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Periclinal chimera can transfer resistance to nematodes in cassava

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ABSTRACT. Screening cassava germplasm for resistance to the root-knot nematode (*Meloidogyne javanica*) showed that periclinal chimera, which came from combination of the wild species *Manihot fortalezensis* and indigenous cultivar UnB 201, resulted in resistance to *M. javanica*. Apparently periclinal chimera acquired resistance from *M. fortalezensis*, with its tissue forming a subepidermis and the internal tissue. Apparently, resistance was due to interaction of DNA in the chimera components, since the DNA moves within all plant tissues. This interaction provided the increased vigor observed in periclinal chimera. This is the first report of transferring resistance to a pathogen by periclinal chimera.

Key words: DNA movement; *Manihot fortalezensis*; Resistance; *Meloidogyne javanica*

INTRODUCTION

Cassava is the most important food for poor people in the tropics and subtropics and feeds almost one billion people (FAO, 2019). Several nematode species are known to attack its roots. The root-knot nematodes (*Meloidogyne* spp.) are recognized as the most important and most threatening to cassava production (Coyne and Namanganda, 1994).

The nematodes attack cassava roots, causing damage that affects the translocation of water and nutrients. *Meloidogyne incognita* and *M. javanica* are considered the most aggressive and damaging nematodes to cassava plantations (Coyne, 1994), causing galls in the roots with consequent yield losses up to 87% in storage roots (Caveness, 1982). Most research on cassava nematode resistance has concerned variety screening for resistance;

little work has been done on developing resistant varieties through classical breeding or other technologies.

We report on a successful trial to use periclinal chimera as an efficient and fast method to transfer resistance to cassava nematodes.

MATERIAL AND METHODS

The assays were carried out under greenhouse conditions at the University of Brasilia Biology Experimental Station and in the Laboratory of Nematology of the Department of Plant Pathology of the University of Brasília.

The *Manihot* accessions evaluated are part of the Cassava Collection of the University of Brasilia. Chimera 1 and its donors (*Manihot fortalezensis* and cv. UnB 201) and the susceptible cultivar Pioneira from Embrapa (control) were challenged with the root-knot nematode *M. javanica*. The assay was conducted in a completely randomized design with four treatments, five replicates each, with two replications in time.

The nematode population was provided by Embrapa Genetic Resources and Biotechnology. Pure inoculum of *M. javanica* was multiplied in tomato plants cv. Santa Clara in a greenhouse and prepared as a nematode egg suspension according to Hussey and Barker (1973), modified by Boneti and Ferraz (1981).

Stem cuttings 15 cm long, from adult plants were planted in 8-liter plastic bags containing autoclaved soil and sand in a proportion of 1: 1, placing one cutting per bag, and irrigated according to plant needs. The plants were kept in a greenhouse for 40 days after planting.

Each cassava plant was inoculated with a nematode suspension of 5000 eggs + eventual second stage juveniles. In the first assay, susceptible tomato cv. Santa Clara served to test inoculum quality, while in the second assay cassava cv. Pioneira known to be susceptible to *M. javanica* (Carneiro *et al*., 2006) was included as positive control. During the experiments temperatures in the greenhouse averaged 24.1°C (17.7 - 30.6°C).

Host reaction to the nematode was evaluated 60 days after inoculation in both assays. The root system of each plant was removed and washed in tap water. Eggs and eventual juveniles were extracted according to the technique of Hussey and Barker (1973), modified by Boneti and Ferraz (1981). The number of eggs + J2 was counted in a Peter's chamber under a light microscope. After counting, the host reaction was evaluated by calculating the reproduction factor (RF). For each treatment the RF was calculated as the final population (fp) divided by the initial population (ip) of 5000 eggs + J2. For the evaluation of host reaction do the nematode, we followed Oostenbrink (1966). Plants with FR equal to zero were considered immune, those with FR higher or equal to 1.0 as susceptible, while those with FR lower than 1.0 but above zero were considered resistant.

The Statistica package software was used for Analysis of variance (ANOVA), and the means were compared using the Tukey test ($p < 0.05$). For data normalization, the following transformations were used in the values for RF to $1 / (x + 1)$ (experiment 1), and $\log (x)$ (experiment 2).

RESULTS AND DISCUSSION

In both assays Chimera 1 behaved as resistant to *M. javanica* (Figure 1A), with a very low reproduction factor (RF = 0.11 and 0.1, respectively). The same behavior was

observed for the wild accession, *M. fortalezensis* (R = 0.12 and 0.08). A susceptible reaction was found for cv. UnB 201 (RF = 4.22 and 3.25, respectively), for cv. Pioneira (Figures 1B, 1C), the positive control (second experiment, RF = 6.38), and for tomato cv. Santa Clara (RF = 21.09 and 20.87). Since the donor cv. UnB had a susceptible reaction and the second parental component *M. Fortalezensis* was resistant, we concluded that resistance to nematodes in Chimera 1 was transferred from *M. fortalezensis* by the technique of periclinal chimera. Apparently, periclinal chimera acquired resistance from *M. fortalezensis* having its tissue forming the subepidermis and the internal tissue. Probably resistance was due to interaction of DNA of the two chimera components since they move within all plant tissues (Stegemann and Bock, 2009).

