Notas Científicas

Resistance to *Fusarium* dry root rot disease in cassava accessions

Saulo Alves Santos de Oliveira(1), Camila Santiago Hohenfeld(2),
Vanderlei da Silva Santos(1), Fernando Haddad(1) and Eder Jorge de Oliveira(1)

(1)Embrapa Mandioca e Fruticultura, Rua Embrapa, s/nº, Chapadinha, CEP 44380-000 Cruz das Almas, BA, Brazil. E-mail: saulo.oliveira@embrapa.br, vanderlei.silva-santos@embrapa.br, fernando.haddad@embrapa.br, eder.oliveira@embrapa.br
(2)Universidade Federal do Recôncavo da Bahia, Rua Rui Barbosa, nº 710, Centro, CEP 44380-000 Cruz das Almas, BA, Brazil. E-mail: chohenfeld@gmail.com

Abstract – The objective of this work was to identify sources of resistance to dry root rot induced by *Fusarium* sp. in cassava accessions. A macroconidial suspension (20 µL) of 11 *Fusarium* sp. isolates was inoculated in cassava roots, from 353 acessions plus seven commercial varieties. Ten days after inoculation, the total area colonized by the pathogen on the root pulp was evaluated by digital image analysis. Cluster analysis revealed the presence of five groups regarding resistance. The root lesion areas ranged from 18.28 to 1,096.07 mm^2 for the accessions BGM 1518 and BGM 556, respectively. The genotypes BGM 1042, BGM 1552, BGM 1586, BGM 1598, and BGM 1692 present the best agronomical traits.

Index terms: *Manihot esculenta*, bioprospection, germplasm bank, screening for resistance.

Resistência à podridão seca das raízes ocasionada por *Fusarium* em acessos de mandioca

Resumo – O objetivo deste trabalho foi identificar fontes de resistência à podridão seca das raízes ocasionada por *Fusarium* sp. em acessos de mandioca. Inoculou-se uma suspensão (20 µL) de macroconídios de 11 isolados de *Fusarium* sp. em raízes de 353 acessos e sete variedades comerciais de mandioca. Dez dias após a inoculação, avaliou-se a área total colonizada pelo patógeno na polpa das raízes, por meio de análise de imagens digitais. A análise de agrupamento revelou a presença de cinco grupos quanto à resistência. As áreas lesionadas das raízes variaram de 18,28 a 1.096,07 mm^2 nos acessos BGM 1518 e BGM 556, respectivamente. Os genótipos BGM 1042, BGM 1552, BGM 1586, BGM 1598 e BGM 1692 apresentam as melhores características agronômicas.

Termos para indexação: *Manihot esculenta*, bioprospecção, banco de germoplasma, seleção para resistência.

Root rot, one of the most destructive diseases of cassava (*Manihot esculenta* Crantz) worldwide, is responsible for severe losses and can be caused by a complex of species, including *Phytophthora* spp., *Scytalidium* spp., *Botryodiplodia theobromae*, *Armillaria mellea*, *Sclerotium rolfsii*, *Nattrassia mangiferae*, and *Fusarium* spp. (Bandyopadhyay et al., 2006; Okechukwu et al., 2009). The two most prevalent pathogen species in Brazil are *Phytophthora drechsleri*, mostly associated with soft root rot, and *Fusarium* spp., causing dry root rot (Bandyopadhyay et al., 2006; Muniz et al., 2006). Under favorable conditions, the losses caused by the disease may reach 100%.

The use of cultivars resistant to different pathogens is, by far, the most effective management practice in cassava production systems because the associated costs are lower than those of chemical pesticides. In general, resistance screening is conducted under field conditions; however, because the disease incidence and severity can only be assessed after harvesting, the process of selection for improved cassava accessions could be very difficult. To solve this problem, different strategies have been used for the identification of resistance in laboratory assays: inoculation of stem cuttings, root slices, and whole root (Onyeka et al., 2005).

The objective of this work was to identify sources of resistance to dry root rot induced by *Fusarium* sp. in cassava accessions belonging to the germplasm bank of Embrapa Mandioca e Fruticultura.

The experiment was carried out at Cruz das Almas, state of Bahia, Brazil, under controlled conditions.

