

The Doubled Haploid Facility

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Introduction

The Doubled Haploid Facility (DHF) is a service facility at Iowa State University, Department of Agronomy, offering the production of doubled haploid lines in maize to researchers and breeding companies.

Materials and Methods

Doubled haploid (DH) technology.

Conventional development of inbred lines in maize through self-pollination takes six to eight generations. DH technology allows the production of completely homozygous lines within two generations, saving time and resources in line development (Figure 1).

Induction crosses and haploid selection. In the first generation, donor plants of maize are pollinated with special haploid inducing genotypes (male). About 8-12 percent of the seed produced on the female plants will have a haploid genome originating from the mother plant. A dominant marker allele—conferring color to the crown and embryo of the seed—allows the discrimination between these haploid seed and the diploid seed resulting from a regular fertilization of the donor by the inducer (Figure 2).

Haploid germination, chromosome doubling, and DH production. Haploid plants are sterile. To produce seed, these plants need to be treated with a chemical for chromosome doubling. The haploid seeds are germinated in the Agronomy greenhouse on ISU campus. At the 2-3 leaf stage, colchicine solution is injected into the stem just above the basal

meristem, to initiate the doubling of meristematic cells. After transplanting to the field, about 15-20 percent of the haploid plants will show partial restoration of their fertility, which allows self-pollination. The seed produced on these partially doubled up plants are completely homozygous.

Since 2010, these induction and haploid nurseries have been grown at the Ag Engineering/ Agronomy Research Farm. About 400,000–500,000 induced seeds for haploid selection are produced every growing season. About 50,000–60,000 haploid seedlings are germinated and transplanted to the haploid nursery for DH production.

Results and Discussion

This research focuses on the two main bottlenecks of the DH production process.

Haploid inducer development. The haploid inducer lines were licensed from the University of Hohenheim. Over the past few years, we were able to develop haploid inducing genotypes with better agronomical adaptation to Midwest growing conditions, with additional selectable marker for the identification of haploid seeds and seedlings, and the ability to overcome a partial dent sterility used in popcorn and organic breeding. These lines can be licensed through Iowa State University (ISURF 04065, ISURF 04099).

Spontaneous haploid genome doubling (SHGD). The germination and treatment of haploid seedlings in the greenhouse and the transplanting not only require resources (greenhouse space, vegetable trans-planter), but also are time consuming and labor intensive. In 2013, haploids derived from a panel of expired PVP lines were screened for their ability to restore fertility. Two genetic backgrounds could be identified, and testing

over the years showed this trait is heritable and controlled by one major locus in the genome. With the SHGD trait added to donor materials, haploids could be directly seeded in the future, saving time and money.

In summer 2017, we produced F1 between these SHGD lines and breeding lines submitted by five commercial breeding companies for an evaluation of the trait in actual breeding materials. Haploids of these crosses will be grown in summer 2018 in a demonstration block.

The revenues of DHF help to support graduate student research. In 2017, four PhD students had their field experiments at the Ag Engineering/Agronomy Research Farm.

In August each year, the DHF hosts a workshop where researchers and breeders have the opportunity to learn “hands-on” how to generate DH lines (Figure 3). During the summer pollination season, guests come from all over the world to learn the technique.

Acknowledgements

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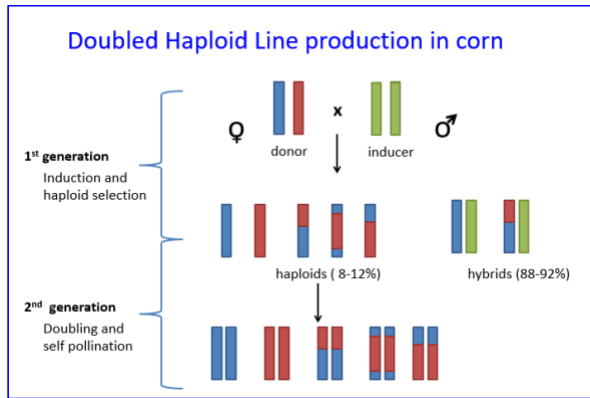


Figure 1. Scheme of DH production in corn.



Figure 2. Haploid selection based on a seed color marker. Induced seed have a colored crown, diploid hybrid seed has a dark embryo, and haploid seed has a clear embryo (fourth from the left).



Figure 3. A graduate student leads a group of students through the haploid nursery.