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Molecular Relationship of Niger Phytoplasma with Pigeonpea and Parthenium Phyllody Phytoplasmas

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ABSTRACT

Phytoplasmas are prokaryotes that lack a cell wall and are the causal agents of numerous plant diseases. Phytoplasmas inhabit sieve elements in the phloem of plants and are transmitted between plants by phloem-feeding insects. Phytoplasmas are unculturable, phytopathogenic bacteria that cause economic losses worldwide. It is belong to the class of Mollicutes. *As unculturable micro-organisms, phytoplasma taxonomy has been based on the use of the 16S rRNA-encoding gene to establish 16SrRNA groups. Pytoplasmas can affect a wide range of plant hosts, including agriculturally and economically important plants, such as agriculture crops, fruit tree, landscape plant and flowers. Since pigeonpea and parthenium generally found in the niger crop ecosystem, also found in naturally infected with phyllody disease. Studies were conducted to know their relationship with the niger phyllody disease. Nested PCR assay of first round PCR product using universal primers R16F2n/R16R2, a PCR product of 1.2 kb specific to the phytoplasmal 16S rDNA regions of pigeonpea and parthenium were obtained, sequenced and compared with niger phyllody 16S rDNA sequence. Upon comparison of phyllody 16S rDNA sequences of niger, parthenium and pigeonpea, it was revealed that, the nucleotide identities among the three phytoplasma ranged from 85-95 per cent. Nucleotide identity of niger with parthenium was 95 per cent and sequence similarity of 85 per cent with pigeonpea. Phylogenetic analysis revealed that niger and parthenium were closely related and clustered together whereas, pigeonpea form a different cluster.*

Key word: *Phytoplasma, Pigeonpea, Niger, Parthenium and Molecular characterization*

INTRODUCTION

Plant-pathogenic phytoplasmas, first described as mycoplasma-like organisms, discovered by a group of Japanese scientists in 1967[3]. Taxonomically, they belong to the class *Mollicutes*, and have been recently classified within the provisional genus "*Candidatus* phytoplasma" based on 16S rDNA sequence analysis[6]. Taxonomy has been based on the use of the 16S rRNA-encoding gene to establish 16Sr groups.

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Phytoplasmas are prokaryotes that lack a cell wall and are the causal agents of numerous plant diseases[9,7]. Phytoplasmas are small enough to pass through bacteriological filters and, like mycoplasmas, are resistant to antibiotics that interfere with cell-wall formation. Phytoplasmas are unculturable, phytopathogenic bacteria that can affect a wide range of plant hosts, including agriculturally and economically important plants, such as fruit tree, landscape plant and flowers cause economic losses worldwide. They restricted to the sieve elements of host plants and are transmitted to other plants *via* sieve-tube sap feeding leafhoppers (*Cicadellidae*), planthoppers (*Cixiidae*) or psyllids (*Psyllidae*) in a persistent manner[4]. Infected plants exhibit symptoms of stunting, shoot proliferation, witches‟ broom of developing tissues (clustering of branches), phyllody (retrograde metamorphosis of the floral organs to leaf like structures), virescence (green coloration of non-green flower parts), formation of bunchy fibrous secondary roots, reddening of leaves and stems, generalized yellowing, decline, phloem necrosis and fasciation that may be due to the imbalance of plant growth regulators[8,1,5,10,4]. The present study was carried out to investigate the molecular relationship of niger phytoplasma with pigeonpea and parthenium phyllody phytoplasmas.

MATERIAL AND METHODS

Source of phytoplasma: Samples from naturally infected niger, pigeonpea and parthenium plants displaying phyllody disease symptoms were collected from Zonal agricultural research station, University of agricultural sciences, GKVK, Bengaluru, Karnataka during 2016.

Extraction of phytoplasma DNA: Niger, pigeonpea and parthenium plants exhibiting characteristic symptoms of phyllody were collected from a field. Nucleic acids were extracted from the midribs of fresh, symptomatic leaves and healthy leaf tissue by as previously described modified Cetyl Trimethyl Ammonium Bromide (CTAB)[15] method and used for PCR amplification by using degenerated oligonucleotide universal primers[2]. The DNA concentrations were measured with Nanodrop Spectrophotometer.

