Identification of sida micrantha mosaic virus as the causal agent of common mosaic in cotton in Goiás

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ABSTRACT

Hoffmann, L.V.; Inoue-Nagata, A.K.; Vaz, L.N.A.; Barroso, P.A.V.; Faria, J.C. Identification of sida micrantha mosaic virus as the causal agent of common mosaic in cotton in Goiás. ***Summa Phytopathologica,*** v.47, n.4, p.222-224, 2021.

Detection and molecular identification of viruses are fundamental to define control strategies against viral diseases, particularly for whitefly-transmitted viruses. Cotton (*Gossypium*) plants showing leaf mosaic symptoms and yield reduction were observed in commercial cultivars (*G. hirsutum*) and in plants of the cotton germplasm collection of Embrapa maintained in the field (*G. hirsutum, G. barbadense, G. mustelinum*). DNA was extracted from cotton plants with symptoms of mosaic and interveinal chlorosis, and a begomovirus-specific genome was amplified with degenerated universal primers, which indicated their association with a begomovirus. This virus was identified as an isolate of sida micrantha mosaic virus (SiMMV) after the amplicon sequencing. The virus could not be transmitted by whiteflies (*Bemisia tabaci* MEAM1) to cotton plants when the latter were used as inoculum source under protected cultivation house, suggesting a complex interaction among viruses, plants and vectors.

Keywords: Whitefly, bolls, SiMMV, begomovirus, geminivirus.

RESUMO

Hoffmann, L.V.; Inoue-Nagata, A.K.; Vaz, L.N.A.; Barroso, P.A.V.; Faria, J.C. Identificação de sida micrantha mosaic virus como o agente causal de mosaico em algodão em Goiás. ***Summa Phytopathologica,*** v.47, n.4, p.222-224, 2021.

A detecção e identificação molecular de v**írus** são fundamentais para se definir estratégias de controle de viroses, tendo uma importancia crucial para v**írus** transmitidos por moscas-brancas. Plantas de algodoeiro (*Gossypium*) com sintomas de mosaico e redução de produtividade foram observadas em cultivares comerciais (*G. hirsutum*) e em acessos do banco de germoplasma de algodão da Embrapa mantidos em campo (*G. hirsutum, G. barbadense, G. mustelinum*). A extração de DNA a partir de plantas com sintomas de mosaico e clorose internerval e amplificação do genoma de begomovírus com primers degenerados e universais mostrou a associação com um begomovírus. Este vírus foi identificado como um isolado de sida micrantha mosaic virus **(**SiMMV) por sequenciamento do amplicon. O vírus não pode ser transmitido para algodão por mosca-branca (*Bemisia tabaci* MEAM1) quando plantas de algodão foram usadas como fonte de inóculo, em casa de cultivo protegido, sugerindo uma complexa interação entre vírus, plantas e vetores.

Palavras-chave: Mosca-branca, capulhos, SiMMV, begomovírus, geminivírus.

Cotton plants of different varieties are frequently observed showing mosaic symptoms in commercial fields. The major symptoms are chlorotic spots evenly distributed in a mosaic pattern all over the leaf, which presents green streaks randomly alternating with chlorotic streaks, neither restricted to veins nor limited by them. Young plants infected with begomoviruses may not develop, showing severe stunting. In mature plants, the symptoms usually appear in only one ramification, possibly due to restricted translocation in the plant. In general, only few infected plants are found, which makes this disease not believed to be responsible for remarkable production losses; however, they were not quantified yet. Symptomatic plants were observed in the Brazilian Cerrado, the biome where around 2 thousand tons of cotton lint are annually produced (1).

Viruses causing mosaic in cotton have been observed since 1954, and the disease is known as common mosaic (5). This disease was correlated with variegated chlorosis in other malvaceous plants, including the abundant *Sida* spp. (6). Intriguingly, whiteflies that had fed on infected sida plants were capable of transmitting the virus to healthy cotton plants, but not from infected cotton plants to other cotton plants (6).

