Reactions of Selected Bean Germ Plasms to Infection by *Fusarium oxysporum* f. sp. *phaseoli*

M. A. PASTOR-CORRALES, Plant Pathologist, Bean Program, Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia, and G. S. ABAWI, Professor, Department of Plant Pathology, New York State Agricultural Experiment Station, Cornell University, Geneva 14456

ABSTRACT

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A Brazilian isolate of *Fusarium oxysporum* f. sp. *phaseoli* (*F. o.* f. sp *phaseoli*) was used to determine the reactions of 66 lines of beans to the Fusarium yellows pathogen in greenhouse tests. The distal 1 cm of root tips of 1-wk-old seedlings grown in sterilized sand were removed, roots were dipped in a spore suspension of *F. o.* f. sp. *phaseoli* for 5 min, and the seedlings were then transplanted into pots filled with pasturized soil. As the concentration of *F. o.* f. sp. *phaseoli* spores was increased from 0 to 1×10^7/ml, incubation time to the first disease symptom was decreased and disease severity ratings (DSR) increased on the field-susceptible cultivar IPA 1. However, only slight disease symptoms occurred at a spore concentration of 1×10^7 on the line HF 465-63-1, known to show resistance under field conditions in Brazil. Severely infected plants became defoliated and often died within 2–3 wk. The dead stem and petiole tissues were covered with pink to orange spore masses. The extent and intensity of vascular discoloration was generally proportional to the overall foliar disease severity. Forty bean accessions evaluated with an inoculum concentration of 1×10^6 spores per milliliter were resistant (DSR 1–3), six were intermediate (DSR 3.1–6.0), and 20 were susceptible (DSR 6.1–9.0). Based on these results, an international nursery consisting of 48 entries of bean germ plasm for evaluation against *F. o.* f. sp. *phaseoli* has been formulated and is available for distribution from the Centro Internacional de Agricultura Tropical (CIAT).

Fusarium oxysporum (Schlecht.) f. sp. *phaseoli* Kendrick & Snyder (*F. o.* f. sp. *phaseoli*) is the causal agent of Fusarium yellows of beans. Recently, Ribeiro and Hagedorn (9) described two pathogenic races of *F. o.* f. sp. *phaseoli* based on the differential reactions of bean germ plasms to three isolates of the pathogen obtained from Brazil, the Netherlands, and the United States. The isolates of *F. o.* f. sp. *phaseoli* from the United States and the Netherlands represented one race, whereas the isolate from Brazil was considered a different race. It was later found (10) that resistance to the Brazilian race of *F. o.* f. sp. *phaseoli* is controlled by a dominant gene, designated *Fop*1, which is present in the bean cultivars Early Gallatin, Tenderette, and Pintado. In contrast, an incompletely dominant gene (*Fop*2) was found in cultivar Preto Uberabinha that confers resistance to the European and North American race of *F. o.* f. sp. *phaseoli*. It was postulated that resistance to both races of *F. o.* f. sp. *phaseoli* was expressed by restriction of growth of the pathogen in infected tissues.

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Severe outbreaks of Fusarium yellows have been reported from many countries in Latin America including Brazil, Colombia, Panama, and Costa Rica (6,7). The authors observed severe epidemics of Fusarium yellows of beans during July and August of 1985 in several states of the northeastern region of Brazil. Losses were especially high in the states of Bahia and Pernambuco during the drought and high temperature that prevailed during this period. The objective of this study was to evaluate selected bean germ plasms against *F. o.* f. sp. *phaseoli* using an accurate and rapid greenhouse inoculation method.

MATERIALS AND METHODS

A highly virulent isolate of *F. o.* f. sp. *phaseoli* (F5) obtained from severely infected plants with typical symptoms of Fusarium yellows near Belem do Sao Francisco, Pernambuco, Brazil, was used. Inoculum of *F. o.* f. sp. *phaseoli* was produced on acidified potato-dextrose agar (APDA) plates incubated at 25 C for 2 wk. Spore suspensions (microconidia, macroconidia, and a few chlamydodpores) were prepared by adding about 5 ml of distilled water to each APDA plate and scraping the culture surface with a glass slide. The suspension was then passed through four layers of cheesecloth, centrifuged at 5,000 rpm, then the pellet was resuspended in distilled water and centrifuged again. This washed spore pellet was resuspended in water, and the concentration was adjusted according to counts with a hemacytometer.

