Progress. Agric. 22(1 & 2): 19 – 25, 2011 ISSN 1017-8139

Research Note:

LIGHT MICROSCOPY AND PCR BASED DETECTION OF THE CAUSAL AGENT OF LEAF MOSAIC OF JUTE

K .M. G. Dastogeer*, M. A. Ali and M. Ashrafuzzaman[1]
Department of Plant Pathology, Bangladesh Agricultural University
Mymensingh-2202, Bangladesh

ABSTRACT

Leaf mosaic transmitted by whitefly is a devastating disease of jute. It is thought to be caused by a virus belonging to begomovirus genus under geminivirus family. To confirm the identity of the causal agent, infected and healthy leaves were studied using light microscope and by using polymerase chain reaction (PCR) technique of DNA. The inclusion bodies were observed under light microscope as large, blue-violet, prominent inclusion bodies in the nucleus of the infected leaf tissues. In molecular detection technique DNA from infected and healthy plants was extracted and analyzed by polymerase chain reaction (PCR) using degenerate primers PALIv1978/PARIc496. PCR fragment of the expected size 1.2kb for the common region (CR) in the geminivirus were obtained from infected plants. DNA collected from healthy plant did not show any band during electrophoresis. Therefore, it can be concluded that leaf mosaic of jute is cause by a virus.

Key Words: Causal agent, Detection, Light microscopy, Mosaic, PCR

Jute (*Corchorus capsularis* L. and *C. olitorius* L) is the most important cash crop of Bangladesh and thus, plays an important role in the economy of the country. Jute was once known as the golden fibre of Bangladesh. Among the jute growing countries of the world, Bangladesh ranks second in respect of production (Islam and Rahman, 2008). In 2010-2011, 8.40 million bales of jute were produced from 1.75 million acres of land (BBS, 2011). The fiber is chiefly used for manufacturing hessian, gunny bags, sacks and carpet. Gunny bags are used for storing and transporting grains, pulses, spices, sugar, cement, fertilizer, minerals, cotton and wool all the world over. The jute sticks are used as fuel and also for making gunpowder charcoal.

Jute plants suffer from different diseases. Among them leaf mosaic has been reported to be the most damaging one. This disease was first reported by Finlow in 1917. The leaf mosaic of jute has wide spread occurrence in the major jute growing countries of the world, like Bangladesh, Burma, India, Nepal and Pakistan (Dempsy, 1975). Leaf mosaic of jute has been considered as important limiting factor for jute cultivation (Harender *et al*., 1993).

*Corresponding Author: E-mail: dastogeer.ppath@bau.edu.bd

The disease is characterized by small yellow flakes on the lamina at initial infection stage which gradually increases in size to form green and chlorotic intermingled patches, produce yellow mosaic appearance. The infection can reduce plant height to the extent of 20% and adversely affect the yield of the fiber (Ghosh *et al*., 2008). The disease has been reported to be transmitted through grafts, seed and pollen (Saha, 2001). Whitefly transmission of the disease has also been reported (Ahmed *et al*., 1980).

It is reported that the causal agent of leaf mosaic of jute is a virus (Mitra *et al*., 1984, Ghosh *et al*., 2008). It is also reported that mycoplasma or rickettsia can also be the causal agent (Rabindran *et al*., 1988 and Biswas *et al*., 1992) or it could be a genetic disorder of the host cell. Identification of the causal agent of leaf mosaic of jute is a national demand to formulate control measures targeting the specific causal agent. There is no systematic study in this aspect in Bangladesh. Therefore, the study was undertaken to explore the identity of the causal agent of leaf mosaic of jute.

The study was carried out at the Molecular Plant Pathology Laboratory at the Division of Plant Pathology, Bangladesh Agricultural Research Institute, Gazipur during March 2011to December 2011.