The total isolated DNA used as a template in first round PCR for amplification with P1/P7 primers[2,14] followed by nested PCR was done by using 2µl of diluted standard PCR product with phytoplasma specific primers R16F2n/R16R2. The first round PCR and nested PCR were carried out sequentially in a final volume of 25µl reactions containing 2.5 µl of 10X PCR buffer, 2.0 µl (25 mM) MgCl2, 0.5 µl (10 mM each) dNTPs, 1.0 µl (10 µM) each primers, 0.2 µl Taq DNA polymerase (5 u/ µl), and 2 µl template DNA (50 ng/ µl). The DNA was amplified by initial denaturation at 95 ºC for 5 minutes followed by 35 cycles of denaturation at 94 ºC for 1 minute, primer annealing at 55 ºC for 1 minute, primer extension at 72 ºC for 2 minute and finally at 72 ºC for 10 min for final primer extension. After completion of the reaction, the products were kept at 4 ºC prior to electrophoresis. The extracted nucleic acids were quantified by agarose gel electrophoresis. The PCR products were analysed by electrophoresis in 1% (w/v) agarose gel and visualized with a UV transilluminator following ethidium bromide staining.

Sequencing of phytoplasma 16S rDNA and Comparative sequence analysis: 16S rDNA from niger, pigeonpea and parthenium plants samples were collected from ZARS, UAS, GKVK, Bengaluru were amplified by PCR using 16S rDNA specific primers R16F2n/R16R2 and obtained 1250 bp product in all samples. The products were sent to Chromous Biotech Pvt. Ltd., Bengaluru for the sequencing by Sanger's primer walking method. Sequencing was done in both directions using forward and reverse primers. The sequences retrieved were subjected to BLAST analysis.

Construction of Phylogenetic tree: The sequence homology obtained in BLAST (www.ncbi.nih.gov /BLAST) and Neighbor joining phylogenetic tree was generated using MEGA 6.06 software tool.

RESULTS AND DISCUSSION

In the present study, efforts were made to establish the molecular relationship of niger phytoplasma with pigeonpea and parthenium phyllody phytoplasmas. Since pigeonpea and parthenium generally found in the niger crop ecosystem, also found in naturally infected with phyllody disease. Studies were conducted to know their relationship with the niger phyllody disease. Nested PCR assay of first round PCR product using universal primers R16F2n/R16R2, a PCR product of 1.2 kb specific to the phytoplasmal 16S rDNA regions of pigeonpea and parthenium were obtained, sequenced and compared with niger phyllody 16S rDNA sequence (Plate 1).

Molecular diversity of phytoplasma infecting niger, pigeonpea and parthenium revealed that the nucleotide identities among the three phytoplasma ranged from 85-95 per cent. Nucleotide identity of niger with parthenium was 95 per cent and sequence similarity of 85 per cent with pigeonpea. Phylogenetic analysis revealed that niger and parthenium were closely related and clustered together whereas pigeonpea form a different cluster together (Table 1 and Fig. 1).