Preliminary tests showed that the root-dip inoculation technique of Ribeiro and Hagedorn (9) with minor modifications was best for infecting bean with *F. o.* f. sp. *phaseoli* and evaluating cultivar reactions. Inoculating bean seeds at planting or drenching seedlings with spore suspensions with and without root injury did not result in consistent disease development. The commercial cultivar IPA 1 and the breeding line HF 465-63-1 (developed by Paulo Miranda, Bean Breeder, Pernambuco State Enterprise for Agricultural Research, Recife, PE, Brazil), which are known to be susceptible and resistant, respectively, in the northeastern region of Brazil, were used to validate the inoculation procedure. Roots of 1-wk-old seedlings growing in sterilized sand were washed in running tap water; about 1 cm was cut off each of the tips, and then the roots were dipped for about 5 min in the spore suspension of *F. o.* f. sp. *phaseoli* at the desired concentration. Inoculated seedlings were then transplanted into 10-cm-diameter plastic pots (generally two seedling per pot) filled with pasteurized soil (60 C for 30 min). Plants were grown in a greenhouse at variable temperature (20–33 C) and relative humidity (35–80%) and fertilized once a week with 50 ml of a 15-15-15 fertilizer suspension (NPK, 3 g/L).

Fusarium yellows severity was recorded at different time intervals using the CIAT scale (2) of 1 (no visible symptoms) to 9 (dead or severely infected plants with 100% of the foliage showing wilting, chlorosis, necrosis, and/or premature defoliation). A rating of 3 indicates that one to three leaves, representing no more than 10% of the total foliage, are wilted and chlorotic. Ratings of 5 and 7 indicate that about 25 and 50% of the leaves and respectively, are wilting and chlorotic. At the end of the test, the taproot and stem tissues of the plant are split open and rated for vascular discoloration (none, light, intermediate, or severe). Germ plasms with mean DSRs of 1–3, 3.1–6.0, or 6.1–9 were considered resistant, intermediate, or susceptible, respectively (2).

The reactions of the 66 bean lines were determined using an inoculum concentration of 1×10^6 spores of *F. o.* f. sp. *phaseoli* per milliliter. Seeds of these lines were obtained from the bean breeders or the Germplasm Bank Unit at CIAT.

