ENTOMON 30(1): 85–90 (2005)
Short Communication No. ent.30112

Planococcus citri (Risso)-an additional mealybug vector of *Badnavirus* infecting black pepper (*Piper nigrum* L.) in India

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ABSTRACT: Citrus mealybug (*Planococcus citri* (Risso), commonly found associated with black pepper (*Piper nigrum* L.) plants in India was shown to transmit *Badnavirus* associated with stunted disease on the basis of symptomatology and polymerase chain reaction using *Badnavirus* specific primers.

KEYWORDS: *Badnavirus*, black pepper, citrus mealybug, *Piper nigrum*, *Planococcus citri*

Many species of mealybugs (Heteroptera: Pseudococcidae), besides being pests of many crops are also vectors of plant viruses, and are known to transmit many badnaviruses, closteroviruses and trichoviruses (Hull, 2002). The important genera involved in transmission of viruses include *Pseudococcus*, *Planococcus* and *Ferrisia* (Roivainen, 1980). Among them, *Planococcus* spp. and *Ferrisia virgata* (Cockerell) are commonly observed to infest black pepper (*Piper nigrum* L.) plants, the dried berries of which form an important item of international commerce for India, earning around Rs. 88 crores annually through export (Selvan, 2002). The crop is mainly grown in Kerala and Karnataka. However, stunted disease caused by viruses is an important production constraint of black pepper in India and other black pepper growing countries ((Duarte *et al.*, 2001; Lockhart *et al.*, 1997; Sarma *et al.*, 2001; Bhat *et al.*, 2003)). The disease is characterized by distortion, reduction in size, mottling and mosaic on leaves along with stunting of the whole plant, short spike length and poor filling of spikes leading to reduction in yield (Fig. 1). Two viruses namely, *Cucumber mosaic virus* (CMV) and *Badnavirus* were found associated with the disease (Sarma *et al.*, 2001; Bhat *et al.*, 2003). As black pepper is vegetatively propagated, the primary spread of the disease occurs through use of infected cuttings for planting and secondary spread in the field takes place through insect vectors. *Badnavirus* infecting

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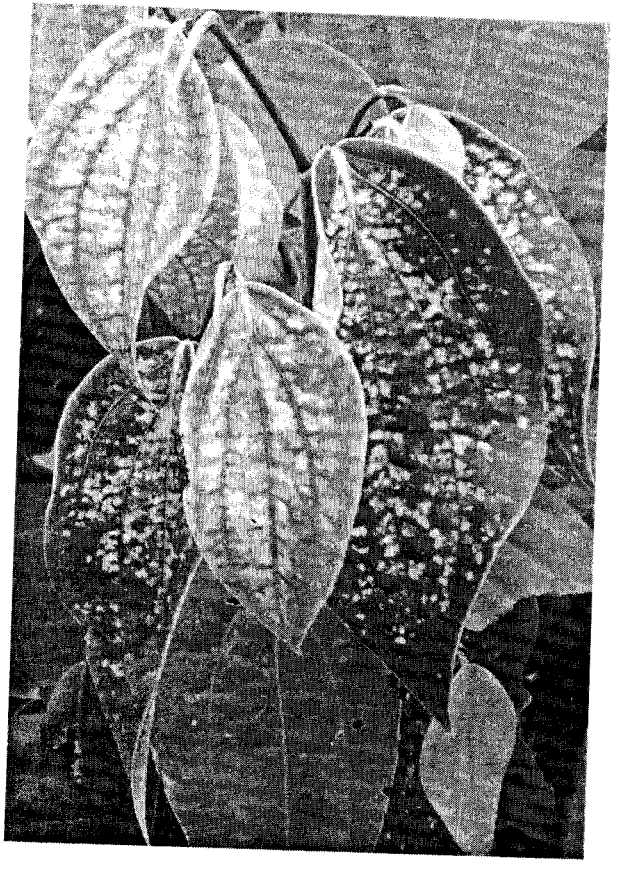

FIGURE 1. A naturally infected black pepper plant showing typical symptoms of *Badnavirus* infection.

black pepper was shown to be transmitted by striped mealybug, *F. virgata* in India (Bhat *et al.*, 2003). This paper reports an additional vector, namely, citrus mealybug, *Planococcus citri* (Risso) involved in the transmission of *Badnavirus* infecting black pepper in India.

