ISSN 0976 – 9080

BIOMIRROR

An Open Access Journal

Rauvolfia Serpentina Showing Viral Infection Symptom In Yogyakarta, Indonesia

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ARTICLE INFO

Received 30 November 2013
Revised 04 January 2014
Accepted 08 January 2014
Available Online 13 January 2014

Keywords:
Rauvolfia serpentina,
symptom,
virus,
infection.

ABSTRACT

Rauvolfia serpentina, family Apocynaceae, is widely cultivated in Asia such as India and adjoining countries for the production of roots used in several herbal formulations. In Indonesia, *R. serpentina* mostly are still as wild crops which grow at the forest in Java, Kalimantan and other islands. In Yogyakarta, some *R. serpentina* planted in experimental plots in 2011 showed some typical viral infection symptoms such as vein banding, severe mosaic, and stunting of the whole plant. The causal agent was transmittable by sap inoculation to indicator plants (*Chenopodium amaranticolor*) which produced chlorotic local lesions. Using the same method, the causal agent was also transmittable to healthy *R. serpentina* and produced vein banding, severe mosaic, and stunting similar to the symptoms of naturally infected *R. serpentina*. TAS-ELISA detection using anti Cucumber mosaic virus (CMV) antibody to the *R. serpentina* leaves showing the typical symptoms resulted in negative reactions. From the results of examinations, it is suggested that the causal agent of the symptom is virus and the virus is not CMV.

Introduction:

Rauvolfia serpentina (L). Benth. ex Kurz. (Apocynaceae) commonly known as *Sarpagandha* (*Pule pandak* in Indonesia) is an important medicinal plant in Indian subcontinent and South East Asian countries. Its roots are used as a remedy for many human illnesses such as high blood pressure, insomnia, anxiety, excitement, schizophrenia, insanity, and others. The plant grows well generally in the region with annual rainfall from 200 to 250 cm and at altitude up to 1000 m, and in deep fertile soil rich in organic matter [1,2, 8].

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Citation: Supyani * (*Rauvolfia Serpentina* Showing Viral Infection Symptom In Yogyakarta, Indonesia) Biomirror: 8-11 / bm-3004081114

So far, reports of viral infection on *Rauvolfia serpentina* are very limited. The single scientific report of viral infection on *Rauvolfia serpentina* we found so far is the report of Raj et al.[6] Raj et al.[6] reported the natural occurrence of CMV (*Cucumber mosaic virus*) infection on *Rauvolfia serpentina* in Lucknow, India. The symptom were severe mosaic and stunting of the whole plant. The virus was transmitted by sap inoculation to indicator plants *Nicotiana tabacum* cv. White Burley, *N. rustica*, and *N. glutinosa*, which produced necrotic local lesions and systemic mosaic. The virus also reacted positively with antiserum of CMV in gel double diffusion tests, indicating the virus was CMV.

In Indonesia, *R. serpentina* are mostly still as wild crops which grow at the forest in Java, Kalimantan and other islands. Recently, some researches initiate to explore and domesticate this plant [7,8]. We observed some plants showing typical symptoms of viral infection such as mosaic, vein clearing and stunting of the whole plant of *R. serpentina* which were planted in experimental plots in Yogyakarta, in 2011.

To infestigate whether the symptoms were due to viral infection, we conducted transmission assay. The result of transmission assay showed that the causal pathogen was transmitted by mechanical inoculation to indicator plants *Chenopodium amaranticolor* which produced chlorotic local lesions. Furthermore, by using the same method, the causal

pathogen was also transmitted to the healthy *R. serpentina* and produced severe mosaic, vein banding, and stunting similar to typical symptoms shown by the naturally infected *R. serpentina*. The results of these two transmission assays indicated that the typical symptoms shown by some *R. serpentina* in Yogyakarta was due to viral infection. Moreover, we would like to investigate what type of virus infecting the *R. serpentina*. Inspired from the report of Raj et al.[6], we performed TAS-ELISA detection assay using anti CMV antibody to the *R. serpentina* leaves showing the typical symptoms to investigate whether the associating virus was CMV. The result of the assay showed negative reactions. From the results of examinations, it is suggested that the causal agents associated with the typical symptoms of some *R. serpentina* in Yogyakarta is virus and the virus is not CMV.

