INOCULATION OF BEAN AND SOYBEAN WITH CLONED BEAN GOLDEN MOSAIC VIRUS (BGMV) DNA USING PARTICLE ACCELERATION*

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ABSTRACT

Seeds of beans (cvs. Carioca and Jalo) and soybeans (cvs. Davis, Doko, Eureka, Cariri, BR-15 and Cristalina) were bombarded with the two cloned components (A and B) of bean golden mosaic virus (BGMV), isolated from Brazil. Beans cultivars Carioca and Jalo, and soybeans cvs. Davis and Cariri developed typical golden mosaic symptoms. The presence of the virus was confirmed by squash blot hybridization.

Key words: bean golden mosaic virus, virus inoculation, particle acceleration.

RESUMO

Inoculação de feijão e soja com o DNA clonado do vírus do mosaico dourado do feijoeiro (BGMV) através da aceleração de partículas

Sementes de feijão (cvs. Carioca e Jalo) e soja (cvs. Davis, Doko, Eureka, Cariri, BR-15 e Cristalina) foram bombardeadas com os dois componentes (A e B) clonados, do vírus do mosaico dourado do feijoeiro (BGMV) isolados do Brasil. Os cultivares de feijão: Carioca e Jalo e de soja, Davis e Cariri, apresentaram sintomas típicos de mosaico dourado. A presença do vírus na planta foi confirmada através de "squash blot hybridization".

Bean golden mosaic virus (BGMV) is a geminivirus infecting common beans (*Phaseolus vulgaris* L.) in Brazil, Argentina, Central America, Caribbean countries and United States, where it causes significant yield losses. This virus possesses twinned (geminate) icosahedral virions and single-stranded DNAs in a bipartite genome. The two distinct DNA components, designated DNA-A and DNA-B, of the Brazilian isolate of BGMV (BGMV-BZ) were recently cloned and sequenced (Gilbertson *et al.*, 1993). The bean golden mosaic virus is transmitted by whitefly (*Bemisia tabaci* G.) and both DNA components are required for plant infection. Some isolates of BGMV, particularly those from Brazil, are not sap-transmitted as virions to beans (Gilbertson *et al.*, 1991b). Recently, it was reported that cloned DNA of BGMV-BZ was introduced into bean and soybean (*Glycine max* (L.) Merril) genome by microprojectiles accelerated using an electrical discharge device (Gilbertson *et al.*, 1991a).

This report describes a procedure to infect bean and soybean with cloned DNAs of BGMV-BZ utilizing two biolistic devices built in our laboratory (Rech *et al.*, 1991; Finer *et al.*, 1992). Two cultivars of bean and six of soybean,

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which have been widely cultivated in Brazil and in other Latin America countries, were utilized in this study.

Full-length DNA components A and B from BGMV-BZ were cloned in the vector pBS(+) at *HindIII* and *AccI* restriction sites, respectively (Gilbertson *et al.*, 1991a).

Mature seeds of bean (cvs. Carioca and Jalo) and soybean (cvs. Davis, Doko, Eureka, Cariri, BR-15 and Cristalina) were surface sterilized with sodium hypochloride (1%) and placed in vermiculite. Germination occurred at 28°C with 16 h photoperiod. Three days after germination, the seeds were immobilized in the center of a Petri plate with 12% xanthan gum (Sigma, G1253). One cotyledon of each germinated seed was removed to expose the primary leaves and the embryonic axes.

For the bombardment, plasmid DNAs containing component A and B were digested with *HindIII* and *AccI*, respectively, to excise the viral inserts and purified with a phenol-chloroform extraction. DNA was coated onto 1.5-3.0 μm average diameter gold particles (Aldrich Chemical Co., Inc.) by adding 1 μg of both viral DNA components to 250 μg of gold particles, as described by Aragão *et al.* (1992).

The particle bombardment was carried out as previously described (Aragão *et al.*, 1992; Aragão *et al.*, 1993), using an electrical discharge (Rech *et al.*,1991) or a low pressure (100 psi) helium-driven device (Finer *et al.*, 1992). After bombardment, the seeds were transferred to soil and maintained in a greenhouse. Symptoms were evaluated 4-6 weeks after the bombardment. Infection of the bombarded plants was confirmed by squash blot hybridization, according to Gilbertson *et al.* (1991b).

