

## The total protein band profile of the green leafhoppers (*Nephotettix virescens*) and the leaves of rice (*Oryza sativa*) infected by tungro virus

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**Abstract.** Sulistiyarsi A, Suranto, Supriyadi. 2012. The total protein band pattern of the green leafhoppers (*Nephotettix virescens*) and the leaves of rice (*Oryza sativa*) infected by tungro virus. Nusantara Bioscience 4: 32-35. Tungro virus is one of most important diseases of rice plants caused by double infection with RTBV and RTSV which is transmitted by *Nephotettix virescens* Distant. The interaction between host and virus-vector are still quitted difficult to understand. The aims of this study were: (i) to know the character of the total protein band pattern of rice plants infected with tungro virus compared to the health one, (ii) to look at the different between the band profiles of total protein of *N. virescens* that consume the host rice plants infected by tungro virus and that of the healthy rice plants. Total protein band profiles of rice plants were identified using SDS-PAGE. To extract the leaves, buffer merchapto-ethanol was used, while the sample extraction of green leafhoppers employed buffer PBS IX, and for staining the protein coomassie brilliant blue was used. Data were analyzed descriptively based on the score of the migration of the band (Rf). The results showed that the protein contains every 0.5 g of healthy leaves and the infected by the virus were 0.567 g and 1.011 g respectively. Clear difference of the protein pattern was found in the healthy plant and the infected one. In general, the entire band in the infected plant was much thicker compared to the infected leaves. Protein bands with a higher quantity were expressed by the protein on the molecular weight of 108, and 117 kDa. These proteins are presumably from the group of  $\beta$ -galactosidase and bovine serum albumin. The function of such proteins is still unknown, but it may be related to the plant's responses to virus infection, because the protein did not appear in the healthy plants. The total protein content of both *N. virescens* which acquired the healthy leaves and the infected one were 0.1395 g and 0.1546 g respectively. Qualitatively, there was no significant difference in the protein expression in those vectors, but slightly thicker band were observed in the infected leaves.

**Keywords:** rice, tungro, *Nephotettix virescens*, protein banding.

**Abstrak.** Sulistiyarsi A, Suranto, Supriyadi. 2012. Pola pita protein total wereng hijau (*Nephotettix virescens*) dan daun tanaman padi (*Oryza sativa*) yang terinfeksi virus tungro. Nusantara Bioscience 4: 32-35. Virus tungro merupakan salah satu penyakit penting tanaman padi disebabkan oleh infeksi ganda RTBV dan RTSV, yang disebarkan oleh *Nephotettix virescens* Distant. Interaksi antara inang dan vektor virus masih sulit dipahami. Penelitian ini bertujuan: (i) mengetahui karakter pola pita protein total tanaman padi yang terinfeksi virus tungro dan tanaman padi sehat, (ii) mengetahui karakter pola pita protein total *N. virescens* yang mengkonsumsi inang tanaman padi terinfeksi virus tungro dan tanaman padi sehat. Pola pita protein total tanaman padi diidentifikasi menggunakan metode elektroforesis dengan SDS-PAGE. Sampel daun diekstraks menggunakan buffer mercapto ethanol, sedangkan sampel wereng hijau diekstraks menggunakan buffer PBS IX; dan pewarnaan pita protein menggunakan coomassie brilliant blue. Data dianalisis secara deskriptif berdasarkan nilai migrasi pita (Rf). Hasil penelitian menunjukkan kadar protein 0,5 g daun tanaman padi sehat dan daun tanaman padi terinfeksi virus tungro masing-masing sebesar 0,567  $\mu$ g dan 1,011  $\mu$ g. Perbedaan pola pita protein secara jelas ditemukan di antara tanaman padi sehat dan terinfeksi. Pada umumnya, pola pita protein pada tanaman padi yang terinfeksi virus tungro lebih tebal dari pada tanaman padi sehat. Pita protein dengan kuantitas lebih tinggi diekspresikan pada protein dengan berat molekul 108, dan 117 kDa. Diduga protein ini dari kelompok  $\beta$ -galaktosidase dan bovine serum albumin. Fungsi protein tersebut belum diketahui, namun diduga berkaitan dengan respon tanaman terhadap infeksi virus, karena protein tersebut tidak muncul pada tanaman sehat. Kadar protein total wereng hijau yang mengkonsumsi daun tanaman padi sehat dan terinfeksi virus tungro masing-masing sebesar 0,1395  $\mu$ g dan 0,1546  $\mu$ g. Secara kualitatif, tidak terdapat perbedaan yang signifikan ekspresi protein pada inang, tetapi pada daun yang terinfeksi cenderung lebih tebal.

