Introduction of inbreeding in cassava genetic improvement

Introduction
Field activities
Tissue culture work
The future

Cassava breeding is difficult because:

- Maintains a large “genetic load” in our populations
- We never “capture” genetic superiority
- We never separate properly SCA (non-additive) from GCA (additive) genetic effects
- Hybrids are not “designed”, just found

Inbreeding offers many advantages for cassava genetic improvement

Exposes recessive traits; eliminate bad ones exploit good ones
Inbreeding offers many advantages for cassava genetic improvement

- Exposes recessive traits: eliminate bad ones exploit good ones
- Leads to a more efficient exploitation of non-additive effects

Results from three diallel studies in different environment

<table>
<thead>
<tr>
<th>Genetic Parameter</th>
<th>Sub-humid</th>
<th>Mid-altitude</th>
<th>Sub-humid</th>
<th>Mid-altitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>σ²A Fresh Root Yield</td>
<td>17.88</td>
<td>1.452</td>
<td>0.0009</td>
<td>0.0015</td>
</tr>
<tr>
<td>Acid Soils</td>
<td>1.485</td>
<td>3.379</td>
<td>0.0025*</td>
<td>0.0009</td>
</tr>
<tr>
<td>Mid-altitude</td>
<td>11.9</td>
<td>1.43</td>
<td>0.0025*</td>
<td>0.0009</td>
</tr>
<tr>
<td>σ²D Thrips SED</td>
<td>23.87*</td>
<td>0.765</td>
<td>0.0027*</td>
<td>0.0011</td>
</tr>
<tr>
<td>Acid Soils</td>
<td>9.028</td>
<td>0.873</td>
<td>0.0025*</td>
<td>0.0011</td>
</tr>
<tr>
<td>Mid-altitude</td>
<td>152.1*</td>
<td>2.47**</td>
<td>0.0018**</td>
<td>0.0001</td>
</tr>
<tr>
<td>Epistasis test Thrips SED</td>
<td>100.40**</td>
<td>4.257**</td>
<td>0.0013</td>
<td>0.0001</td>
</tr>
<tr>
<td>Acid Soils</td>
<td>15.054**</td>
<td>0.872</td>
<td>0.0014</td>
<td>0.0001</td>
</tr>
<tr>
<td>Mid-altitude</td>
<td>168.9**</td>
<td>-0.32</td>
<td>0.0014</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Dominance effects cannot be transmitted

Imagine that the phenotypic values for the three possible genotypes in a given locus are

- AA = 2
- Aa = 5
- aa = 2

When a plant produces gametes the special phenotypic effect (value = 5) that resulted from the specific combination of alleles A and a is lost.

Gametes transmit alleles, not allelic combinations...
Epistatic effects cannot be transmitted

Complementary gene action is a typical example of simple epistatic effects between two loci in Mendelian genetics.

Aa Bb

Self pollination

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Genotype</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AA BB</td>
<td>= 18</td>
</tr>
<tr>
<td>2</td>
<td>AA Bb</td>
<td>= 18</td>
</tr>
<tr>
<td>1</td>
<td>AA bb</td>
<td>= 6</td>
</tr>
<tr>
<td>2</td>
<td>Aa BB</td>
<td>= 18</td>
</tr>
<tr>
<td>4</td>
<td>Aa Bb</td>
<td>= 18</td>
</tr>
<tr>
<td>2</td>
<td>Aa bb</td>
<td>= 6</td>
</tr>
<tr>
<td>1</td>
<td>aa BB</td>
<td>= 5</td>
</tr>
<tr>
<td>2</td>
<td>aa Bb</td>
<td>= 5</td>
</tr>
<tr>
<td>1</td>
<td>aa bb</td>
<td>= 2</td>
</tr>
</tbody>
</table>

Epistatic effects cannot be transmitted

Complementary gene action is a typical example of simple epistatic effects between two loci in Mendelian genetics.

