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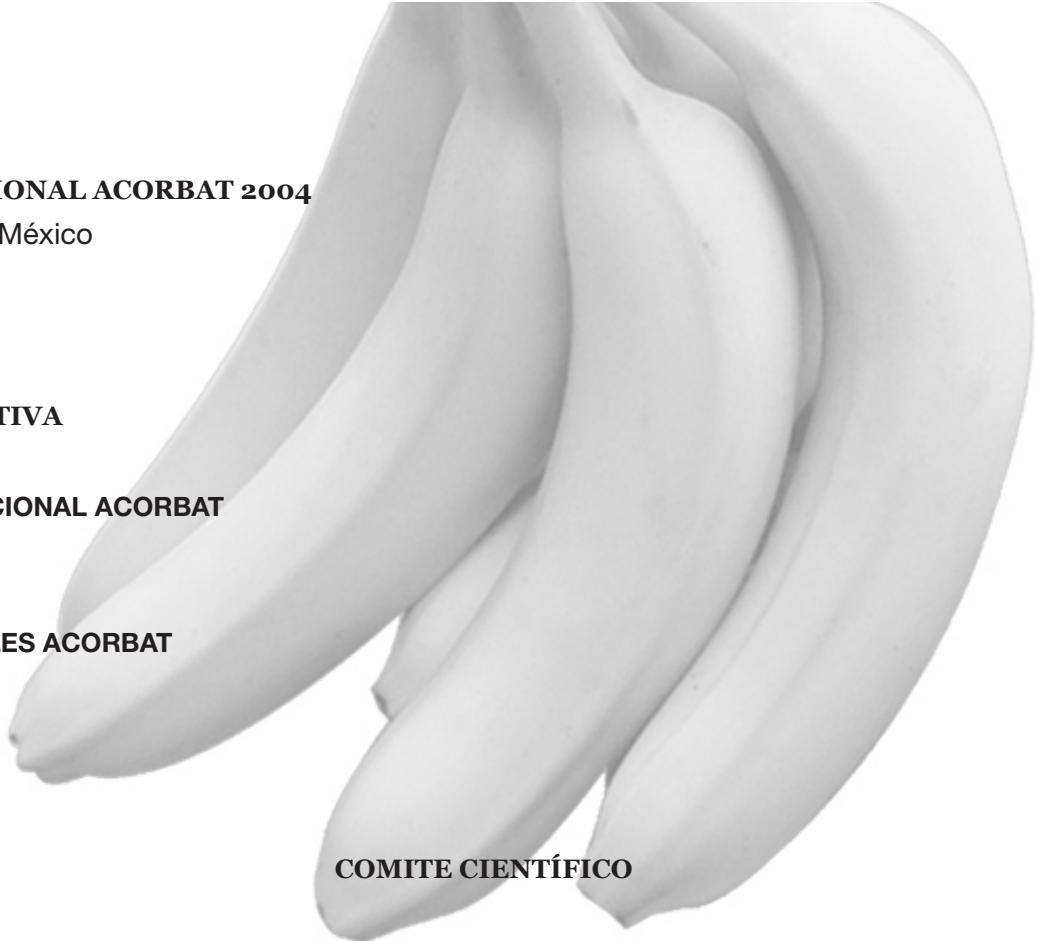

