Heterotic Grouping & Hybrid Breeding in Tropical Maize

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Product Profile Based Breeding for Increased Genetic Gains
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Outline

• Overview major maize heterotic groups in the world
  – Concept of heterotic groups and patterns
  – Maize heterotic groups in CIMMYT
  – Strategies for the establishment of heterotic groups

• Hybrid Breeding in Tropical Maize
  – Key decisions in hybrid development
  – Testing inbred lines in hybrid combinations
  – Identification of testers
Introduction

• Shull (1908, 1909) and East (1908) conducted experiments on heterosis and inbreeding ↔↔. The pure-line hybrid concept

• They observed that when maize plants are selfed, their vigor and grain yield declines rapidly. However, when two inbred lines are crossed, both vigor and grain yield of the F1 hybrid often exceeds the mean of the two parents.

• To exploit heterosis in hybrid breeding, the concept of heterotic groups and patterns was suggested

• **Heterotic group** is related or unrelated genotypes from the same or different populations, which **display a similar combining ability and heterotic response** when crossed with genotypes from other genetically distinct germplasm groups.

• **Heterotic pattern** specific pair of two heterotic groups, which may be populations or lines, **that express in their crosses high heterosis and consequently high hybrid performance.**
Introduction

• The concept of heterotic patterns includes the subdivision of the germplasm available in a hybrid breeding program in at least two divergent populations, which are improved with inter-population selection methods.

• Two populations of a specific heterotic pattern are typically improved as follows:
  – Progenies are generated within the same heterotic pool.
  – The progenies are then evaluated for their yield performance when test-crossed with a tester from the opposite heterotic pool.
  – Lines showing superior testcross performance are inter-mated to form the next cycle of selection.

• Heterosis has been extensively studied in maize because of
  – its large expression for grain yield (100-200%),
  – its intensive exploitation in hybrid breeding of maize, and
  – ease of both self- and controlled cross-fertilization.
Maize Heterotic Groups in USA

- Hybrid breeding was started in the 1920s.
- In 1924: the first varietal crosses sold in USA (Crabb, 1947).
- In 1930s: first successful **double-cross hybrids**.
- In the late 1950s, transition to more productive **single-cross hybrids** marked the inception of two so-called heterotic groups, **Iowa Stiff Stalk Synthetic (SS)**, and **Non-Stiff Stalk (NSS)**, which today constitute genetically distinct breeding pools providing superior hybrid performance.
- Corn Belt Dent varieties **Reid and Lancaster** were in widespread use in the USA before the onset of the hybrid maize era and have emerged as opposing components in the dominant heterotic pattern in the USA. **Iodent** is commonly accepted as a strain of Reid.
- Heterotic patterns gained recognition with the advent of commercial single crosses in the U.S. Corn Belt in the mid 1960’s and the need for **female parents that could produce amounts of seed to economically support the commercial use of single crosses**.
- Commercial companies expanded the male pool as they developed hybrids that brought unique combinations of traits to the market or exploited unique environmental niches. Thus, **the male pool became to be more unique**.
Central and Western Europe

• High yielding **U.S. dent lines** were crossed with adapted European flint lines.
• Dent inbreds from the U.S. were primarily selected for earliness.
• Parental flint inbreds were developed by selfing from a few European OPVs.
China

- Hybrid breeding was started in China in the 1930s.
- The basic profile of heterotic patterns in maize used in China is **domestic** and **abroad**. The domestic group consists of lines selfed from local OPVs.
- The abroad group consists of Lancaster Sure Crop (LSC), Reid Yellow dent (RYD).
- In the **North Spring Maize Region**, the major pattern of heterotic groups is **domestic × LSC**.
- While in the Huanghuaihai **Summer Maize Region** the major pattern is **domestic × PN** (Li et al., 2004).
Tropical germplasm

- Several promising heterotic patterns have been described by Wellhausen (1978), Goodman (1985), and Vasal et al. (1999).
- Tuxpeno, N3, Kitale II, Pool 9A,
- ETO, Ecuador 573, SC, Cuban Flint, Caribbean Flint, Katumani, K64R, Tiko, Suwan
- SR52 commercialized in 1960, which out yielded Hickory King by 46%.
- The inbred line SC was extracted from a landrace which was grown on Mr Southey’s farm hence it was designated “Southern Cross”
- The N3 was derived from the landrace Salisbury white grown in Salisbury (now Harare) before the advent of hybrid maize in 1960
- SR52 has also been used as a parent for several three-way cross hybrids, particularly in Kenya.
- Consequently SR52 became the basis of hybrid breeding programs in central, eastern and southern Africa.
Backgrounds of Heterotic grouping in CIMMYT