Materials and Methods:

Maintenance of *R. serpentina* and Indicator Plants:

R. serpentina and indicator plants were maintained at 28ºC in the insect proof screen house in Yogyakarta. Healthy and diseased plants were maintained separately in different screen house.

Mechanical Transmission Assay:

Leaves tissue of diseased *R. serpentina* was harvested and homogenized (1:3 w/v) in sterilized pre-cold pestle and mortar in chilled 0.05M Potassium Phosphate buffer, pH 7.2 containing 1% Sodium sulphite (Na_2SO_3) and was strained/passed through double layer of muslin cloth as described by Hill [4]. The mechanical inoculation was carried out according to the protocol described by Green [3] with modifications. The indicator plants (*C. amaranticolor*) at 2-3 two leaf stages were rubbinoculated on the upper surface of the leaves with the slurry (inoculum), using 600 mesh carborundum powder as abrasive. The inoculum was also inoculated to the healthy *R. serpentina*. After inoculation, the inoculated plants were rinsed with distilled water to remove superfluous inoculum and kept in an insect proof screen house. The uninoculated plants (bufffer inoculated plants) of each test plants were maintained as control.

Symptom Observation:

Plants were observed daily for the symptom development from1 to 4 weeks post inoculation (wpi) as described by Green [3]. Symptom was observed both on mechanically inoculated indicator plants and *R. serpentina*. The typical symptom observed were: local lesions, mosaic, vein banding, chlorosis, and stunting.

Immunoassay

Immunoassay was performed utilizing Triple Antibody Sandwich Enzyme-Linked Immunosorbent Assay (TAS ELISA) format using PathoScreen® Kit from Agdia Inc., following protocol from the manufacturer. Three leaves of sample plants were grind in extraction buffer at a 1:10 ratio (w/v). In brief, after sampel wells were coated using polyclonal antibody, 100 µl of prepared samples were dispensed into sample wells. The plate was incubated for 2 hours at room temperature, followed by 7 times washing with washing buffer. Here, monoclonal antibody mixture specific for general CMV was used as detection antibody. 100 µl of alkaline phosphatase conjugate was dispensed per well, then incubated for 2 hours at room temperature, followed by 8 times washed with washing buffer. 100 µl of PNP substrate was dispensed into testwell followed by incubation for 60 minutes (in dark). The developing color were observed by eyes, and the color intensity (absorbance value) were measured on an ELISA plate reader at 405 nm.

Results and Discussions:

Symptom Observation on Natural infected plant

Around 200 pots of *R. serpentina* were planted in experimental plots in Yogyakarta. In 2011, we observed that around half of them were shown typical symptoms of virus infection, including mosaic, vein banding and stunting (Fig 1).

Fig 1. *R. serpentina* naturally showing typical symptoms of viral infection. Left a, healthy (normal) ; Left b, showing stunt and chlorotic symptoms. Right, clear vision pattern of vein banding symptom.

A literature investigation revealed that there is only one report of occurrence of virus on *R. serpentina* whose was reported by Raj et al. [6]. They reported that *R. serpentina* was naturally infected by CMV and caused severe mosaic and stunting of the whole plants which were growing in experimental plots, in India. Furthermore, they also said that it was the first record of natural occurrence of CMV on *R. serpentina*. Here, we would like to confirm whether the typical symptom showed by some *R. serpentina* in Yogyakarta was due to viral infection by mechanical transmission assay.

Mechanical Transmission and Symptom Observation

We transmitted the possibly causal agent of the typical simptoms by mechanical inoculation of sap of the *R. serpentina* showing typical symptoms to indicator plants *C. amaranticolor*. At 1 week after inoculation (wai) the indicator plants showed chlorotic local lesion symptom, indicating typical virus infection (Fig 2). This result was also comparable to the result of Raj et al. [6]. They reported that the causal pathogen (CMV) in *R. serpentina* in India was transmitted by sap inoculation to indicators plants *Nicotiana tabacum* cv. White Burley, *N. rustica*, and *N. glutinosa*, which produced necrotic local lesions and systemic mosaic.

Furthermore, we also transmitted the sap of the *R. serpentina* showing typical symptoms to some normal looked (healthy) *R. serpentina*. At 4 wai, the mechanically inoculated *R. serpentina* showed typical symptoms similar to the symptoms showed by *R. serpentina* from where the sap was prepared. The symptoms were mosaic, vein banding (Fig 3), and some of plants showed stunting (unpublished data).