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PLANT BREEDING FOR RESISTANCE TO BACTERIAL WILT IN MUSACEAE

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RESUMEN

El moko del banano es causado por la bacteria *Ralstonia solanacearum*, raza 2. La enfermedad constituye una seria barrera para el cultivo de la región Amazónica y representa una amenaza para otras regiones productoras. El objetivo de este trabajo es presentar las alternativas diferentes usadas en Brasil en el orden generar nuevas variedades de banano y plátanos, a saber, la obtencion de variedad por mejoramiento convencional y las tecnologías de la transformación genéticas. Las evaluaciones de los genotipos fueron conducidas inicialmente en condiciones de campo en un área con alta infestación del agente causador de la enfermedad y posteriormente mediante la inoculación del patógeno en invernaderos. Fueron seleccionados como resistentes los genotipos F2P2, SH3362, 1319-01, 1741-01, 4215-02, 7343-01 y Burmanica. En una segunda etapa, 49 genotipos (incluyendo los resistentes) fueron inoculados con diferentes aislados de la bacteria. Todos los genotipo se mostraron susceptibles a por lo menos, uno de los cinco aislados de *R. solanacearum* utilizados, inclusive los diplóides F2P2, SH3362, 1319-01, 1741-01, 4215-02, 7343-01 y Burmanica, hasta entonces considerados resistentes, los cuales presentaron reacciones de susceptibilidad a *R. solanacerarum* aislada del banano del tipo Bluggoe. Los híbridos 1319-01 y F2P2 también fueron susceptibles a *R. solanacearum* aislada de Nanicão. Los híbridos 1741-01 y SH3362 también fueron susceptibles a los aislados de Prata y de Caipira respectivamente. Se observó que el 7343-01 fue susceptible al aislado de Maçã en tanto que el híbrido 4215-02 fue susceptible a *R. solanacearum* aislada de Nanicão siendo que ambos habían sido evaluados anteriormente como resistentes. Con relación a la sobrevivencia de *R. solanacearum* en el suelo, los resultados mostraron que la bactéria puede sobrevivir el suelo por hasta diez meses en latosuelos amarillos y por hasta ocho meses en podzólicos indicando la necesidad de por lo menos 10 meses de restricción al cultivo de plantas del orden *Zingiberales* en áreas de banano con precedentes de la enfermedad. Utilizando diplóides previamente seleccionados para resistencia al Moko (F2P2, SH3362, 1741-01 y 1319-01, 1741-01 y 4214-02) fueron realizadas 223 polinizaciones en Cruz das Almas (BA), siendo producidas 1,643 semillas, que resultaron en la obtención de 986 híbridos. Han sido realizados en el 2003 cuarenta y tres cruzamientos incluyendo el híbrido 1741-01 y los genotipos tetraplóides (YB42-21 FHIA-01 FHIA-18 y PA12-03) y los cultivares (Prata Anã, Prata Común, Prata Ponta Aparada, Prata Santa Maria y Pacovan) y 40 entre el F2P2 y los mismos genotipos tetraplóides y cultivares mencionados siendo producidas 20 semillas. Además, como una alternativa para generar una nueva fuente de resistencia, estudios en transformación genética usando un gen del peptide antibacteriano también estan siendo conducidos

ABSTRACT

Moko disease of banana, caused by *Ralstonia solanacearum* race 2 (Smith) constitutes a major drawback for the culture of banana and plantains in the Amazon Region and a menace to other banana producing regions. The objective of this work is to present the different approaches used in Brazil in order to generate new varieties of banana and plantains, namely, conventional plant breeding and genetic transformation technologies. In order to achieve these results, plants were evaluated and selected in highly infested fields. Field selected plants were evaluated by inoculation under greenhouse conditions. Studies on the bacterial survival in the soil were also carried out. Thirty diploid, 34 improved diploid, two triploid varieties and 16 tetraploid hybrids were evaluated in moko-infected field. In a second phase of the experiments, forty six genotypes were evaluated by inoculation with different isolates of R. solanacearum. All genotypes tested were susceptible to at least one of the five isolates of *R. solanacearum.* Although apparently resistant in the field, the selected genotypes were also susceptible to at least one of the isolates. The analysis of the bacteria survival in the soil showed that *R. solanacearum* can survive up to 10 months in yellow latosoil and up to 8 months in podzolic soil. A total of 223 pollinations were conducted using the field selected genotypes producing 1,643 seeds. Eighty three crosses involving two selected diploids with four tetraploids and five triploid cultivars produced 20 seeds. In addition, as an alternative to generate a new source of resistance, studies on plant genetic transformation are also conducted using an antibacterial peptide gene.

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INTRODUCTION

Bananas and plantains (*Musa* spp.) are the most important tropical fruits. They are staple food in the Brazilian tropical areas for both urban and rural population. Banana is the second most produced fruit in the world with a total area of 4.2 million hectares distributed in 124 countries and an annual production of 69 million ton. The main banana producer countries are India, Equador, Brazil, China and Phillipines, representing 58% of world banana production (FAO, 2002).

