

Original Research Article

<https://doi.org/10.20546/ijcmas.2020.902.361>

## Genetic Variability of Rice Phytoplasma by using Heteroduplex Mobility Assay (HMA)

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### ABSTRACT

#### Keywords

Candidatus  
Phytoplasma,  
mycoplasma-like  
organisms,  
heteroduplex

#### Article Info

Accepted:  
25 January 2020  
Available Online:  
10 February 2020

Heteroduplex mobility assay (HMA) is based on the principle that DNA heteroduplexes formed between related sequences have a reduced mobility in polyacrylamide gels proportional to their degree of divergence. HMA has been reported as a reliable means for identification and classification of phytoplasmas. Recently, the 16/23S spacer region has been demonstrated to be the best choice, when using the heteroduplex mobility assay (HMA) to differentiate closely related phytoplasmas and to group field isolates into well characterized phytoplasmas. The rice samples of varieties BPT 5204, White Ponni, CO 39 and ADT 43 produced no heteroduplex with assay, signifying that they are very closely related to each other.

### Introduction

Since their discovery in 1967 as ‘mycoplasma-like organisms’, the phytoplasmas have quickly become established as a unique group of plant pathogens. Diseases, frequently called ‘yellows’, have been known since the late 1800s; originally thought to be associated with viruses, many are now known to be caused by phytoplasmas. During the 1970s, research centred on diagnosis using symptoms and electron microscopy to visualize the phytoplasmas in the phloem sieve cells of

their hosts, transmission by insect vector and studies on the spread of the diseases they caused. The biology and taxonomy of these obligate pathogens were still shrouded in mystery. It was the advent of the molecular biological revolution in the 1980s that saw the introduction of techniques such as nucleic acid purification, DNA hybridization and the polymerase chain reaction, which with the secrets of these fastidious bacteria begin to emerge. In the 1990s the term phytoplasma had been proposed, and by 2004 a distinct taxonomic group, ‘Candidatus Phytoplasma’,

was defined. The evolution of molecular techniques has led to more information and paradoxically, less clarity in grouping different phytoplasma 'taxa'. As of today there are hundreds of diseases caused by phytoplasmas and about 100 known insect vectors.

Since their identification (Doi *et al.*, 1967), phytoplasmas have been identified as pathogens in numerous plant genera and in some cases have caused severe epidemics in major crops such as grapevine, sugarcane and coconut. Phytoplasmas are vectored by phloem-feeding leafhoppers, planthoppers and psyllids (Weintraub and Beanland, 2006), and the contemporaneous presence of phytoplasma, weed reservoir and vector has often been the cause of severe losses, especially in countries with weak rural economies. As phytoplasmas have still not been cultured *in vitro*, their diagnosis relies mainly on molecular techniques such as PCR, usually followed by RFLP for assignation to a '*Candidatus* (*Ca.*) Phytoplasma' species or to a 16S rDNA group.

Heteroduplex mobility assay (HMA) was first developed for the detection and estimation of genetic divergence between human immunodeficiency virus (HIV) strains (Delwart *et al.*, 1993). Recently, HMA has been employed to detect and to differentiate phytoplasmas (Zhong and Hiruki, 1994) and all results for differentiation of phytoplasmas from HMA were in agreement with those obtained by PCR and in particular RFLP analysis.

It has been demonstrated that HMA provided sensitive differentiation of phytoplasmas when other methods such as RFLP were not readily applicable to differentiate between very closely related 20 phytoplasmas (Ceranica-Zagorac and Hiruki, 1996). Obviously, HMA combined with PCR will be a very simple,

fast, sensitive and reliable method for detection and classification of different strains and groups of phytoplasmas.

HMA has been used for differentiation of phytoplasmas in the aster yellows group and clover proliferation group (Wang and Hiruki, 2001); elm yellows group (Angelini *et al.*, 2003) and australian grapevine phytoplasmas (Constable and Symons, 2004). The investigation on the genetic variability of various isolates of African LYD phytoplasmas associated with Cape St Paul Wilt disease (CSPWD, Ghana), lethal disease (LD, Tanzania) and lethal yellowing (LYM, Mozambique) were done and were also compared to the Caribbean phytoplasma associated with lethal yellowing in cross-linked gels.

