



**Comment by Nagib Nassar on article: Introgression of genes from wild
cassava ,Doi 10.1007/s 10681-9685-6 :
Euphytica, 2008**

Author of the comment : Nagib M.A. Nassar, Professor, Universidade de Brasilia, Brazil

Keywords: Cassava, dry matter, interspecific hybridization, gene markers

The following comment was submitted to Euphytica. It was reviewed by the same journal as seen below. We feel free to publish both of the comment and the review. Readers are invited to comment. We welcome different opinions. It is our policy to have Geneconserve as an educative forum of discussion.

This particular paper tries to give an account of using interspecific hybridization within the genus of cassava (*Manihot* spp.). It is good to try interspecific hybridization with cassava. Its wild relatives possess valuable genes to improve the crop but they were neglected in the past. However, it is difficult to do the job. Interspecific barriers, hybrid sterility, and severe seed dormancy are some obstacles among others. This comment may be useful to both readers and authors as well.

1. Frequently cassava is pollinated by tiny insects of Coleoptera (Nassar and Carvalho, 1990). Because of their very small size they enter in sacs which protect cassava panicles. It seems to readers that this fact did not receive sufficient attention. Interspecific hybridization with cassava has been dealt as it is the case of other crops where there is no problem of these insects. Because of this obstacle, we normally manage it by using gene markers and using wild species as maternal parent since it possess recessive genes. There are many morphological gene markers presented in section photos gallery of www.geneconserve.pro.br <http://www.geneconserve.pro.br/> [http://www.geneconserve.pro.br/>](http://www.geneconserve.pro.br/) It may be useful visiting this page. Only in case of using gene markers one can trust that interspecific hybridization has happened (Nassar, 1999, 2006). Gene markers must be reported in such work. Their photos must be presented too. *M. pseudoglaziovii* which is reported to have been used as a parent exhibits moderate barriers between it and cassava, so there is possibility of these progenies no more than auto pollination within cassava plants itself. It is likely that protection by sacs did not impede these tiny polinator insects of Coleoptera

from pollinating cassava.

2- *Manihot pseudoglaziovii* exhibits severe dormancy of seed (Nassar, 1999, Nassar and Ortiz, 2008). If *pseudoglaziovii* used as maternal parent as is explained above, progeny seed will show severe dormancy and will be very difficult to grow unless breaking dormancy has been done (Nassar, 2006, 2007). It is noted that no mention to break dormancy in the article. Available literature (Nassar 2007, Nassar et al., 2007, Nassar and Ortiz, 2008) referred to importance of breaking dormancy to grow progeny of interspecific hybrids.

3. As it is explained above, *Manihot pseudoglaziovii* exhibits moderate barriers between it and cassava. Trials to break these barriers should be done. Some methods were used and proved efficient to achieve this breakthrough (Nassar et al. 1996, Nassar, 1999). This factor is really an important one. It may puzzle who is familiar with interspecific hybridization that crosses done without considering it. Normally it is recommended making new trials using one of methods mentioned in above literature.

4. There are many wild species of *Manihot* species, almost 100 (considering new species described in the last years). Many material used by some breeders are no more than escapees, no more than weeds. They do work well as a source of certain useful genes, but not genes normally donated by the wild progenitor. Because of this reason a precise classification and description must be done by an expert in this field. The material should be photographed, described. Both photos and description must be included in the article. This will guarantee for reader a correct idea about material used in the experiment.

5. *M. tristsis* is a doubtful species as this been raised by Rogers and Appan (1973). probably it is no more than scape of cassava or an extreme segregation of it. In this case hybridization which was made is no more than crosses within cassava gene pool itself (Nassar, 2006) Nassar and Ortiz, 2008, Nassar et al, 2007, Rogers and Appan, 1973).

6. Increasing dry matter content does not mean increasing starch content. Since crosses have dealt with wild species, it is likely that what has been increased is fiber content!! Fibers and starch are principal contents of dry matter in cassava root. Readers may have a better idea if starch and fibers contents were estimated separately in the selected introgressed root. HCN is also an aspect that should be considered. It is expected that it would have been astronomically increased, because HCN content in *M. tristsis* is more than 1000 mgm/kgm (Nassar and Souza, 2006).

