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Cassava periclinal chimeras: Synthesis feasibility, genotype compatibility and combining ability

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ABSTRACT. Periclinal chimera are made constituted of two genotypes growing side by side. One of these genotypes makes up the epidermis, the second forms the internal tissues. As a nonconventional method to improve cassava, it brought its productivity to an extraordinary level that has never been reported before. In previous experiments the chimera were synthesized by hormone treatment, which was applied to the surface of the grafts to promote callus formation. We propose here a simple method that significantly increases the induction of periclinal chimera. It is principally to make grafts in which scions are cut in a slanted position close to a bud and the rootstock cut in the opposite direction. The scion and the rootstock are placed in close contact, having the juxtaposition of the scion and the rootstock so that the buds can make contact with each other. A cello tape is used to fasten and hold them together. We also interpret what has been noted of exceptional chimera productivity based on combining ability between genotypes of multiple ploidy levels and the movement of DNA from one periclinal chimera layer to another. *Manihot fortalizensis* showed the highest combining ability and the highest compatibility with all cassava cultivars tested. Before synthesizing periclinal chimera it is

recommended to examine combining ability between candidate species and varieties.

Key words: Tallus; DNA movement; Graft; Hybrid vigor; Heterosis; Polyploidy

INTRODUCTION

Cassava is a food for more than one billion people in the tropics and subtropics (Nassar and Ortiz, 2010; FAO, 2019). It is a principal source of energy for people of northeast and north Brazil, including more than one hundred million people in this country (IBGE, 2018).

The introduction of periclinal chimera technique has opened a new era of cassava breeding

since it offers a a simple and easy method for achieving high productivity and transferring useful characters within one generation (Nassar and Bomfim 2013, Bomfim and Nassar, 2014; Gakpetor et al., 2017).

Four interspecific periclinal chimera varieties have been produced by Nassar and coworkers in the last 10 years using hormone treatment. We report here an easy method without any chemicals that gives a high percentage of success. We also present a theory to interpret high productivity and vigor noted in periclinal chimera plants.

MATERIAL AND METHODS

This work began in August 2018 and continued in 2019 at the Experimental Station of the Universidade de Brasília. Cuttings of 30 cm used as scions or stocks from the following material (of our living collection) were joined in different combinations. In each combination, 50 grafts were made.

These were *Manihot fortalizensis* · UnB 338, *M. fortalizensis* · UnB 201, *M. fortalizensis* · UnB 031, *M. fortalizensis* · UnB 205, Hybrid *M. glaziovii* · UnB 360, Hybrid *M. glaziovii* · UnB 310, UnB 360 · UnB 530 and UnB 360 · UnB 220.

M. fortalizensis (Nassar et al., 2011) is believed to be a recently evolved species that came from natural hybridization of *M. glaziovii* with cassava (Nassar, 2006). The hybrid of *M. glaziovii* used in this experiment is a polyploid type of interspecific hybridization of *M. glaziovii* with cassava (Nassar, 2006), *M. pohlii* is a wild species native to Bahia state, Brazil. It behaves as a weedy plant in several manners such as growing in disturbed habitats. It also propagates easily by cuttings, indicating it had hybridized in the past with a cassava variety. UnB 338 is a second generation of backcrossing an interspecific hybrid UnB 300 with cassava. It is characterized by high protein content (5%) and rich in essential amino acids that are normally absent in cassava but it roots with difficulty (Gomes and Nassar, 2013). We used it in a periclinal chimera synthesis to obtain a chimeral type of it with *M. fortalizensis* that can root easily. The cultivar UnB 360 is an interspecific hybrid of *M. aesculifolia* with cassava, followed by polyploidization. Cultivar UnB 228 is a second generation of interspecific hybridization of *M. glaziovii* with cassava. The morphology of the leaves of every cultivar and interspecific hybrid can be used as a marker to identify the chimeral type formation.

UnB 205 was also used in combination with *M. fortalizensis* because of its high carotene contents. We plan to determine if its high carotene can be transferred through periclinal chimera synthesis.

To make grafts, 50 scions and 50 stocks in each trial were used. Scions were cut in a slanted position close to a bud and the rootstock cut in the opposite direction. The scion and the rootstock were placed in close contact having the juxtaposition of the scion and the rootstock so that both buds could make contact with each other. A common cellophane tape from stationary shops was used to fasten and hold them together (Figure 1).

