SHORT COMMUNICATION

PHYTOPLASMA DISEASE ASSOCIATED WITH *CROTON BONPLANDIANUM* WEED IN ANDHRA PRADESH, INDIA

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Abstract: Phytoplasma was detected in *Croton bonplandianum* weed by direct and nested PCR 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 this weed.

Keywords: *Croton bonplandianum*, Nested PCR, 1% AGE, Phytoplasma specific primers

INTRODUCTION

During 2014 phytoplasma disease symptoms were observed on *Croton bonplandianum* weed at road side of S.V. Agricultural College, Tirupati, Andhra Pradesh. These weed more common throughout agriculture and bare lands in Rayalaseema area of A.P. Phytoplasmas are wall less prokaryotes. They are bounded by a "unit" membrane, and have cytoplasm, ribosomes and nucleic acid. 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, yellowing and virescence, collect infected leaf samples from *Croton bonplandianum* weed (Fig. 1). To investigate the possibility of a phytoplasma association with this weed, total DNA was isolated from infected and healthy weed plant using the CTAB method (Doyle & Doyle, 1990). *DNA isolation:*-Infected plant material (0.5g) was ground in a pre-sterilized pestle and mortar with liquid nitrogen until a fine powder was obtained and transferred to sterile eppendorf tube. To this added 1ml of pre-heated (65ºC) extraction buffer (1M Tris (pH 8.0), 5 M NaCl, 0.5M EDTA, 2% CTAB, 1% PVP, 0.1 % Mercaptoethanal) and incubated for 1 hour in water bath at 65ºC. Then tubes were centrifuged (Refrigerated Eppendorf centrifuge) at 10,000 rpm for 10 min at room temperature and the supernatant was collected into eppendorf tubes. To this added equal volumes of phenol-chloroform (1:1) mixed and centrifuged the tubes at 10,000 rpm for 10 min, transfer the supernatant to the fresh eppendorf tube and added equal volumes of chloroform and Isoamyl alcohol (24:1) mixed well and then centrifuged the tubes at 10,000 rpm for 10 min, collected the supernatant in to separate eppendorf tube and added 0.1 volume of 3M sodium acetate (pH 4.8) and 0.6 volume of ice cold isopropanol then incubated at -20ºC for overnight.

After incubation, the tubes were taken out and centrifuged at 13,000 rpm for 20 min at 4ºC. The supernatant was discarded and the pellet was washed with 70% alcohol and again centrifuged at 13,000 rpm at 4ºC for 10 min, discarded the supernatant, air dried the pellets and dissolved in 50µl of sterile distilled water. 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). The conditions for amplification of phytoplasma gene are; 1 cycle of 94°C for 4min , 35 cycles of 94°C for 30 s , 55°C for 1min(56°C for P_1/P_7 primers) ,72°C for 2 min and 1 cycle of 72°C for 10 min. Running of 1% agarose gel electrophoresis for confirmation of phytoplasma associated with this weed.

RESULT

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 sample (Fig. 2), but not from healthy weed samples.

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Fig. 1. phytoplasma infected weed (left) and healthy weed plant (right).

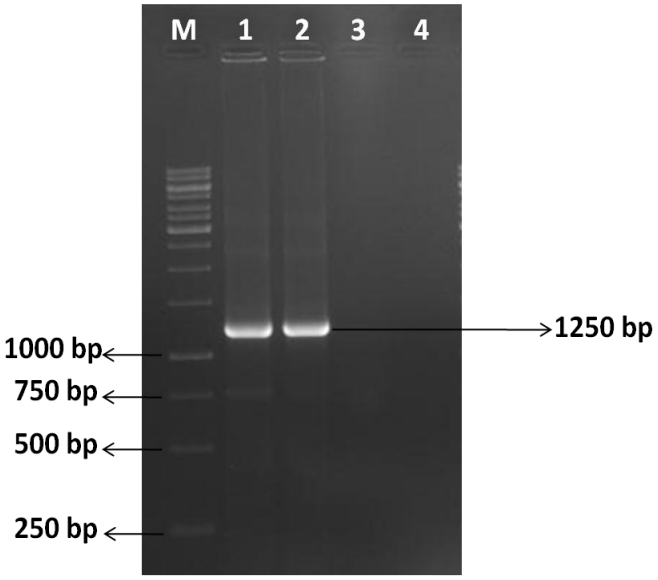

Fig. 2. M- Gene 1kb ruler (0313) 1, 2- phytoplasma infected *Croton bonplandianum* weed, 3, 4- healthy *Croton bonplandianum.*

CONCLUSION

Phytoplasma cause the diseases on various weeds and crops in Andhra Pradesh. *Croton bonplandianum* is a common weed in A.P; it may act as an alternate host to phytoplasma. I concluded that the diversity of the potential reservoir of phytoplasma 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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