

Detection of Bean golden mosaic virus in Fabaceae family plants

Detecção de Bean golde nmosaic vírus em plantas da família Fabaceae

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ABSTRACT

The Bean golden mosaic virus (BGMV) is the causal agent of the golden mosaic of bean, one of the main diseases of common bean (Phaseolus vulgaris). This virus is transmitted by the whitefly [Bemisia tabaci (Gennadius)], an insect of great agricultural importance due to its polyphagous behavior. This study aimed to identify plants of the Fabaceae family, host of BGMV. Sixteen plant species belonging to the Fabaceae family were inoculated with BGMV by whitefly. Fifteen days after inoculation, the plants were evaluated for the manifestation of the mosaic symptom, and leaves were collected for molecular detection of the virus. The experiment was carried out in a greenhouse, with a completely randomized design, five replicates, and one virus-free control for each species. The presence of BGMV was confirmed in Lathyrus sativus, Glycine max, Canavalia ensiformis, Vigna unguiculata and Macroptilium atropurpureum, in addition to beans (Phaseolus vulgaris).

Keywords: BGMV, Host Plants, *Bemisia tabaci*, Infection.

RESUMO

O Bean Golden mosaic vírus (BGMV) é o agente causal do mosaico dourado do feijoeiro, uma das principais doenças do feijão comum (*Phaseolus vulgaris*). Esta virose é transmitida pela moscabranca [Bemisia tabaci (Gennadius)], um inseto de grande importância agrícola devido ao seu comportamento polífago. Este estudo objetivou identificar plantas da família Fabaceae, hospedeiras de BGMV. Dezesseis espécies de plantas pertencentes à família Fabacea e foram inoculadas com BGMV por meio de mosca-branca. Quinze dias após a inoculação, as plantas foram avaliadas quanto à manifestação do sintoma de mosaico, e folhas foram coletadas para a detecção molecular do vírus. O experimento foi realizado em casa de vegetação, com delineamento inteiramente casualizado, cinco repetições, e uma testemunha livre de vírus para cada espécie. A presença de BGMV foi confirmada em Lathyrus sativus, Glycine max, Canavalia ensiformis, Vigna unguiculatae Macroptilium atropurpureum, além do feijão (Phaseolus vulgaris).

Palavras-chave: BGMV, Plantas Hospedeiras, Bemisia tabaci, Infecção.

1 INTRODUCTION

The golden mosaic is the main virus that affects the common bean culture. This disease, caused by the Bean golden mosaic virus (BGMV), is considered the most harmful to bean culture in the tropical and subtropical regions of the Americas (Costa, 1987; Lemos et al., 2003; Wendland et al., 2016), causing losses of 40 to 100% in grain production (Wendland et al., 2016).

BGMV belongs to the genus Begomovirus, Geminiviridae family, has twinted icosahedric particles, and each monomer is between 18 and 20 nm (Lazarowits, 1992). The genetic material of this virus consists of two components of circular single-stranded DNA, called DNA-A and DNA-B. DNA-A contains genes related to viral replication, synthesis of the protein coat, regulation of gene expression and viral particles encapsidation, while DNA-B has genes related to viral movement and symptoms expression (Timmermans, Das & Messing, 1994; Brown, 1997).



The most prevalent symptoms caused by BGMV are intense yellow mosaic throughout the leaf blade, dwarfism, shortening of between nodes, loss of apical dominance and superbrotation of axillary gems (Furlan, 2004). According to Bianchini, Menezes and Maringoni (1989) the symptoms expressed by theplant, when infected with this virus, can be grouped into two types: wrinkling (or severe deformations) and mosaic. Plants that present wrinkling undergo a drastic reduction in size, and may occur excessive lateral shoots and resulting in the symptom called superbrotation. Mosaic plants, on the other hand, have a less accentuated reduction than in development (Wendland et al., 2016).

The BGMV is not transmitted by seeds, or mechanically, the only form of dispersion in nature is through the vector insect, the whitefly [Bemisia tabaci (Gennadius)] (Hemiptera, Aleyrodidae) (Dhar&Sinhg,1995), with persistent circulating non-propagating transmission modality (Costa, 1965). In this case, the virus isacquired by the vector over a long feeding period in the phloem, circulates in the insect body, reaches the salivary glands, and is subsequently injected into the healthy plant at the feeding time (Rezende & Kitajima, 2018; Glosh, Rao & Baranwal, 2019).

