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Cassava Periclinal Chimera Vigor: A theory on its origin

N.M.A. Nassar

Departamento de Genética e Morfologia, Universidade de Brasília, Brasília, DF, Brasil

Corresponding author: N.M.A. Nassar
E-mail: Nagibnassar@geneconserve.pro.br

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ABSTRACT. Cassava Periclinal chimera plants could be synthesized by a very easy grafting method. Thanks to this technique we can produce vigorous plants during a very short period. This can be achieved by grafting two types that have high combining ability. The most striking feature is that we can obtain enormous roots up to five times the common ones. It is suggested this vigor is due to gene action of overdominance and there is DNA movement from epidermis (one cell layer tissue) to internal tissues. Previous results are explained in view of this present one.

Key words: Grafting; Combining ability; Hybrid vigor; DNA movement; Resistance transference

INTRODUCTION

Chimera is constituted from two different tissues in plant as well as animal or any seres vivos (Tilney-Bassett, 1986). Chimera attracts plant breeders because of its potentiality to combine two different forms within a very short time. It arises normally from a bud either apical or lateral. It is formed from three layers which are the outer layer referred to by L1, the second inner layer L2, the third and inner layer L3 which gives the central tissues (Marcotrigiano and Gradziel, 1997; Chen et al., 2006; Ohtsu and Kuhara, 1994).

Chimera is classified into three types: sectorial, mericlinal and periclinal (Marcotrigiano, 1997) Sectorial is composed of two different sectors extending on the longitudinal part of the whole plant. Periclinal has the outer (epidermis) layer different genetically from the whole inner tissues. Mericlinal chimera has part of one layer, normally the outer layer different from the whole plant tissues.

Periclinal chimera is the unique stable while the other two types are unstable, and is responsible to commercial varieties of chimera. It is perpetuated because its lateral buds are the same structure of the apical one. Vegetative reproduction by farmers permits also this maintenance.

Recent research of Nassar and coworkers (Nassar and Bomfim, 2013, 2014, 2016; Bomfim and Nassar, 2014) exposed and showed clearly the use for interspecific chimera to develop a new useful cultivar. One of the most striking features is what proved by Nassar and coworkers (Ferreira et al., 2021) that periclinal chimera can be used to transfer resistance to diseases and consequently this can be applied to other characters.

Periclinal chimera can be synthesized within a very short time compared to several generations of backcrosses needed in classical breeding methods which consume various years of hybridization. Moreover, as shown by authors in the last twelve years, the use of periclinal chimera may enable perpetuate what observed of vigor and consequent high productivity (Gakpetor et al., 2017).

In the University of Brasília, it could be synthesized periclinal chimera by a very simple grafting (Gakepetor et al., 2017; Nassar, 2019). This excluded totally hormone use. This can be done simply by using a whip method where buds of both scions and rootstock are being cut to half, then wrapped by a tape. This assures formation of callus in both sides of graft and scion giving rise to future periclinal chimera.

Morphological criteria are used to identify periclinal chimera based on the fact that L2 forms a subepidermal layer which is responsible to flower and fruit formation. Interaction of two layers of different origin leads to new forms of leaves (Pratt and Einser, 1975). The fact L3 is responsible to form the whole internal tissues, including the cylindrical vascular and form the roots too permit us know to what genotype L3 belongs to. This can be done by chromosome counting (Stewart and Derme, 1979; Sugawara et al., 2002; Nassar and Bomfim, 2010). This chromosome counting of the roots may shed light on constitucion of the periclinal chimera in case of differences in chromosome number of the two parents (Gakpetor et al., 2017).

Feasible synthesis of chimera

In the beginning, chimera noted arising from adventitious shoots at graft union and was called graft hybrids since it believed they were the result of cell fusion. Later it was discovered it belonged to two different species.

Very few chimera shoots could be obtained in the past until technique of Nassar and coworkers (Gakpetor et al., 2017) where they grafted by whip method two manihot species noting the buds be cut to half of both stock and graft. This stimulated callus tissue between the two buds from which arises chimera.

Estimated chimera formation was calculated up to 15% compared to 1% in the chimera grafting and use of hormone method (Nassar and Gakpetor, 2021) Grafts should be done prior to bud sprouting and the graft should be positioned so that both buds of scion (donor) and root stock are in close physical contact (Figure 1A; Figure 1B).