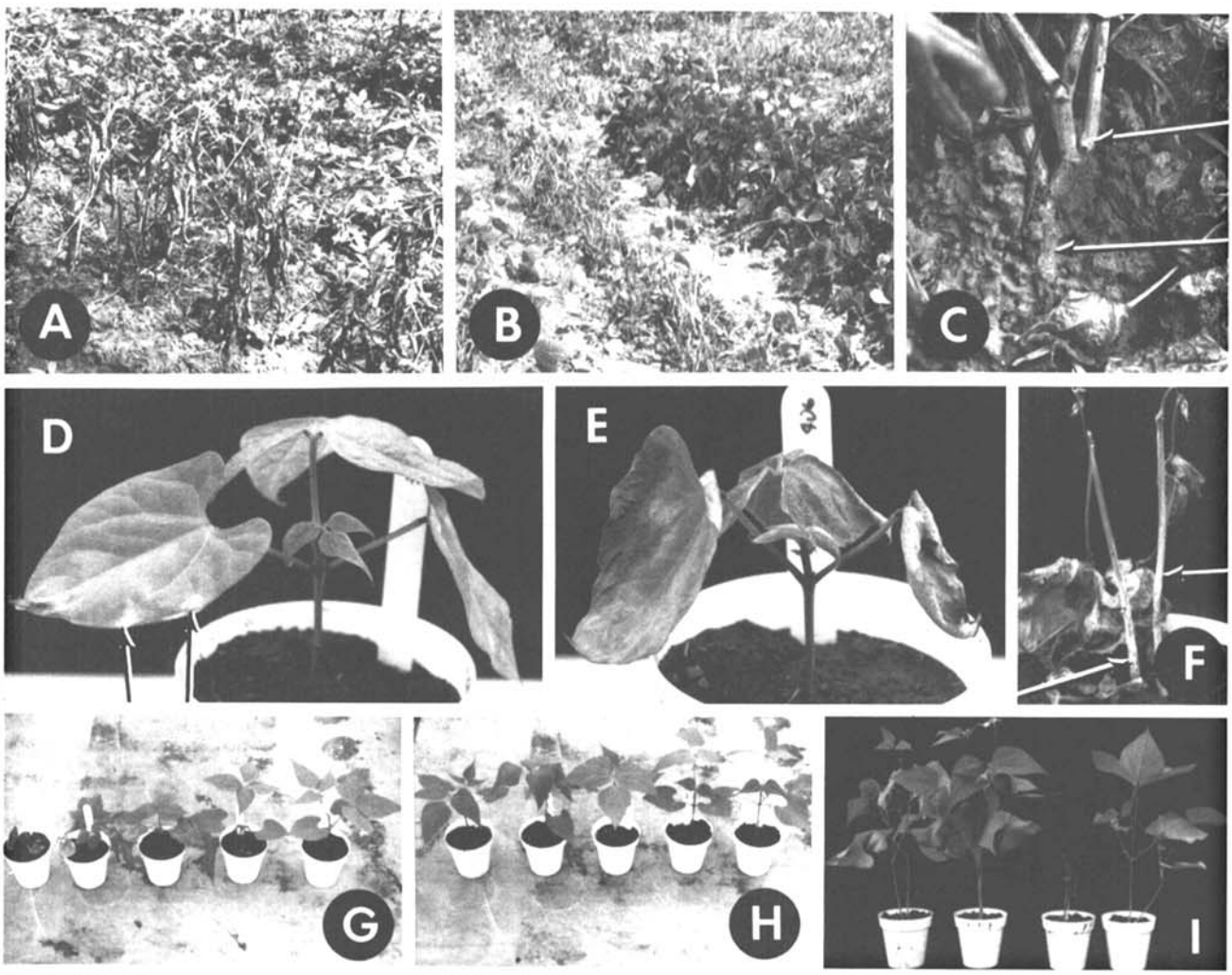

Fig. 1. Symptomatology and reactions of bean germ plasms to infection by *Fusarium oxysporum* f. sp. *phaseoli* under field and greenhouse conditions. **(A)** Severe Fusarium yellows incidence in a field near Belem do Sao Francisco, PE, Brazil, with extensive necrosis and premature defoliation. **(B)** Section of germ plasm evaluation trial adjacent to the field illustrated in A, showing the reaction of the resistant line HF 465-63-1 (right row) and the susceptible cultivar IPA 1 (left row). **(C)** Pink-orange spore masses on stem and petiole tissues of naturally infected plants (arrows). **(D)** Initial symptoms of *F. o.* f. sp. *phaseoli* on IPA 1 plant inoculated with a suspension containing 1×10^6 spores per milliliter after 9 days of incubation. Notice chlorosis and water-soaked lesions on the margins of the primary leaves (arrow). **(E)** Severe symptoms of epinasty, chlorosis, necrosis, and wilting incited by *F. o.* f. sp. *phaseoli* on IPA 1 at 15 days postinoculation. **(F)** Complete defoliation and death of IPA 1 infected by *F. o.* f. sp. *phaseoli* 22 days after inoculation with spore masses on the stem tissues (arrow). **(G and H)** Plants of IPA 1 and HF 465-63-1, respectively, inoculated with spore suspensions containing (left to right) 1×10^7, 1×10^6, 1×10^5, 1×10^4, and 0 spores of *F. o.* f. sp. *phaseoli* per milliliter. **(I)** Reactions of BAT 1400 and ABA 2 to infection by *F. o.* f. sp. *phaseoli* at 22 days postinoculation. (Left to right) BAT 1400 inoculated, BAT 1400 uninoculated, ABA 2 inoculated, and ABA-2 uninoculated.