The *Badnavirus* infected black pepper isolate collected during October 2003 from Indian Institute of Spices Research (IISR), Experimental Farm, Peruvannamuzhi, and maintained by vegetative propagation under insect proof glasshouse conditions was used as source for transmission studies. Mealybug transmission tests were done using *P. citri* commonly seen on shoots of black pepper plants (Fig. 2). The adults were collected from black pepper plants in the field (from Wyanad District, Kerala) and reared on mature pumpkins in the laboratory. After three generations on pumpkin, the non-viruliferous young adult female mealybugs were given a 24 h acquisition access on symptomatic black pepper leaves (on the lower surface) kept in petri plates lined

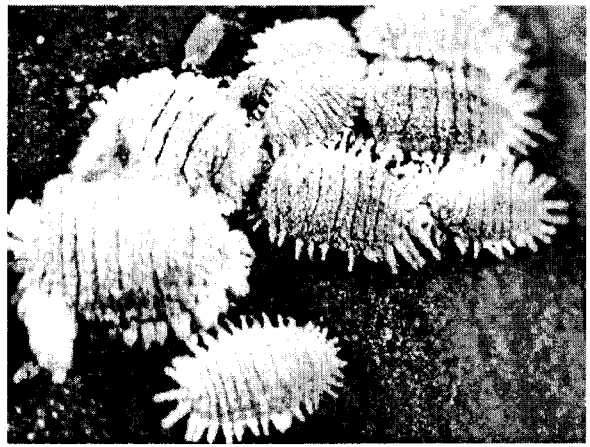

FIGURE 2. Citrus mealybug, *Planococcus citri* used in the transmission tests.

with moist filter paper and covered with black cloth. Healthy black pepper seedlings were raised from seeds under insect-proof conditions inside the greenhouse. Fifteen mealybugs each were then transferred to 30 day old healthy test seedlings of black pepper (20 seedlings of cv. Karimunda and 10 seedlings of var. Panniyur-I) at four leaf stage kept in a cage covered with black cloth. After an inoculation access period of 24 h, the plants were sprayed with chlorpyriphos @ 0.075% and were then removed from the cages and kept for observation in the insect proof glasshouse maintained at about 28 °C.

To confirm the presence of virus in the plants exhibiting symptoms of virus disease, total nucleic acids extracted from the plants were subjected to polymerase chain reaction (PCR) using *Badnavirus* specific primers. The protocol of de Silva *et al.* (2002) was used for isolation of total nucleic acids from plants. The primer pair derived from the hypothetical protein gene sequence of *Badnavirus, Piper yellow mottle virus* (PYMV) (de Silva *et al.*, 2002) was used to prime the amplification. The genome sense primer 5′ CTCCTTCATCTCCTCAAGAAGCCT 3′ was derived from the beginning of the first 24 bases of the coding region. The genome antisense primer, 5′ CACCCCCGGGCCAAAGCTCTGATACCA 3′ represented the last 27 bases of the coding region of the hypothetical protein gene (Gen Bank accession number AJ626981). The PCR reaction (50μl) contained 200 ng each of the primer, 2.5 units Taq polymerase (Genei, Bangalore), 1×PCR buffer (Genei, Bangalore), and 10μM each of the dNTPs (Finnzymes OY, Finland). PCR mix (40μl) containing the above components was added to the tubes containing the template DNA (10μl) resulting in a final reaction volume of 50μl. Amplification was performed in an automated thermal cycler (Eppendorf Master Cycler Gradient) programmed for 5 cycles of 94°C for 30 sec, 37°C for 30 sec, and 72°C for 2 min, followed by 25 cycles of 94°C for 30 sec, 58°C for 30 sec and 72°C for 2 min. Following PCR, reaction products