Moko disease of banana, caused by *Ralstonia (Pseudomonas*) *solanacearum* race 2 (Smith), incites wilt of the leaves, starting from the young ones, and necrosis of the candle leaf as well. Immature fruits of infected plants show yellowish color and dry rot of the pulp. Early infection, prior to flowering, causes abnormal development of the bunch, fruit rot before ripening, and some plants may not yield a bunch. The moko disease can be disseminated either by insects, through infested soil or by root contact. These characteristics associated with unavailability of resistant cultivars and low production technology make the moko disease a very serious problem for the banana crop (Buddenhagen, 1961; Takatsu, 1986; Stover, 1972; Matos et al., 1996).
R. solanacearum, race 2, was first reported in Brazil in the Amazon Region, State of Amapá (Tokeshi, 1976). Currently this disease is also present in the States of Amazonas, Pará and Acre, all of them located in the Amazon Region (Takatsu, 1986). According to diagnostic surveys the number of banana orchards affected by the A strain of *R. solanacearum*, race 2, in the Amazon Region has increased in the past years (Matos et al, 1996; Pereira et al, 1997).

Several recessive genes are involved on banana resistance to moko (Vakilii, 1965; Rowe Richardson, 1975). Results reported by Stover (1972), showed several levels of susceptibility to moko in banana cultivars, pointing out that the cultivar Pelipita (ABB) is highly resistant to the pathogen, thus indicating the genetic resistance as a viable control measure for moko in regions where banana crop is cultivated under very low production technology (INIBAP, 1994).

Despite that possibility, no moko resistant germplasm was found when tetraploids (AAAB), such as PV03-44, JV03-15, PA03-22, Pioneira; triploids (AAA) Caipira, Nam, Nanica and Nanicão, (AAB) Pacovan, Prata, Prata Anã, Mysore, Thap Maeo and Ouro da Mata; and plantains (AAB) Pacovi, Pacovan (plantain) and Bluggoe (ABB) , were planted in naturally infested soil (Silva et al., 1998).

Different strategies have been proposed in order to improve resistance to bacterial diseases in plants by genetic engineering, including producing antibacterial proteins of non-plant origin, inhibiting bacterial pathogenicity or virulence factors, enhancing natural plant defenses and artificially inducing programmed cell death at the site of infection (Morgues et al., 1998). The constitutive expression of antibacterial peptides from insects is the strategy that has shown the most promising results for control of phytopathogenic bacteria (Jaymes et al. 1993, Sharma et al., 2000; Kobayashi et al., 2001). Therefore, transgenic banana plants expressing antibacterial peptides could be an alternative in generating moko resistant plants as well as new material source to be incorporated in the breeding programs.

The objective of this work is to present the different approaches used in Brazil in order to generate new varieties of banana and plantains, namely, conventional plant breeding and genetic transformation technologies.

METHODOLOGY

Conventional plant breeding field evaluation. Plant material was multiplied at Embrapa Cassava & Fruits (Cruz das Almas, BA, Brazil). Plants were evaluated under field conditions in moko-infected fields at Embrapa Occidental Amazon (Manaus, AM, Brazil). Experiments were installed in the year 2001 using 15 plants/genotype of 30 diploid genotypes (Burmannica, Calcutta, Perak, Birmania, Khae Prae, Malbut, Madu, Monyet, Pa Rayong, Borneo, Khai Nai On, Mambee Thu, S/N 2, TA, Pa Abissinea, Pa Musore 2, Niyarma Yik, Malaccensis, PA Songkla, Raja Uter, Tuu Gia, Sowmuk, Thong Dok Mak, Tjau Lagada, Selangor, SA, IDO 110, Akondro Mainty, Modok Gier and Khae), 34 improved diploids (TH03-01, 0116-01, 0304-02, 0323-03, 0337-02, 1304-04, 1304-06, 1318-01, 1319-01, 1741-01, 2803-01, 4154-01, 4154-06. 4154-08, 4215-02, 4223-03, 4223-06, 4249-04, 4249-05, 4252-03, 4252-04, 4279-06, 4279-10, 4279-13, 4285-02, 5012-02, 5854-03, 7341-01, 7341-03, F2P2, DM 18, M-53, SH3263 e SH3362), two triploid varieties (Grande Naine and Figue Pomme Naine) and 16 tetraploid hybrids (Calypso, Bucanner, Ambrosiam, FHIA-01, FHIA-03, FHIA-18, YB42-21, PV42-53, PV42-68, PV42-81, PV42-85, PV42-129, PV42-142, ST12-31, ST42-08, SH3640).