Pesq. agropec. bras., Brasília, v.48, n.10, p.1414-1417, out. 2013
DOI: 10.1590/S0100-204X2013001000014

A mini-core cassava germplasm collection was characterized as to resistance or susceptibility level. For that, 353 accessions (from a total of 1,291) and seven commercial varieties (BRS Aipim Brasil, BRS Kiriris, BRS Verdinha, Caravela, BRS Aramaris, Mani-Branca, and Fécula-Branca) were inoculated with a mix of 11 *Fusarium* sp. isolates. Assessments of resistance level were conducted using the method modified from Onyeka et al. (2005), with whole-root inoculation and evaluation of the final lesion area ten days after inoculation, by means of digital image analysis.

Entire cassava roots (from plants produced from 2011–2012), with diameter between 6–10 cm, were washed, disinfected with 0.5% hypochlorite solution, and placed on sterile filter paper to dry. For inoculation, 3–4 mm depth wounds were produced in the central region of the root using a metal punch (Ø 0.6 cm). The roots were inoculated with a 20 µL drop of *Fusarium* sp. suspension, which was achieved by mixing all 11 isolates and adjusted to $2x10^5$ macroconidia mL^{-1}; a mock inoculation (only water) was also used, according to the methodology described by Onyeka et al. (2005), with modifications. The samples were maintained in a moist chamber at 26°C and evaluated every two days up to ten days after inoculation, to estimate the area under the disease progress curve, and, ten days after inoculation, to quantify the final lesion. The roots were cut longitudinally and assessed to determine the total area colonized by the pathogen on the root pulp using photography and digital image analysis. The lesion area was measured with the software UTHSCSA ImageTool (University of Texas Health Science Center, San Antonio, TX, USA).

Differences in the resistance level were determined based on the area under the disease progress curve and on the final lesion area obtained ten days after inoculation. A completely randomized design was used, with five replicates for each isolate and cassava accession. The data were subjected to analysis of variance, using the R software, version 2.15.1 (R Development Core Team, Vienna, Austria). Pearson's correlation test was conducted to evaluate the associations between the area under the disease progress curve and the final lesion area (F-LA) values. Cluster analysis was carried out based on Mahalanobis distance in order to classify the accession according to resistance to root rot disease.

A strong positive correlation was observed between the area under the disease progress curve and the F-LA values at ten days after inoculation ($r = 0.967$, $p < 0.001$). Due to its positive correlation with the area under the disease progress curve, F-LA was considered the most suitable variable for the assessment of disease resistance because only a few roots and replicates are needed.

Cluster analysis revealed the presence of five major groups defined by F-LA at ten days after inoculation (Figure 1). Group 1 contained the ten accessions with the lowest F-LA values (<35 mm^2), which were classified as resistant. Within this group, the accession with the lowest F-LA value was BGM 1518, with a mean value of 18.28 mm^2, followed by BGM 1586 and BGM 1589, with F-LA values of 20.75 and 22.81 mm^2, respectively.

This group comprises some accessions with good agronomic traits (Table 1). Half of them (BGM 1042, BGM 1552, BGM 1586, BGM 1598, and BGM 1692) had high agronomic potential in terms of fresh root yield (>43.0 Mg ha^{-1}) and starch yield (>12.0 Mg ha^{-1}); good multiplication rate, evidenced by the number of cuttings per plant (>8.0 stakes); and good harvest index (>50% from accessions BGM 1692, BGM 1598, and BGM 1552). In contrast,

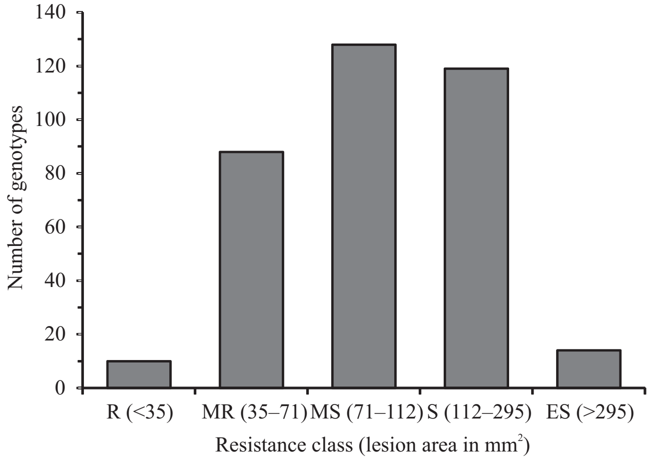

Figure 1. Frequency of cassava genotypes in each class of resistance, based on the final lesion area. R, resistant; MR, moderately resistant; MS, moderately susceptible; S, susceptible; ES, extremely susceptible.

