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MANAGEMENT CHILLI VEINAL MOTTLE VIRUS IN CHILLI USING PSEUDONOMAS FLOURESCENCE

C. RANASINGHE [1], P.M. MALLIKAARACHCHI [2], D.M. GUNASEKERA [1] AND V. NAVAGAMUWA [1]

[1] *Plant Virus Indexing Center, Homagama, Sri Lanka*
[2] *Faculty of Agriculture, University of Ruhuna, Matara, Sri Lanka*

EXTENDED ABSTRACT

Chilli is an important and essential component of daily Sri Lankan diet. This crop is susceptible to wide range of virus diseases which resulting for heavy crop losses. Among these, Chilli Veinal Mottle Virus (ChiVMV) is one of the major prevalent virus (Anon, 2001). Viruses differ from other plant pathogens and pests because they cannot be eradicated chemically. Utilization of beneficial microbes isolated from rhizosphere, referred to as Plant Growth Promoting Rhizobacteria (PGPR), might offer a promising viral disease control strategy. *Pseudomonas fluorescence,* a PGPR, commonly used as bio control agents, is reported to apply against several viral diseases (Maurhoper *et al.*, 1994; Elbadry *et al.*, 2006; Rakib *et al.*, 2012). However, no detail studies have been conducted on the application of *P. florescence* against ChiVMV in chilli in Sri Lanka. This study was conducted to evaluate the effectiveness of *P. fluorescence* against ChiVMV in chilli. Specific objectives are the isolation of *P. fluorescence* from chilli rhizosphere, identification and confirmation of isolates as *P. fluorescence,* and testing the efficacy of *P. fluorescence* in inducing resistance in chilli against ChiVMV.

Rhizosphere soil adhered to the roots of the healthy chilli plants were collected from the chilli cultivations located at Mahailluppallam, Hambanthoa, Matale and Athurigiriya areas. Bacteria were isolated from the rhizosphere soil by dilution plate count technique on King's B (KB) media (King *et al.*, 1954). Bacterial colonies grown on KB medium were observed under Ultra Violet (UV) light and fluorescent colonies were isolated. All isolated colonies were then subjected to biochemical assays (Gram reaction, catalase test, semi solid medium test, starch hydrolysis test, methyl red vogas proskauer (MRVP) test) according to Bergey's Manual for Determinative Bacteriology (Breed *et al.*, 1989).

Efficacy of the isolates of *P. fluorescence* was separately tested by seed treatment and soil drench method. In seed treatment, chilli seeds (variety MI-2) were soaked for 18 hrs in each bacterial suspension namely, M1, M2, ATC2, MTC3, and H4, separately and

in soil drench method, one month old chilli plants of variety MI-2 were dipped in each bacterial suspension. Seeds and plants treated with water was used as a control. In both method bacterial concentration $1x10^8$ cfu/ml was used. Five weeks old treated plants were mechanically inoculated with ChiVMV infected sap (1:10w/v) in phosphate buffer pH 7.0. Each isolate (treatment) was replicated four (three plant/replicate) times and the plants were arranged according to the complete randomized design.

To examine the effect of isolates of *P. fluorescence,* plant height, fresh and dry weights of the shoots and roots were measured at the end of the experiment. Disease severity rating was made from one week to eight week after post inoculation by using the following rating scale on the leaves. 0- No symptoms, 1- <10 % canopy infection, 2-11-25 % canopy infection, 3- 26-50 % of canopy infection, 4- 51-75 % canopy infection, 5- >75 % canopy infection. Accumulation of ChiVMV in foliar tissues was determined by double antibody sandwich Enzyme Linked Immunosorbance Assey (DAS-ELISA) using commercially available antiserum for ChiVMV. For each experiment, disease severity was measured by the area under disease progress curve (AUDPC). Data were analyzed using analysis of variance (ANOVA) in SAS. Means were compared using Least Significant Different (LSD).

A total of 25 soil samples were collected and 5 isolates were identified as *P. fluorescence* using culture characters on KB and biochemical tests. There was a significant difference (P=0.0001) among all five isolates compared with the control in both application methods. All isolates in both application methods showed lesser disease severity compared to untreated plants. Furthermore, isolate M2 showed the least disease severity in both application methods. Similar results were demonstrated by Damayanti and Katerina (2008) in which rhizobacteria treatment on hot pepper inoculated with tobacco mosaic virus (TMV) and chilli veinal mottle virus (ChiVMV) exhibited milder symptom expression compared with control plants. There was a positive correlation of disease severity with time in all isolates in both application methods. In seed treatment method, isolates MTC3, ATC2 and M2 showed less disease severity with time while in soil drench method, isolates MTC3 and M2 only showed less disease severity with time compared to untreated plants.

