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Periclinal Chimera: A New Efficient Plant Breeding Technique

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Abstract

Periclinal chimera plants could be synthesized by a very easy grafting method. Thanks to this technique we can transfer useful characters from one genotype to another, we also can produce vigorous plants during a very short period. Recently in cassava, resistance to nematode could be transferred, and could develop vigorous plants by combining 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. By using this type of chimera we can replace traditional hybridization and no need to recurrent crosses cycles to transfer useful characters, reducing to a very short period developing a new variety and perpetuating it too.

Keywords

Grafting, Combining Ability, Hybrid Vigor, DNA Movement, Resistance Transference

1. Introduction

Chimera term refers to fusion of two different forms in plant as well as animal or any *seresvivos* [1]. 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. But normally 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 [2] [3] [4].

Chimera is classified into three categories genotypes: sectorial, mericlinal and periclinal [5]. 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 chimeras have 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 for commercial varieties of chimera. Periclinal chimera 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 [6] [7] [8] [9] exposed and showed clearly the use for interspecific chimera for developing a new useful cultivar. One of the most striking features is what proved by Nassar and coworkers [10] that periclinal chimera can be used to transfer resistance to diseases and consequently this is applied to other characters.

This can be done within a very short time compared to several generations of back crosses needed in classical breeding methods which consume various years of hybridization. Moreover, as shown by various authors in the twelve years, the use of periclinal chimera may enable perpetuate what observed of vigor and consequent high productivity in addition to genetic effect that results from interaction between the two genotypes forming the periclinal chimera [11].

In the University of Brasília, periclinal chimera could be synthesized by a very simple grafting [11] [12]. This excluded totally hormone use. This simply uses 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.

To identify and confirm periclinal chimera formation, morphological and cytogenetics criteria are used. This is based on ontogenetically information. Because we trace epidermis layers in leaf and young stem surfaces and can differentiate L1 derived cell types epidermal cells, trichomes, and guard cells [13].

Morphological criteria are used too based on the fact that L2 forms a subepidermal layer which is responsible for flower and fruit formation. Interaction of two layers of different origin leads to new forms of leaves [14]. The fact L3 is responsible to form the whole internal tissues, including the cylindrical vascular and form the roots too, we can know to what genotype L3 belongs by chromosome counting [15] [16]. This chromosome counting of the roots may shed light on constitution of the periclinal chimera in case of differences in chromosome number of the two parents.

2. How to Synthesize Chimera?

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

Some scientists since 1930 tried synthesizing periclinal chimera by simple grafting, but it was not assured and probably ignored the importance of buds fusion and callus formation. [17] was the first to develop a method of chimeration formation from grafting two species after union of the graft parts, he cut it though transversely. Callus then developed, from which adventitious shoots formed. Yet he didn't note the importance of buds fusion and callus formation

and so didn't note the following researchers.

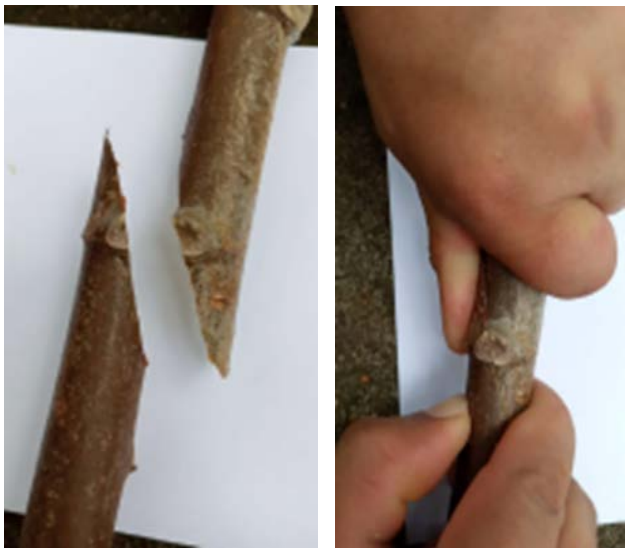
Very few chimera shoots could be obtained in the past until technique of Nassar and coworkers in 2010's [11] where they grafted by whip method two Manihot species noting the buds be cut to half of both stock and graft. This stimulated callus tissue between the two buds form which arises chimera.