**Kata kunci:** padi, virus tungro, wereng hijau, *Nephotettix virescens*, pola protein

### INTRODUCTION

Tungro virus is one of the most important diseases of the rice plants in South and Southeast Asia causing significant economic losses. The disease is caused by double infection of *rice virus tungro bacilliform virus* (RTBV) and *rice virus tungro spherical virus* (RTSV),

which is transmitted by green leafhoppers (*Nephotettix virescens* Distant) (Muralidharan et al. 2003; Tyagi et al 2008). This vector is the most effective in transmitting the viruses on the rice plant and the very dominant species in the tropics (Rachim 2000). *N. virescens* is the most effective vector in transmitting the tungro virus (Supriyadi et al. 2004, 2008; Widiarta 2005) and have also been

recorded its population is more dominant than other vectors in the field (Himawati and Supriyadi 2003; Supriyadi et al. 2004; Widiarta 2005).

A healthy rice plant uninfected by tungro virus contains much chlorophyll and therefore can be used for photosynthesis and producing food for the plant. Accordingly, leaves of rice plants do not contain lots of protein so that the amino acid content of the plants is low. On the rice plants infected by the tungro virus, the DNA from the virus will then infect the plant cells and take over the functions of DNA of the plants in order to synthesize the protein, which is used by the viruses to replicate the viral DNA. Therefore, it is necessary to test the protein band profiles of healthy rice plants and infected ones. RTSV particles themselves do not play a role in transmitting the RTBV, but the one which has a role is the helper factor protein, that is a product of the interaction between RSV with the infected host plants (Hibino and Cabauatan 1986).

The process of tungro virus transmission by vectors has been known to involve the helper component which serves in binding virus particles on the mouth of the vector. Helper components are thought to be specific proteins which are important for virus absorption at the vector stylet (Supriyadi et al. 2004). According to Hibino (1996) protein as a helper component of the vector is produced in the body that is secreted into the mouth of the stylet. Helper proteins of the vector of *N. virescens* are produced by means of his own mouth (stylet) or the thorax which is secreted into the mouth of the tool, so it requires identification of the total protein.

Based on the above background the researcher raised several issues, namely the study of the total protein profile of host plants of the rice infected by tungro virus and total protein profiles of individuals of *N. virescens*. The research aimed: (i) to know the character of the total protein band profiles of rice plants infected with tungro virus and that of healthy rice plants, (ii) to know the character of the band profiles of total protein of *N. virescens* that consume the host rice plants infected by tungro virus and that of the healthy rice plants.

## MATERIALS AND METHODS

### Place and time

The observation on the rice plants infected by the tungro virus was done in the Laboratory of Science and Plant Diseases, Faculty of Agriculture, Sebelas Maret University (UNS) Surakarta, while the total protein band profile analysis of host plants infected by rice tungro virus and the total protein band profiles of green leafhopper *N. virescens* was conducted at the Laboratory of Microbiology, IUC, Gadjah Mada University, Yogyakarta. The study was conducted from February to July 2010.