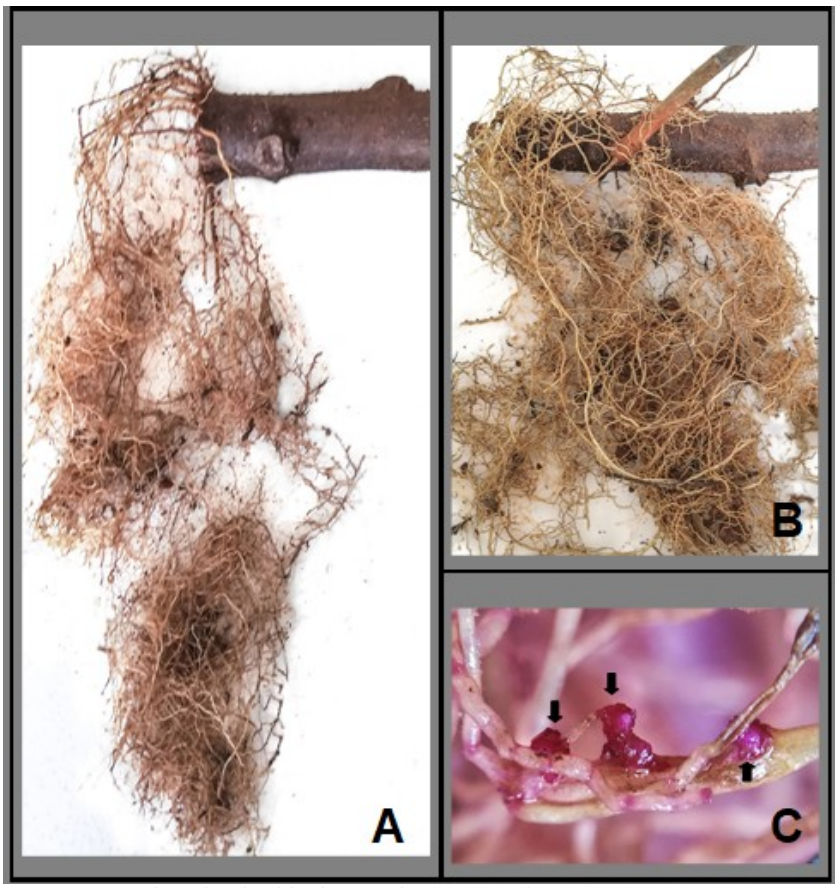

Figure 1. Cassava roots inoculated with the root-knot nematode (*Meloidogyne javanica*). A. Chimera 1 (resistant); B. Cultivar Pioneira (susceptible); C. root of cv. Pioneira with nematode egg masses stained with phoxin B (arrows).

There is almost no published information on the incorporation of parental resistance to the chimeric component. This is probably due to the very recent technique of the periclinal chimera, introduced only a few years ago by Nassar *et al*. (2016). The only reported case involved resistance to insects by periclinal chimera through its epidermal layer. It was resistance to the potato aphid (*Macrosiphum euphorbiae*) conferred by

glandular trichomes in *Lycopersicon pennellii*. This resistance was acquired by *L. esculentum* by the production of an interspecific chimera with an L1 layer of *L. pennelli* (Goffreda et al., 1990).

This resistance found in *L. pennelli* is due to the sugar esters produced by the trichomes. The chimeras with an *L. pennellii* L1 layer had the same type of trichomes and produced sugar esters with similar compositions and concentrations to *L. pennellii*. Apparently, where disease or insect attacks are prevented by an outer layer, there is a good possibility of manipulation of the periclinal chimera.

Periclinal chimera should receive breeder's attention due to the short time need for synthesis; normally decades or more would be needed for developing varieties by classical methods of hybridization (Nassar and Bomfim, 2013; Bomfim and Nassar, 2014; Gakpetor *et al*., 2017), especially for perennial plants where reproduction cycles normally take several years.

The resistance of cultivars UnB 220 and UnB 360 (Ferreira, 2019), which came from wild cassava *M. glaziovii* and *M. aesculifolia*, respectively supports the importance of wild plants as a source of resistance. This resistance was acquired and maintained along the years through processes of evolution and natural selection.

Many researchers have emphasized this phenomenon from Harlan (1976) to Loskutou and Rines (2011). The identification of wild relatives is the most important step in this process. As reported by IDRC (2010), efforts to improve one of the world's most resilient staples, cassava, have paid off, with lasting and, in some instances, dramatic benefits. Plant breeding has increased this starchy root's nutritional value and resistance to disease, saving countless lives as a result. Wild *Manihot* species collected by Nassar in Brazil were used by the International Institute of Tropical Agriculture in Nigeria to breed hybrids that are resistant to cassava mosaic disease, which is caused by a virus transmitted by whiteflies. Without the cultivars called MS, developed from those hybrids, Nigeria and Uganda, the world's leading cassava producers, would have suffered greatly from mosaic disease.

CONCLUSIONS

The manipulation of a wild relative or its hybrid by periclinal technique appears to be the most efficient approach for incorporating resistance, since it saves time consumed in hybridization and backcrosses and avoids the difficulty of hybridization itself due to genetic barriers.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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