Evaluation under greenhouse conditions. In a second phase of the experiments, plants were evaluated by inoculation with different isolates of R. solanacearum. Ten plants of each diploid (AA) genotype were inoculated with the biovar 1 of *R*. *solanacearum*, race 2, by injecting 1mL of a bacterial suspension, concentration of 10^8 CFU. mL^{-1} into the pseudostem at 10 cm from the soil level. Forty six banana genotypes, among them 31 AA diploid (Burmannica, PA Musore, Calcutá, F2P2, SH3362, 1319-01, 1741-01, 4215-02, 7343-01, 0304-02, 0323-03, 0337-02 0116-01, 1318-01, 1304-06, 4223-02, 4249-05, 4279-06, 4249-04, 4223-06, 4154-01, 4252-03, 4279-13, 3422-01, 4285-02, 4279-13, 4154-08, 5012-02, 7341-03, 4285-02, TH03-01), 12 AAAB tetraploid (Bucanner, Calypso, Ambrosia, PV42-53, PV42-68, PV42-81, PV42-85, PV42-142, ST42-08, ST12-31, PC42-01 and SH3640), two AAB tripoid (Yangambi 02 and Figue P.Naine) and one ABB triploid (Pelipita), were inoculated with *R. solanacearum* race 2. The isolates of *R. solanacearum* were obtained from infected plants of the cultivars Prata, Maçã, Nanicão, Caipira and Figo.
External symptoms were evaluated at a week interval, based upon the following disease ratting scale:

1. No symptoms
2. Necrosis of the candle leaf
3. Yellowing of 2 – 3 leaves
4. Buckle of the petiole
5. Death of the plant

Plants without disease symptoms, eight weeks after inoculation, were considered resistant to moko disease.

***R. solanacearum* survival in high land (non-flooding land) soils.** The experiments were conducted using two different soils of high land ecosystem, yellow latosoil and podzolic soil. The soils were infested with naturally infected rhizomes of the cultivars Maçã, Nanica, D'angola and Prata (equal amount of each cultivar). Pseudostems were incorporated in the soils 30 cm depth. Prata Anã and PV03-44 were used as susceptible index plants. For each evaluation period six plants were planted (1.0 m x 0.5 m spacing) for each cultivar. Planting were conducted at 0, 60, 120, 180, 240, 300 and 360 days after soil infestation.

Crossing. During the years 2001 to 2003, in order to generate diploid hybrids, diploid plants (Burmannica, F2P2, SH3362, 1319-01, 1741-01 and 4215-02) previously selected for moko resistance and other agronomical traits were intercroosed. In addition, in the year 2003, forty three crosses using the hybrid 1741-01 with tetraploid genotypes (YB42-21, FHIA-01, FHIA-18 and PA12-03) and AAB cultivars (Prata Anã, Prata Comum, Prata Ponta Aparada, Prata Santa Maria and Pacovan). Forty crosses using F2P2 with the above genotypes were also performed.

Banana Plant Genetic Transformation. The experiments on plant genetic transformation are conducted using the cultivars Pacovan (the most important cultivar in the Northern Brazil), Pacovan-Ken (a Pacovan hybrid resistant to black sigatoka, yellow sigatoka and Panama disease) and the diploids Calcuta and Ouro (resistant to black sigatoka). Plant genetic transformation procedures will be carried out using both *Agrobacterium tumefaciens*-mediated and particle delivery methods. Gene constructions contain either antibiotic or herbicide resistance genes as selectable markers plus the gene of interest (antibacterial peptide).

RESULTS AND DISCUSSION

Evaluation under field conditions. The majority of the eighty tested genotypes were susceptible to moko. Only five diploid hybrids (AA) were resistant.

Although resistance to moko disease has not been detected in triploid and tetraploid commercial varieties so far (Vakilii, 1965; Silva et al., 1998), the results presented in this work show the occurrence of genetic variability among diploid (AA) banana genotypes able to express resistance to *R*. *solanacearum*, race 2.
The detection of resistance to moko disease in diploid (AA) genotypes opens up a real possibility of creating resistant commercial varieties, through conventional breeding techniques. Considering that only a small number of genotypes was evaluated, it is expected that new sources of resistance to *R*. *solanacearum*, race 2 would be detected as evaluations continue.