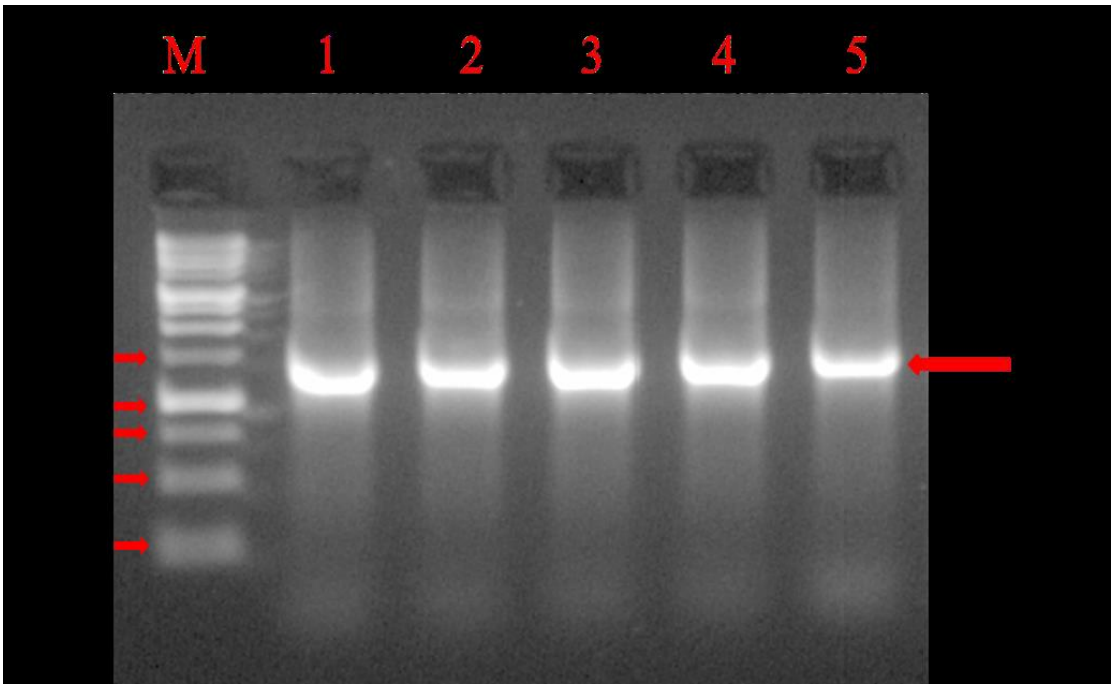

Plate 1: Nested- PCR amplification of 16S rDNA of niger, pigeonpea and parthenium phyllody phytoplasma

Lanes:

Lane M: 1.0 kb Ladder
Lane 1: Niger phyllody phytoplasmal DNA
Lane 2: Pigeonpea phyllody phytoplasmal DNA
Lane 3: Parthenium phyllody phytoplasmal DNA
Lane 4: Positive sample (Aster phyllody)
Lane 5: Positive sample (Periwinkle phyllody)

Table 1: Phylogenetic analysis of niger 16S rDNA with different phytoplasmal strains

Parthenium	Identity		
Niger	95 %	Identity	
Pigeonpea	90 %	85 %	Identity
Crops	**Parthenium**	**Niger**	**Pigeonpea**