Fig 2. The result of mechanical transmission assay. Indicator plants (*C. amaranticolor*) were mechanically inoculated with sap of *R. serpentina* showing typical symptoms. At 1 wai, the leaves of *C. amaranticolor* showed chlorotic local lesions

Fig 3. The result of mechanical transmission assay. Normal looked (healthy) *R. serpentina* was mechanically inoculated with sap of the *R. serpentina* showing typical symptoms. At 4 wai, the leaves of the mechanically inoculated *R. serpentina* showed typical symptoms (chlorosis, mosaic, and vein banding). Left, uninoculated plant; Right, inoculated plant.

According to the results of mechanical transmission assay to indicator plants and *R. serpentina*, it is suggested that the causal agent associate with the typical symptoms on *R. serpentina* in this research is virus. Moreover, using the report of Raj et al. [6] as consideration, it is suggested that the causal agent associate with the typical symptoms on *R. serpentina* in this research is *Cucumber mosaic virus*. To confirm the notion, we conducted immunoassay.

Immunoassay

Immunoassay was performed utilizing TAS ELISA format using monoclonal antibody specific for general CMV. The result of assay showed that all of *R. serpentina* including the naturally normal looked (healthy) plants, the naturally showing typical symptoms plants, and the mechanically inoculated plant reacted negatively with the antiserum of general CMV. This results indicated the absent of CMV in the plants (Table 1).

Table 1. Mean of ELISA values (0D 405) of duplicate samples for extracts of leaves from *R. serpentina*.

Samples[a]	Absorbance Value at 405 nm[b,c]	Description
Naturally normal looked (healthy) plants	0.0875 ± 0.0007	CMV undetected
Naturally showing typical symptoms plants	0.095 ± 0.0078	CMV undetected
Mechanically inoculated plants	0.065 ± 0.0021	CMV undetected
Positive control	0.759 ± 0.0255	CMV detected
Negative control	0.038 ± 0.0410	CMV undetected

[a]Extracts prepared in extraction buffer at tissue : buffer ratio of 1:10 (w/v).
[b]Loading diagram following the manufacturer (TAS-ELISA) is explained in the text.
[c]Mean ± standard deviation.

Regarding the experiment of Raj et al.[6], they reported that both naturally infected and artificially transmitted *R. serpentina* plants reacted positively with antiserum of CMV indicating the presence of CMV. In this research, based on the results of the assays, it can be conluded that the causal agent associate with the typical symptoms on *R. serpentina* in Yogyakarta mentioned in this research was virus. Furthermore, the virus was not CMV.

Acknowledgement:

This research was founded by *DIPA Universitas Sebelas Maret* through Contract No: 2342/UN27.16/PN/2012, under skema of research *Penelitian Unggulan Perguruan Tinggi*.

References:

1) Dey A and De JN (2010) *Rauvolfia serpentina* (L). Benth. ex Kurz. Review. Asian J Plant Sci 9(6): 285-298.
2) Dey A and De JN (2011) Ethnobotanical aspects of *Rauvolfia serpentina* (L). Benth. ex Kurz. in India, Nepal and Bangladesh. Review. Journal of Medicinal Plants Research 5(2) : 144-150.
3) Green SK (1984) Guidelines for diagnostic work in plant virology. Technical Bulletin No 15. The Asian Vegetable Research and Development Center. Shanhua, Taiwan, ROC.
4) Hill SA (1984) Methods in plant virology. Volume (I), Published by British Society for Plant Pathology by Blackwell Scientific Publications, ISBN 0-632-00995-0.
5) Pakrashi S, Akkhari B (1968) Rauwolfia alkaloids. Farmatsiia 17(4): 64-82.
6) Raj SK, Kumar S, Pratap D, Vishnoi R and Snehi, SK (2007) Natural occurrence of *Cucumber mosaic virus* on *Rauvolfia serpentina*, A New Record. Plant Disease 91 (3): 322.
7) Sulandjari (2006) Hasil akar dan reserpina pule pandak (*Rauvolfia serpentina* Benth.) pada media bawah tegakan berpotensi alelopati dengan asupan hara. Biodiversitas 9(3): 180-183.
8) Wijayakusuma HM H (1994) Tanaman berkhasiat obat di Indonesia. Pustaka Kartini, Jakarta: 110-112.