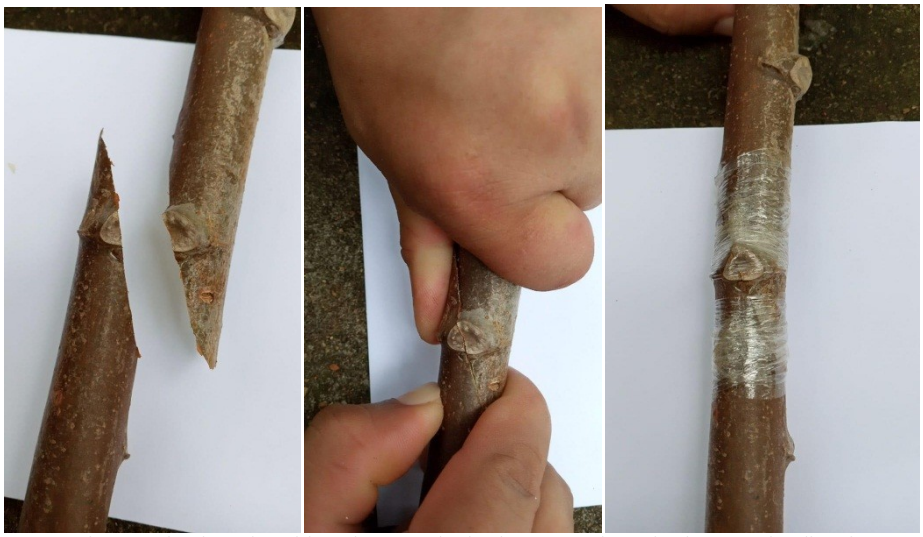

Figure 1. A: scions cut in slanted position close to a bud. The rootstock cut in the opposite direction. B: Scions placed in close contact having juxtaposition of scions and rootstock so that both buds make contact with each other. C: A cellophane tape was used to fasten and hold scion and rootstock together

Any auxiliary shoots and adventitious shoots sprouted from any place except from the graft's union buds were removed. Chimera induction rates were estimated. Periclinal chimera were identified according to stem growth, leaf morphology (shape and form).

RESULTS AND DISCUSSION

Graft combinations were carried out to develop periclinal chimeras. To achieve success in producing chimera without hormones, a new technique was applied. This involves cutting buds of both grafted varieties in half before holding them together, permitting meristematic callus tissue to form, from which periclinal chimera may sprout. Results of the grafts were as follows:

As noted from the Table, the combinations with *M. fortalizensis* (Figure 2). showed a notable success when grafted with UnB 031, UnB 338 and UnB 201, respectively. Clearly cutting the bud in half was essential to achieve this result. Apparently cutting the buds in half stimulated formation of callus of both buds of the grafted plants, which developed into a chimera. The callus constituted by meristematic cells of both of the tissues gave rise to the

periclinal chimera. Grafting *M. pohlii* gave a good percentage of success compared to using hormones without bud contact.

Table 1. Percentage of periclinal chimera graft success using different cassava cultivars with the crop wild relative *Manihot fortalizensis* compared to a check of: UnB 360 and 530 - Hybrid *M. glaziovii* with cassava (n = 50 for each comination).

Periclinal chimera graft	%
M. fortalizensis □ UnB 338	12
M. fortalizensis □ Unb 031	14
M. fortalizensis □ UnB 201	6
M. pohlii x UnB 201	5
M. fortalizensis □ UnB 205	0
M. glaziovii □ UnB 360	0
M. glaziovii □ UnB 310	0
UnB 360 □ UnB 530	0
UnB 360 □ UnB 220	0

Figure 2. Manihot fortalizensis leaf.

M. fortalizensis showed the highest compatibility when grafted with UnB 031, UnB 338 (Figure 3) and UnB 201 while other material of UnB 360, or *M. glaziovii* failed in compatibility with cassava to give periclinal chimera. UnB 360 is constituted from hybridization of cassava with *M. aesculifolia*. It is really an interspecific hybrid and so is the *M. glaziovii* interspecific hybrid. Nassar (1981) reported that the success of grafting in

Manihot depends on phylogenetic relationships and may be used as measure of how much distance is between species. Periclinal chimera however were not tried at that time.

Figure 3. UnB 338 leaf.

Periclinal chimera vigor

Periclinal chimera formed from *M. fortalizensis* with cassava cultivar UnB 338 (Figure 4) was very vigorous and reached 3 meters height in 10 months compared to cultivar UnB 338 which reached only 1 meter height in the same period. *M. fortalizensis* reached 2 meters height in the same period. Productivity of periclinal chimera formed from this species with either cultivar UnB 201 or UnB 031 was very productive (Figure 5 and 6) and reached 120 tons per hectare in case of periclinal chimera with UnB 201 and 88 tons per hectare in case of periclinal chimera with UnB 031 (Bomfim and Nassar, 2014; Gakpetor et al., 2017). These gave exceptional production that has never been documented before. In the other case, periclinal chimera formed from *M. pohlii* with UnB 201 gave woody roots (Figure 7) (Gakpetor et al., 2017). This phenomenon of high productivity noted in every case where *M. fortalizensis* was used with cassava cultivars 031 and UnB 201 may be attributed to the classic theory of combining ability (Hayman, 1954; Griffing, 1956).

