Molecular Characterization of Phytoplasma Associated With Amaranthus (Amaranthus Cruentus)

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Abstract- *Amaranthus (Amaranthus cruentus) is an annual or a short- lived perennial plant belongs to the Amaranthaceae family. Recently, symptoms of little leaf, severe stunting and phyllody were observed in amaranthus fields in the Hessaraghatta area of Bangalore with incidence of 5-7%.Leaf samples taken from Amaranthus plants showing witches' broom symptoms were tested by PCR followed by sequencing. Using phytoplasma specific primers P1/P7 DNA fragment of the expected size was amplified from symptomatic and not from asymptomatic plants. Phylogenetic analysis of the 16S rDNA sequence clustered the amaranthus phytoplasma detected in India within the peanut witches' broom (16SrII) group ('Candidatus Phytoplasma australasiae). The phytoplasma detected in the present study infects amaranthus comes under 16Sr II-D.*

Keywords- Amaranthus, Candidatus Phytoplasma australasiae, 16SrII-D.

I. INTRODUCTION

Amaranth originated in south-eastern Asia and Central Southern America but now days occurs worldwide. Approximately 60 species of amaranthus genus are cultivated for their leaves in Central America, south Asia and for grain production in central and South America. Amaranth is used as forage and green manure and in food and medicines. But most of the species are summer annual weeds. Most cultivated amaranth species are grow well in poor or saline soils and have a relatively high tolerance to pathogens and insect pests, resistant to drought conditions (Andrasofszky et al.1998) .Amaranth grains are rich source of protein, dietary minerals, manganese, magnesium, iron and selenium. Amaranth leaves are a good source of vitamin C, A, calcium, and folate.Amaranthus infected by many fungal disease up to now. Recently we observed phytoplasma disease from farmer's field around Hessaraghatta area, Bangalore. Ten samples collected from different locations to determine the presence of phytoplasma disease.

II. MATERIAL AND METHODS

Total DNA was extracted from midribs of infected as well as healthy amaranthus plants using modified CTAB method (Swarnalatha et al., 2013). For detection of phytoplasma, the isolated genomic DNA was used as template in PCR by using phytoplasma-specific 16S rDNA universal primers P1/P7 (Deng and Hiruki, 1991). Bands of expected size 1.7 were produced in all samples from symptom-bearing plants, but not for the symptomless ones. To review the taxonomic positions of amaranthus phytoplasma isolates, full length 16Sr RNA sequences derived were queried using iPhyClassifier online tool (Zhao et al., 2009). Searches for sequence similarity to the available sequences in the database were performed using BlastN (http://www.ncbi.nlm.nib.gov/). The amaranthus phytoplasma sequence identity matrixes were generated using Bioedit Sequence Alignment Editor (version 5.0.9) and simultaneously to estimate evolutionary distances between all pairs of sequences phylogenetic tree was generated by MEGA7 software (Kumar et al., 2016) using the neighbour joining method with 1000 bootstrapped replications.

III. RESULTS AND DISCUSSION

Total DNA from symptomatic amaranthus leaves and midribs collected from field as template DNA, fragments of expected size of 1.7 kb were obtained from PCR amplification with the primer pair P1/P7.No amplification was observed in healthy amaranthus plants (Fig. 1).Three PCR products amplified with P1/P7 samples were selected and purified (Gel Extraction kit, Merck KGaA. germany) and directly sequenced. BLAST analysis showed the highest sequence identity (99%) with the Papaya yellow crinkle phytoplasma strain Y10097 designated as 'Ca. Phytoplasma australasia' that belongs to subgroup 16SrII-D. To confirm the identity of this phytoplasma found in amaranth, we analyzed its 16S rRNA gene sequence phylogenetically. A phylogenetic tree was constructed using the minimum evolution approach of the MEGA7 program (Kumar et al., 2016).Phylogenetic analysis clearly demonstrated that this phytoplasma closely clustered with peanut witches broom group (16SrII).The resultant 16S rDNA sequences showed 99% with '*Candidatus* Phytoplasma australasiae' 16Sr II group.