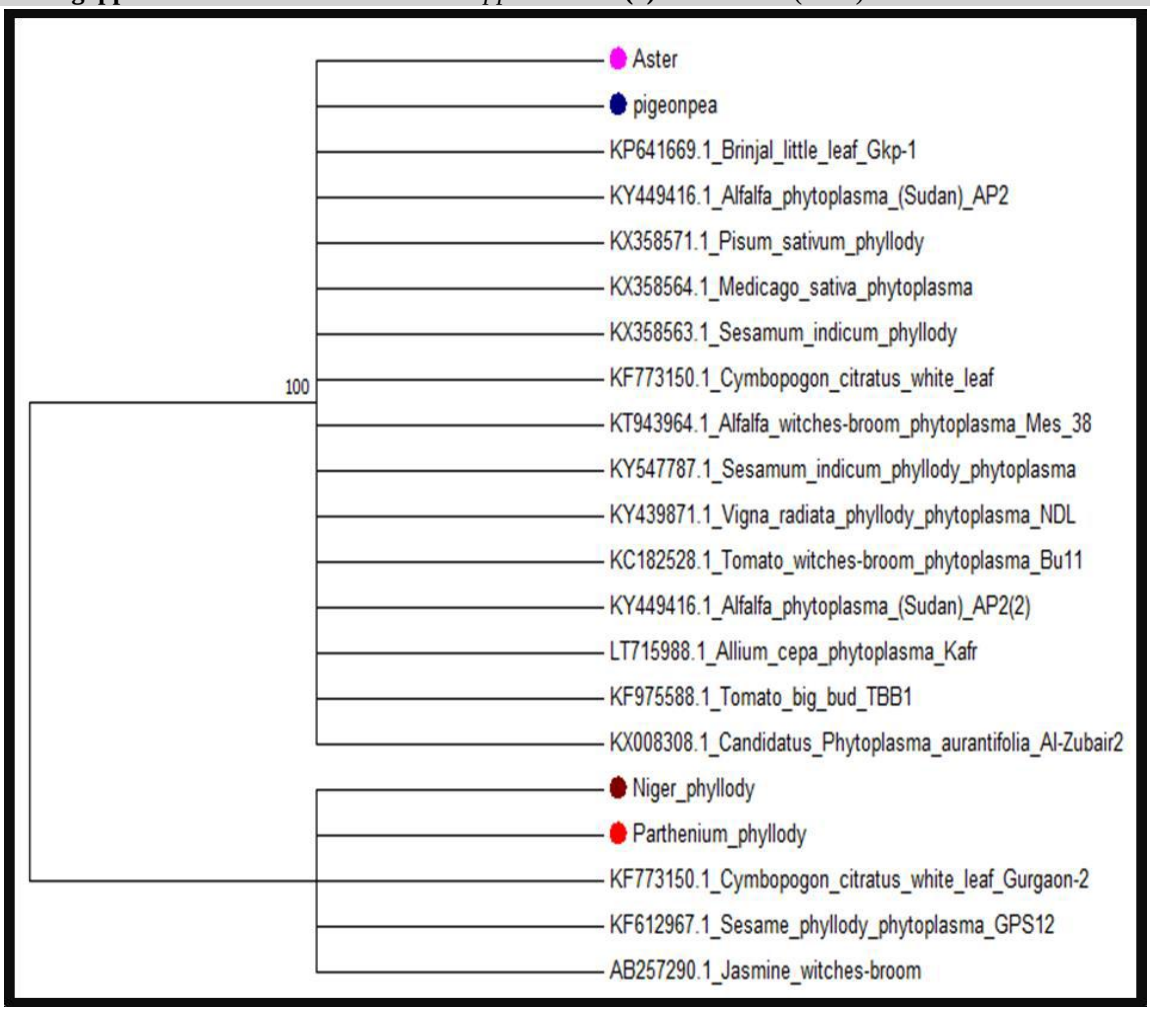

Fig. 1: Phylogenetic tree constructed by maximum parsimony method using 16S rDNA sequences of niger, pigeonpea and parthenium phyllody phytoplasma and other phytoplasmal strains

Since pigeonpea as a main crop and parthenium as an important ubiquitous weed are generally found in the niger crop ecosystem. The phyllody symptoms were observed and confirmed on both pigeonpea and parthenium under natural condition and they can be a potential collateral hosts. Both pigeonpea and parthenium were found in niger crop fields naturally infected with phyllody disease. Hence, the molecular characterization was done to know the molecular relationship between the phytoplasmas infecting pigeonpea, parthenium and niger. Nested PCR assay of first round PCR product using universal primers R16F2n/R16R2, a PCR product of 1.2 kb specific to the phytoplasmal 16S rDNA regions of pigeonpea and parthenium were obtained, sequenced and compared with niger phyllody 16S rDNA sequence. These results are in agreement with the earlier work of Raj *et al*.[12], Molecular relationship of niger, pigeonpea and parthenium phyllody phytoplasma revealed that, the niger phyllody closely related to parthenium phyllody by showing a sequence similarity of 95 per cent, whereas niger and pigeonpea phyllody showing a sequence similarity of 85 per cent. Phylogenetic analysis, showed that niger and parthenium clearly separated from pigeonpea to form a distinct cluster of its own. Furthermore, the phylogenetic tree constructed also showed that niger and parthenium phyllody phytoplasma clustered with *Cymbopogon citratus* white leaf, sesame phyllody phytoplasma GPS12 and Jasmine witches-broom phytoplasma whereas pigeonpea phyllody phytoplasma clustered with Brinjal little leaf Gkp-1, Alfalfa phytoplasma(Sudan) AP2, *Pisum sativum* phyllody, *Medicago sativa* phytoplasma,

Sesamum indicum phyllody, Alfalfa witches'-broom Phytoplasma Mes 38, Tomato big bud TBB1, *Candidatus* Phytoplasma aurantifolia Al-Zubair2 and Tomato big bud TBB1 phytoplasma. These results are in agreement with earlier work of Schneider *et al.* 1995 who reported 16S rDNA sequence similarity of Brinjal little leaf and ash yellow is 97.2 per cent and that between Brinjal little leaf and elm yellows subgroup of the elm yellows strain was 96.5 to 97.4 per cent.