The wild diploid (AA) Burmannica and diploi hybrids (AA) F2P2, SH3362, 1319-01, 1741-01, 4215-02 and 7343-01 were selected as moko resistant.

Evaluation under greenhouse conditions. All forty six genotypes tested were susceptible to at least one of the five isolates of *R. solanacearum* used in our experiments (Table 1). The diploids F2P2, SH3362, 1319-01, 1741-01, 4215-02, 7343-01 and Burmannica which showed moko resistance under field conditions were susceptible to the Figo (Bluggoe ABB) isolate. 1319-01, 4215-02 and F2P2 were also susceptible to the Nanicão isolate. 1741-01 and SH3362 were also susceptible to bactéria isolates from 'Prata' and 'Caipira', respectively. 7343-01 was susceptible to 'Maçã' isolate (Table 1).

***R. solanacearum* survival in high land (non-flooding land) soils.** The analysis of bacteria survival demonstrated that *R. solanacearum* can survive up to 10 months in the yellow latosoil and up to 8 months in podzolic soils. The results indicate that cultivation of plants from the *Zingiberales* Order should be avoided in infected fields for at least 12 months (Table 2).

Crossing. A total of 223 crosses involving six diploid genotypes (AA) were conducted. The crosses included a wild species (Bumannica) and five improved genotypes (F2P2, SH3362, 1319-01, 1741-01 e 4215-02) which showed some degree of resistance to moko (Table-3). Hundreds of seeds were obtained from theses crosses. Table 4 shows the results of seed production and hybrid plants generated from ten crosses 1741-01x1319-01, fifteen 1741-01x SH3362 and fourteen 1741-01x F2P2. A total of 4,479 flowers were pollinated producing 1,643 seeds. Currently, plants resulted from these crosses are in the greenhouse (126), nursery (4) and in the fields (269) (Table 4).

During the year 2003, forty three crosses were conducted involving the hybrid 1741-01 with tetraploid genotypes (YB42-21, FHIA-01, FHIA-18 and PA12-03) and cultivars (Prata Anã, Prata Comum, Prata Ponta Aparada, Prata Santa Maria and Pacovan). Also, forty crosses of F2P2 with the above genotypes and cultivars. The crosses produced 20 seeds (Table 5).

Banana Plant Genetic Transformation. Currently, the results of experiments are preliminary and restricted to the establishment of plant regeneration protocols.

TABLE 1- Disease reaction of banana genotypes to different isolates of *R. solanacearum* race 2. Embrapa Occidental Amazon, 2001.

Genotypes	Isolate				
	Figo	Prata	Maçã	Nanicão	Caipira
F2P2	S	R	R	S	R
SH3362	S	R	R	R	S
1319-01	S	R	R	S	R
1741-01	S	S	R	R	R
Burmannica	S	-	-	-	-
4215-02	S	-	-	S	R
7343-01	S	-	S	-	R
0304-02	-	S	S	-	S
0323-03	-	S	S	-	S
0337-02	-	S	S	-	S
0116-01	-	S	S	-	S
1318-01	-	S	-	-	S
1304-06	-	-	-	-	S
4223-02	-	-	-	-	S
4249-05	-	-	-	-	S
4279-06	-	-	-	-	S
4249-04	-	-	-	-	S
Bucanner	S	-	-	-	S
Calypso	S	-	-	-	S
Ambrosia	S	-	-	-	S
Figue P.Naine	S	-	-	-	S
PV42-53	-	S	S	-	S
PV42-68	-	S	S	-	S
PV42-81	-	S	S	-	S
PV42-85	-	S	S	-	S
PV421-42	-	S	S	-	S
ST42-08	-	S	S	-	S
ST12-31	-	S	S	S	S
PC42-01	-	S	S	-	S
SH36-40	-	S	-	-	S
4223-06	-	S	-	-	S
PA Musore	S	R	-	-	S
4154-01	-	S	-	-	S
Calcutá	-	-	-	-	S
4252-03	-	S	-	-	S
4279-13	-	S	-	-	S
3422-01	S	-	-	-	S
Yangambi 02	-	S	S	-	-
4285-02	-	S	-	-	S
4279-13	-	S	-	-	S
4154-08	-	S	-	-	S
5012-02	-	S	-	-	S
7341-03	-	S	-	-	S
4285-02	-	S	-	-	S
Pelipita	S	R	R	-	-
TH03-01	-	S	-	-	S

TABLE 2- Banana plants infected with *Ralstonia solanacearum*, expressed in percentage. Embrapa Occidental Amazon, 2001.