M. fortalizensis is a new *Manihot* species believed to be recently evolving from interspecific hybridization of M. *glaziovii* with cassava (Nassar et al. 2011). It is apomictic and has 2n = 54.

Combining ability must have occurred by genetic interaction of genes of the two grafted parents involved in forming periclinal chimera; *M. fortalizensis* and cassava cultivars. In the last two decades various reports confirmed RNA transference through the plant vascular system. The most striking feature came from Stegemann and Bock (2009), who reported gene transfer in the contact zone between scion and rootstock. In the case of periclinal chimera, the contact zone is extended in all plants.

Ohata (2004) reported chromatin transfer from dying stock cells through the vascular system across the graft union to the growing points on the scion and how the process causes transformation in the fast dividing scion flower primordia. He suggested genetic material leak between cellular components, with DNA transfer between the cells of grafted plants.

Figure 4. Periclinal chimera (*Manihot fortalizensis* - UnB 338).

Figure 5. UnB 338

Figure 6. Periclinal chimera of *Manihot fortalizensis x* UnB 031

From what notes of periclinal chimera vigor (of M. caerulescens and UnB 031. (Figure 4)). So, we can deduce that genes of M. fortalizenzis have contact with genes of genotypes 031 or 021 that form the inner layers. The genes achieve complementation with other genotypes layer in periclinal chimera and this may lead to express vigor.

Tsaftaris et al. (2007) reported that if there is over dominance combined with additive genes, which will lead to the expression of more heterosis. A similar theory has been adopted and presented by Hull (1945). In case of M. fortalizensis which is 3x or 4x, the interaction will be between a high number of alleles which reach 4 or 3 alleles of M. fortalizensis with 2 alleles of the combining variety. Total of alleles should be 5 alleles in case of triploid M. fortalizencis (3+2) and it is 6 alleles in case of M. fortalzensis 4x.

The fact of having an increased number of loci seen in triploid or tetraploid results in increasing quantitatively higher genetic expression and inducing hybrid vigor (Tsaftaris et al. , 2000).

A striking feature is the compatibility seen in certain combinations such as that of M. fortalizensis with UnB 338, UnB 031 and UnB 201 against incompatibility seen in the case of UnB 205. The incompatibility in the latter could be due to the fact the cultivar evolved through hybridization with wild species distant genetically from M. fortalizensis.