Light microscopic study

The leaves were collected from young growing tips of infected and healthy jute plant.The tissue were prepared for staining in Azure- A (Cristie and Edwardson, 1967) by abrading with sand paper (600 mess). The chlorophyll was removed by placing the abraded tissue in 2-methoxyethanol for 15-30 minutes and then the tissue were stained in 0.1% Azure-A stain for 15-30 minutes. The tissues were washed sequentially in 95% ethanol and 2-methoxy ethyl acetate for 15-30 minutes each to remove the stain, blotted dry and mounted in a drop of Euparal on a glass slide, and cover slip before viewing under a light microscope. The specimens were then examined under the microscope at magnification ranging 100X to 1000X. The type, color and the location of inclusion body were then described.

Detection of the causal agent using PCR technique

Leaves from infected and healthy jute plants were collected and preserved in the laboratory at normal room temperature. DNA from each leaf sample was extracted following the protocol as described by Rojas *et al*., (1993). Approximately 25mg leaf tissue were taken in a mortar and ground with pestle in 300 µl extraction buffer solution and taken in 1.5 microfuge tube. The ground samples were vortexed (Vortex-Mixture: VM-2000, Taiwan) for 20 seconds for proper mixing. The samples were incubated at 65^{o}C for 10 minutes in water bath (WB-2400, Taiwan) and then centrifuged for 10 minutes at 10000g. The supernatant fluid (approx.250µl) was transferred to a clean microfuge tube and 50µl isopropanol was added. The tubes were vortexed and centifuged for 10 minutes at 10000g and supernatant fluid was removed. The pellete was washed with 200µl of 70% ethanol and centrifuged for 3 minutes at 10000g. The supernatant was discarded completely without disturbing the DNA pellete and dried for 5 minutes in a Speed Vac-drier. The

pellete were re-suspended in 300µl of distilled water. Finally the DNA samples were stored in a refrigerator at -20ºC. To get the PCR product begomovirus-specific degenerate primers (Rojas *et al*., 1993) were used to amplify the corresponding genomic fragments of the virus. Primer PAL1v1978 (5′-GCATATGCAGGCCCACATYGTCTTYCCNGT-3′) was designed to anneal to the complementary sense strand of the replicative form AL1 sequence encoding the derived amino acid sequence ThrGlyLysTh-rMet TrpAla, which was a conserved, putative NTP-binding site present in viral replication associated proteins. Primer PAR1c496 (5′-AATACTGCAGGGCTTYCTRTACATRGG-3′)was designed to anneal to the viral sense strand of the AR1 ORF sequence encoding for the conserved, derived amino acid sequence ProMetTyrArgLysProArg, which was located near the amino terminus of the coat protein. During the experiment, PCR buffer, dNTPs, and primer solution were thawed from frozen stocks, mixed by vortexing and placed on ice. DNA samples were also thawed out and mixed gently. The primers were pipetted first into PCR tubes compatible with the thermocycler used (0.2 ml). A pre-mix was then prepared in the following order: buffer, dNTPs, DNA template and sterile distilled water. Taq DNA polymerase enzyme was then added to the pre-mix, mixed well and aliquoted into the tubes containing primers. The tubes were sealed and placed in thermo-cycler. The cycling was started immediately. PCR amplification was carried out with 150 ng of each sample extract DNA. The presumed viral DNA was amplified under the following conditions: denaturation at 95°C for 3 minutes followed by 35 cycles at 95°C for 50 s, 55°C for 50 s and 72°C for 1 min with a final extension step of 72°C for 10 min. Amplified fragments were subjected to electrophoresis in 1% agarose gel and stained with 0.5 µg ml-1 ethidium bromide. The gel was placed under UV illuminator inside of a gel documentation system. DNA bands were observed, focused and the photograph was taken.

Inclusion bodies

Inclusion bodies were observed in the nucleus of host cell of the infected leaves. The inclusion bodies were large, black or blue-violet structure and were visible in the phloem parenchymatous cells of the mosaic infected leaves. Inclusion bodies in the nucleus of expanded mature cells were minimal. Nuclei in which numerous inclusion bodies were visible become hypertrophied. Inclusion bodies were scattered which can be seen under light microscope (100X) (Fig. 1a). The inclusion bodies were not uniformly distributed throughout the vascular system of leaf. The resolution of the stained inclusion bodies was confirmed at a higher magnification (Fig. 1b-1d). The tissue of healthy plants was free from any kind of inclusion body (Fig.1e-1f). Inclusion bodies appeared similar in the leaf samples of the infected plants of all cultivars. In some cases, quantitative differences in inclusions between the cultivars were noted. Inclusion bodies were also visible in parenchyma cells immediately outside the phloem.