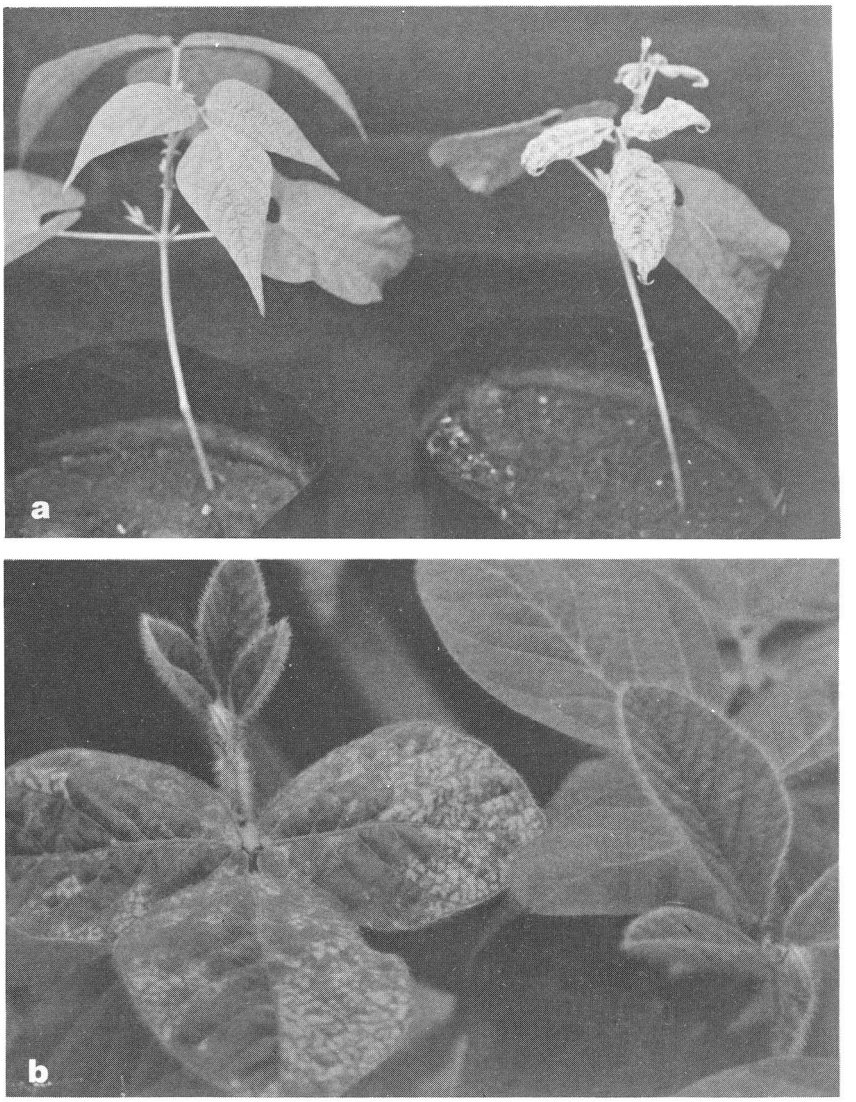

FIG. 1 - Plants one month after inoculation by electric particle acceleration with the cloned components A and B of BGMV-BZ. a) Bean cv. Jalo, with golden mosaic (right) and the control plant (left).; b) soybean cv. Davis, plant with golden mosaic symptoms (left) and the control plant (right).

Three to four weeks after the bombardment, typical golden mosaic symptoms appeared in trifoliolate leaves of Carioca and Jalo bean cultivars. Infected plants showed reduced and distorted growth (Fig. 1). The presence of the virus was confirmed by squash blot hybridization (data not shown). The primary leaves did not develop symptoms of the disease. Virus symptoms were less severe in the cultivar Carioca.