RESULTS

Effects of inoculum density on disease incidence and severity. Severity of Fusarium yellows on the known field-susceptible cultivar IPA 1 was closely related to the inoculum density of *F. o.* f. sp. *phaseoli* (number of spores per milliliter of suspension) used for inoculation (Fig. 1G). Plants of this cultivar inoculated with a suspension containing 1×10^6 spores of *F. o.* f. sp. *phaseoli* per milliliter died within 2–3 wk of inoculation (Table 1). In contrast, plants of the known field-resistant line, HF 465-63-1, showed only slight symptoms 25 days after inoculation with 1×10^7 spores per milliliter (Fig. 1H). Plants of this line inoculated with 1×10^8 spores per milliliter were stunted but otherwise showed only slight symptoms. The concentration containing 1×10^6 spores per milliliter was used in all subsequent tests.

Symptomatology. Initial symptoms on susceptible plants appeared about 5–9 days after inoculation as transient wilting, especially on lower leaves. At 9 days after inoculation, the primary leaves showed epinasty symptoms and chlorotic water-soaked areas appeared at their margins (Fig. 1D). Symptom expression progressed rapidly as almost all the leaves showed epinasty, chlorosis, and wilting by about 14 days after inoculation (Fig. 1E). Plants at this stage of infection also showed pronounced stunting. Severely infected plants of susceptible cultivars such as IPA 1, Rio Negro, or Roshina G2 often were completely defoliated 3 wk after inoculation (Fig 1F,I). At this stage of disease development, dying or dead stem and petiole tissues of susceptible cultivars were covered with the characteristic pink-orange spore masses of *F. o.* f. sp. *phaseoli* (Fig. 1F). The typical symptoms of Fusarium yellows observed in greenhouse tests were similar to those we observed on *F. o.* f. sp. *phaseoli*-infected plants in many areas of northeastern Brazil (Fig. 1A–C). Vascular discoloration was evident on all susceptible plants and was first detected after the initial appearance of foliar symptoms. The extent and intensity of vascular discoloration was generally proportional to the severity of foliar symptoms. Vascular discoloration extended upward throughout the plant in susceptible cultivars such as IPA 1.

Reactions of selected bean germ plasms to *F. o.* f. sp. *phaseoli*. Large differences were found in the reactions of the 66 selected bean entries to infection by *F. o.* f. sp. *phaseoli* (Table 2). Forty selections were considered resistant under the severe disease pressure provided in this study. Only six entries showed an intermediate reaction, whereas 20 entries were highly susceptible.

The inoculation procedure proved efficient, rapid, and highly effective (Fig 1I). Reactions of most of the selections were evident 15 days after inoculation and sometimes earlier. A high correlation was found between the field reaction of the cultivars, where known, and that determined by the greenhouse procedure. Figure 1B shows the reactions of IPA 1 and HF 465-63-1 in a heavily infested field near Belem Sao Francisco, Pernambuco, Brazil, whereas Figure 1G and 1H show the reactions of IPA 1 and HF 465-63-1 in the greenhouse at CIAT, respectively.

Table 1. Influence of inoculum density of *Fusarium oxysporum* f. sp. *phaseoli* on severity of Fusarium yellows in field-susceptible (IPA 1) and field-resistant (HF 465-63-1) bean germ plasm in the greenhouse

Inoculum density (spores/ml)	Disease severity rating (1–9)[a]	
	IPA 1	HF 465-63-1
0 (water check)[b]	1.00	1.00
1×10^4	2.67	1.00*[c]
1×10^5	7.00	1.00*
1×10^6	9.00	1.17*
1×10^7	9.00	3.67*

[a] Disease severity ratings were recorded 25 days after inoculation, using a scale of 1 (no visible symptoms) to 9 (dead or severely infected plants with 100% of the foliage showing wilting, chlorosis, necrosis, and/or premature defoliation). Ten replicate plants were included per treatment.

[b] Data of the checks were not included in the analysis of variance.

[c] There was a significant interaction between inoculum density and two bean genotypes (F= 20.331, df = 3, 40, P <0.001). * = Significantly different from the corresponding treatment (IPA 1) by Tukey's t test (P=0.05).

