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Ranunculus Phyllody Diseases in north-eastern Uttar Pradesh, India associated with Phytoplasma

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ABSTRACT: In present time, incidence of Phyllody Diseases in *Ranunculus* plant are dramatically increases in north-eastern part of Uttar Pradesh. Taking an account of this disease, here we were try to indentify that Phytoplasma which is associated with this disease by transmission electron microscopy (TEM) study of infected plant and through isolation of nucleic acid of causative pathogen and PCR amplification of their genetic material by using suitable primer. Present work is the first report of ranunculus phyllody from India. Insect transmission, molecular biology, sequencing and phylogenetic analysis studies are now under way.

Keywords: *Ranunculus scelratus*, Phytoplasma, Phyllody, 16S rDNA, North-eastern.

INTRODUCTION

Ranunculus (*Ranunculus scelratus*, L.) commonly known as Jaldhania is an important aquatic weed throughout found in all aquatic places In India, during winter season ranunculus is grown as aquatic weeds. Ranunculus production is affected by two diseases, shoot and rot caused by *Pseudomonas marginalis* pv. *marginalis* (Brown) Stevens and mottle caused by a potyvirus (Fujimori et al.,1996). Some varieties of ranunculus has been known to develop the symptom proliferation or phyllody in Gorakhpur, in Eastern U.P. The disease has not been consistently associated with nutritional and environmental factors, nor have fungal or bacterial pathogens have not been isolated from the affected plants. The authors, therefore, suspected a phytoplasma to be the causal agent. Characteristic symptoms of this disease become apparent at the flowering stage of plant development. For the detection and identification of phytoplasmas, polymerase chain reaction (PCR) techniques have been increasingly

employed. Ahrens and Seemuller (1992), Vibio, M., *et al*., 1995 reported that the 16S rRNA gene of phytoplasmas could be amplified by PCR using specific primers. Namba et al. (1993) described the detection of phytoplasmas and that phylogeny by PCR analysis. By this study, we report the results of electron microscopic observations and molecular detection of the pathogen causing proliferation or phyllody of *Ranunculus scelratus* first time in India.

MATERIALS AND METHODS

Figure-1 (A-C): Symptoms of ranunculus phyllody, PCR amplification of 16SrDNA sequences from the phytoplasma.