Since the 1950s, the existence of B. tabaci biotypes has been proposed to distinguish populations that differed in biological characteristics in relation to the host range, adaptability to the host plant, variability in the transmission efficiency of viruses, and ability to cause phytotoxity (Brown 2000). In Brazil, the occurrence of biotypes A (New World - NW), B (specie Middle East-Asia Minor 1 - MEAM 1), Q (specie Mediterranean - MED) and NW2 (New World2) has already been observed (Marubayashi et al., 2013; Barbosa et al., 2014), while in the Paraná state the biotype B prevails (Walz, 2017), although biotype Q has also been identified (Moraes et al., 2018).

B. tabaci is a highly polyphagous insect, which causes several damages in more than 600 plant species, responsible for the transmission of several plant viruses (Brown, Frohlich & Rosell, 1995; Perring, 2001). Due to its polyphagous character, the chances of BGMV transmission by B. tabaci for non-cultivated plants species that are close to the cultivation of common bean is very large (Ribeiro et al., 2003).

Among the plant species reported as BGMV hosts in Brazil, constituting an important pathogen source to be transmitted to the common bean crop, those belonging to the Fabaceae family stand out (Pinto et al., 2016). Thus, this study aimed to identify host species of Bean golden mosaic virus belonging to the Fabaceae family.



2 MATERIAL AND METHODS

HOST PLANTS

Sixteen plant species belonging to the Fabaceae family were used in this study: common bean (*Phaseolus vulgaris* L.) cultivar Carioca, lima-beans (*Phaseolus lunatus*), grass pea (*Lathyrus sativus*), black gram (*Vigna mungo* L. Hepper), soybean (*Glycine max* L. Merrill), Jack beans (*Canavalia ensiformis*), cowpea (*Vigna unguiculata*), crotalaria (*Crotalaria spectabilis, Crotalaria ochroleuca, Crotalaria juncea, Crotalaria incana*), lablab (*Lablab purpureus*), siratro (*Macroptilium atropureum*), common vetch (*Vicia sativa*), white lupine (*Lupinus albus*), and velvet bean (*Mucuna pruriens*).

The experimental design was completely randomized, with five replications, and a virus-free control plant for each plant species tested. The experiment was conducted in a greenhouse at the Institute of Rural Development of Paraná IAPAR-EMATER (IDR-Paraná), Londrina, Parana, Brazil.

BEAN GOLDEN MOSAIC VIRUS TRANSMISSION

The BGMV isolate came from the research station of IDR-Paraná, Londrina, Paraná, Brazil. It was collected from a common bean plant (*Phaseolus vulgaris*) cultivar Carioca, presenting typical symptoms of BGMV infection (mosaic and wrinkling). The plants were inoculated with the viral isolate by vector insect *B. tabaci* (whitefly) transmission. The insects were kept in cages coated with anti-aphids, in symptomatic BGMV isolate presence for a period of 48 hours for the acquisition of the virus. After this period, seedlings aged seven days from germination, of the species belonging to the Fabaceae family selected for this study, were placed in the cage for the virus transmission. After 24 hours of exposure to the source of inoculum with whitefly, the seedlings were removed from the cage, and insecticide was applied for the removal of the vector insect. The seedlings were transplanted to pots containing autoclavated clay soil.

SYMPTOM ASSESSMENT AND SAMPLE COLLECTION

The plants were visually evaluated for mosaic and wrinkling symptoms (presence or absence), fifteen days after inoculation. To confirm BGMV infection, after symptom evaluation, top leaves were collected individually from each plant, packing it in foil and ice. The samples were taken to the Virology laboratory of IDR-Paraná and then submitted to DNA extraction and conventional polymerase chain reaction (PCR) detection.