Soybean cultivars Davis and Cariri developed mild golden mosaic symptoms with yellow-gold flecks on trifoliolate leaves and little or no leaf distortion. The presence of the virus in these plants was confirmed by squash blot hybridization (Fig. 2). The probe used was the component A excised with *Hind*III from the plasmid pBS(+). As in bean, primary leaves did not develop any symptoms. None of the plants of the other soybean cultivars presented symptoms or were positive for the presence of total nucleic acids in their leaves (Table 1). The fact that some cultivars were not infected does not necessarily means that they are resistant to BGMV. Other studies should be conducted to evaluated this possibility.

As shown in Table 1, there was no difference in using an electrical discharge or a helium-driven device. Similar results were found by Garzón-Tiznado *et al.* (1993) bombarding *Capsicum annum* with cloned DNA with infectious clones of a new geminivirus (PHV), using a high-pressure helium device.

These results have demonstrated that particle acceleration is an efficient method for the introduction of cloned BGMV DNA in Brazilian cultivars of bean and soybean. The procedure described has several attractive features: (a) is rapid, (b) requires relatively small amounts of DNA and (c) is an alternative method to *Agrobacterium*-mediated inoculation of plants with cloned viral DNA. Moreover, particle

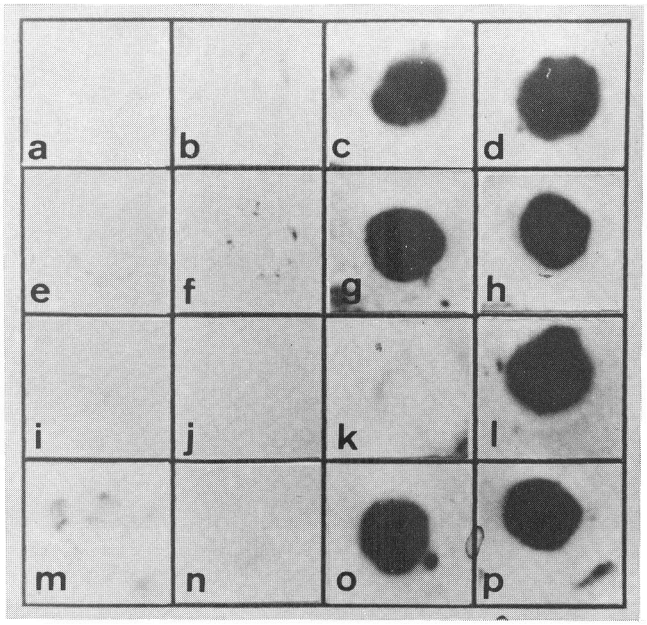

FIG. 2 - Squash blot hybridization of soybean leaf tissue inoculated with BGMV by particle acceleration. Plants developed symptoms four weeks after bombardment a-b: cv. Davis (ns); c-d: cv. Davis (s); e-f: cv. Cariri (ns); g-h: cv. Cariri (s); i-j: cv. Doko (ns): k: negative control (non-infected); l: positive control (infected bean); m-n: cv. Eureka (ns); o: soybean infected in the field; p: bean inoculated with whitefly in the greenhouse. (s: plants with symptoms. ns: plants without symptoms).

acceleration is a way to deliver cloned DNA into hosts which the virus can not be sap-transmitted. Also, this method can be utilized as a rapid procedure to determine the host range of the virus and might contribute to study the molecular aspects of the virus.

TABLE 1 - Infection of bean and soybean with BGMV-BZ by particle acceleration. Symptoms were evaluated 4-6 weeks after inoculation. Controls did not develop any symptoms.

Cultivar	Number of independent experiments	Plants with symptoms/number of inoculated plants	
		ED	HD
Bean			
Carioca	7	59/70	77/90
Jalo	5	44/55	38/50
Soybean			
Davis	3	12/30	9/20
Doko	3	0/25	0/25
Eureka	3	0/15	0/15
Cariri	3	15/30	10/25
BR-15	3	0/15	0/20
Cristalina	3	0/20	0/15

ED:Electrical device; HD: Low-pressure helium device.

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