DISCUSSION

The inoculation procedure and inoculum density of 1×10^6 spores per milliliter used in this study were highly effective in determining the reactions of bean germ plasms to *F. o.* f. sp. *phaseoli*. These results agree with those reported previously by Ribeiro and Hagedorn (9,10) regarding the conditions for evaluation of bean germ plasm for resistance against *F. o.* f. sp. *phaseoli*. Of special interest was the close relationship found between the reaction of bean to *F. o.* f. sp. *phaseoli* in the greenhouse inoculation procedure and that observed for natural infection in the field. IPA 1 and HF 465-63-1 were highly susceptible and resistant, respectively, in both greenhouse and field evaluations. In addition, disease syndromes produced in the greenhouse were remarkably similar to those produced under field conditions. In both situations, the pink-orange abundant spore masses on infected stem and petiole tissues were highly diagnostic. However, disease progress in the field on infected plants is generally slower and thus appears much later in the growing season on older plants. In this test, all roots of seedlings were injured and immediately dipped in a highly concentrated spore suspension, thus depositing inoculum directly to the vascular system of the taproot as well as the lateral roots. The *F. o.* f. sp. *phaseoli* inoculum in naturally infested fields is unevenly distributed, and thus, only a small portion of the root system of any plant may become infected. Accordingly, it may take considerable time for infections on lateral roots to progress into the taproot and the stem areas, especially under fluctuating environmental conditions. Severe symptoms become obvious only after many of the xylem vessels become plugged (7).

These findings show that large-scale evaluation of bean germ plasm under greenhouse conditions can be conducted with confidence. Such an evaluation will eliminate the susceptible lines that otherwise would be evaluated in the field and thus improve speed and efficiency of the breeding program.

A high proportion of the germ plasm evaluated (40 of 66 lines) was resistant to *F. o.* f. sp. *phaseoli* in this study. However, this is not surprising because the germ plasm evaluated was not selected randomly but was suggested by bean breeders and agronomists at CIAT based on documented history of superior field performance and productivity. For example, BAT 477 and San Cristobal 83 consistently performed well compared with other bean lines in the northeastern areas of Brazil. These same lines were found highly resistant in this study to *F. o.* f. sp. *phaseoli* and *Macrophomina phaseolina* (Tassi) Goid. (8) and are drought-tolerant (1). Echandi (5) concluded that all commercial bean cultivars that were evaluated in Costa Rica were susceptible to *F. o.* f. sp. *phaseoli*. Several cultivars including Manteigao Preto, Manteigao Lustroso, Manteigao 41, Pintadinho Precoce, Suieu, Cherokee wax, Processor, Contender, and Rosinha Sem Cipo were reported resistant to *F. o.* f. sp. *phaseoli* in Brazil and other areas (3,5,9–11). Dongo and Muller (4) identified resistant red-seeded bean cultivars that possess many strong lateral roots. Ribeiro and Hagedorn (9,10) showed that a single gene controlled the resistance of several bean lines to *F. o.* f. sp. *phaseoli*. A dominant gene designated *Fop*1 was effective against the Brazilian race of *F. o.* f. sp. *phaseoli* and was present in the cultivars Tenderrette, Pintado, and Early Gallatin. Resistance to the European and North American race of *F. o.* f. sp. *phaseoli* was controlled by an incompletely dominant gene designated *Fop*2, which was found in the cultivar Prato Ubershinha.

Only one aggressive isolate of *F. o.* f. sp. *phaseoli* was used in the evaluation of bean germ plasm in this study. There is a need to conclusively determine the races of *F. o.* f. sp. *phaseoli* that may exist in

Table 2. Reactions of selected bean germ plasms to inoculation with *Fusarium oxysporum* f. sp. *phaseoli* in the greenhouse