### Procedures

The rice cultivar tested in this study was of Ciherang type. Rice plant samples were taken from endemic areas in Yogyakarta, Indonesia. The rice plants were planted in pots placed in a greenhouse. Samples of *N. virescens* from the

field were grown in a rice box breeding nursery in our campus. Tungro virus transmission was conducted in a greenhouse. Samples for total protein electrophoresis profiles using the leaves of rice plants in both healthy and the rice infected by the tungro virus. As for the total protein profile analysis of green leafhoppers, samples were taken from the head and thorax of green leafhoppers that attacked both healthy rice plants and the plants infected by the tungro virus. Observation of the rice plant is described qualitatively to identify the healthy rice plants and the tungro virus- infected rice plants.

Total protein profile analysis was conducted to identify differences and/or total protein band profile similarity between the rice plants infected by tungro virus and healthy rice plants and also between green leafhoppers that consumed healthy rice plants and the plants infected by tungro virus. Profile analysis of total protein band was done with the technique of electrophoresis on SDS-PAGE with several stages, adopting the method of Wongsosupantio (1992), Coats et al. (1990), and Cruz et al. (1998). Leaf samples of rice plants employed mercaptoethanol extract buffer, while the green leafhoppers samples employed IX PBS buffer extract. Each procedure requires 0.5 g of sample. The concentration of acrylamide for *stacking gel* was 3%, while for *gradient gels* was 10%. Electrophoresis was run at a constant voltage of 100 VA, until the bromphenol blue traveled near the bottom of the gel. Gels were fixed and then staining was done in one night with a solution of Coomassie brilliant blue R-250. Staining process was followed by destaining using a solution consisting of methanol, acetic acid, and water (40:40:20) which were all shaken until the protein bands appeared. Total protein electrophoresis results were documented in a digital photograph.

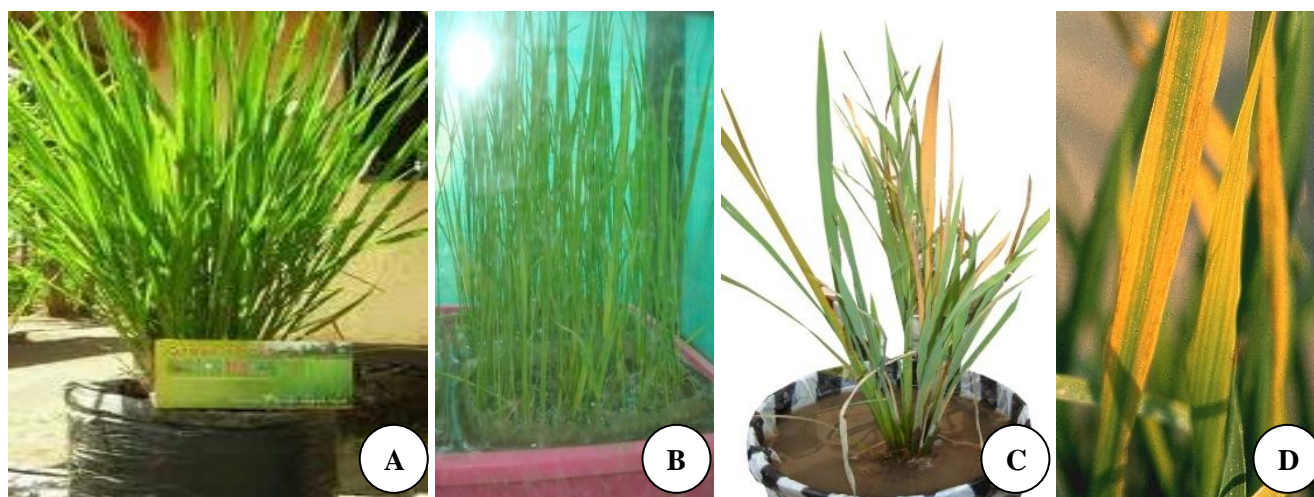
The data of the total protein band pattern of the rice and *N. virescens* were analyzed by zymogram to observe similarities or differences in the total protein band profiles between rice plants infected with tungro virus and healthy rice plants.