- CIMMYT’s breeding efforts in the early 1960s and 1970s were focused on intra-population improvement via RS.
  - More than 100 populations and 30 genetically broad-based backup pools were established (Vasal et al., 1999).
- Objective was development of adapted OPVs with multiple traits (without consideration to heterotic pattern) for the tropical highlands and lowlands, as well as subtropical regions of the developing world (Vasal et al., 1982).
- The initial gene pools were classified by grain types (dent or flint), maturity (early, intermediate, and late), and grain colors (white and yellow).
- CIMMYT started hybrid maize breeding program in 1985.
  - Inbred line development and assignment germplasm into different heterotic groups
- CIMMYT has developed and released 603 CMLs.
Six races have achieved global economic importance

- More than 300 maize races are maintained at CIMMYT.
- Morphological descriptions of races or group(s) of related individuals with enough characteristics in common to permit their recognition as a group.
- **Mexican Dents, Corn Belt Dents (CBD), Tusons, Caribbean Flints, Northern Flints and Flours, and the Catetos (Argentinean Flints),** although several other races are important regionally (Goodman 1978).
Heterotic grouping in CIMMYT

Heterotic Partners of CIMMYT gene pools

<table>
<thead>
<tr>
<th>Gene Pools</th>
<th>Partner</th>
<th>Gene Pool</th>
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<td>Tropical</td>
<td>TEW A &amp; B</td>
<td>G15 &amp; G16</td>
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<tr>
<td></td>
<td>TEY A &amp; B</td>
<td>G17 &amp; G18</td>
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<tr>
<td></td>
<td>TIW A &amp; B</td>
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<tr>
<td></td>
<td>TIY A &amp; B</td>
<td>G21 &amp; G22</td>
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<td></td>
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<td>G23 &amp; G24</td>
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<td>TLY A &amp; B</td>
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<td>G27 &amp; G28</td>
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<tr>
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<td>SEY A &amp; B</td>
<td>G29 &amp; G30</td>
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<tr>
<td></td>
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<td></td>
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<td>G33 &amp; G34</td>
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<td></td>
<td>HLW A &amp; B</td>
<td>G9 &amp; G10</td>
</tr>
<tr>
<td></td>
<td>TZLY A &amp; B</td>
<td>G11 &amp; G12</td>
</tr>
</tbody>
</table>

Figure 4. Maize gene pools bred at CIMMYT and their heterotic partners according to their maturity, grain color and target agro-ecology. TEW and TEY, TIW and TIY, and TLW and TLY indicate tropical early white and yellow, tropical intermediate white and yellow, and tropical late white and yellow, respectively. SEW and SEY, and SIW and SIY indicate subtropical early white and yellow, and subtropical intermediate white and yellow, respectively. HEW and HEY, HLW and HLY, and IHW and IHY indicate tropical highland early white and yellow, tropical highland late white and yellow, and tropical intermediate highland white and yellow, respectively.

Ortiz et al 2010
Maize Heterotic grouping in CIMMYT

- Heterotic group 'A' as germplasm heterotically similar to Tuxpeño (Pop 21, Pop 49, 43 LPS), N3(Salisbury white), Kitale II, M37, Reid(stiff stalk);
  - Pop 21: a collection of 7 Tuxpeño race white
  - Pop 49 is Tuxpeno-Crema C17
  - Pop 43 (LPS) : 16 lines from Tuxpeno recombined and improved for resistance for MSV in Nigeria
    - CML78, CML197, CML206, CML312, CML442 (derivative M37W/ZM607), CML536 (derivative of 197 and 442), CML539 (derivative of 312 with SR)
Maize Heterotic grouping in CIMMYT

- Heterotic group 'B' resembles SC (Southern cross landraces from Mr. Souhey’s farm), ETO (Pop32), Ecuador 573, Lancaster, (Mo17), Coastal Tropical Flints, Caribbean, K64R, Suwan DMR and is complementary to 'A'.
  - CML202 (ZSR), CML395 (IITA), CML444 (Pop 43C9), CML543 (derivative of 395/202, CML566 (444*/LPS, CML569 (395*/LPS)
SR52 - the first single Crosse hybrids commercialized in Africa