Germ plasm[a]	DSR (1-9)[b] 15	DSR (1-9)[b] 22	Vascular discoloration[c]	Reaction class[d]	Germ plasm	DSR (1-9) 15	DSR (1-9) 22	Vascular discoloration	Reaction class
RIZ 21	8.4[e]	9.0	VS	S	AFR 159	1.4	2.6	S	R
RIZ 30	9.0	9.0	VS	S	AND 286	1.4	1.0	ND	R
					AND 313	1.0	...	...	...
BAT 76	8.8	9.0	VS	S	AND 323	1.0	1.0	ND	R
BAT 1297	8.4	9.0	VS	S	AND 357	1.0	1.0	ND	R
BAT 1298	8.4	9.0	VS	S					
BAT 1393	9.0	9.0	VS	S	Argentino	1.0	1.0	L	R
BAT 1592	5.6	6.1	VS	S	Bayo Criollo de los llanos	1.6	5.4	S	I
BAT 336	1.6	1.0	ND	R	Bayo Rio Grande	1.0	1.0	ND	R
BAT 477	1.0	1.0	ND	R	Blue Bush Lake 274	7.0	8.3	VS	S
BAT 1385	1.0	1.0	ND	R	Cacahuete 72	1.0	1.0	ND	R
BAT 1400	1.0	1.0	ND	R	Calima	1.0	1.0	ND	R
G 4000 (NEP Bayo 22)	1.0	1.0	ND	R	Carioca	5.2	6.7	VS	S
G 5059 (H6 Mulatinho)	1.0	3.4	L	I	Chiapas 7	2.4	4.8	VS	I
					Durango 5	2.8	4.2	L	I
A 211	9.0	9.0	VS	S	Durango 222	1.0	1.0	L	R
A 170	1.0	2.3	L	R	Ecuador 605	1.0	1.0	ND	R
A 55	1.0	1.0	L	R	Ecuador 1056	1.0	1.0	ND	R
A 107	1.0	1.0	L	R	Garboncillo Zarco	2.6	5.2	VS	I
A 195	1.0	1.0	L	R	Ica Pijao	7.4	8.7	VS	S
A 295	1.3	1.0	L	R	Ica Tui	4.0	7.5	VS	S
A 300	1.0	1.0	ND	R	Ipa 1	7.8	8.9	VS	S
A 301	1.0	1.0	ND	R	Jamapa	5.8	8.4	VS	S
					Mortino	1.0	1.0	ND	R
LM 21525	1.0	1.0	ND	R	Nima	1.0	1.0	ND	R
V 8025	1.0	1.0	L	R	Porrillo Sintetico	8.2	9.0	VS	S
WAF 4	1.0	1.0	ND	R	Puebla 152	5.2	5.6	S	I
WAF 9	1.0	1.7	S	R	Rio Negro	9.0	9.0	VS	S
EMP 81	1.3	1.3	L	R	Rio Tibagi	4.0	6.7	VS	S
ABA 2	8.6	9.0	VS	S	Rosinha	8.0	8.9	VS	S
					Rosinha G 2	9.0	9.0	VS	S
XAN 112	1.0	1.0	ND	R	San Cristobal 83	1.0	1.0	MD	R
XAN 195	1.0	1.0	ND	R	Sanilac	1.0	1.8	L	R
					Top Crop	1.0	1.0	ND	R
MCD-025	1.0	1.0	ND	R	Tundama	1.0	1.0	L	R
MCD 254	1.0	1.0	S	R					
HF 465-63-1	1.0	1.0	ND	R	$LSD_{0.05}$	1.04	1.14		

[a] All of the germ plasms evaluated are in the bean collection at CIAT and can be obtained by the designation as listed.
[b] Disease severity ratings were recorded 15 and 22 days after inoculation, using a scale of 1 (no visible symptoms) to 9 (dead or severely infected plants with 100% of the foliage showing wilting, chlorosis, necrosis, and/or premature defoliation). Ten replicate plants were included per germ plasm. Uninoculated control plants (dipped in water) of all germ plasms remained free of Fusarium yellows.
[c] Vascular discoloration was rated at the end of the test as light (L), severe (S), very severe (VS), or none (ND).
[d] Reaction class was based on the CIAT DSR scale (2), where 1.0–3.0 = resistant, 3.1–6.0 = intermediate, and 6.1–9 = susceptible.

Brazil and other bean-growing areas. Many isolates of *F. o.* f. sp. *phaseoli* have been obtained from the bean-growing areas in the northeastern regions of Brazil, Costa Rica, Peru, and Colombia and are now being used to determine the possible existence of races of *F. o.* f. sp. *phaseoli* in Latin America. Results of this study will be used to formulate future screening strategies to develop multirace-resistant cultivars, if needed.

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