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Morphological Characteristics and Pathogenicity of *Synchytrium psophocarpi* (Rac.) Baumann Associated With False Rust on Winged Bean

Abdollah Karami, Zainal Abidin Mior Ahmad and Kamaruzaman Sijam
Department of Plant Protection, Faculty of Agriculture,
University Putra Malaysia, 43400 UPM, Serdang, Selangor Darul Ehsan, Malaysia

Abstract: Problem statement: Winged bean (*Psophocarpus tetragonolobus* L.) is an important tropical legume in countries like Malaysia due to its potential as a high protein value crop. However, bright-orange pustules observed on the veins of young leaves, stems, pods and sepals depict symptoms of false rust disease on beans. The causal agent of this disease has been found to be *Synchytrium psophocarpi*. Currently, there is no published literature of this or other related species in Malaysia. Thus, there is a very serious lack of knowledge on the taxonomic characterization and pathogenicity of the local fungus. Therefore, there is an important need for this microorganism to be documented. **Approach:** This study was reported based from samples obtained from infected winged bean plants found in the fields around the University Putra Malaysia campus in Serdang, Selangor, Malaysia. The morphological characteristics were studied using dark field and scanning electron microscope. Meanwhile, pathogenicity test was carried out using two methods which were moist chamber and on Petri dish. **Results:** The sporangia were spherical to ovoid in shape and approximately 20.69 µm in diameter. The average diameter for spore measured was 2.02 µm and the flagella were 10.75 µm in length. Positive disease development with false rust disease symptoms was observed in both methods of inoculation practiced. It confirmed the pathogenicity of the fungus as the causal pathogen with the appearance of clear disease symptoms. **Conclusion:** This research finding is the first detailed report for *Synchytrium psophocarpi* associated with false rust disease of winged bean in Malaysia. It described the morphology, zoospore production and pathogenicity of the causal fungal organism. This information would be very useful for the studies involving this pathogen in future.

Key words: Winged bean, S*ynchytrium psophocarpi*, false rust, pathogenicity

INTRODUCTION

The winged bean is a tropical legume plant also known by other names such as asparagus pea, goa bean, manila bean and locally called kacang botol. It belongs to the family Leguminosae[10]. The winged bean is fully appreciated since it is known to be completely not poisonous in all parts from the shoots, flowers, roots, leaves, pods to seeds. The plant grows in abundance throughout the year in Southeast Asia and East Africa[11].

In Malaysia, winged bean has been known as an important source of vegetable. It is suitable for human consumption at all stages of its life cycle with high protein content. It is prone to attack by false rust or orange gall disease caused by *Synchytrium psophocarpi* (Rac.) Baumann. This disease was previously reported in South East Asia, Africa and Philippines[1,8], Indonesia[9] and Papua New Guinea[5]. *S. psophocarpi* is a force parasite that has no resting spore[4]. The sporangia are spread by wind, insects and other natural agents [3]. The disease symptoms are the appearance of bright orange pustules along the veins of young leaves and stems, pods and sepals of the flowers. This limits plant growth and reduces the quality of pods. The disease appears as yellow lumps on plant parts and may cause bending. The fungus grows on both sides of the leaf. Temperatures between 25-28°C, high relative humidity and moisture are favorable conditions for disease development in young leaves.

The present investigation was aimed for identification and confirmation of pathogenicity of *S. psophocarpi* associated with false rust on winged bean in Malaysia.

MATERIALS AND METHODS

Sampling of diseased winged bean plants: False rust infected leaves, stems and pods were sampled from

Corresponding Author: Zainal Abidin Mior Ahmad, Department of Plant Protection, Faculty of Agriculture, University Putra Malaysia, 43400, Malaysia Tel: +60 3 89467243 Fax: +60 3 86560698

winged bean plants found growing in the fields around the University Putra Malaysia campus in Serdang, Selangor. The diseased samples were collected and stored in a refrigerator at 5°C prior to further studies.

Morphological characteristics of sporangia and zoospores: Fungal sporangia were removed from infected plants and immersed in a suspension of sterile distilled water contained within a dropping bottle for 30 min. The suspension was shaken and a drop was placed on a clean glass slide and covered with a glass slip. The sporangia were observed at 400 and 1000 times magnification using dark field microscopy. Zoospores were liberated from the sporangia following the method described previously [2]. Sporangia from infected plant parts were placed on a watch glass containing sterile distilled water and incubated at room temperature (26±2ºC) for between 1 and 2 h. The duration was sufficient to allow the release of zoospores. Zoospore counts were made by placing a drop of suspension on a haemocytometer and observed under a light microscope. Zoospores were examined by dark field microscopy and SEM. The normal procedures involved for the fixation of plant tissues for SEM examination were done for observing zoospores released from sporangia on plant parts of infected winged bean.

Pathogenicity tests on winged bean: Two methods of inoculation were conducted for the tests as follows:

- Seedling inoculation in a moist chamber: Winged bean seeds were sown in a soil mixture containing sand, clay and peat in the ratio of 2:1:3 respectively sterilized in an autoclave at 1.05 kg cm^{-2} for 90 min. Germination occurred at 5 days after sowing in a shade house. Sporangia suspension were prepared and sprayed onto ten leaves of 2 week old plants kept within a framed polyethylene enclosure (1× 0.5×0.5 m) constructed on a wooden frame fitted over an aluminum tray 6cm in height. This was placed on a bench in the shade house. Inoculated plants were incubated for 48 hrs at a temperature of 28ºC and relative humidity of 98% maintained in the chamber by spraying with sterile water to run off twice daily at 0800 and 1400 h respectively. The tray was filled with 1 cm depth of water regularly. The experiment was repeated three times. Plants were assessed daily for disease symptoms based on the method reported previously[1]
- Detached leaf inoculations on a Petri dish: Ten winged bean leaves (two leaf pieces per petiole) of four week old plants grown in the shade house were randomly selected and inoculated with sporangia suspension. Two leaf pieces were placed on a Petri dish with the petiole ends inserted in absorbent paper moistened with sterile distilled water. The lids were then sealed with parafilm and incubated at room temperature (26±2ºC). The leaves were examined daily for disease symptoms

RESULTS

Morphological characteristics of sporangia and zoospores: Dark field microscopy revealed the presence of orange colored sporangia that were spherical to ovoid in shape (Fig. 1). The mean diameter of each sporangia measured approximately 20.69 µm.