To identify the virus that causes common mosaic in cotton plants and to estimate the consequent losses, plants showing mosaic and interveinal chlorosis symptoms were collected from central Brazil, in a Cerrado area (Goiás State). Samplings involving commercial varieties, breeding lineages and germplasm collection entries were carried out from naturally infected plants in the field (Figure 1).

Figure 1. *Gossypium hirsutum* plants showing leaf chlorotic spots in growing areas in central Brazil.

Part of the begomovirus genome was amplified from DNA extracts from symptomatic leaves using the begomovirus universal primers pAL1v1978 and pAR1c715 (9), which were expected to amplify part of the coat protein, the coding regions of Rep protein and the entire intergenic region from the component A of the genome of a bipartite begomovirus. PCR amplicons of ~1.3 kb were obtained from 22 out of 69 samples from cotton genotypes including the varieties BRS293, Deltapine Acala 90 and breeding lineages, in addition to native Brazilian cotton (*G. mustelinum*), collected in Bahia, and *G. barbadense* (3), collected in Maranhão and Minas Gerais, and sown at Santo Antonio de Goiás for multiplication and maintenance of genetic resources. Five samples were randomly selected, eluted from the agarose gel, purified, sequenced and compared with other begomovirus sequences using the blastN algorithm (7).

The obtained five sequences shared 94% to 97% nucleotide identity with those of sida micrantha mosaic virus (SiMMV). Among those five sequences, one was from the commercial variety BRS293 of *G. hirsutum*, collected in Santa Helena de Goiás, Goiás State. The other four were from the germplasm collection field: two plants of the native Brazilian cotton *G. mustelinum*, and two of *G. hirsutum* germplasm, LA RN 910 and 'Plains'. The virus isolated from cotton genotypes LA RN 910 and 'Plains' shared 95% nucleotide identity with SiMMV isolated from common bean GO60 (GenBank: KC706535.1 and HM357459.3). This virus was originally found in *Sida micrantha* plants (8), soybean (7) and okra (4) in Brazil.

Symptomatic plants produced fewer bolls than healthy plants. This was estimated using twenty pairs of diseased/healthy plants in the same field plot. Asymptomatic plants produced, on average, 28.5 bolls per plant, significantly higher than the quantity produced by infected plants (t test, p= 0.0054): an average of 17.2 bolls.

To verify whether SiMMV from cotton plants could be transmitted to other cotton plants by whiteflies, two inoculation trials were carried out in net protected cultivation houses. The whiteflies (*Bemisia tabaci* MEAM1) used for transmission tests were grown in an insect-proof cage in healthy cotton plants. Non-viruliferous whiteflies were fed on symptomatic and PCR-positive cotton plants of the commercial cultivar BRS 293 and of the Brazilian native species *G. mustelinum* for 48 hours. After the acquisition access period, 10 (first trial) or 20 (second trial) whiteflies were transferred to cotton plants (cultivars FM966 and BRS 293 in the first trial and DP1231 B2 RF and Buriti in the second trial) in individualized pots inside an aphid-proof cage. For each of the four cultivars, 10 plants were inoculated, and 10 plants remained as non-inoculated controls. The inoculation access period was 72 hours for the first trial or seven days for the second trial, during which most whiteflies remained alive within the cages. Then, the whiteflies were eliminated by application of a systemic insecticide. The plants were monitored and no symptom was observed after 28 days. DNA was extracted from each of the 80 individual plants and used for the detection test, as described above. None of the inoculated or non-inoculated plant samples resulted in the amplification of a begomovirus by PCR, indicating absence of infection, while the source plant was positive.

This is the second begomovirus identified in cotton in Brazil. Another begomovirus, *Cotton chlorotic spot virus* (CCSV) (2), was detected in cotton plants native to Northeast Brazil, in the Caatinga Biome. Whitefly control has become more difficult with the dispersion of polyphagous populations, also in cotton fields, to which losses are caused especially by honeydew deposited on the fibers. Honeydew-contaminated fibers may cause severe damage to gins and mills which are, therefore, rejected by the industry. Worldwide, the major problem associated with cotton viruses is caused by a begomovirus complex, c*otton* leaf curl *viruses* that are found in the Old World (10). We concluded that SiMMV is associated with cotton mosaic symptoms in cotton in Goiás.

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