## RESULTS AND DISCUSSION

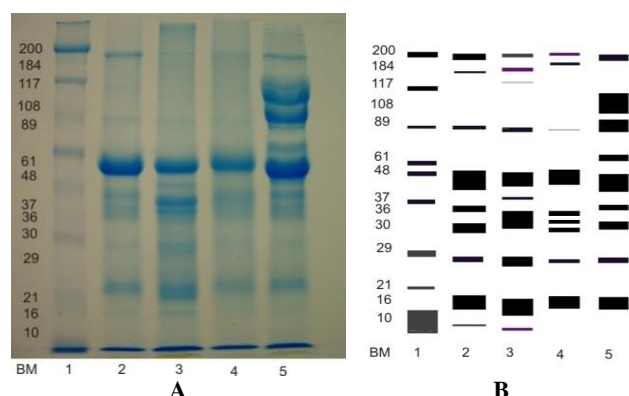
### Description of the rice plants infected by tungro virus

The rice plants uninfected by the tungro virus grew well the leaves were green and plants were relatively high. The rice plants infected by the tungro virus grew somewhat stunted; the young leaves turn yellowish from the tip, and the yellow leaves appear somewhat twisted; the older leaves look yellow to orange; the offsprings were few, and the heights of the plants were hardly even. In the breeding process, the tungro virus transmission appears on the third leave which looks somewhat twisted (Figure 1).

According to Gnanamanickam (2009), the general symptoms of rice plants infected by tungro virus are leaf discoloration that begins from leaf tip and extends to the blade or the lower leaf portion; infected leaf sometimes also show molted or striped appearance and stunting. The presence of tungro virus can be confirmed by some serological tool based on protein characteristics (Suparyono et al. 2003).



**Figure 1.** Rice plants. A. Healthy plants, B. The breeding of the tungro virus-infected ones, C-D. Adult plants infected by tungro virus



**Figure 2.** The results of the total protein band profiles of rice leaf samples by SDS-PAGE electrophoresis. A. Protein banding pattern profiles, B. Zymogram, 1 = marker, 2 = leaves of healthy rice plants: 30 mg (12 mL), 3 = leaves of healthy rice plants: 40 mg (16 mL), 4 = healthy leaves of rice plants: 20 mg (8 mL), 5 = leaves of the rice plants infected by the tungro virus: 40 mg (6.6 mL).

#### Total protein band profiles of the healthy rice plants and the ones infected by the tungro virus

The results of measurements with a spectrophotometer at a wavelength of ( $\lambda$ ) = 595 nm indicated that the protein content of the healthy rice plants and the infected one were 0.567  $\mu$ g and 1.011  $\mu$ g respectively. Protein levels are used to determine the concentration of the sample used in the process of running electrophoresis.

The electrophoresis results of the protein band profiles of the healthy rice plants and the ones infected by green leafhoppers at a certain range of molecular weight showed a very thick gene expression, which was not expressed on the healthy rice plants (Figure 2). Profile of protein bands that appeared on the BM (16, 30, 47, 61, 89 and 200) kDa were expressed by the healthy rice plants and the rice plants infected by the tungro virus, which means both the healthy rice plants and the ones infected by the tungro virus

expressed the same protein band profile, which was based on markers consisting of protein glutamate dehydrogenase, ovalbumin, carbonic anhydrase, myoglobin, lysozyme, and aprotinin. On the tungro virus-infected plants, more quantity of expressed proteins was seen on the protein bands which looked thicker.