Phylogenetic analysis supported BLAST comparisons the amaranthus phytoplasma clustered with 'Ca. Phytoplasma australasia' (Fig. 2). IPhyClassifier (Zhao et al., 2009) was used to perform sequence similarity and generate virtual restriction fragment length polymorphism (RFLP) profiles. RFLP profiles of amaranthus 16S rDNA sequence exhibited identical to those of the reference strain Y10097, 'Ca. P. australasia', subgroup 16SrII-D (Fig.3). In china the amaranth phytoplasma strain was related to Candidatus Phytoplasma ziziphi belonged to the 16SrV-B subgroup (Yang et al., 2011).Amaranthus phytoplasma in mexico belong to the Candidatus Phytoplasma aurantifolia. The phytoplasma detected in the present study infects amaranthus comes under 16Sr II-D in India.

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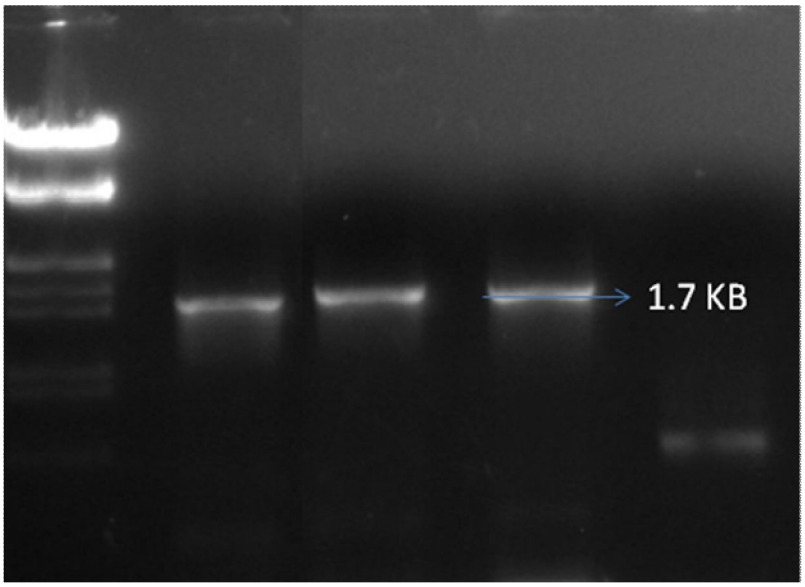

Fig.1.Gel picture showing PCR amplification of phytoplasma samples (1.7 kb)
M: Molecular weight marker
Lanes 1-3 Amplification of Phytoplasma
Lanes 4: Healthy sample