TOTAL DNA EXTRACTION AND VIRAL DETECTION BY PCR

For DNA extraction was followed by the cetyl trimethyl ammonium bromide (CTAB) method, proposed by Murray and Thompson (1980), with some modifications according to Freitas-Vanzo et al. (2020). The BGMV detection from the total DNA extracted from the plants was performed by conventional PCR, using the oligonucleotides PAL1v 1978/PAR1c 496 (Rojas et al. 1993). The reaction was prepared in a volume of 25 μL, containing 10 ng of DNA; 0.3 μL of each oligonucleotide at 20 μM; 0.75 μL of MgCl₂; 1 μL of dNTPs at 5μM;2.5 μL of buffer 10XTaq DNA Polymerase Buffer; 1U of Taq DNA Polymerase; and water MiliQ to complete the final volume of 25 μL. The reaction occurred in thermocycler, with initial denaturation at 94 °C for 5min, followed by 35 cycles of denaturation at 94 °C for 45s, annealing at 60 °C for 30s and extension at 72 °C for 45s, ending with an extension at 72 °C for 20 min. The PCR product was stained with SYBR Gold and separated electrophoretically, using 1% agarose gel in TBE buffer [Tris base 10.8 g, 5.5 mL boric acid and 4 mL of EDTA 0.5 mol. L⁻¹(pH 8.0)]. The marker of molecular weight 1kb DNA Ladder Plus was used as standard. After electrophoresis, the gel was analyzed using the Photodocumenter L-PIX Molecular Imaging (Loccus).

3 RESULTS

Among the sixteen species used in this study, *Bean golden mosaic virus* (BGMV) infection was confirmed in seven species, they are: common bean (*Phaseolus vulgaris* L.) cv. Carioca, limabeans (*Phaseolus lunatus*), grass pea (*Lathyrus sativus*), soybean (*Glycine max*), Jack beans (*Canavalia ensiformis*), cowpea (*Vigna unguiculata*) and siratro (*Macroptilium atropureum*) (Table 1; Figure 1).

Table 1. List of plant species of the Fabaceae family used in the study, result of the *Bean golden mosaic virus* (BGMV), and detection by PCR of the DNA-A coat protein gene.

Species	Symptom	Detecção
Phaseolus vulgaris L.	M/W	Positive
Phaseolus lunatus	M	Positive
Lathyrus sativus	NO	Positive
Vigna mungo L. Hepper	NO	Negative
Glycine max L. Merrill	M	Positive
Canavalia ensiformis	M	Positive
Vigna unguiculata Crotalaria spectabilis Crotalaria ochroleuca	M NO NO	Positive Negative Negative
Crotalaria juncea	NO	Negative
Crotalaria incana Lablab purpureus	NO NO	Negative Negative
Macroptilium atropurpureum	M	Positive

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Negative

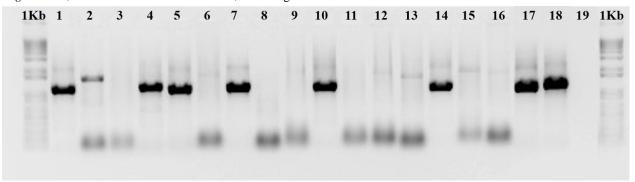


Mucuna pruriens

Vica sativa	NO	Negative
Lupinus albus	NO	Negative

- M - Mosaic; W - Wrinkling; NO - Not observed.

Figure 1. Agarose gel electrophoresis of PCR products to detect *Bean golden mosaic virus*, by DNA-A coat protein amplification, producing a 1.100 bp amplicon. The samples were stained with SYBR® Gold nucleic acid gel stain. The lines correspond to: 1 - Phaseolus vulgaris L. cv. Carioca; 2 - Lablab purpureus; 3 - Vigna mungo L. Hepper; 4 - Glycine max L. Merrill; 5 - Canavalia ensiformis; 6 - Lupinus albus; 7 - Phaseolus lunatus; 8 - P. vulgaris cv. Carioca negative control; 9 - Crotalaria spectabilis; 10 - Lathyrus sativus; 11 - Crotalaria ochroleuca; 12 - Crotalaria incana; 13 - Crotalaria juncea; 14 - Macroptilium atropureum; 15 - Vicia sativa; 16 - Mucuna pruriens; 17 - Vigna unguiculata; 18 - Positive controlto BGMV; 19 - Negative controlto BGMV.



The manifestation of typical symptoms of common bean golden mosaic was observed in the species of common bean, lima-bean, soybean, Jack bean, cowpea and siratro (Table 1; Figure 2).

Figura 2. Plants species of the Fabaceae family host of *Bean golden mosaic virus*, with manifestation of golden mosaic symptoms; (a) *Phaseolus vulgaris* L. cv Carioca; (b) *Phaseolus lunatus*; (c) *Glycine max* L. Merrill; (d) *Canavalia ensiformis*; (e) *Vigna unguiculata*; (f) *Macroptilium atropurpureum*.