Planting period (days after soil infestation)	Soil					
	Yellow Latosoil			Podzolic soil		
	Prata Anã	PV03-44	Mean	Prata Anã	PV03-44	Mean
0	100	100	100	100	100	100
60	100	100	100	100	100	100
120	100	100	100	100	80	100
180	80	100	90	80	80	80
240	80	80	80	20	20	20
300	40	60	50	0	0	0
360	0	0	0	0	0	0

TABLE 3. List of crosses conducted with six genotypes selected for moko resistance. Embrapa Cassava & Fruits, 2001-2003 .

Parental		Number of crosses
Female	**Male**	
Burmannica	F2P2	3
Burmannica	SH 3362	2
Burmannica	1319-01	2
Burmannica	1741-01	2
Burmannica	4215-02	4
Burmannica	7341-01	5
Burmannica	Pa musore 3	-
Burmannica	Burmannica	1
F2P2	7341-01	1
F2P2	Burmannica	6
F2P2	SH 3362	5
F2P2	1319-01	5
F2P2	1741-01	8
F2P2	4215-02	5
F2P2	Pa musore 3	-
F2P2	SH 3362	-
SH 3362	Burmannica	-
SH 3362	F2P2	3
SH 3362	1319-01	4
SH 3362	1741-01	3
SH 3362	4215-02	3
SH 3362	7341-01	-
SH 3362	SH 3362	-
SH 3362	Pa musore 3	-
1319-01	Burmannica	7
1319-01	SH 3362	8
1319-01	1741-01	11
1319-01	F2P2	9
1319-01	7341-01	4
1319-01	Pa musore 3	-

1319-01	1319-01	-
1319-01	4215-02	8
1741-01	Burmannica	12
1741-01	F2P2	12
1741-01	SH 3362	16
1741-01	1319-01	12
1741-01	4215-02	13
1741-01	7341-01	6
1741-01	1741-01	11
1741-01	Pa musore 3	1
4215-02	Burmannica	3
4215-02	F2P2	3
4215-02	SH 3362	5
4215-02	1319-01	4
4215-02	7341-01	-
4215-02	4215-02	-
4215-02	Pa musore 3	-
4215-02	1741-01	6
Total		**223**

TABLE 4. List of progenies from crosses of genotypes selected for moko resistance. Embrapa Cassava & Fruits, 2001-2003

Cross	C	PF	Seeds			PC	PT	PA
			SR	SE	SG			
1741-01 x 1319-	1	1,093	297	230	-	3	-	132
1741-01 x SH	1	1,572	334	31	200	73	-	-
1741-01 x F2P2	1	1,814	355	96	100	50	4	137
Totais	**3**	**4,479**	**986**	**357**	**300**	**126**	**4**	**269**

CE: Number of crosses; FP: Number of pollinated flowers; SR: Number of non-viable seeds; SE: Number of embryos recued in vitro; SG: Number of seeds under greenhouse conditions; PC: Number of seedlings in the greenhouse; PT: Number of seedlings in the nursery; PA: Number of plants in the field.

TABLE 5. List of crosses using two diploid selected for resistance to moko with triploid and tetraploid from the germoplasm bank at Embrapa Cassava & Fruits, 2003.

Female Parental	Number of crosses	
	1741-01	F2P2
YB42-21 (tetraploid)	1	3
FHIA-01 (tetraploid)	5	2
FHIA-18 (tetraploid)	3	3
PA12-03 (Pioneira)	2	1
Prata Anã	19	20
Prata Comum	2	1
Prata Ponta Aparada	4	5
Prata Santa Maria	4	1
Pacovan	3	4
Total	**43**	**40**

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