Estimated chimera formation was calculated up to 15% compared to 1% in the chimera grafting and use of hormone method [13] 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 1(a)** and **Figure 1(b)**).

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.

3. Efficiency in Plant Breeding

Periclinal chimera of two genotypes can be considered a new variety with its new characteristics. These characteristics come from either of the two parents and are imposed on to the other genotype. Examples from this author and coworkers demonstrate that synthetic chimera could be an important source of new phenotype and can transfer genotype from either two parents [10]. 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.



(a) (b)

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.

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 [13]. In the past, periclinal chimera had contributed a lot in diversification of fruit chimera that arose in nature [18]. 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 [10]. 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 such as cassava where vegetative propagation is predominant the new characters obtained by periclinal chimera method can be perpetuated. This is an advantage not possible 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 species *M. fortalizensis* Nassar *et al.* 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 (**Figure 2**).

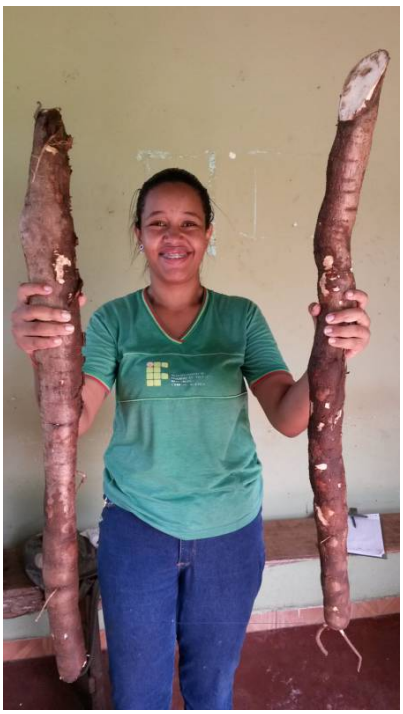


Figure 2. Periclinal chimera of. *M. fortalizensis* × UnB 031.

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 [19].

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.

M. fortalizemsis is a new Manihot species named by Nassar and coworkers [19]. 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 [20] 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.

Before Stegemann, almost half a decade, Ohata 2004 [21] 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, Ohata suggested genetic material might move between cellular components.

The periclinal chimera vigor noted by us every time using *M. fortalizensis* as a parent against poor performance everytime used another parent such as *M. pohlii* bring deduction that genes of *M. fortalizenzis* may have contact with genes of common cassava genotypes that form the inner layers. And it achieves complementation with other genotypes layer in periclinal chimera and this may lead to express vigor.

Heteroses has been well explained by Tsafaris 2008 [22]. He reported that if there is over dominance combined with additive genes it will lead to the expression of more heterosis. Hull 1945 [23] adopted a similar theory. In case of *M. fortalizensis* which is 3× or 4× 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* 4×.

According to Tsaftaris and Polideros, 2008 [22] 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 striking 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 [24] [25]. 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 [26].

This interpretation finds support in what was seen when *M. glaziovii* was grafted with cultivar developed by hybridizing cassava with *M. aesculifolia*, *i.e.* the first generation of interspecific hybridization that further when polyploidized gave the above mentioned type [13].

4. Conclusions

The conclusion reached from these examples is that vigor of root formation in periclinal chimera depends on combining ability of the two genotypes grafted to form the chimera. 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 [20] [21].

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 Nassar and co-laborators [19]. 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. Apparently, resistance was due to interaction of DNA of the chimera components since they move within all plant tissues [20].

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 by Nassar and coworkers [9].

The only case reported resistance to insects by periclinal chimera through its epidermal layer came from Goffreda and coworkers in 1990 [27]. 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* [27].

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.

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Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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