Induction Gene-based DH Breeding for Multicrops

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Outlines

- Introduction
- DH breeding in maize
- Induction gene-based DH breeding in multicrops
- Summary
For protection of variety’s right in main crops, genetic **homozygous** lines are essential for DUS test.
Hybrid crops

Breeding of hybrid variety in maize needs male and female homozygous parent inbred lines.
For non-hybrid crops, it needs to be self-pollinated generations to obtain homozygous lines for conventional variety.
How to develop homozygous lines in breeding

**Conventional method vs. DH technology**

1. **Conventional method**
   - **Parent**
     - A × B
   - **Selfing**
     - F₁
     - F₂
     - Fₙ
     - Fₙ
   - **homozygosity**
2. **DH technology**
   - **Parent**
     - A × B
   - **Inducer or other ways**
     - F₁
   - **Haploid**
   - **Chromosome doubling**
     - DH line
   - **100% homozygosity**

1. Tradition way needs continuous selfing about 8 or more generations
2. DH way can achieve the homozygous lines in only 2 generations via haploid and chromosome doubling.
   ---- Accelerating breeding cycle
The haploid generation pathways in plants

**Haploid Induction**

- **In vitro**
  - Anther pollination
  - Ovule

- **In vivo**
  - Partheno-genesis
  - Interspecies hybridization
  - Intraspecies hybridization

**Chromosome doubling**

**Embryo rescue**

**Haploid identification**

- CENH3
- Ig
- Stock 6

**DH**
Part II  DH technology system in maize (*in vivo*)

Advantages

- Maternal origin
- Genotype-independent
- Easy operation
- High efficiency
- High homozygosity
- Accurate phenotypic evaluation
Key progress: High induction rate haploid inducers

Induction rate $>10\%$

Induction rate $8\%-10\%$

Inducers as male parent can trigger maternal haploid in large scale in different breeding germplasms

Kernel oil content $\sim 8-10\%$
Key progress2: High efficiency identification systems

Maternal haploid

Diploid

Colorless embryo

Purple embryo

Purple aleurone

Purple aleurone

Germplasms (Female)

Haploid inducer (male)

Maternal haploid

Colorless embryo

Purple aleurone

Purple embryo

Purple aleurone

Diploid

R-nj color system, Sarkar and Coe, 1966
Kernel oil marker and automatic screening system

Common kernel and High-oil kernel

High-oil inducer (KOC >8%)

The automatic haploid screening system

With high oil haploid inducers, haploids can be screened by automatic system based on the oil content in the crossed seeds.
Key progress 3: Large-scale DH line production

DH production cycle

April (Planting)

June (Pollination)

July (Embryo Isolation)

August (Transplanting to field)

September (Pollination)

October (Embryo Isolation)

November (Transplanting to field)

December (Pollination)

March (Pollination)

April (Pollination)

June (Pollination)

July (Embryo Isolation)

August (Transplanting to field)

September (Pollination)

October (Embryo Isolation)

November (Transplanting to field)

December (Pollination)
DH technology in commercial maize breeding

1. Seed companies using DH technology

2. DH service

DH technology has been successfully used in large scale and hybrids from DH lines have replaced the traditional ones rapidly in maize breeding over the past decades.
Key progress 4: Cloning of induction genes

The finding above confirmed that the system is actually “Induction gene-based DH system”

**ZmPLA1**

Contributes to about 90% of HIR

**ZmDMP**

Liu et al. 2017; Zhong et al. 2019
Part III  Induction gene-based DH technology in multicrops
The induction gene has homologous gene in different monocot cereal crops like wheat etc.

### MTL/PLA1/NLD-based DH system in cereal crops

<table>
<thead>
<tr>
<th>Crops</th>
<th>Amino acid sequence identity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorghum</td>
<td>90.39</td>
</tr>
<tr>
<td>Millet</td>
<td>84.32</td>
</tr>
<tr>
<td>Indica rice</td>
<td>77.05</td>
</tr>
<tr>
<td>Wheat</td>
<td>76.78</td>
</tr>
<tr>
<td>Japonica</td>
<td>76.16</td>
</tr>
</tbody>
</table>
**MTL/PLA/NLD-based DH system in wheat**

Haploid identification
Accuracy ~ 97.7%

Chromosome doubling
Doubling rate > 90%

Wheat haploid induction
HIR ~ 10-20%

Liu et al., 2019
Qi et al., 2023
**MTL/PLA1/NLD-based haploid induction in rice and millet**

Rice
HIR ~ 2-6%

Foxtail millet
HIR ~ 2.8%

Yao et al., 2018

Cheng et al., 2021
Species with *DMP* gene amino acid sequence identity higher than 60%.
**DMP-based DH system in tomato**

### Female parent

<table>
<thead>
<tr>
<th>Seed setting rate (%)</th>
<th>Total seeds</th>
<th>Haploids</th>
<th>HIR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24.70</td>
<td>29,397</td>
<td>509</td>
<td>1.94 ± 0.74</td>
</tr>
</tbody>
</table>

Zhong et al., 2021

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**Legend**

- **Red seed**
- **White seed**
- **Haploid**
- **Diploid**

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**Female parent**

<table>
<thead>
<tr>
<th>Generation</th>
<th>Seed setting rate (%)</th>
<th>Total seeds</th>
<th>Haploids</th>
<th>HIR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>24.70</td>
<td>29,397</td>
<td>509</td>
<td>1.94 ± 0.74</td>
</tr>
</tbody>
</table>

**Male parent**

- **AC-dmp**

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**DH**

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**Wild type**

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**sldmp**

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**Figure a**

- **Wild type**
- **sldmp**

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**Figure b**

- **Wild type**
- **sldmp**

---

**Figure c**

- **Wild type**
- **sldmp**

---

**Figure d**

- **Wild type**
- **sldmp**

---

**Figure e**

- **Wild type**
- **sldmp**

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**Figure f**

- **Wild type**
- **sldmp**

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**Figure g**

- **Wild type**
- **sldmp**

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**Figure h**

- **Wild type**
- **sldmp**

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**Figure i**

- **Wild type**
- **sldmp**

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**Figure j**

- **Wild type**
- **sldmp**
**DMP-based haploid induction in rapeseed and tobacco**

- Arabidopsis: HIR ~ 2.2%
- Rapeseed: HIR ~ 2.6%
- Tobacco: HIR ~ 1.1%

Zhong et al. 2020, 2022
Part IV Summary

1. The induction gene-based DH breeding system has obvious advantages and has been successfully used in maize.

2. The DH system has confirmed effective in multicrops and pave the way to accelerate practical breeding.

3. The high homozygosity of DH lines is beneficial for the protection of variety right.