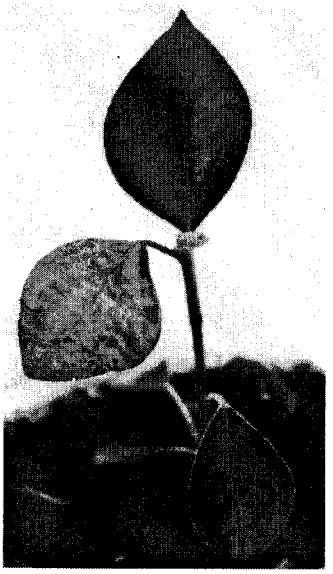

FIGURE 3. *Planococcus citri* transmitted black pepper seedling exhibiting initial symptoms of the disease.

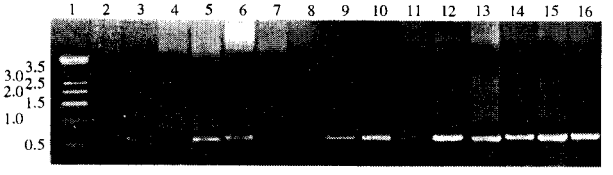

FIGURE 4. Agarose gel electrophoresis of polymerase chain reaction (PCR) products. Lane 1: 500 bp Marker DNA ladder; Lane 2: PCR product from uninoculated black pepper plant (negative control); Lanes 3–15: PCR product from mealybug transmitted black pepper plants; Lane 16: PCR product from badnavirus infected black pepper (positive control). Numbers on the left indicate MW of marker DNA bands in kb.

(20μl) were analysed by 1% agarose gel electrohoresis in Tris-acetate EDTA (TAE) buffer containing ethidium bromide. DNA was visualized and photographed using a UV transiluminator and a gel documentation apparatus (Alpha Innotech Corporation, USA). 500 bp ladder (Genei, Bangalore) was used as a size standard.

The *Badnavirus* could easily be transmitted by *P. citri* from naturally diseased black pepper to healthy seedlings of both cv. Karimunda and var. Panniyur-I. The initial symptoms of the disease like vein clearing and chlorotic mottle could be seen 4 weeks after inoculation (Fig. 3). Six out of 10 seedlings of Panniyur-I and 13 out of 20 seedlings of cv. Karimunda showed symptoms of the disease. The symptoms were more prominent on cv. Karimunda compared to var. Panniyur-I. Under natural

conditions also, disease symptoms are more prominent and severe on cv. Karimunda and related accessions while var. Panniyur-I shows mild symptoms. Total nucleic acid extracted from these plants when subjected to PCR, gave an expected PCR product of about 700 bp thus confirming the presence of virus in the plants (Fig. 4). No such band was seen in healthy seedlings. The specificity of the 700 bp band was confirmed by cloning and sequencing (data not shown).

Badnavirus is known to be associated with black pepper in many South East Asian countries and Brazil (Lockhart *et al.*, 1997; Duarte *et al.*, 2001; de Silva *et al.*, 2002; Bhat *et al.*, 2003). The disease was shown to be transmitted by *P. citri* in Indonesia, Philippines, Sri Lanka and Thailand (Lockhart *et al.*, 1997; de Silva *et al.*, 2002), *Pseudococcus elisae* in Brazil (Duarte *et al.*, 2001) and *F. virgata* in India (Bhat *et al.*, 2003). Thus, *P. citri* reported in this paper is an additional vector for the transmission of *Badnavirus* infecting black pepper in India. Nine species of mealybugs have been reported to be associated with black pepper in India (Devasahayam, 2000). Among them, *F. virgata, P. citri* and an undescribed *Planococcus* sp. are very common and abundantly seen. *F. virgata* and *P. citri* generally infest foliage and shoots while the undescribed *Planococcus* sp. exclusively infests roots.