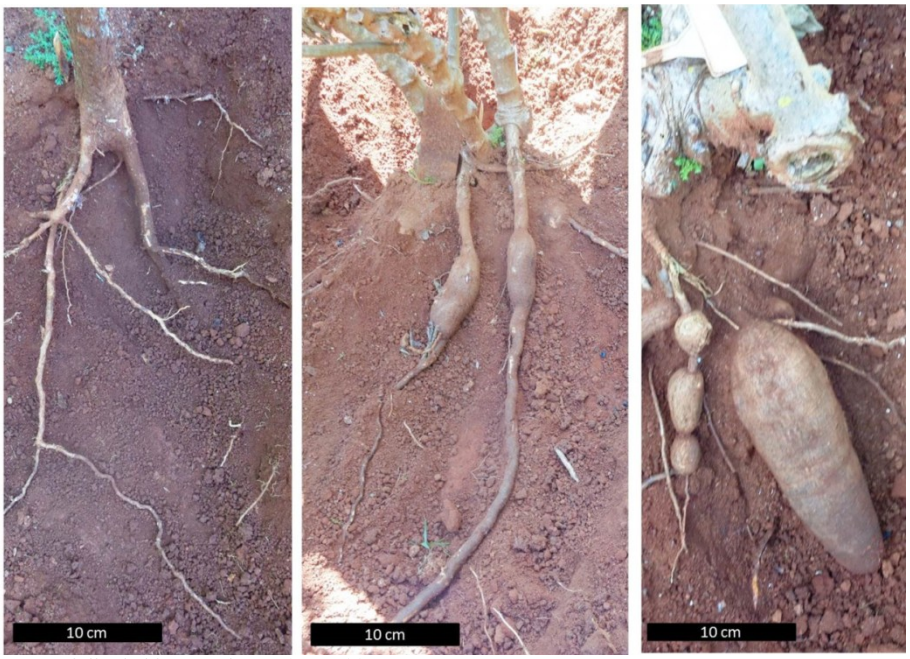

Figure 7A. Periclinal chimera of *Manihot pohlii* x UnB 201

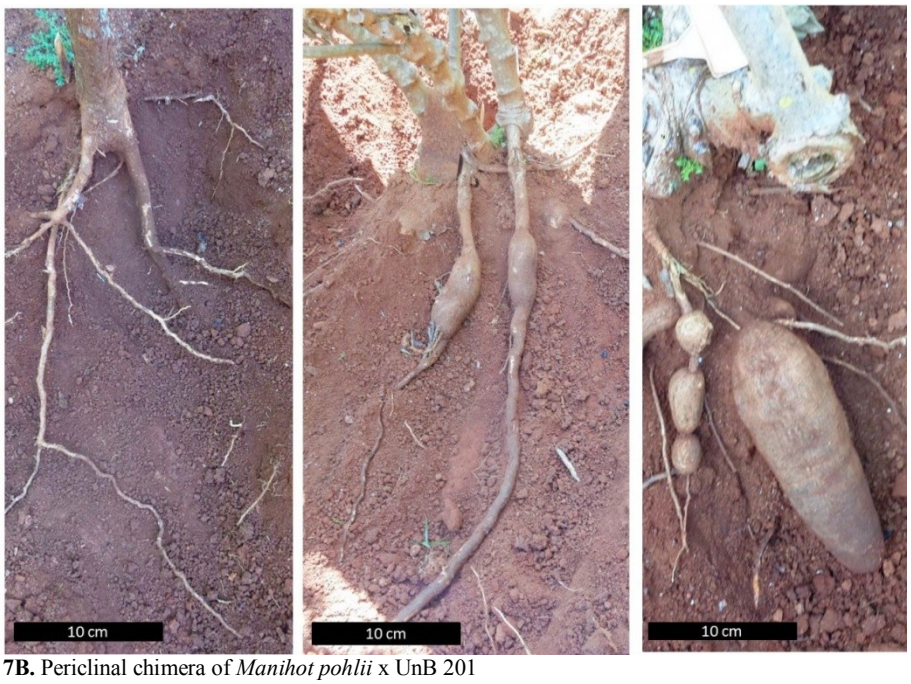

Figure 7B. Periclinal chimera of *Manihot pohlii* x UnB 201

This is confirmed by what is seen when M. glaziovii was grafted with UnB 360. This cultivar UnB 360 had been developed by us by hybridizing cassava with M.

aesculifolia. It is the first generation of interspecific hybridization that further polyploidized using the type used here.

We can hypothesize from these results that root formation vigor in periclinal chimera depends on combining ability of the two genotypes grafted to form the chimera. There is gene movement along the two layers in contact within the chimera. Moreover, vigor is enhanced when chimeras form between polyploid species (Figure 8).

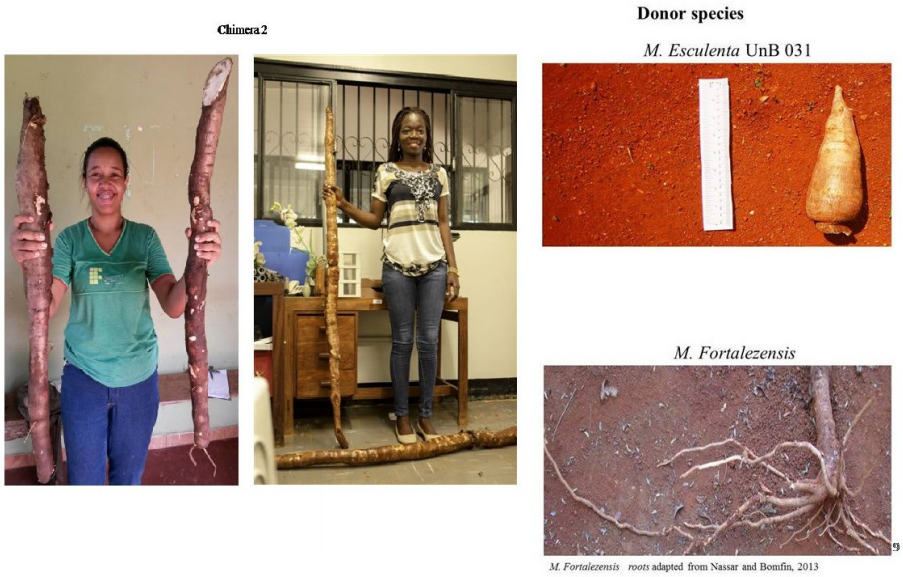

Figure 8. Cassava root compared to chimeras (scale 15 cm)

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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