All five tested bacterial isolates showed their ability to enhance plant growth in both methods. There was no significant difference (0.17) between the two application methods with respect to plant height. However, there was a significant difference among the treatments (0.0001). Furthermore, there was an interaction between the application methods and treatments for plant height (0.0001). In seed treatment method, there was no

significant difference in plant height compared to the control. However, a significant difference in plant height compared to the control was observed in the soil drench method. This result indicates that application of *P. flourescence* as a soil drench has increased the plant height.

The fresh weights of the roots were significantly different (0.0156) between two methods of application as well as among the treatments (0.0209). Further, Root fresh weight is higher in seed treated plants than the plants of soil drench method. There is an interaction between application methods and treatments (P=0.035). In seed treatment method, root fresh weights were higher in all the isolates than that in untreated plants. However, in soil drench method, there was no significant difference among treatments. This clearly indicates that seed treated with *P. flourescence* has increased the root growth.

When consider shoot fresh and dry weights, there was a significant difference between the two application methods and among the treatments. But root dry weight differed among treatments. There is no interaction between the application methods and treatments in above three growth parameters. Shoot fresh weight was higher in ATC2, M1 and M2 isolates than that in control. Comparatively, shoot dry weight was high in the isolates; ATC2 and M1. When consider root dry weight, it was high in the isolates ATC2, M1, M2 and H4 compared to control. This is in agreement with the results of Parmer and Dadarwal (1999), where PGPR treatment of groundnut plants increased the dry weight of root significantly.

There is a significant difference (P=0.02) in virus concentrations between the two application methods and between treatments (P=0.0066) based on DAS-ELISA test results. Seed treated plants showed lesser virus than soil drench plants. Furthermore, there is no interaction between the treatments and application methods (0.189). the mean ELISA absorbance values of the plants treated with bacterial isolates MTC3, ATC2 and M2 showed negative reaction to the ELISA test as well as low virus titer compared to the untreated plants. These results showed that treating plants with *P.fluorescence* enhanced the plant growth while reducing virus titer. Furthermore, some of the bacterial treatments could maintain better plant growth characters than the control plants, even when they were infected by ChiVMV. These results were in agreement with Damayanthi *et al.* (2007) which evaluated the application of root colonizing bacteria against tobacco mosaic virus in hot pepper. Accordingly, treatment of hot pepper seeds and plants with rhizobacteria improved hot pepper health and its productivity through the promotion of

host nutrition and growth and stimulation of plant host defenses rather than antagonism. In this study, it is demonstrated for the first time that *P. fluorescence* is effective in management of ChiVMV in chilli.

P. fluorescence reduced the severity of ChiVMV disease in chilli and enhanced the plant growth in both seed treatment and soil drench application methods. Further, individual treatment of *P. fluorescence* resulted in different level of ChiVMV control in chilli. Further studies are recommended to increase the efficacy of management of ChiVMV in chilli using *P. fluorescence*.

REFERENCES

Anonymous. 2001. Identification of RADP markers linked to PVMV and CVMV resistance gene(s) in pepper. Asian Vegetable Research and Development Centre, Progress Report, p 126-128.

Damayanthi, T.A. and T. Katerina. 2008. Protection of hot pepper against multiple infections of viruses by utilizing root colonizing bacteria. Journal of ISSAAS. 14(1): 92-100.

Damayanthi, T.A., H. Pardede and N.R. Mubarik. 2007. Utilization of root colonizing bacteria to protect hot pepper against Tobacco mosaic tobamovirus. HAYATI Journal of Bioscience. 105-109.

Elbadry, M., R.M. Taha, K.A. Eldougdoug and H.G. Eldin. 2006. Induction of systemic resistance in faba bean (*Vicia faba* L.) to bean yellow mosaic poty virus via seed bacterization with plant growth promoting rhizobacteria. Journal of Plant Diseases and Protection, 113:247-251.

Maurhofer, M., C.Hase, P. Meuwly, J.P. Metraux and G. Defago. 1994. Induction of systemic resistance of tobacco necrosis virus by the root colonizing *P. fluorescence* strain CHAO influence of the gac a gene and of Pyoverdine production. Pytopathology 84: 139-146.

Parmer, N. and K.R. Dadarwal. 1999. Stimulation of nitrogen fixation and induction of flavonoid-like compounds by rhizobacter. Journal of Applied Microbiology 14: 340-358.

Rakib, A., A. Ani, and M.A. Adhab. 2012. Protection of melon plants against Cucumber mosaic virus infection using *P. fluorescence* biofertilizer. African Journal of Biotechnology 11: 16579-16585.