PHYTOPLASMA DISEASES ASSOCIATED WITH *CLEOME VISCOSA* AND *BORRERIA HISPIDA* WEEDS IN ANDHRA PRADESH, INDIA

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Abstract: Phytoplasma was detected in *Cleome viscose* and *Borreria hispida* weeds by direct and nested polymerase chain reaction using universal primers P1/P7 and R16F2n/R16R2 specific to 16SrRNA gene of phytoplasma. Running of 1% agarose gel electrophoresis for confirmation of phytoplasma associated with these two weeds.

Keywords: Nested PCR, *Cleome viscosa*, *Borreria hispida,* Phytoplasma specific primers, 1% AGE

INTRODUCTION

During 2014 phytoplasma disease symptoms were observed on *Cleome viscosa* and *Borreria hispida* weeds at field of Regional Agricultural Research Station, Tirupati, Andhra Pradesh. These two weeds are more common throughout agriculture and bare lands in A.P. Phytoplasmas are wall less prokaryotes. They are bounded by a "unit" membrane, and have cytoplasm, ribosomes, and both DNA and RNA. In ultrathin sections, they appear as a complex of multibranched, beaded, filamentous or polymorphic bodies ranging from 175-400 nm in diameter for the spherical and oblong cells and up to 1700 nm long for the filamentous forms (Waters and Hunt, 1980). Phytoplasmas are generally present in phloem sieve tubes and in the salivary glands of insect vectors. While phytoplasmas are multiply in the phloem, little is known about its mechanism. Most phytoplasmas are transmitted from plant to plant by leafhoppers and plant hoppers (Purcell, 1982).

MATERIAL AND METHOD

Phytoplasma infected weed samples shows that little leaves, short internodes, stunted growth and virescence, collect infected leaf samples from *Cleome viscosa* and *Borreria hispida* weeds (Fig. 1). To investigate the possibility of a phytoplasma association with these two weeds, total DNA was isolated from 100 mg leaf midribs from infected and symptomless plant samples using the CTAB method (Doyle & Doyle, 1990). Running of nested PCR by using phytoplasma specific primers P1/P7 for first round of PCR (Deng & Hiruki, 1991) and R16F2n/R16R2 for nested PCR (Gunderson *et al*, 1996). Running of 1% agarose gel electrophoresis for confirmation of phytoplasma associated with these two weeds. Expected size of amplicons are 1.8 kb after first round PCR and 1.25 kb after second round PCR, the DNA amplified only from symptom bearing weed samples (Fig. 2), but not from the symptomless plant samples.

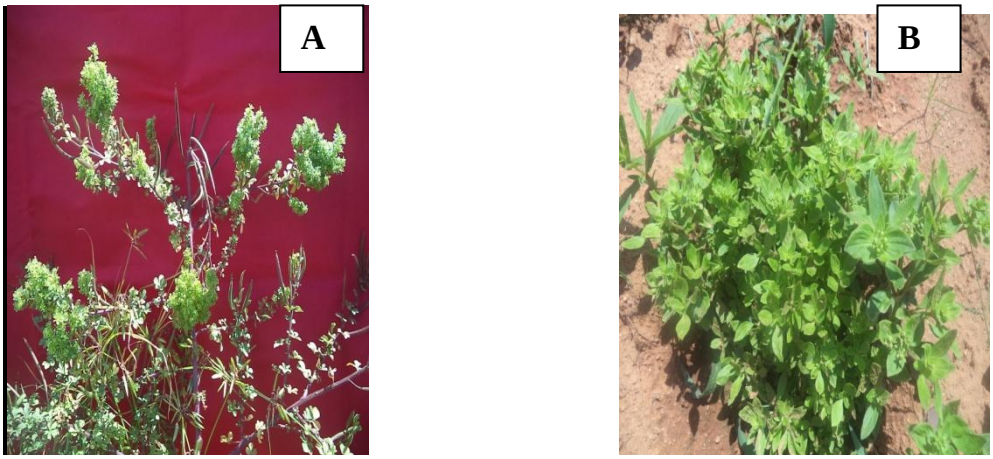

Fig. 1. A. Phytoplasma infected *Cleome viscosa* weedand **B**. *Borreria hispida*. Little leaves, virescence and stunted growth of weeds.

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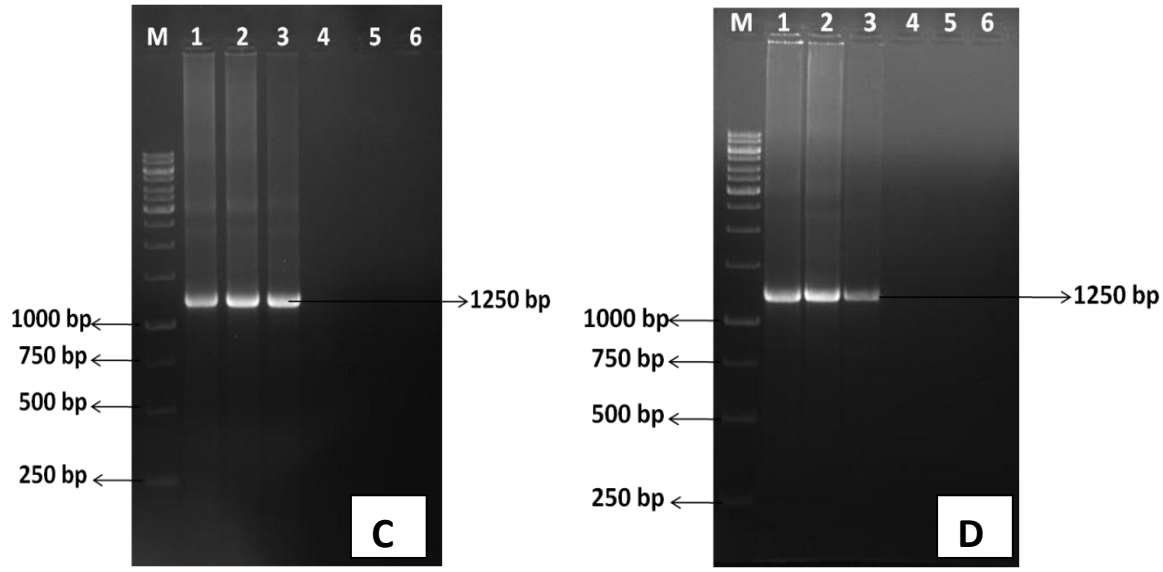

Fig 2. Amplification of phytoplasma 16S rDNA by Nested PCR using the phytoplasma specific primers R16F2n/R16R2 from infected plants. **C.** Lanes: M- gene 1kb ruler (0313) 1, 2 & 3- phytoplasma infected *Cleome viscosa* weed, 4, 5 & 6- healthy *Cleome viscosa.* **D.** Lanes: M- gene 1kb ruler (0313) 1, 2 & 3- phytoplasma infected *Borreria hispida* weed, 4, 5 & 6- healthy *Borreria hispida.*

CONCLUSION

In India so far various weeds are identified having phytoplasma infections. I concluded that the diversity of the potential reservoir of disease has been increased with the discovery of new phytoplasmas hosts. Hence, it would be importance to study the diverse nature of phytoplasmas.

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