CIMMYT Maize DH Facilities & DH Line Development Service to Partners

Cost-effective DH line production requires technical knowhow, laboratory and field facilities, and trained work force, which may not be available in all the maize breeding institutions. To enable partners in Latin America and Africa to avail the benefits of the DH technology, CIMMYT established DH facilities in Mexico and Kenya, respectively. Through these facilities, NARES and private seed companies are availing DH line production service on a cost-recovery basis. With the establishment of the maize DH facility at Kunigal in Karnataka, India, in collaboration with University of Agricultural Sciences-Bengaluru, maize breeding programs in South Asia can benefit from DH development service offered by CIMMYT at an economy of scale.

Maize DH Facility at Agua Fría Research Station, Mexico

- CIMMYT’s first DH facility was established at the Agua Fría Research Station in the state of Hidalgo, Mexico.
- During initial years (2007 to 2011), DH program focused on developing tropical haploid inducers and developing protocols for DH line production in the tropics.
- Large scale DH line production started in 2011-12 to serve breeding programs of CIMMYT, NARES & private companies in Latin America.
- By 2020, 114,544 DH lines were delivered from 786 maize populations.
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Maize DH Facility at Kiboko, Kenya

- DH facility at Kiboko, Kenya was established in 2013 in partnership with KALRO, and with financial support from Bill & Melinda Gates Foundation.
- The facility offers DH line development service to the public and private sector maize breeding programs in Africa.
- 245,448 DH lines delivered between 2015 and 2020 to 1468 populations.

Maize DH Facility at Agricultural Research Station, Kunigal, Karnataka, India

- Established In 2021 in partnership with University of Agricultural Sciences-Bengaluru, and with financial support from CGIAR Research Program on Maize (MAIZE).
- Aims to strengthen maize breeding program of NARES and seed company partners in South Asia with DH line development service.
- DH development service to be offered on a cost-recovery model similar to the DH facilities in Kenya and Mexico.

DH Development Service Offered by CIMMYT in Latin America and Africa

- About 50% of the populations were received from NARES and private company partners since 2017.
- There is a strong demand for DH development service from NARES and private seed company partners.

To request DH development service from CIMMYT, please contact:

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DH Technology Drastically Reduces Time Taken to Develop Homozygous Maize Lines

DH lines improve breeding efficiency and reduce costs.
- Simplified breeding operations due to handling of lesser number of finished lines (e.g., seed shipments, nursery preparation, inventory management, reduced cold room space etc.).
- Efficient use of breeder’s time – breeders can focus more on selection of best lines and hybrids than resource intensive inbreeding.

DH lines are best suited for variety registration.
Unlike conventional inbreds, DH lines do not exhibit
• inbreeding depression
• genetic segregation
• masking of gene action

Use of DH lines, especially in combination with molecular markers or genomic selection, results in increased selection gain through:
• Reduced cycle time
• Maximum genetic variance among lines
• High heritability in per se and test cross evaluations
• No genetic drift during line development

DH lines are ideal for molecular marker applications
• 100% homozygosity results in higher genotype-to-phenotype association.
• DH lines needed to be genotyped just once for molecular marker-based selection, unlike selfing progenies (e.g., F3s, F4s).
• DH enables genetic selection, which is more efficient than progeny selection. Hence, less sample size needed to fix multiple loci in homozygous condition.

Advantages of Using DH Lines in Maize Breeding

DH line development

DH lines are developed by recurrent self pollinations, by 2020, 114,544 DH lines were delivered from 786 maize populations.

Advantages of Using DH Lines in Maize Breeding

- Developed by recurrent self pollinations.
- Takes ~8 crop seasons (28-42 months) to derive inbred lines.
- Inbred lines not 100% homozygous.

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DH Technology in Maize Breeding

Homogenous inbred parents are essential for developing maize hybrids. Hybrid maize cultivars are critical for enhanced yields and stress resilience. Inbred lines are traditionally produced by 6-8 generations of recurrent self pollinations. Doubled haploid (DH) technology is an alternative to the time-consuming and cumbersome process of selling to produce inbred lines.

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Maize DH Development Process

DH line production in maize involves: 1) in vivo haploid induction using maternal haploid inducers; 2) haploid identification at seed/seedling stage; 3) doubling the chromosomes in putative haploid seedlings; and 4) production of seed from fertile doubled haploid plants.

Haploid Induction and Identification
- To produce haploid seed, source population from which DH lines are desired is crossed with pollen from maternal haploid inducers.
- Haploids are identified by using R1-nj anthocyanin marker.
- Diploids express anthocyanin marker on both endosperm and embryo while haploids express only on endosperm.

Chromosome Doubling of Putative Haploids
- Haploid seed germinated in greenhouse.
- Haploid seedling roots and crown region submerged in a mitotic inhibitor chemical (colchicine) to achieve chromosome doubling.
- Treated haploid seedlings are transplanted in the field.
- False positives are eliminated in the field based on plant vigor and anthocyanin coloration on the stem.
- Fertile plants are self-pollinated.
- Under best agronomic conditions, only a few of the surviving haploids produce pollen, and upon selfing few of those plants produce D1 (DH) seed.

For more details on maize DH line production, please refer to:
- Prasanna BM, Chaikam V and Mahuku G. (2012). Doubled Haploid Technology in Maize Breeding: Theory and Practice. CIMMYT, Mexico D.F.

Tropicalized Haploid Inducers Developed by CIMMYT

Haploids are induced in maize from source populations (F1 or F2) by crossing the plants (as female) to unique genetic stocks called “maternal haploid inducers”. Maternal haploid inducers in maize have mutations in ZmMTL/ZmPLA1/ZmNLD gene, which cause abnormalities in pollen/sperm development leading to induction of haploids. For obtaining DH lines, having a haploid inducer with high haploid induction rate (HIR) and adaptation to target environment is critical. Prior to 2011, inducer lines adapted to tropics were not publicly available. To address this critical gap, CIMMYT in collaboration with University of Hohenheim, Germany, developed first-generation tropicalized haploid inducer lines (TAILs). To further improve the inducers, CIMMYT developed second-generation TAILs (CIM2GTAILs) which are currently used in CIMMYT’s DH pipelines and are widely distributed to partners.

Agronomic Performance of Different Haploid Inducers in Tropical Environments

<table>
<thead>
<tr>
<th>Temperate haploid inducers</th>
<th>TAILs</th>
<th>CIM2GTAILs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haploid induction rate ~8-10%</td>
<td>Haploid induction rate ~6-8%</td>
<td>Haploid induction rate ~8-13%</td>
</tr>
<tr>
<td>Exhibit poor vigor in the tropics, besides severe susceptibility to tropical maize diseases.</td>
<td>Better vigor and disease resistance than temperate inducers</td>
<td>Resist to major tropical diseases.</td>
</tr>
<tr>
<td>Seed production difficult from these lines when grown in tropical regions.</td>
<td>Released in 2012 and distributed to 24 institutions.</td>
<td>Released in 2017 and distributed to 33 institutions globally.</td>
</tr>
</tbody>
</table>

For more information on CIM2GTAILs, please see: https://www.cimmyt.org/news/second-generation-haploid-inducers-now-available/

Public/private sector organizations can avail CIM2GTAILs under specific terms and conditions. To avail CIM2GTAILs, please contact:
- Dr. B.M. Prasanna
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