Figure-1A: Healthy plant of *Ranunculus sceleratus L.*

Figure-1B: Phyllody diseases in *Ranunculus sceleratus L.*

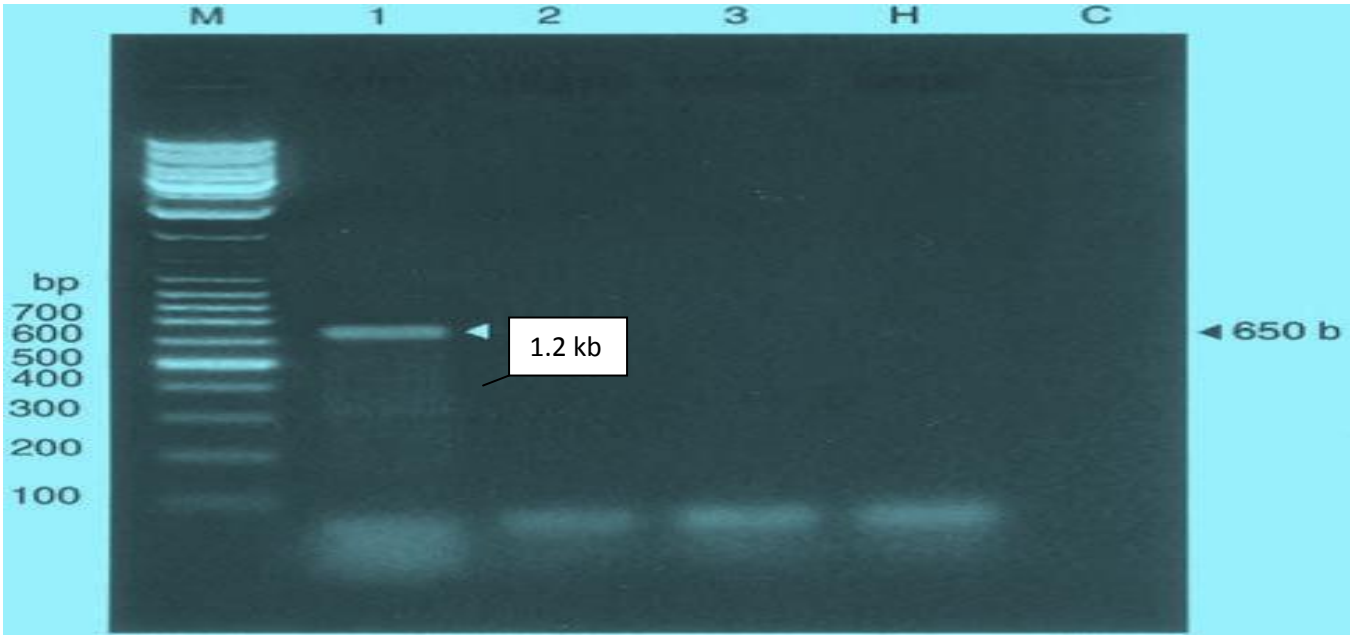

Figure-1C: PCR amplification of 16S rDNA sequences from ranunculus phyllody phytoplasma. Agarore gel electrophoresis of nestad PCR products obtainted for DNA samples isolated from *Ranunculus sceleratus* using primers :R16F2n/R16R2, M ,H ,C- molecular markers :(lane 1) nested PCR products using DNA from infected plants, (lane 2, 3) showing from healthy tissue.

Plant materials: Leaves, petioles and flowers from ranunculus plants bearing proliferation or phyllody symptoms and as well as from asymptomatic plants were collected from green houses in Gorakhpur in winter season 2010-2012.

Electron microscopy: For transmission electron microscopy (TEM), small segments were cut out from leaves, stalks and flowers of diseased and asymptomatic plants and immersed immediately in 2% glutaraldehyde in 0.1M phosphate buffer, pH 7.0, on ice. After 2-16hr, samples were rinsed in the buffer, postfixes 2hr in 1% buffered OsO4, dehydrated in a graded series of ethanol and embedded in epoxy resin. The resin blocks were polymerized at 80°C for 8hr.Ultrathin sections were cut with a diamond knife set on an ultra microtome, stained in 2% uranylacetate solution and then counterstained in lead citrate solution. The sections were examined with a TEM model 1200E transmission electron microscope at 80kV.

DNA isolation and PCR amplification: Total extracted DNA used in the PCR was isolated from leaves, stems and flowers of symptomatic and asymptomatic R. scelratus using the CTAB method. Nucleic acid concentration was determined both spectrophotometrically (Gene Quant, Pharmacia) and in 1% agarose gels in 0.5•~TBE (45mM Tris-borate, 1mM EDTA, pH 8.0) using maker DNA as a standard. After flowering, plant bulbs were also examined by PCR analysis. The universal primer pair used to screen all ranunculus samples was P1/P7 (Deng and Hiruki, 1991), which amplified approximately 1.2kbp of the 16S rRNA gene. A second set of primers was which R16F2n (Gundersen and Lee, 1996) specifically amplified 1.2kb of the 16S rRNA gene of phytoplasma). Amplified DNA fragments were electrophoresis in 1.2% agarose gels, stained with ethidium bromide and visualized by UV illumination.

RESULT AND DISCUSSION

Electron microscopy: Characteristic symptoms of ranunculus phyllody are shown in Figure 1. At flowering stage the stigmas of diseased plants developed into proliferation and petals became green and leaf-like. Sometimes all petals changed into leaf-like structures. More than 90% of the plants were symptomatic at each location. Many significant differences were observed between healthy and diseased plants during the vegetative growing stage. No bacterium or fungus was consistently isolated from either diseased or healthy plants. Examination of ultrathin sections revealed phytoplasmas in sieve elements from stems of diseased plants (Figure: 1-2). The phytoplasmas pleomorphic in shape about 1µm in diameter and were bound by unit membranes. In some preparations, only a few sieve elements were infected. Phytoplasmas were not observed in tissues from asymptomatic plants.

DNA isolation and PCR amplification: Using the universal primer and phytoplasma-specific primer sets, about 1.2 kb DNA fragments were amplified in diseased plants from each location (Figure: 1A-C), but not in the samples from asymptomatic plants. In some samples, phytoplasmas were detected even when diluted 100 fold with sterilized distilled water. Template DNA isolated from diseased fresh bulbs was also amplified with each 1.2 DNA fragments. Berticcini et al. (1988) first reported phyllody and virescence disease of ranunculus in Italy. Stunting or rosette-like symptoms were also observed, but the vector was not described. They found that the

phytoplasma was transmitted to healthy *Ranunculus scelratus* L. by the leafhopper, but the symptoms which appeared in ranunculus phyllody in our study. Our results strongly suggest that the symptoms of ranunculus found in India are caused by a phytoplasma. In present work, we made attempt to identify phytoplasma associated with ranunculus phyllody from India in *Ranunculus sceleratus.*

Our present work is first report of ranunculus phyllody from India in *Ranunculus sceleratus.* In past time, phytoplasma reported was in Japan (Kanerhira, et al., 1997) in *R. asiaticus and* Virulence disesae on ranunculus from Itali (Parella, et al., 2008).

Conclusion

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