When a plant produces gametes the special phenotypic effect (value = 18) that resulted from the specific combination of alleles A and B is lost.

Gametes transmit alleles, not allelic combinations...

Aa Bb

Self pollination

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Genotype</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>A- B-</td>
<td>= 18</td>
</tr>
<tr>
<td>3</td>
<td>A- bb</td>
<td>= 6</td>
</tr>
<tr>
<td>3</td>
<td>aa B-</td>
<td>= 5</td>
</tr>
<tr>
<td>1</td>
<td>aa bb</td>
<td>= 2</td>
</tr>
</tbody>
</table>

How does maize manage to show progress?

Line A₀

(♀)

Line B₀

(♂) x

Good Hybrid A₀B₀

Line A₁

x

Line B₁

Better Hybrid A₁B₁

Line A₂

x

Line B₂

Even Better Hybrid A₂B₂

Line A₃

and so on...

Line B₃

How does the system work?

Additive effects come in lines with few 'bad' alleles.

Dominance 'hides' the genetic load of inbred
How does the system work?

Inbreeding offers many advantages for cassava genetic improvement

Exposes recessive traits: eliminate bad ones exploit good ones

Leads to a more efficient exploitation of non-additive effects

Enhances the value of different traits: backcross scheme

Enhances exchange of germplasm: reduced phytosanitary regulations

Facilitates cleaning of elite clones: remake hybrids through pollinations

Facilitates conservation of germplasm through botanical seeds

Facilitates genetic/molecular studies providing homozygous parents

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Understanding inbreeding depression and inheritance of relevant traits in cassava

Several hundreds of self-pollinated seed from nine elite clones produced

From each elite parent 100 S1 clones were planted in trials

Each trial involved three replications, with three plants/replication

Evaluation conducted in a single location
A group of S3 plants has been analyzed using SSR markers to measure average degree of homozygosity. Results are very satisfactory. One of these plants will be used for full genome sequencing in a project financed by the USA. DNA is currently being extracted and in vitro plants produced so everybody can have access to this reference genotype.

Inbreeding offers many advantages for cassava genetic improvement:
- Exposes recessive traits: eliminate bad ones exploit good ones
- Leads to a more efficient exploitation of non-additive effects
- Enhances the value of different traits: backcross scheme
- Enhances exchange of germplasm: reduced phytosanitary regulations
- Facilitates cleaning of elite clones: remake hybrids through pollinations
- Facilitates conservation of germplasm through botanical seeds
- Facilitates genetic/molecular studies providing homozygous parents

IT TAKES TOO LONG!!
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Cassava microspore derived callus

Proof of concept
Non-reproducible
No plant regeneration attained

Cassava Tetrad Developmental Stages

At cytokinesis

Within the anther

Tetrahedral arrangement

Before releasing microspores

Cassava Pollen Development

Early Uninucleate

Mid Binucleate

Early Binucleate

Pollen Grain
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Field activities
- Pollen development
- Tissue culture work
- Microspore isolation

The future

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Clean isolated microspore suspension by blending method (adapted from Lui et al., 2002) and using Percoll 50%-60%-70% (adapted from Kyo and Harada, 1986).

(A) Upper centrifugation band showing tetrads and uninucleate microspores.
(B) Middle centrifugation band rich in uninucleate microspores.

Cassava microspore division and microcallus formation

Clean isolated microspore suspension by blending method (adapted from Lui et al., 2002) and using Percoll 50%-60%-70% (adapted from Kyo and Harada, 1986).

(A) Upper centrifugation band showing tetrads and uninucleate microspores.
(B) Middle centrifugation band rich in uninucleate microspores.
Work ahead

• Develop a system that will allow detect cell division in spite of thick wall

• Continue the screening of culture media and pre-treatments that will induce cell division

• Once calli from microspores are developed work out the protocol for regeneration

• Continue self-pollinations of elite germplasm and landraces in search of useful traits and to reduce inbreeding depression in cassava