4 DISCUSSION

A total of sixteen samples were tested for transmission and susceptibility to BGMV in this study, we observed that in addition to common bean (*Phaseolus vulgaris* L.), lima-bean (*Phaseolus* lunatus), grass pea (Lathyrus sativus), soybean (Glycine max), Jack beans (Canavalia ensiformis), cowpea (Vigna unguiculata) and siratro (Macroptilium atropurpureum) are susceptible to BGMV (Figure 1; Table 1), corroborating the data presented by Wendland et al. (2016), which observed that the circle of BGMV host plants is apparently restricted to the Fabaceae family, especially of the genus Phaseolus, in addition to Glycine max, Calopogonium mucunoides, Canavalia ensiformes, Vigna spp. and Macroptilium spp.

The compatible interactions between host species and the virus are characterized by the establishment of the infection, indicating the presence of proviral cellular factors and resources necessary for infection and movement of the virus (Calvo, Malinowski & Garcia, 2014; Calvo, Martinez-Turino Garcia, 2014; Lv et al., 2017; Otulak-Koziel, Koziel Lockhart, 2018).

On the other hand, plant-virus combinations can result in incompatibility. These incompatible interactions occur between a virus and a non-host plant, being characterized by the absence of virus infection, which can be explained by the lack of cellular factors essential for replication or viral movement, antiviral defense or a combination of both (Lellis et al., 2002; Jaubert et al., 2011), as observed for the species Vigna mungo, Lupinus albus, Crotalaria spectabilis, Crotalaria incana, Crotalaria juncea, Crotalaria ochroleuca, Lablab purpureus, Vica sativa and *Mucuna pruriens* (Figure 1; Table 1).

In addition to the detection of BGMV, in the present study it was also possible to observe that some host species were symptomatic to this virus, exhibiting the mosaic phenotype (Table 1; Figure 2). The occurrence of symptoms during viral infection is the result of a complex interaction between the virus and its host plant (Zanardo, Souza & Alves, 2019). In the case of the expression of the mosaic symptom, the modulation of photosynthesis seems to be a conserved strategy for most viruses toimprove their fitness (reproduction and experience), while drastic changes in the components and architecture of chloroplasts, caused by this microorganism, lead to the occurrence of this symptom (Zhao et al., 2016).

It is also important to consider that in the present study we found that the transmission of BGMV to P. lunatus, L. sativus, G. max, C. ensiformis, V. unguiculata and M. atropurpureum through whitefly was efficient. This insect is sucking type, and is very important in agricultural terms, due to its ability to infest more than 600 plants species (Glosh et al., 2019). The virus transmission occurs during feeding, which will cause uneven plant development and drop in productivity (Tomquelski et al., 2020).



According to Watanabe et al. (2019) there is no competition between the different whitefly biotypes in the field, between soybean, beans, cotton, tomato and pepper crops, and the food preference of each biotype to these crops, has caused great concern to farmers, since the occurrence of the pest can become constant in the production fields.

Besides having a large number of hosts, the whitefly also has great ability to reproduce, adapt to adverse conditions, and develop resistance to pesticides. This has made the control measures for this pest do not provide the desired efficiency (Lacerda&Carvalho, 2008). However, there are some control alternatives that can be associated with keeping the whitefly at levels thatdo not harms cultures. In general, integrated pest management should be adopted, where cultural, chemical and biological control methods are involved (Silva et al., 2017).

5 CONCLUSIONS

The species *Phaseolus lunatus*, *Lathyrus sativus*, *Glycine max*, *Canavalia ensiformis*, *Vigna unguiculata* and *Macroptilium atropurpureum*, are hosts of *Bean golden mosaic virus*.



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ABBREVIATIONS

BGMV, Bean golden mosaic virus; CTAB, cetyltrir methyl ammonium bromide; DNA, deoxyribonucleic acid; DNA-A, deoxyribonucleic acid A; DNA-B, deoxyribonucleic acid B; dNTPs, phosphate deoxyribonucleotides; EDTA, ethylenediaminetetraacetic acid; H₂O, water; MEAM 1, Specie Middle East-Asia Minor 1; MED, Mediterranean specie; NaCl, sodium chloride; NW2, new world 2; PCR, polymerase chain reaction; PVP, polyvinylpyrrolidone.