- SR52 commercialized in 1960, which out yielded Hickory King by 46%.
- Hickory King is U.S. varieties imported in 1900 and 1905 and distributed to farmers (Weinmann, 1972).
- SR52 was developed from two highly homozygous and divergent maize inbred lines, namely N3-2-3-3 and SC5522 ("N3" and "SC").
- GD between the parents 0.342 (1242 SNPs)
- The inbred line SC was extracted from a landrace which was grown on a Mr Southey’s farm hence it was designated “Southern Cross”.
- The N3 was derived from the landrace Salisbury white grown in Salisbury (now Harare) before the advent of hybrid maize in 1960.
- SR52 has also been used as a parent for several three-way cross hybrids, particularly in Kenya.
- Consequently SR52 became the basis of hybrid breeding programs in central, eastern and southern Africa.
Clustering of all 498 accessions based on 1,041 SNPs.

https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0032626
Mid-altitude Maize Heterotic Groups Identity

• More concerted and systematic efforts by breeders are needed to more clearly separate the HG of tropical maize in Africa for higher heterosis in hybrids

Source: Semagn et al., BMC Genomics 2012 13:113
Heterotic Group comparison

Temperate lines in red, tropical lines in green
Heterotic patterns of US maize germplasm

SSS Female gene pool

Hybrid formation

NSSS Male gene pool

Seed parent line development

Pollen parent Line development

Cooper et al., 2014
## Genetic similarities among CIMMYT inbreeds

- We have ample genetic variability among CIMMYT elite lines
- Opportunity to develop high yielding variety

<table>
<thead>
<tr>
<th>Heterotic group</th>
<th>Line 1</th>
<th>Line 2</th>
<th>Line 3</th>
<th>Line 4</th>
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<tr>
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<td>CML411</td>
<td>69%</td>
<td>100% CML269</td>
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<td>B</td>
<td>CML269</td>
<td>70%</td>
<td>70%</td>
<td>100% CML311</td>
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<td>CML311</td>
<td>71%</td>
<td>71%</td>
<td>71%</td>
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<td>A Tester</td>
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<td>69%</td>
<td>69%</td>
</tr>
<tr>
<td>A Tester</td>
<td>CML312</td>
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<td>70%</td>
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<tr>
<td>B</td>
<td>CML511</td>
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<td>CML421</td>
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<td>71%</td>
<td>71%</td>
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<tr>
<td>A Tester</td>
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<td>68%</td>
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<tr>
<td>A</td>
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<td>B Tester</td>
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<td>72%</td>
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<td>B Tester</td>
<td>CML395</td>
<td>68%</td>
<td>71%</td>
<td>69%</td>
</tr>
</tbody>
</table>
Strategy for establishment of heterotic patterns

Strategy MELCHINGER and GUMBER (1998):

1. Group germplasm based on its cross performance

2. Identified groups are the two heterotic groups

Strategy CRESS (1967):

1. Combine germplasm in one synthetic; random mating

2. Randomly create two populations used as heterotic groups
Introgression of temperate materials into tropical background

**TEMPERATE (NSS)**
- L, IO, Mn13, Oh7-
- Midland, Oh43, W117

**TEMPERATE (SS)**
- Am, B73, B14, B37, B47, Reid

**PROJECTS:**
- Line devel., testers
- Line devel., Testers
- Pollen parent gene pool
- Seed parent gene pool

**PROJECTS:**
- Line devel., testers

**TROPICAL**
- B, LSC, ETO, Eucador 573, Katumani, SC, K64R, Tiko, Suwan

**TROPICAL**
- A, Tuxpegno, I137, M37W, N3, Kitale II, Pool 9A,
Hybrid Breeding in Tropical Maize
Why hybrid maize?

• The most productive innovation in plant breeding
• It started a revolution in
  • Agricultural productivity
  • Uniformity of products
  • Fixing specific traits that serve as trade marks
  • Seed production and marketing
  • Catalyst for the establishment private sector
• In USA maize yield increase 1.3 t/ha in 1930 to 7.5 t/ha in 1985 because of the use of hybrids, greater use of fertilizers and herbicides, Higher plant densities, and other improved cultural practices.
• 1984 spread to Europe
• 1960 and 1970 in Eastern and Southern Africa
Hybrid maize breeding

1. Maize has a convenient reproductive organization with separate male and female flowers on the same plant:
   – enabling both inexpensive self-pollination for inbred line development and
   – controlled cross-pollination for hybrid seed production

2. Efficient hybrid breeding requires methods that
   – quickly generate homozygous and homogeneous lines and
   – enable cost efficient seed production.
   – Inbred lines developed by continuous self pollination have and by doubled haploid (DH) technology
Hybrid seed production

1. **Manual detasseling** of seed parents was employed to maximize hybrid purity and to avoid hand pollinations in hybrid seed.