A critical point was made here to cut both of the two in contact buds to half before they positioned in graft. This improved notably the percentage of periclinal chimera obtained because it enhanced formation of callus from which the adventitious branch shall sprout giving the periclinal chimera.

Efficiency in plant breeding

Periclinal chimera of two genotypes can be considered a new variety with its new characteristics. Examples came from this author and co workers demonstrated that synthetic chimera could be an important source of new phenotype and can transfer genotype from either two parents (Ferreira et al., 2021). The most important advantage of this new breeding method is the short time of synthesis of periclinal chimera during very few months compared to several years necessary for producing a new variety in conventional methods. In cassava, which is a very efficient plant in transferring solar energy to carbohydrate this means economizing much time to gain energy and food.

The recent discoveries by Nassar and coworkers on periclinal chimera properties made it possible to replace traditional methods in addition to feasibility of synthesis by simple modification of grafting methods (Nassar and Gakpetor, 2021). In the past, periclinal chimera had contributed a lot in diversification of fruit chimera that arose in nature (Hocquigny et al., 2004). The most important of these is that periclinal chimera can transfer resistance to disease from any of its two parents producing a resistant type within a very short time not pass very few months (Ferreira et al., 2021). In traditional methods of plant breeding it needs hybridization between resistant types and susceptible one followed by backcrosses that normally consume several years. In perennial plants where vegetative propagation is predominant the new characters obtained by periclinal chimera method can be perpetuated. This is impossible to do in other techniques.

Third advantage is obtaining the vigor of a hybrid when using two varieties of high combining ability to synthesize a new periclinal chimera. For example, periclinal chimera formed from species *M. fortalizensis* with cassava cultivar UnB 338 was very vigorous and reached 3 meters height in 10 months compared to common cassava cultivar cultivar which reached only 1 meter height in the same period under the same experimental conditions.

If species *M. fortalizensis* left to grow normally it would reach a maximum of 2 m height in the same period. Productivity of periclinal chimera formed from this species with either cassava cultivar was very productive reaching 120 ton per hectare compared to 20 ton productivity for the best common cassava cultivars (Gakepetor et al., 2017, Figure 2).

This productivity of cassava periclinal chimera has never been reported in any cassava literature up to this moment. On the other hand when synthesized periclinal chimera from cassava with another species, *M. pohlii* it gave fibrous roots with no any edible roots (Nassar et al., 2011). This result supports the idea of combining ability and overdominance responsible to periclinal chimera vigor.

This phenomenon of high productivity of certain parents against very low productivity of other two genotypes in periclinal chimera may be interpreted by classic theory of combining ability (Hayman, 1954; Griffings, 1956).

M. fortalizensis is a new Manihot species named by Nassar and co workers (Nassar et al., 2011). It is believed to be a recent evolving species collected from Fortaleza region in Ceara state Brazil. Probably came by interspecific hybridization of M. glaziovii and cassava.

Combining ability can be interpreted by genetic interaction of genes of the two grafted parents involved in forming periclinal chimera: *M. fortalizensis* and common cassava cultivars. Various reports confirmed RNA transference of the plant vasculars. Probably the most striking feature came from Stegemann and Bock (2009) who reported gene transfer in the contact zone between scion and rootstock. In case of periclinal chimera, the contact zone is extended in all plants since the epidermis layer extends to the whole plant and it is formed from one cell depth.

Before Stegemann, almost half a decade Ohta (2004) reported chromatin transfer from stock cells through the vascular system across the graft union to the scion and he explained clearly how this chromatin transference causes transformation in scion flower primordia. Moreover, Ohta suggested genetic material might move between cellular components. Taller et al. (1998) showed introduction of some characteristics of stock into progeny when it selfed and obtained seed that were grown. They also noted the appearance of a novel in germinating seed.

Apparently there were change in heritable characters where Taller et al. worked on, but there is doubt on the plant they worked on if it was simple graft or it was in fact a periclinal chimera. In that time, periclinal chimera was not a focus in grafting work neither it was mentioned in the works of Taller or Ohta. It is believed that their plant was in fact a periclinal chimera produced accidentally while applying the graft and this is most probably to happen while applying whip graft technique. Formation accidently of periclinal chimera permitted the translocation of DNA from stock to scion and produces the novel characters. This is because periclinal chimera is constituted by a layer of one cell belong to a variety extends along the whole plant and surrounding internal tissues of the second variety in the whole stem.