Light microscopic techniques was tried to observe the inclusions associated with the presumed viral infection of the mosaic affected jute leaf. The result gave the excellent indication of the association of virus with the mosaic infected jute leaf. Conspicuous nuclear inclusions were observed in the mosaic infected jute leaf which is the diagnostic

character of geminivirus infection as described by Cristie *et al*., 1986. Similar inclusions were observed earlier by Schneider (1959) and were interpreted as viral inclusions. Similar result was obtained by Kim *et al*. (1979) from bean leaf tissue infected by bean golden mosaic virus and Lastra and Gil (1981) from tomato leaf infected with tomato yellow leaf curl geminivirus. The microscopy techniques described have several significant advantages over other procedures. Perhaps, most importantly, the infection can be detected within minutes, whereas even the relatively rapid serology procedures described in the companion paper normally require at least 24 hours. These microscopy procedures also provide physical information about the location of the causal agent within the host. The detection of stained inclusions by light microscopy requires no antiserum and only simple laboratory equipment. However, this is the first experiment of this kind with mosaic infected jute plant.

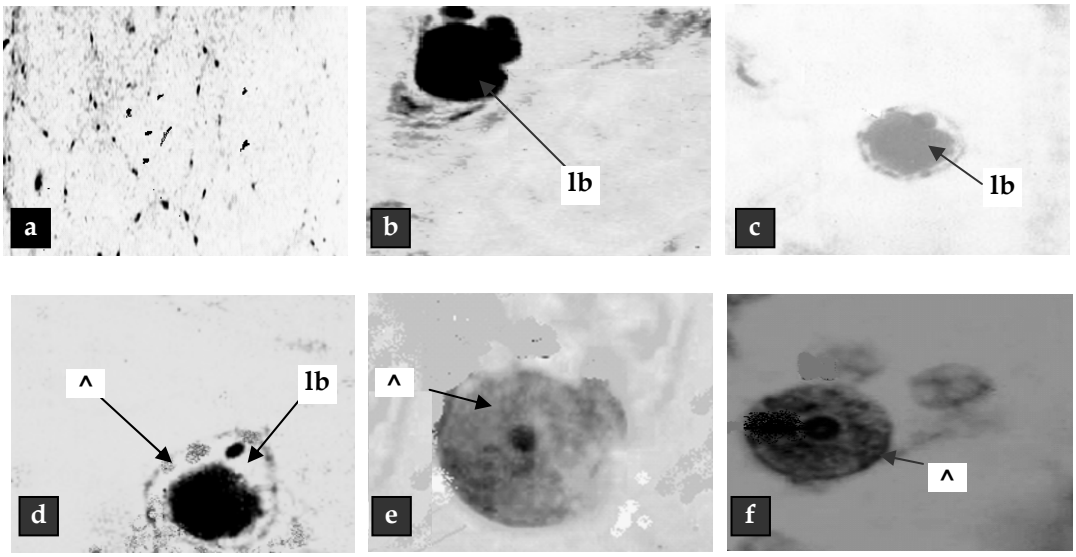

Fig. 1. Light micrograph, N= Nucleus, Ib = inclusion body (a) Distribution of inclusion bodies in the nucleus (100X), (b-d): Nuclear inclusion body (1000X) in the mosaic infected leaves, (e-f): Healthy leaf sample showing no inclusion body (1000X)

Detection of the causal agent of leaf mosaic of jute

A total of seven samples were collected all but one of which were from infected plants and rest one was from healthy plant. DNA fragment of Approximately 1.2 kb amplified by PCR using primers PAL1v1978 and PARIc496 was observed on 1% agarose gels for the sample corresponding to infected plants, while no amplification products were obtained from nucleic acids extracted from healthy plants and distilled water control (Fig.2). This result is in accordance with the result reported by Ghosh *et al*. (2008) who used the same primers and reported 1.2 kb amplification of DNA from the infected leaves. These primers have been used extensively for the identification of begomoviruses in a wide range of crop plants and their vector *B. tabaci* previously (Maruthi *et al*., 2006; Narayana *et al*., 2007;