DOI: 10.1590/S0100-204X2013001000014

the remaining resistant accessions – BGM 1546, BGM 1589, BGM 1518, and BGM 1491 – did not show good agronomic characteristics, but can be used in programs of recurrent selection to incorporate their resistance genes into elite cassava varieties.

Group 2, with 88 genotypes, was considered moderately resistant. This cluster contained accessions that were resistant under field conditions, such as cultivar BRS Kiriris (Fukuda et al., 2002), with F-LA of 70.52. This is a great indicative of the presence of resistance sources within the Brazilian germplasm bank, considering the genotypes with the lowest F-LA values (Figure 1).

A total of 128 genotypes with some level of resistance, but larger F-LA values, varying from 71 to 112 mm^2, was clustered into group 3 and classified as moderately susceptible. Two commercial cultivars used as standards, Caravela (F-LA = 72.31 mm^2) and Mani-Branca (F-LA = 111.63 mm^2), were also grouped into this cluster; both express intermediary field resistance to root rot disease.

With F-LA ranging from 112 to 295 mm^2, the susceptible cluster (group 4) comprised 119 genotypes. Despite their different field behavior, four commercial or traditional varieties were considered susceptible: BRS Aipim Brasil, BRS Verdinha, Fécula-Branca, and BRS Aramaris. Notably, 'BRS Aramaris' (F-LA = 260.12 mm^2) is considered resistant under field conditions in Northeast Brazil (Fukuda et al., 2002). The mean F-LA of the extremely susceptible group (group 5), with 14 genotypes, was higher than 295 mm^2. BGM 1484 showed the lowest F-LA for this group, of 315.27 mm^2, whereas the highest F-LA (1,096.1 mm^2) was observed for accession BGM 556.

Differences on resistance reactions are expected in experiments using wounding procedures; therefore, resistance could be linked with pre-formed barriers that prevent pathogen penetration such as biochemical and structural compounds (Prusky & Keen, 1993). This could explain why one genotype (BRS Aramaris) showed good field resistance to root rot disease, but was grouped together with susceptible varieties. The same can be applied to the reaction of 'BRS Kiriris', which was clustered as moderately resistant, but expresses a resistant phenotype in the field.

No complete resistance was registered among the germplasm accessions, although different degrees of resistance were observed. In comparison, the resistance to *P. tropicalis*, another cassava root rot pathogen, has been reported to be controlled by QTL (Alvarez et al., 2002): different degrees of resistance were found for this causal agent, and it is possible that the same occurs with *Fusarium* spp. Therefore, the basis of the resistance to dry root rot must be investigated.

Sources of genetic resistance to dry root rot, caused by *Fusarium* sp., were found in the cassava germplasm bank of Embrapa Mandioca e Fruticultura. The resistance levels were sufficiently high, and the superior genotypes could be used in the development of new cassava populations, mainly the ten most resistant to root rot disease, among which BGM 1042,

Table 1. The ten most resistant cassava genotypes of germplasm bank of Embrapa Mandioca e Fruticultura, their agronomic traits(1) and qualitative descriptors.