This coincides with what Stegemann and Bock reported that genetic exchange can occur in both directions , but only involves the transfer of plastid DNA between cells and is limited to the site around the graft i.e. the site where exists contact between the cells from the grafted genotypes. The transference of DNA between cells around the graft is possible in the whole epidermis layer covering internal tissues, but in a simple graft it is limited to the site around the graft.

Synthesizing periclinal chimera in our method explained above show it is very similar to common graft and differs only in connecting the two buds of stock and scion together. This may be had been done in the published works of Taller et al. and Ohta et al. without paying attention to arising periclinal chimera type in place of expected simple graft. This idea may receive support of fact that only one plant was studied in case of Taller et al.

The periclinal chimera vigor noted by us every time using M. fortalizensis as a parent against poor performance when using another parent such as M. pohlii let us deduce that genes of M. fortalizenzis may have contact with genes of common cassava genotypes that form the inner layers and they together achieve complementation or overdominance and this may have lead to express vigor.

Heterose has been well explained by Tsafaris et al. (2008). He reported that if there is overdominance combined with additive genes it will lead to the expression of more heterosis. Hull (1945) adopted a similar theory. 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. fortalizensis* (3+2) and it is 6 alleles in case of *M. fortalzensis* 4x.

According to Tsaftaris and Polideros (2008) clearly the increased number of loci seen in triploid or tetraploid will result in increasing quantitatively higher genetic expression and induces hybrid vigor.

Probably the most important feature of our results is the compatibility seen in certain combinations such as that of *M. fortalizensis* with UnB 338, UnB 031 and UnB 201 against extreme incompatibility seen in case of grafting *M. fortalizensis* with UnB 205 (Nassar et al., 2010; Nassar, 2000). The incompatibility in the latter case could be attributed to the fact that the cultivar has evolved through hybridization with wild species distant genetically from *M. fortalizensis*. Frequent hybridizations between Manihot species and cassava do happen in nature as confirmed recently (Bredeson et al., 2016).

This interpretation finds support in what was seen and found incompatible *M. glaziovii* was grafted with cultivar developed by hybridizing cassava with M. aesculifolia (Nassar and Gakepetor, 2021). The conclusion reached from these examples is that: 1. Vigor of root formation in periclinal chimera depends on combining ability of the two genotypes grafted to form the chimera. 2. There is gene movement along all the two layers in contact with the chimera. Moreover, vigor is enhanced when chimera is developed by grafting two polyploidy forms.

Apparently there is transference from DNA of epidermis composed from a genotype to internal tissues developed from another type. This phenomenon of DNA translocation within grafted plants has been confirmed by several authors in the last few years (Stegemann and Bock, 2009; Ohta, 2004).

The transference of DNA of epidermis to internal tissues led to reaching the most important plant breeding phenomenon which is transference of resistant to diseases from paternal genotype to periclinal chimera plant, producing new resistant cultivar. This result was confirmed by what found by Nassar and collaborators (Ferreira et al., 2021). In this work resistance to nematode was transferred from the resistant genotype M. fortalizensis by the technique of periclinal chimera. The final product, periclinal chimera acquired resistance from M. fortalizensis having its tissue forming the sub epidermis and the internal tissue.

Publication of Nassar and co-workers is the first information on the incorporation of parental resistance to the chimeric component. This probably due to the very recent technique of the periclinal chimera, introduced only a few years ago (Bomfim and Nassar, 2014).

The only case reported resistance to insects by periclinal chimera through its epidermal layer came from Goffreda and co workers in 1999. It was resistance to the potato aphid conferred by glandular trichomes in Lycopersicon pennellii. This resistance was acquired by *L. esculentum* by the production of an interspecific chimera with L1 layer of L. pennelli (Goffreda et al., 1990).

Periclinal chimera will draw attention of crop breeders due to the short time needed for synthesis and reproduction vegetatively; normally this period extends to decades in case of improving varieties by classical methods. Moreover it brought to reality breeders dream of perpetuating hybrid vigor achieved by combing ability.

Figure 1. (A) Scions cut in slanted position close to a bud. The rootstock cut in opposite directions. (B) Scions placed in close contact having juxtaposition of scions and rootstock so that both buds make contact with each other.

Figure 2. Periclinal chimera of *Manihot fortalizensis x* UnB 031

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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DEDICATION

Dedicated to the memory of F. A. M. Duarte, Editor, Scientist, and Grand pioneer of Genetics journalism in Brazil.