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Cassava Breeding by Unconventional Method Using Periclinal Chimera

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Abstract

Manihot glaziovii Muell is a wild cassava species native to Brazil and known as a solid source of resistance to cassava mosaic and brown streak mosaic, the most divesting diseases now of cassava in East, central Africa and south east Asia. It is a source of resistance to meadow bug too. Classical method of hybridization with wild species *M. glaziovii*, is very much troubling and for this reason were neglected by concerned breeders. We present here an efficient and very rapid alternative technique which is synthesizing periclinal chimera from *M. glaziovii* or with its interspecific hybrid. It enabled obtaining hybrids during few months compared to several years in traditional sexual hybridization. Somatic hybridization was confirmed by morphological marker genes of winged fruits having white line. Obtained periclinal chimera enabled establishing varieties expected to have combined favored characters of the two parents. In addition it may exhibit hybrid vigor in relation to higher productivity as other periclinal chimera did in last cases.

Keywords

Somatic Hybridization, Cassava Mosaic Brown Streak Mosaic Meadow Bug

1. Introduction

Cassava is principal food for more than one and half billion poor people in humid tropics of Africa, Asia and South America as shown in **Table 1**. From the table it is noted that Mosaic and brown streak mosaic which are principal diseases destroy more than 70% of plant production in infected areas. Meadow bug; coccinella manihotis; an insect which causes large losses in Nigeria where seven million hectars are planted. A sum of thirteen million hectares in Africa are attacked in the whole continent.

Table 1. Cassava areas and production in 2022.

COUNTRY	AREA	PRODUCTION	T/HA
NIGERIA	7,402,563	60,835,540	6.07
CONGO	4,397,970	48,774,623	20.3
BRASIL	1,343,000	17,648,564	13.1
TANZANIA	1,432,409	6,354,439	7.06
INDONESIA	657,000	13,574,000	17
WORLD	28,563,942	208,627,012.53	11.7

The damage of crop in some regions of south east Asia and Africa reached maximum in Thailand, Uganda and Tanzania where it threatens all country by famine [1] [2].

A situation similar to today's occurred in Kenya and Tanzania some 90s ago, *i.e.* 1930s [3], However, it was saved from femine thanks to efforts of Storey and Nichols [4]. These scientists localized sources of resistance in the wild species; *Manihot glaziovii Muell*, and they successfully developed resistant varieties came from interspecific hybrids of cassava and its wild relative *M. glaziovii*. It conferred solid resistance to cultivated cassava against the two diseases. They reported that within survey of resistance in all wild cassava relatives, only *M. glaziovii* exhibited robust resistance. *M. glaziovii* also possess resistance to insect meadow bug (*Coccinella manihotis*) because the insect cannot adhere on its smooth branches; a caracter not found in other wild cassavas normally infected by insect. No any author tried interspecific hybridizton of *M. glaziovii* and cassva probably because of growth habit. It grows vertical up to 10 metres where it ramificate and form flowers. It is impossible for this reason to collect flowers. Moreover it has habit of flowering only in fifth year. If hybridizations of cassava with *M. glaziovii* was tried in 1990, they would have saved for Nigeria and Congo so millions of dollars that spent on importing parasites and establishing it there to control meadow bug. These exotic insects transformed after few years to another danger on cassava itself.

Old cultivars developed by Storey and Nichols cannot be reintroduced into production. This is due to two reasons: first, accumulation of pests and viruses in plants over 90 years of vegetative propagation, and second, mutability of the virus which has overcome resistance forms, rendering current plantations highly susceptible.

Therefore, it is necessary to reintroduce resistance genes once again from *M. glaziovii*. Trials of synthesizing classical interspecific hybrids resistant to cassava mosaic and brown streak mosaic are absent judging from present scenario of publications in Africa, Asia, and South America. For this reason, this researcher has taken advantage of *M. glaziovii* interspecific hybrids that were bred by him during many years [5] [6] and used them to develop periclinal chimeras between *Manihot glaziovii* hybrids and cassava cultivars.

2. Material and Methods

Interspecific hybrids of Cassava with *M. glaziovii* were produced systematically every year in period 1978 to 1982. *M. glaziovii* was used as pollinator. Its stem was cut several times to enforce it produce lateral branches which grow vertically and they were cut down in following year. Cassava was used as female parent. This is the only way to overcome its pollination incompatibility. Collected hybrid seed suffer sever dormancy which overcome by storing seed for 4 months' period.

To produce periclinal chimera graft technique was applied (**Figure 1**). It was used here interspecific hybrid of cassava with *M. fortalizensis* as rootstocks and cassava selected cultivars as scions. A hybridization made in the year before with one of related wild species *M. fortalizensis* gave a huge root (**Figure 2**) and revealed hybrid vigor, …we tried with *m. glaziovii* this vigor associated with periclinal chimera. This hybrid showed root seen in **Figure 2**. It was the first alert to hybrid vigor in chimera periclinal.

Figure 1. Scions cut in slanted position close to a bud. The rootstock cut in the opposite direction.

Figure 2. Periclinal chimera root of *M. fortalizensis* with cultivar UnB33, 20 months old.

Figure 3. Interspecific hybrids cassava with *M. glaziovii*.

Figure 4. The living collection of Manihot species at the Universidade de Brasilia.