2. **Mechanical detasseling**, or a combination of mechanical followed by manual detasseling for isolation fields.

3. **Cytoplasmic male sterility (cms)** in the 1950s to 1970s, but gained renewed importance with the advent of **Southern corn leaf blight**, which eliminated the use of T-cytoplasm as a primary cms source for hybrid seed production.

4. **Seed Production Technology for Africa (SPTA)**
Major sources of cms

• Three major sources of cms have been recognized: cms-T (Texas), cms-C (Charrua) and cms-S (USDA) (Gabay-Laughnan and Laughnan 1994).

• While cms is caused by defects in mitochondrial DNA and, thus, maternally inherited, fertility in hybrids needs to be restored.

• This is accomplished by crossing cms females with males, carrying matching genic inherited restoration of fertility (Rf) genes. Rf1 and Rf2 restore the fertility of cms-T, Rf3 the fertility of cms-S, and Rf4 and Rf5 the fertility of cms-C (Gabay-Laughnan and Laughnan 1994).

• While actual seed production using cms is less costly compared to mechanical detasseling, both cms and Rf genes need to be introduced into the respective female and male parents, respectively.
Seed Production Technology for Africa (SPTA)

**Benefits:**
- Improved yield under stress conditions (5 – 15%)
- Improved seed purity of hybrids reaching farmers
- Production cost savings to seed companies

**Ms44 SPT Construct (PHP70533):**
- Ms44 microRNAi
- PG47PRO: Bt1TP-AA
- LTP2PRO: Ds-RED
- Fertility restoration
- Pollen Ablation
- Transgenic kernel selection

1. **Parent Seed Increase**
- Ms44/Ms44 PP Inbred Transgenic
- Ms44/Ms44 NPP Female Inbred
- Ms44/Ms44 NPP Female Inbred Non-GMO

2. Small amounts of non-GMO NPP female seed will be sent (at cost) to licensed seed companies.

3. **Single cross production**
- Ms44/Ms44 NPP Female Inbred A
- ms44/ms44 Male Inbred B
- Ms44/ms44 F1 NPP Hybrid

4. **Three way hybrid production**
- Ms44/Ms44 F1 NPP Hybrid
- ms44/ms44 Male Inbred C
- 3-way Hybrid 1:1 PP:NPP

**Partners**

- CIMMYT
- KALRO
- CORTEVA agriscience
- ARC LNR
Major steps in hybrid maize development

1. Development of inbred lines and classifying them into heterotic groups

2. Testing inbred lines in hybrid combinations

3. Testing and identifying superior hybrids for commercial seed production and use by farmers
Criteria for Choosing parents to develop breeding populations

Development and selection of elite lines that perform well *per se* and *in cross combinations* are the key factors for success.

1. **Show superior performance for the traits of interest,**
   - Early testing method proposed by Jenkins (1935): the inbred lines acquired their individuality as parents of top crosses very early in the inbreeding process and remained relatively stable thereafter.
   - S1 lines with above average testcross performance selected for further inbreeding

2. **Maximize within-population variance for the traits of interest, and**
   - Heterosis depends on the presence of differences in the allele frequencies and dominance effects between the parents of a cross (Falconer, 1981).

3. **Preserve heterotic patterns for maximum heterosis in hybrid development.**
   - Breeding starts within each heterotic group
**Separating inbred lines into heterotic groups**

1. Heterotic pattern provide defined structures to breeding materials
2. Simplify management of germplasm
3. Provides suitable tester for assessing the breeding value of exotic germplasm
4. Facilitate selection of parents for developing
   - Bi-parental crosses for inbred line development (same heterotic group)
   - Hybrid combinations for testing (opposite heterotic groups)
Developing good inbred lines

- Developing superior lines is not just a matter of selfing but also of germplasm improvement.