The zoospores were first released in water by about 90 min after sporangial submersion. The morphology of the zoospores derived from winged bean in Serdang, Selangor were similar to the descriptions as reported previously[1,6,7]. Figure 2 shows the scanning electron microscopy of two zoospores. The average spore diameter measured 2.02 µm and the flagella were 10.75 µm in length.

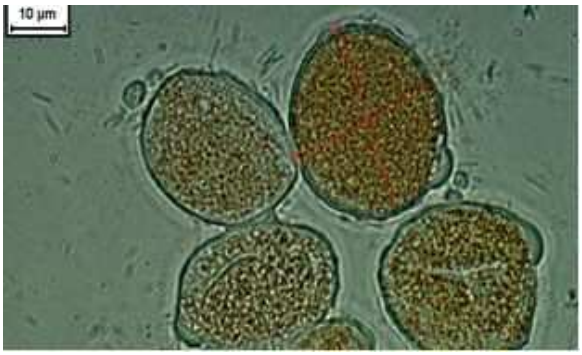

Fig. 1: Sporangia of *S.psophocarpi* observed under dark field microscopy at 400x magnification

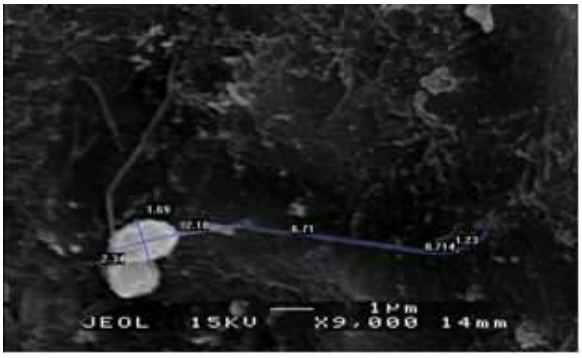

Fig. 2: Scanning Electron Microscopy (SEM) micrograph of zoospores of *S. psophocarpi* (Bar = 1 µm)

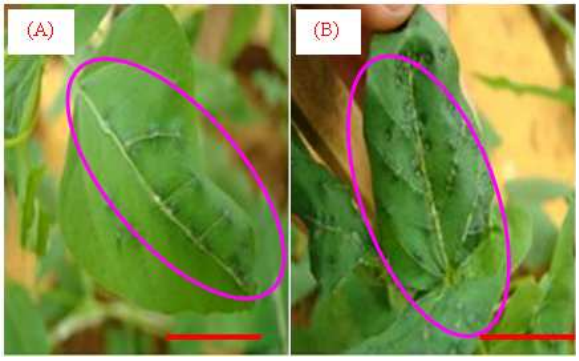

Fig. 3: The development of false rust disease on winged bean plants (A) 8 days after inoculation and (B) 14 days after inoculation in mist chamber, (Bar = 10 mm)

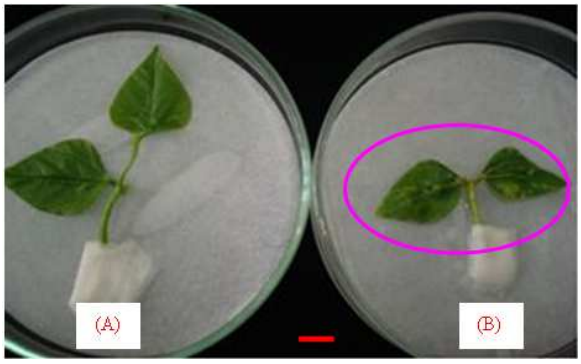

Fig. 4: Pathogenicity test results on detached winged bean leaves showing (A) negative control and (B) positive disease development at 7 days after inoculation on Petri dish, (Bar = 10 mm)

Pathogenicity tests on winged bean: Both methods of inoculation gave positive disease development with false rust disease symptoms appearing 8 days after inoculations by moist chamber method and 7 days after inoculations by Petri dish method. The appearance of orange gall was observed on 70% of leaves inoculated in the moist chamber (Fig. 3A and B) and 90% of leaves inoculated on Petri dish (Fig. 4A and B). Orange colored sporangia were similarly confirmed on leaf tissues by both methods, while none were found on the negative controls.

DISCUSSION

This study confirmed the similarities in morphology of the isolates from false rust infected winged beans in Serdang, Selangor with those previously described in literature for *Synchytrium psophocarpi* (Rac.) Baumann. It also confirms the pathogenicity of the fungus as the causal pathogen with the appearance of clear disease symptoms. Young winged bean leaves were found to be very susceptible and orange gall symptoms mainly concentrated along the veins on the lower surface of leaves similar to the previous study[3]. The leaves also become curled. By utilizing the methods successfully used for developing disease on winged bean from this work, it will be possible to conduct further investigations to elucidate the influence of environmental factors on the disease and to determine host range.

CONCLUSION

The results presented herein report taxonomic characterization and pathogenicity of *Synchytrium psophocarpi* associated with false rust disease of winged bean in Malaysia. This detailed report on the morphology, zoospore production and pathogenicity of the causal fungal organism would be very useful for the studies involving this pathogen in future.

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