Genotype	Origin (municipality, state)	F-LA	FRY			HI			NS			SY			Morphological descriptors(2)		
			$\bar{X}$	LIIC	LSIC	$\bar{X}$	LIIC	LSIC	$\bar{X}$	LIIC	LSIC	$\bar{X}$	LIIC	LSIC			
BGM 0126	Unknown	29.83	-	-	-	-	-	-	-	-	-	-	-	-	LBR	WH	WH
BGM 1042	Tianguá, Ceará	32.56	43.85	32.47	50.22	47.70	37.98	52.44	8.34	7.81	10.26	12.34	8.90	13.95	DBR	PP	WH
BGM 1491	Jaguarari, Bahia	27.99	4.50	10.85	42.23	18.37	24.19	53.29	1.37	7.01	10.23	1.60	3.02	11.73	-	-	-
BGM 1518	Cafarnaum, Bahia	18.28	33.09	19.14	40.07	48.62	37.44	54.85	9.06	8.23	10.77	11.18	6.35	12.28	WH	YE	CR
BGM 1546	Santa Maria da Vitória, Bahia	33.91	14.50	9.73	34.36	52.17	37.83	58.95	5.16	6.54	9.36	3.42	2.18	9.87	-	-	-
BGM 1552	Angical, Bahia	25.17	50.04	34.02	52.55	56.06	44.85	60.01	9.04	8.21	10.75	13.83	9.18	14.45	DBR	WH	WH
BGM 1586	Itaueira, Piauí	20.75	44.61	23.56	44.49	44.66	34.18	51.59	11.99	7.82	10.64	12.61	6.51	12.89	DBR	PP	CR
BGM 1589	Nazaré do Piauí, Piauí	22.81	29.34	26.76	46.41	43.28	41.35	57.56	7.57	8.46	11.13	6.51	7.14	12.72	-	-	-
BGM 1598	Barro Duro, Piauí	27.5	45.46	32.97	51.56	50.03	38.92	54.15	9.11	8.57	11.12	14.03	9.98	15.26	WH	YE	CR
BGM 1692	Gandu, Bahia	35.17	43.91	33.26	46.63	56.81	46.29	56.96	10.98	10.53	12.37	12.44	9.06	12.88	DBR	PI	CR

(1)FRY, fresh root yield; HI, harvest index; NS, number of stakes per plant; SY, starch yield per hectare. $\bar{X}$, phenotypic means; LIIC and LSIC, lower and upper limits of the confidence interval, respectively. (2)Morphological descriptors measured as color. LBR, light brown; DBR, dark brown; WH, white; PP, purple; YE, yellow; CR, cream; PI, pinkish. F-LA, final lesion area.

BGM 1552, BGM 1586, BGM 1598, and BGM 1692 present the best agronomical traits.

Acknowledgements

To Embrapa and Inter-American Development Bank, for financial support; and to Fundação de Amparo à Pesquisa do Estado da Bahia (Fapesb), for scholarship granted.

References

ALVAREZ, E.; LOKE, J.; RIVERA, S.; LLANO, G.A. Genética de la resistencia a pudrición causada por *Phytophthora tropicalis* en dos poblaciones segregantes de yuca (Manihot esculenta Crantz). **Fitopatologia Colombiana**, v.26, p.61-66, 2002.

BANDYOPADHYAY, R.; MWANGI, M.; AIGBE, S.O.; LESLIE, J.F. *Fusarium* species from the cassava root rot complex in West Africa. **Phytopathology**, v.96, p.673-676, 2006. DOI: 10.1094/PHYTO-96-0673.

FUKUDA, W.M.G.; SILVA, S. de O.; IGLESIAS, C. Cassava breeding. **Crop Breeding and Applied Biotechnology**, v.2, p.617-638, 2002.

MUNIZ, M. de F.S.; ANDRADE, F.W.R. de; QUEIROZ, F.M.; MOURA FILHO, G.; MENEZES, M. Caracterização de isolados de *Phytophthora drechsleri*, agente causal da podridão mole de raízes de mandioca. **Tropical Plant Pathology**, v.31, p.195-198, 2006. DOI: 10.1590/S0100-41582006000200013.

OKECHUKWU, R.U.; DIXON, A.G.O.; AKORODA, M.O.; MWANGI, M.; BANDYOPADHYARR, R. Root rot resistance in new cassava varieties introduced to farmers in Nigeria. **Experimental Agriculture**, v.45, p.15-24, 2009. DOI: 10.1017/S0014479708006777.

ONYEKA, T.J.; DIXON, A.G.O.; EKPO, E.J.A. Assessment of laboratory methods for evaluating cassava genotypes for resistance to root rot disease. **Mycopathologia**, v.159, p.461-467, 2005. DOI: 10.1007/s11046-004-6156-z.

PRUSKY, D.; KEEN, N.T. Involvement of preformed antifungal compounds in the resistance of subtropical fruits to fungal decay. **Plant Disease**, v.77, p.114-119, 1993. DOI: 10.1094/PD-77-0114.

Received on June 27, 2013 and accepted on September 9, 2013