Sharma *et al*., 2009 and Mahesh *et al*., 2010). However, this result is in opposition with the result obtained by Zaman and Albrechtsen (1999) who tried extract virus particle by ultracentrifugation and partial purification and failed to locate the causal agent. A Probable reason might be presumption that the causal agent as RNA virus. But a recent finding suggests that the causal agent of leaf mosaic of jute to be DNA containing begomovirus (Ghosh *et al*., 2008).

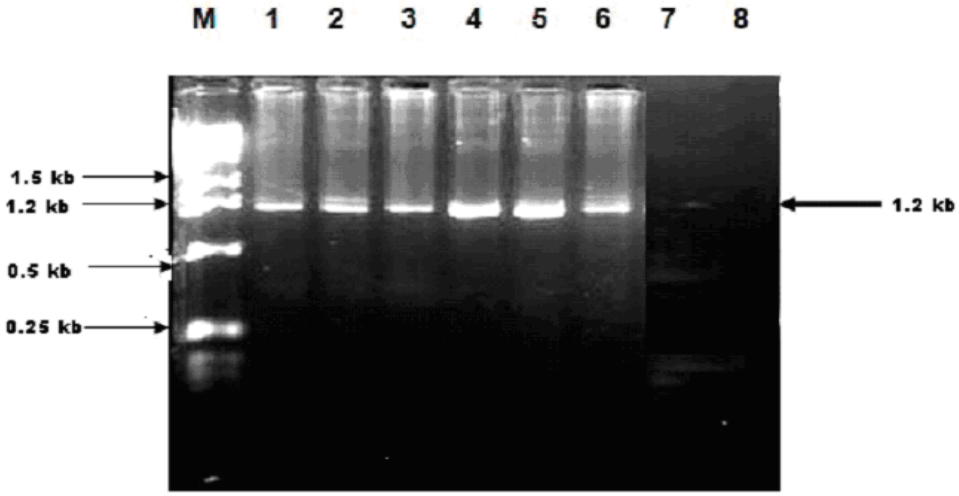

Fig 2: Agarose gel electrophoresis illustrating begomovirus-specific PCR products obtained using the primers PAL1v1978 and PAR1c496. Lanes: 1--4: field infected jute leaf samples, Lanes 5--6: whitefly inoculated samples, lane: 7 healthy jute leaf sample and lane 8: distilled water control, M: DNA 1 kb DNA ladder (Fermentas, Germany)

In conclusion the present experiment clearly indicates a DNA containing begomovirus under the family geminiviridae is the causal agent of the leaf mosaic of jute disease. However, further research can be undertaken to find out the whole genome sequence and genome organization of the virus causing leaf mosaic in jute.

This research was carried out with the financial assistance from, Bangladesh Agricultural University under the project entitled "Detection of the causal agent of leaf mosaic of jute."

REFERENCES

Ahmed, Q. A., Bishwas A. C., Farukuzzaman A. K. M., Kabir M. Q. and Ahmed N. 1980. Leaf mosaic disease of jute. Jute and Jute Fab. Bangladesh. Pp. 9-13.

BBS, 2011. Statistical Year Book of Bangladesh. 22nd Edition, Statistics Division. Ministry of Planning, Govt. of the People's Rep. of Bangladesh, Dhaka. pp. 97-98.

Biswas, A. C., Sultana, K., Begum, H. A., Iqbal. S. and Farukuzzaman, A. K. M. 1992. Diseases of Kenaf (Hibiscus canabinas) and Mesta (Hibiscus sabdariffa) recorded in Bangladesh. *Bangladesh Journal of Jute Fibre Research*, 17: 103-109.

Cristie, R. G. and Edwardson, J. R. 1967. Light and Electron microscopy of plant virus inclusions. Florida Agricultural Experiment Station. Monogr. Ser. pp. 9.