- The choice of germplasm for different traits (DTP, MBR, KS23 etc.)

- Improvement of germplasm source (e.g., by recurrent selection)

- Improved procedures and methodologies to assist in making effective selections (sampling, testing, etc.)
Testing inbred lines in hybrid combinations

• Goal of inbred line development is to identify lines that produce **high-yielding hybrids**.
• Development of a large number of inbred lines with desirable agronomic features is relatively easy, but the main concern is adequate testing of lines to identify **superior genotypes in hybrid combinations**
• The correlation between traits of the inbred lines and their hybrids is weak
  • Empirical and simulation studies show correlations of less than 0.40
• Inbred lines should thus be tested in crosses to identify promising parents of productive hybrids
The object of the maize breeder should not be to find the **best pure line**, but to find and maintain the **best hybrid combination**

Both **GCA** and **SCA** are important to determine the performance of lines in hybrids

Testing a large number of inbred lines in all possible combinations is not practically feasible

**Formula for all possible single crosses** – \( \frac{n(n-1)}{2} \)
- \( n = 10, \) 45 hybrids
- \( n = 20, \) 190 hybrids
- \( n = 100, \) 4950 hybrids
- \( n = 500, \) 124750 hybrids

Cross all inbred lines to a common parent referred to as a tester
Choice of tester

• New inbred lines are crossed to a common parent referred to as a tester
  • This approach is called top-cross or testcross evaluation
  • With such a common tester parent, any difference in hybrid performance can be ascribed to differences in the combining ability of the inbred lines

• Several consideration when selecting a tester, such as
  – broad genetic base vs. narrow genetic base,
  – high gene frequency vs. low gene frequency,
  – general combining ability vs. specific combining ability,
  – high yield vs. low yield, and
  – several testers vs. one tester.
  – The concept of early testing involves a progeny test.
An ideal tester

• An ideal tester maximizes differences among the genotypes being tested
  – Best testers have high TC and high μT
  – Simplicity in use,
  – Provides information on the correct ranking of the
  – Relative merit of the lines under test, and
  – Maximizes genetic gain
# Development of new testers: Performance at Kiboko Demo, 2018

<table>
<thead>
<tr>
<th>Entry NO.</th>
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<tr>
<td>1</td>
<td>CML322/CML543</td>
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<td>2</td>
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<td>5</td>
<td>CML584/CML312</td>
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</table>
GCA vs SCA

• **Additive genetic variance** (or general combining ability) is the main component of the total genetic variance.

• **Nonadditive genetic variance** (or specific combining ability) usually is small, with the level of dominance in the partial to complete dominant range.

• **For previously selected lines**, specific combining ability have greater effects on the determination of yield differences.

• **For unselected lines**, genes affecting general combining ability are more important.
Hybrid performance—hypotheses and prediction

1. Dominance hypothesis, attributes heterosis to the **masking of effects of deleterious alleles by dominant** or partially dominant alleles, with each inbred line providing its own complement of dominant, favorable alleles.

2. Overdominance hypothesis: superior performance in hybrids was caused **by heterozygosity itself**, which acted as a physiological stimulus.

3. Epistasis has often been described as an additional mechanism.

4. Recent genomic data suggested that multigenic nature of heterosis
   - High level of presence/absence variation (PAV) in which sequences found in one inbred are lacking in another
   - More genes were actively expressed in hybrids than in their inbred parents.
Changes in hybrid yield, inbred yield, heterosis and percent heterosis along year of hybrids

![Graph showing changes in hybrid yield, inbred yield, heterosis, and percent heterosis over the years.]
A) Depiction of relationship between recurrent population improvement projects and line development projects

B) Depiction of maize hybrid development as consisting of parallel line development pipelines (red and yellow) within heterotic groups and a hybrid evaluation and commercialization pipeline (green). Lines advanced to late stages with desirable attributes are used in crossing nurseries to recurrently initiate the development of novel replicable lines;
Hybrids breeding design

C) Depiction of maize hybrid development pipelines modified to include trait introgression within heterotic groups. New pipeline segments to accommodate marker-assisted introgression of transgenic events from poorly adapted, but transformable, lines.

D) Depiction of maize hybrid development pipelines modified to include introgression of non-negotiable traits for hybrid sales and rapid cycling through genomic selection for population improvement. Adapted from (Gaynor et al. 2017)
Thank you for your interest!