amplified the 16SrDNA region of the organism. All the positive samples were gene sequenced and sequences were submitted to similarity search (BLAST) in the NCBI (National Center for

Biotechnology Information). Based on the E value, local similarities were identified with the different phytoplasma strains in NCBI data base with highest identity value.

Two hundred and forty eight mature seeds were collected from the infected vines and out of that 183 seeds were allowed to germinate. Because other seeds were in abnormal shape. One hundred and twenty seeds germinated and they were subjected to PCR while 83 seedlings gave positive results for phytoplasma with for P1/P7 PCR reaction. They were used as templates in a nested PCR, using primers R16F2n and R16R2. Visible bands matching, the size of the 16Sr DNA gene segment was observed from the infected seeds.

The observed bands resulting from the nested PCR were of phytoplasma origin and they were DNA sequenced. Thirteen seedling samples which represented each mother plant were sequenced and BLAST results from the sequences of products from seedlings showed alignment with the different phytoplasma strains. (16SrDNA gene of Candidatus phytoplasma aurantifolia isolate KU 052822.1/ Candidatus phytoplasma isolate KU 052830.1 etc). Therefore, designing a specific primer for phytoplasma in bitter gourd based on the results of this study can be useful to identify the pathogen by molecular basis.

CONCLUSION

With the overall results obtained from this study, it is possible to conclude that phytoplasma belonging to the ribosomal groups in bitter gourd can be transmitted through seeds from infected mother plants. But, further studies should be carried out to identify relationship between phytoplasma infection in seeds and their germination percentage.

REFERENCES

Ahrens, U. and E. Seemuller. 1992. Detection of plant pathogenic mycoplasma like organisms by a polymerase chain reactionthat amplifies a sequenceof the 16SrRNA gene.Phytopathology. 82(8):828-832

Cordova, I., P. Jones, N.A. Harrison and C. Oropeza. 1999. In situ PCR detection of phytoplasma DNA in embryos from coconut palms with lethal yellowing disease. Molecular Plant Pathology. pp. 99-108.

Jomantiene, R., R.E. Davis, J.L. Mass and E.L. Dally. 1998. Classification of new phytoplasma associated with diseases of strawberry in Florida, based on analysis of 16RrRNA and ribosomal protein gene operon sequences. International Journal of System Bacterial. 48: 269-277.

Khan, A.J., S. Botti, S. Paltrinieri, A.M. Al–Subhi, and A.F. Bertaccini. 2002. Phytoplasmas in alfalfa seedlings: infected or contaminated seeds. International Organization of Mycoplasmology Conference. pp.148 .

Kirkpatric, B.C 1992. The Prokaryotes, Mycoplasma like organism plant and invertebrate pathogens, A. Balows, H.G. Truper, M. Dworkin, W. Harder and K.H. Schleifer. Springer- Verlag, New York. pp 4050-4067.

Zumbo, P. 2011. Phenol- chloroform Extraction of Nucleic Acid. http//physiology.med.cornell.edu/faculty/mason/lab/zumbo/files/rna_alcohol_precipitation.pdf .(Accessed on 04. 08.2015)