Cristie, R. G., Ko, N. J., Falk, B. W., Hiebert, E., Lastra, R. and Kim, K. S. 1986. Light microscopy of geminivirus-induced inclusion nuclear inclusion bodies. *Phytopathology*, 76: 124-126.

Dempsy, J. M. 1975. Fibre crops. University Press, Florida. USA. pp. 147.

Finlow, R. S. 1917. Annual Reports of the Department of Agriculture. Bengal "chlorosis. *Bengal Agricultural Journal*, 4: 118.

Ghosh, R., Paul, S., Das, S., Palit, P., Acharyya, S., Das, A., Mir, J. I., Ghosh, S. K. and Roy, A. 2008. Molecular evidence for existence of a New World begomovirus associated with yellow mosaic disease of Corchorus capsularis in India. *Australasian Plant Disease Notes*. 3: 59-62.

Harender, R., Bhardwaj, M. L., Sharma I. M. and Sharma, N. K. 1993. Performance of commercial okra (Hibiscus exculentus) varieties inrelation to disease and insect pests. *Indian Journal of Agricultural Science,* 63(11): 747-748

Islam, M. M. and Rahman, M. M. 2008. Hand book on agricultural technologies of Jute, Kenaf and Mesta crops. Bangladesh Jute Research Institute (BJRI). Dhaka, pp. 02.

Kim, K. S., Shock, T. L. and Goodman, R. M. 1978. Infection of phaseolus vulgaris by bean golden mosaic virus: ultrastructural aspects. *Virology*, 89: 22-23.

Lastra, R. and Gil, F. 1981. Ultrstructural host cell changes associated with tomato yellow mosaic. *Phytopathology*, 71: 236-241.

Mahesh, Y. S., Shankarappa, K. S., Rangaswamy, K. T., Prameela, H. A. and D. S. A. Narayana, D. S. A. 2010. Detection and characterisation of a begomovirus associated with leaf curl disease of ornamental croton (Codiaeum variegatum). J. Hort. Sci. Biotechnol., 85: 101-105.

Maruthi, M.N., Rekha, A.R. and Muniyappa, V. 2007. Pumpkin yellow vein mosaic disease is caused by two distinct begomoviruses: Complete viral sequences and comparative transmission by an indigenous Bemisia tabaci and the introduced B- biotype. *EPPO Bull*., 37: 412-419.

Mitra, D. K., Ahmed, Q. A. and Prakash, N. 1984. Electron microscopy of the virus causing leaf mosaic of jute in leaf exudates. *Science and Culture*, 50: 127-128.

Narayana, D. S. A., Rangaswamy, K. T., Shankarappa, K. S., Maruthi, M. N. and Reddy, C. N. L. 2007. Distinct begomovirus closely related to cassava mosaic viruses cause Indian jatropha mosaic disease. *Int. J. Virol.,* 3: 1-11.

Rabindran, R., Samy, K. P., Subbalaksmi, B, Jeyarajan, R. and Doraisamy, S. 1988. Phyllody a new disease of jute (cotchorus olitorius L.). *Review of Plant Pathology*, 69(6): 383.

Rojas, M. R., Gilbertson, R. L., Russell, D. R. and Maxwell, D. P. 1993. Use of degenerate primers in the polymerase chain reaction to detect whitefly transmitted geminiviruses. *Plant Dis*., 77: 340-347.

Saha, R. 2001. Study on the transmission of jute leaf mosaic causal agent. M. S. thesis. Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Sharma, A., Shankarappa, K. S., Rangaswamy, K. T., Dubey, R. K. and Maruthi, M. N. 2009. First record of duranta leaf curl, a begomovirus associated disease in India. *Ind. J. Virol*., 20: 88-89.

Zaman, M. A. and Albrechtsen. 1999. Studies on the seed borne leaf mosaic of jute. Danish Govt. Inst. of Seed Pathology for the Developing Countries. 78, Ryvangs Alle', DK-2900 Hellerup, Copenhagen, Denmark.