## **Materials and Methods**

### **Heteroduplex Mobility Assay (HMA)**

HMA analysis was adapted for the study of genetic variability between isolates of phytoplasma (Constable and Symons, 2004; Wang and Hiruki, 2005). The 16S rRNA gene amplified by PCR from phytoplasma infected rice samples were analyzed by HMA. Five  $\mu$ l of each PCR product amplified from the four isolates of rice infected samples with the respective phytoplasmas was taken.

In each combination, 2  $\mu$ l of annealing buffer (100 mM Tris - HCl at pH 8.0, 20 mM EDTA and 1 M NaCl) was added. One drop of mineral oil was overlaid on the reaction mixture. Samples were then denatured at 98 °C for 4 min, rapidly cooled to 4 °C and then placed on ice for 20 min. Samples were electrophoresed on 1.5 % agarose gels in 1X TAE buffer at 80 V for 1.5 h at room temperature. DNA bands were stained with ethidium bromide and visualized under a UV transilluminator (Marinho *et al.*, 2006).

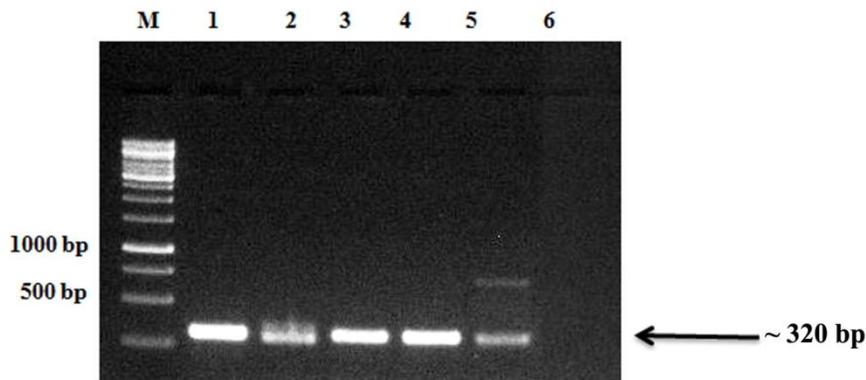
## Results and Discussion

The 16S rRNA gene of phytoplasma associated with rice orange leaf infected samples was studied for heteroduplex mobility assay. The rice samples of varieties BPT 5204, White Ponni, CO 39 and ADT 43 produced no heteroduplex with assay, signifying that they are very closely related to each other.

The coconut root (wilt) phytoplasma produced heteroduplexes at different mobility, suggesting that phytoplasma infecting coconut belongs to different group from the one infecting rice crop (Plate 1).

Heteroduplex mobility assay (HMA) is based on the principle that DNA heteroduplexes formed between related sequences have a reduced mobility in polyacrylamide gels proportional to their degree of divergence. HMA has been reported as a reliable means for identification and classification of phytoplasmas (Wang and Hiruki, 2001). Recently, the 16/23S spacer region has been demonstrated to be the best choice, when using the heteroduplex mobility assay (HMA) to differentiate closely related phytoplasmas and to group field isolates into well characterized phytoplasmas (Wang and Hiruki, 2005).

### Plate.1 Genetic variability of rice phytoplasma by using Heteroduplex Mobility Assay (HMA)



- M- 1 kb DNA ladder
- 1- Rice (Erode- BPT 5204)
- 2- Rice (Coimbatore-White Ponni)
- 3- Rice (Coimbatore- CO 39)
- 4- Rice (Coimbatore- ADT 43)
- 5- Positive control (Coconut root wilt )
- 6- Negative control (Healthy rice sample)

This technique has been developed for phytoplasmas, based on the 16S-23S spacer region and was used to analyse variability between 62 phytoplasmas collected from

North America, Europe and Asia (Wang and Hiruki, 2005). The results were useful for detecting subgroups and provided a rapid and sensitive test as an alternative to RFLP

analysis. In the present study, the results showed that there was no heteroduplex and hence no variation was observed from the rice samples. Similarly there was no any subgroup observed in the phytoplasma which infects rice.

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### How to cite this article:

Valarmathi, P. and Ladhakshmi, D. 2020. Genetic Variability of Rice Phytoplasma by using Heteroduplex Mobility Assay (HMA). *Int.J.Curr.Microbiol.App.Sci*. 9(02): 3124-3217. doi: <https://doi.org/10.20546/ijcmas.2020.902.361>