Badnaviruses have been reported banana from (Anonymous, 1995), citrus (Ahlawat *et al.*, 1996), rice (Dasgupta *et al.*, 1996) and sugarcane (Viswanathan *et al.*, 1996) from India. In India, black pepper is grown as a mixed crop along with crops like banana, coffee, cocoa, citrus and tea, especially in Kerala and Karnataka. *P. citri* is also known to infest all these crops except tea and its main hosts include coffee, citrus and cocoa. These crops are grown to a large extent in Wyanad in Kerala where the incidence of viral disease on black pepper is also high (Bhat *et al.*, 2005). The present study suggests that *P. citri* found on these crops can also act as vector for the transmission of *Badnavirus* in black pepper.

REFERENCES

Ahlawat, Y. S., Pant, R. P., Lockhart, B. E. L., Srivastava, M., Chakraborthy, N. N. and Varma, A. (1996) Association of badnavirus with citrus mosaic disease in India. *Plant Disease* **80**: 590–592.

Bhat, A. I., Devasahayam, S., Sarma, Y. R. and Pant, R. P. (2003) Association of a badnavirus in black pepper (*Piper nigrum* L.) transmitted by mealybug (*Ferrisia virgata*) in India. *Current Science* **84**: 1547–1550.

Bhat, A. I., Devasahayam, S., Venugopal, M. N. and Bhai, R. S. (2005) Distribution and incidence of viral diseases of black pepper in Karnataka and Kerala, India. *Journal of Plantation Crops* (in press)

Dasgupta, I., Das, B. K., Nath, P. S., Mukhopadhyaya, S., Niazi, F. R. and Varma, A. (1996) Detection of rice tungro bacilliform virus in field and glasshouse samples from India using the polymerase chain reaction. *Journal of Virological Methods* **58**: 53–58.

de Silva, D. P. P., Jones, P. and Shaw, W. W. (2002) Identification and transmission of *Piper yellow mottle virus* and *Cucumber mosaic virus* infecting black pepper (*Piper nigrum* L.) in Sri Lanka. *Plant Pathology* **51**: 537–545.

Devasahayam, S. (2000) Insect pests. In: *Black Pepper Piper nigrum*, Ravindran, P. N. (Ed). Harwood Academic Publishers: Amsterdam, 309–334.

Duarte, M. L. R., Albuquerque, F. C. and Chu, E. Y. (2001) New diseases affecting black pepper crop in Brazil. *International Pepper Bulletin* April–December: 51–57.

Hull, R. (2002) *Matthews' Plant Virology*, 4th edn. Academic Press: New York, 1001.

Lockhart, B. E. L., Kirtisak, K. A., Jones, P., Padmini, D. S., Olsziewski, N. E., Lockhart, N., Nuarchan, D. and Sangalang, J. (1997) Identification of *Piper yellow mottle virus*, a mealybug-transmitted badnavirus infecting *Piper* spp in southeast Asia. *European Journal of Plant Pathology* **103**: 303–311.

Roivainen, O. (1980) Mealybugs. In: *Vectors of Plant Pathogens*, Harris, K. F. and Maramorosch, K. (Eds). Academic Press: New York, 15–38.

Sarma, Y. R., Kiranmai, G., Sreenivasulu, P., Anandaraj, M., Hema, M., Venkataramana, M., Murthy, A. K. and Reddy, D. V. R. (2001) Partial characterization and identification of a virus associated with stunt disease of black pepper (*Piper nigrum*) in South India. *Current Science* **80**: 459–462.

Selvan, M. T. (2002) *Arecanut and Spices Database*, Directorate of Arecanut and Spices Development: Calicut, 117.

Viswanathan, R., Alexander, K. C. and Garg, I. D. (1996) Detection of sugarcane bacilliform virus in sugarcane germplasm. *Acta Virologica* **40**: 5–8.

(Received 27 September 2004; accepted 20 December 2004)