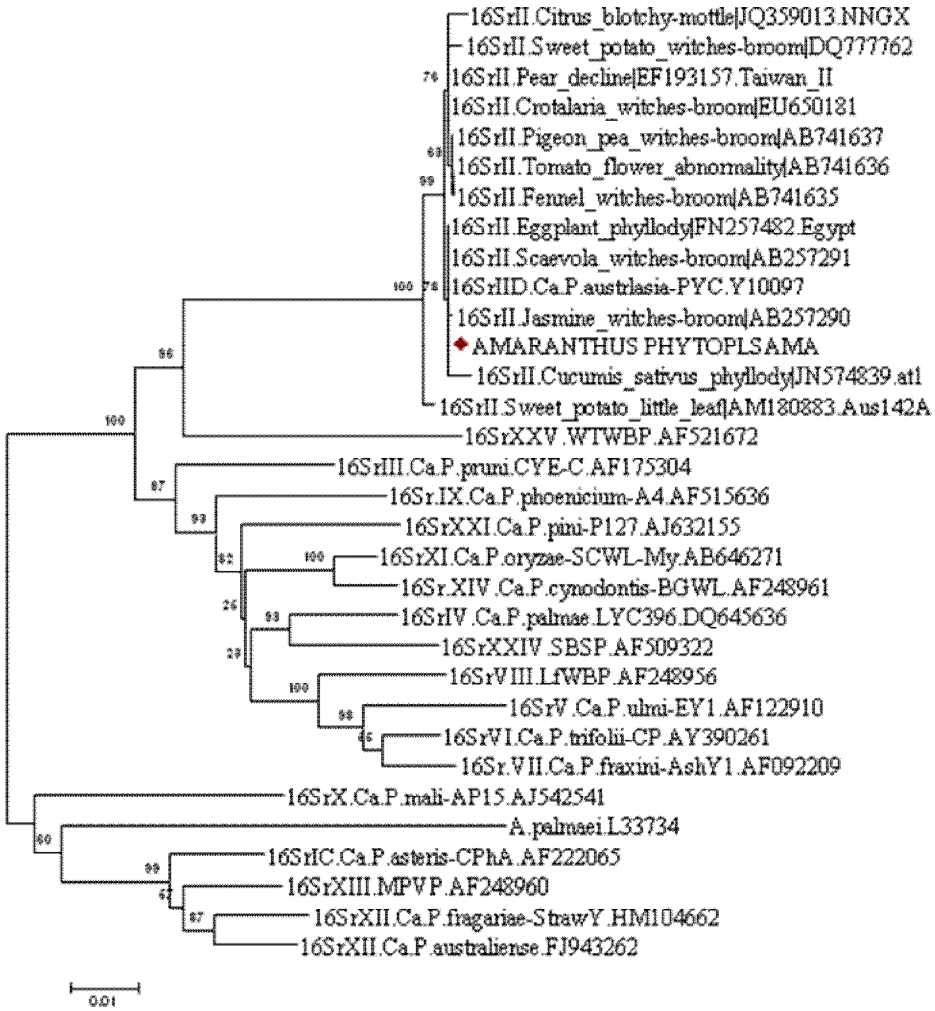

Fig. 2. Phylogenetic tree obtained by the Neighbor-Joining method of 16S -23S rRNA spacer region sequences from phytoplasmas belonging to different groups and a clone of the Amaranthus phytoplasma. Roman numerals and letters represent 16S rDNA RFLP groups and subgroups, respectively.

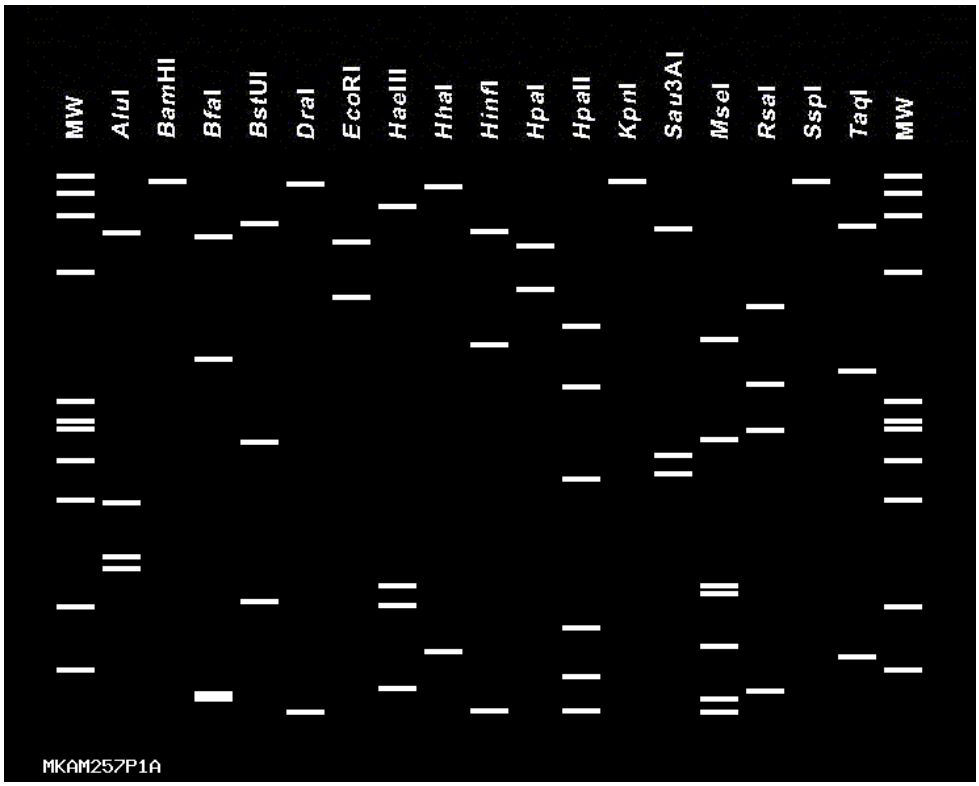

Figure 3**:** Virtual RFLP patterns derived from *in silico* digestions of 1.2 kb 16S rDNA fragments of amaranthus with 17 restriction endonucleases. RFLP fragments were resolved by *in silico electrophoresis* through 3% agarose gel. MW, ΦX174 DNA-*HaeIII* digestion.