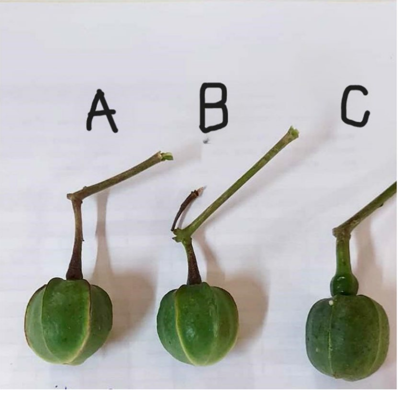

Figure 5. Morfological marker gene of white line on winged fruit indicates soatic hybridization. A fruit of *M. glaziovii* intrspecifi hybrid, B fruit f periclinal chiera, c: uit of cultivar 110.

Cultivars used in grafting to produce periclinal chimera last year are UnB 205, Unb 110, UnB 220 Twenty grafts each with *M. glaziovii* interspecific hybrid were used as stock. Scions cut in slanted position close to a bud. The rootstock cut in opposite direction. The scion and 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.

Any auxiliary shoot and adventitious shoots sprouted from any place except from the graft's union buds were removed. Periclinal chimera were identified according to stem growth compared to wild habit (**Figure 3**, **Figure 4**), leaf morphology; shape and form, and fruit form (**Figure 5**).

3. Results and Discussion

Success of periclinal chimera graft varied from cultivar to another.

It could obtain one plant of trials cultivars 110 and 220 while cultivar 205 did not give any chimera, probably this is due to distant genetic relationship, *i.e.* compatibility between cultivars and interspecific hybrid of *M. glaziovii* [7]. Chimera 9 months old was double in case of cultivar 110 as it was 2.2 kg for chimera against 1 kg of edible roots in cultivar 110. This result confirm what reported by Nassar and Ortiz [8] and Gakepetor *et al.* [9] and finding of chimera synthesized from *M. fortalizensis* with cassava cultivar unb 33, since it gave 8 kg after 18 months compared to 3 kg common cultivar.

Grafting scion of tree like wild species onto stock of cassava have increased root yield up to 7 folds. It results in enlarging root up to a very big size that reaches frequently 8 kg roots per plant. This technique when applied by small farmers in Indonesia enabled preserving wild species and using the vegetative growth as a source of new grafts. Indonesian farmers apply frequently this technique and it is called by them MUKIBAT. Thanks to it productivity of cassava per hectar in Indonesia increased and reached 20 ton per hectar compared to 12 in Colombia and 9 in Nigeria. Using this technique raised important questions about possibility of maintaining this effect in certain cultivar. This author tried to respond this question during the following last 10 years and reached form of elaborating periclinal chimera.

Study of DNA movement within plant tissues has shed light on what may occur and final results of reaction of DNA particularly mRNA of different genotypes when encountered and moved between different layers in cartain plant; and it may result in the exchange of DNA pieces or entire plastid. Researchers suggested graft inheritance and formation of pericinal chimera may be the cause [10].

Genetic alterations and vigorisity of morphological parts of chimera and roots was reported by Wang *et al.* [11]and attributed by them to overdominance gene action in case of cassava roots and morphological growth too.

To have hybrid vigor induced, it must consider DNA movement e consequent contact in the two genotypes cells. This what various scientists in decade 1990s and 2000s reported on movement of DNA masses from tissue to another in grafted plants such as ohata [12]; Stigmann *et al.* [13]. However, no one of them attributed this phenomenon to periclinal chimera which may had been formed accidently in their work and was not noted by anyone of them. As we explained in material and method buds of scion and graft must be in contact and morphological gene marker be noted.

The work of Ferreira *et al.* [14] is the first case of synthesizing periclinal chimera for obtaining resistance to diseases. Last year we presented this case of resistance to nematode due to periclinal chimera. This phenomenon may have arisen and attributed to DNA movement within chimera cells layers.

Should this case prove resistance to mosaic and brown streak mosaic as all references refer to, it will open a door for notable success in cassava and perennial plants breeding due to easy application of periclinal chimera in short time.

For Periclinal chimera use in plant breeding cassava, Nassar and coworkers reported for the first time high vigor accompanying movement of DNA in plant tissue. Earlier in decades 2010s Li *et al.*, [15] reported transformation due to grafting.

Nassar *et al.* as mentioned before reported periclinal chimera has transferred resistance to Nematode in cassava. This confirmed hybrid vigor obtained by him earlier due to periclinal chimera. Later He presented interpretation to both of Hybrid vigor and transference of resistance and attributed both to DNA movement through plant tissues. The present case may offer strong proof on transference of resistance to virus diseases from cultivar to another.

In this work, periclinal chimera is systematically synthesized manually without any hormone and we have had reasonable percentage of success. In previous work, of decade 1940s, movement of DNA is attributed to be through grafts. This was overlooked by Ohata and Stigeman but confirmed by others. Michurin in decade 1940s [16] emphasized on grafts that enabled transference of genetic material to another and ignored totally it has occurred periclinal chimera, probably because it was not detected by him. The result of our trials with different cultivars shows genotypes of cassava respond and exhibit hybrid vigor when there is ability of combination between them.

4. Conclusions

The use of periclinal chimera in transferring resistance to diseases from wild species to cultivated varieties is very promising and probably the most important technique ever developed in the last century because it saves many years wasted in sexual hybridization and backcrosses. In addition, it enables taking benefits of hybrid vigor phenomenon. It also avoids segregation and enables obtaining the whole superior genotype.

NOTE: All of the above mentioned chimeras are available for any interested researcher from East and Central Africa, and south east Asia too. are invited to experiment these chimeras for resistance to mosaic and brown streak mosaic without paying any money. It is requested only a simple acknowledgment to researcher contribution.

Acknowledgement

The above mentioned living collection of Manihot species had been established at the Universidade de Brasilia with help of CNPq Brasilia and the International Development Research Center IDRC, in 1979. Kuwait prize received by senior author in 2013 from KFAS enabled maintaining this program.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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