REFERENCES

1. Bertaccini, A., Phytoplasmas: diversity, taxonomy and epidemiology. *Front Biosci*., **12:** 673-689 (2007).
2. Deng, S. and Hiruki, D., Amplification of 16Sr DNA genes from culturable and non-culturable mollicutes. *J. Microbial. Methods*, **14:** 53-61 (1991).
3. Doi, Y., Teranaka, M., Yora, K. and Asuyama, H., Mycoplasma group like group like microrganisms found in the phloem elements of plants infected with mulberry dwarf, potato witches‟ broom, aster yellows or Pawlownia Witches‟ Broom. *Japanese. J. Phytopathol*., **33:** 259-266 (1967).
4. Hogenhout, S. A., Oshima, K., Ammar, D., Kakizawa, S., Kingdom, H. N. and Namba, S., Phytoplasmas: bacteria that manipulate plants and insects. *Mol. Plant. Pathol.,* **9:** 403-423 (2008).
5. Hoshi, A., Oshima, K., Kakizawa, S., Ishii, Y., Ozeki, J., Hashimoto, M., Komatsu, K., Kagiwada, S., Yamaji, Y. and Namba, S., A unique virulence factor for proliferation and dwarfism in plants identified from a phytopathogenic bacterium. *Proc. Natl. Acad. Sci* ., **106:** 6416- 6421 (2009).
6. IRPCM, Phytoplasma/Spiroplasma Working Team-Phytoplasma Taxonomy Group., *Candidatus* Phytoplasma, a taxon for the wall-less, non-helical prokaryotes that colonize plant phloem and insects. *Int. J. Syst. Evol. Microbiol*., **54:** 1243-1255 (2004).
7. Kirkpatrick, B. C., Mycoplasma-like organisms: plant and invertebrate pathogens. *The Prokaryotes*, pp. 4050-4067 (1992).
8. Lee, I. M., Davis, R. E., and Gundersen, R. D. E., Phytoplasma: Phytopathogenic mollicutes. *Annu. Rev. Phytopathol*., **54:** 221-255 (2000).
9. Mccoy, R. E., Cauldwell, A. and Chang, C. J., Plant diseases associated with mycoplasma-like organisms. *The Mycoplasmas*, **5:** 545- 640 (1989).
10. Omar, A, F., Dewir, H.Y. and El-Mahrouk, M. E., Molecular identification of phytoplasmas in fasciated cacti and succulent species and associated hormonal perturbation. *J. P. Interactions.,* **9:** 632-639 (2014).
11. Omar, A. F., Detection and molecular characterization of phytoplasmas associated with vegetable and alfalfa crops in Qassim region. *J. Pl. Interations.,* **12(1):** 58-66 (2017).
12. Raj, S. K., Khan, M. S., Snehi, S. K., Srivastava, S. and Singh, H. B., *Candidatus* Phytoplasma *asteris* isolate associated with a little leaf disease of pigeon pea in India. *Pl. Pathol.,* **55:** 823 (2006).
13. Schneider, B., Aherns, U., Kirkpatrick, B.C. and Seemuller, E., Classification of plant-pathogenic mycoplasma-like organisms using restriction site analysis of PCR-amplified 16S rDNA. *J. Gen. Microbiol.,* **139:** 519-527 (1993).
14. Smart, C. D., Schneider, B., Blomquist, C. L., Guerra, L. J. And Harrison, N. A. Ahrens, U., Lorenz, K. H., Seemuller, E. and Kirkpatrick, B. C., Phytoplasma Specific PCR primers based on sequences of the 16S-23S rRNA spacer region. *Appl. Envi. Microbiol*., **62(8):** 2988-2993 (1996).
15. Sunard, M., Ben Khalifa, M., Marrakehi. and Fakhfakh., Detection of phytoplasma associated with periwinkle virescence. *Egyp. Pl. Pathol*., **7(1):** 92-97 (1991).