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Comment of Reviewer of EUPHYTICA :Manuscript Number: EUPH2654R3

In general the comments on the manuscript recently published raise relevant issues, which are important but do not necessarily affect the quality of the original article. My comments to each point raised by Nassar are below:

1) The revision makes two major assumptions that are not necessarily true:

a) The tiny insects that the author found in Brasilia are not necessarily found in Cali and b) the bags protecting the inflorescences will allow insects to go through. However, these are considerations that the authors/readers of the comment may find useful for future work. The use of morphological markers, although very useful for genetic studies, would be cumbersome in a breeding project to introgress useful genes. Besides, the inheritance of these markers is not always fully understood. Therefore, the recommendation that "Gene markers must be reported in such work" is too strong. It may be recommendable to do it, but it is not a "must".

2) Point well taken. Perhaps the authors found dormancy, broke it and failed to report it in the original article.

3) The fact that inter-specific crosses may expose moderate barriers is not new. The original article was a study to introgress genes found in wild *Manihot* species. It was more about breeding than about genetics and feasibility of inter-specific crosses. The commentator assumes that the authors did not consider these issues but seems to be an unfounded assumption.

4) As true as this may be, the commentator needs to prove his assessment that what breeders use "are no more than escapees, no more than weeds". This comment seems to me disrespectful to scientists that, in the breeding activity may not be expert taxonomists but rely on people that, one assumes, have the capacity to identify and distinguish different *Manihot* species. The original work was conducted at a place that holds the world cassava collection, has a qualified botanist as curator. It seems to me cumbersome and expensive to be publishing photographs to demonstrate that the parents of a given inter-specific cross were indeed different species. One has to assume that giving a scientific name should be enough "for the reader a correct idea about the material used in the experiment".

5) *Manihot tristis* may or may not be a different species. The authors of the original paper report *M. tristis* and the readers will understand what this is. If *M. tristis* is found not to be a different species, or a sub-species, or just a special type of cassava everybody will understand what the authors used as a progenitor in the original study.

6) Point well taken. Dry matter and starch content are not the same. There is a very tight correlation, which may have held or not in the study. The authors did not measure starch content, so we'll never know. HCN faces a similar problem.

I think that there are some relevant issues raised by the commentator, but also some unfounded assumptions. I would recommend that the comments are published but after considerable editing and corrections. I would also recommend that the references listed are drastically reduced to no more than 5.

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Answer to review by Nagib Nassar

1.If tiny Coleoptera insects are not found in Cali, Is there any publication? Is there any research done to show this? There is a logical question: Why a survey on pollinators of cassava at this place not has not been done before begining its program of plant breeding?, or at least its project of interspecific hybridization ?!!

With respect to morphological markers, they are preliminary principles taught to under graduate students in any undergraduate plant breeding program. If the inheritance of cassava gene markers is not known for authors, why they did not research it before they begin their program of interspecific hybridization? The first lesson given at a plant breeding course is to determine morphological gene markers of a certain crop !! Please note that a lot of cassava morphological gene markers are published at photos gallery of www.geneconserve.pro.br for example

2. Good that this topic is taken, I add: if dormany had been broken, it must have been reported ?!!! It is an essential informations and limiting factors for success on any interspecific hybridization. . Readers must be informed how it been broken, and how much was the success, otherwise they take a wrong idea that there was no any dormancy.

3.If the existence of interspecific barriers is admitted by authors, why not reported? This is one of the most difficult work in cassava interspecific hybridization for which any author is credited. Any reader familiar with these barriers may wander why no any mention to it?

4. Identifying the material, and describing it is one of the essential pre requisite of any publication. Any researcher cannot omit it. In this case of cassava interspecific hybridization, Not describing the material opens door for many enquiries and many questions: The most simple one of them is: The material used was`nt an extreme segregate of cassava itself? This assumption my be supported by absence of seed dormany, absence of barriers and absence of gene markers!! Photos of Leave, Inflorescence and Fruit form

5. I disagree , and find such statement unfair. Readers will not understand what material been used, or its nature. Any person will repeat the work must know exactly what kind of material had been used

6. Good that this point is taken.

7. I disagree on reducing references because of a simple reason: All of them are cited in the text , and, all of them needed to understand the text.

Comments of Scientists :
Nagib;

I am not an expert in this area, but what you have said seems very fair.

Best, Darna
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Nagib:

Excellent and let time and wisdom give the truth

Larry Daley

Larry Daley
Professor Emeritus
Oregon State University, USA
(Vegetable Crops)

Nagib

I am of the strong opinion that scientifically, professionally and socially this is important issue for many countries in the world. Such issues must be high lighted and need immediate attention for publication.

Shyam Yadav
Chief Scientist, National Agricultural Research Institute (NARI),
Papua New Guinea