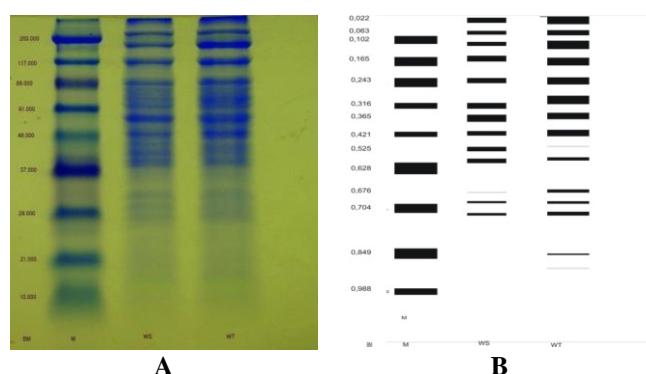
The tungro virus-infected rice plants showed a specific protein band profile which was in the range of protein bands close to 108 MW and 117 kDa which was displayed with a thick band. Protein bands on the range of molecular weight are typical proteins in the rice plants infected by the tungro virus. Based on the marker, both proteins were alleged to be  $\beta$ -galactosidase and bovine serum albumin expressed by the rice plants because of a virus infection. The function of both types of proteins is not known with certainty, but it is allegedly associated with the plant responses to virus infection as a form of self-defense, because both types of proteins were not expressed on the healthy rice plants, thus there is a need for further research. According to Sereikaite et al. (2004) bovine serum albumin usually serves as a nutrient in microbial cells, and supports the growth of cells and is used for immunoblots and is correlated to the immunosorbent of the enzyme test.

In the total protein expression profile in rice plants infected by the tungro virus, it was difficult to distinguish between a viral protein and the protein of rice plants. With the addition of SDS in the electrophoresis, the protein of the plants and of the virus will be cut into polypeptide chains. It is suspected that the polypeptide of the virus protein has a molecular weight similar to that of the polypeptide of the plants so that they settle in the same location, or that the levels and types of viral polypeptides are relatively few compared to the polypeptide of plants so it is difficult to identify. This result has differed to Oluwafemi et al. (2007) which indicated that plants infected with *maize streak virus* (MSV) had protein patterns different from healthy plants. Perhaps, in this study, the isolated viral proteins had very little concentration.

### The profile of protein bands of the green leafhoppers of the rice plants infected by the tungro virus

No significant difference of the profile of the protein bands (MW  $\geq$  200, 117, 89, 61, 48, 23, 29) was shown by *N. virescens* that also attacked the healthy rice plants and the rice plants infected by the tungro virus (Figure 3). On the green leafhoppers that ate rice plants infected by the tungro virus, the protein bands that appeared were slightly thicker which showed more protein concentration, which was the accumulation of the protein of tungro virus-infected plants that was also consumed. Whereas on the lower molecular weight, the protein banding patterns that emerged showed no much difference. The presence of viral proteins on vector species commonly observed, even several pathogenic virus can mediate manipulation of vector behavior may facilitate pathogen spread (Yamagishi and Yoshikawa 2009).

The above phenomenon could be explained that the more insect *N. virescens* feed the infected leaves, the more interaction between the protein of plant host and the protein of vector. This occurrence may stimulate the production of virus protein which expressed in the insect vector. Therefore, if the insect tissue was extracted, more concentration of the protein on the gel occurred. It was interesting to look at, that the lower band both samples did not different. It could be one to the fact that small size of protein could not be stained intensively.



**Figure 3.** The results of total protein band profiles of the green leafhoppers with SDS-PAGE electrophoresis. A. Protein banding pattern profiles, B. Zymogram, M = Marker, WS = green leafhoppers on the healthy plants, WT = green leafhoppers on the plants infected by the tungro virus

### CONCLUSION

The profile of the total banding patterns of the protein of the rice plants infected by the tungro virus was in

contrast to one of the healthy plants having a molecular weight of 108 and 117 kDa. The observation results on the banding pattern profiles of the protein of *N. virescens* that consumed the healthy rice plants and of the *N. virescens* that consumed the tungro virus-infected rice plants showed a difference in quantity as indicated by the thick-thin profile of protein bands at the molecular weight of 200, 117, 89, 61, 48, 23, 29 kDa.

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