

# Accelerating Breeding in Maize: Haploid × Haploid Crosses After Genomic Selection

Ursula K. Frei—research scientist, Department of Agronomy

Doubled haploid (DH) technology reduces the time to generate completely homozygous lines in maize to just two generations. As part of the OREI COOP project, a two-generation rapid cycling breeding scheme, based on crosses between marker-selected haploid plants in the first generation and haploid induction in the second, was proposed to further speed up the breeding cycle.

The scheme exploits the ability of haploids with the trait of spontaneous haploid genome doubling (SHGD) in their genetic background, to produce viable pollen and fertile ears in high percentages. In combination with genomic selection, haploids with favorable alleles can be directly crossed to generate F1 seed for induction crosses in the off-season (Figure 1).

An initial experiment was performed to show that haploid × haploid crosses are possible, and yield sufficient seed for subsequent induction of generated F1.

## Materials and Methods

Haploids generated in 30 different families generated in the background of the BS39 population with the added trait for SHGD, were seeded in three delayed sets. While one set was strictly self-pollinated, as many cross-pollinations as possible were attempted within and between the other two sets, using each haploid only once as a male. At harvest, individual ears were harvested, shelled, and the seed set determined.

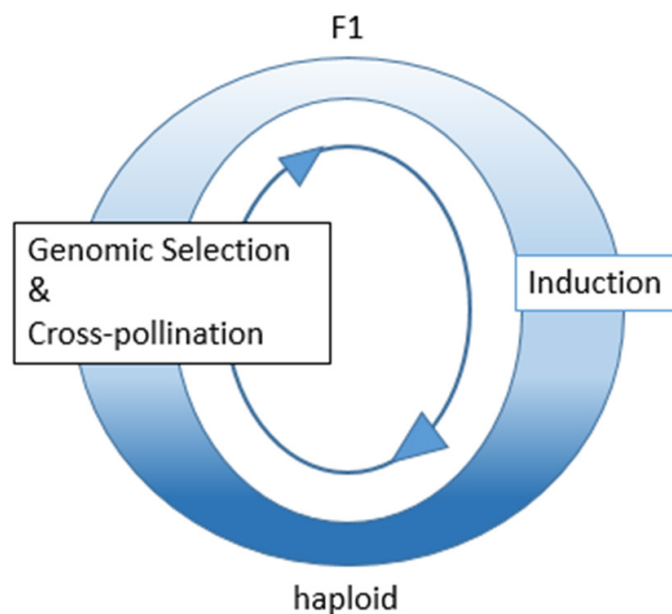


Figure 1. Rapid cycle breeding scheme using haploid × haploid crosses.

## Results and Discussion

On average, 19.6 plants per donor family, or ca. 590 haploids total were present in each set. About 39% of the haploid plants showed restored fertility and were used in self- or cross-pollinations.

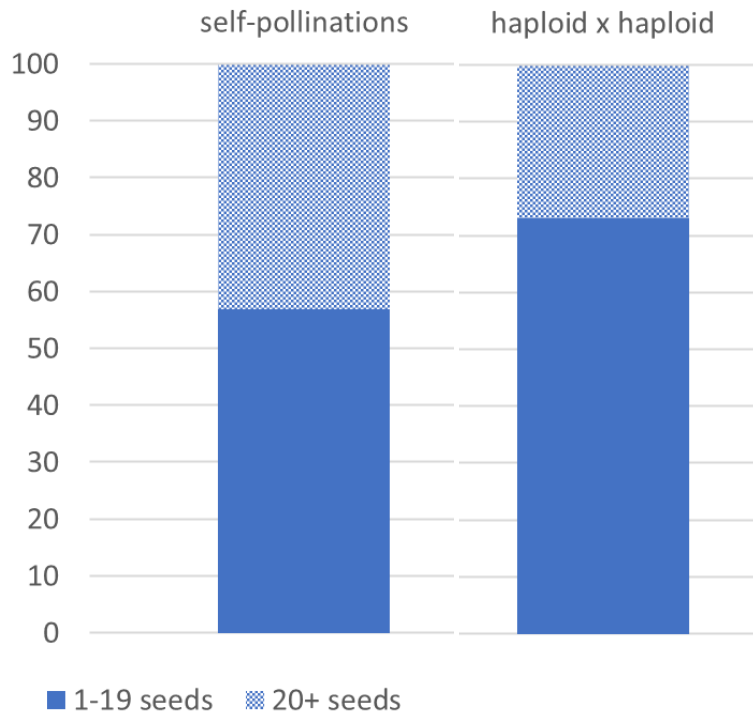
Seed set in cross-pollinations was at 65% of the attempted pollinations, which was higher than strict self-pollinations (54%), since wide anther-silking intervals in the haploids often are a restricting factor in self-pollination, but not for the cross-pollinations. Depending on the number of seed produced per ear, the harvested ears were divided in two groups (1: 1-19 seeds, 2: 20+ seeds).

A larger percentage of the ears generated in crosses fell into the groups with less than 20 seed per ear (73.1% versus 56.8%), whereas the self-pollinated ears dominated in the group with higher seed set (Figure 2). In the cross-pollinations, any haploid plant showing silk was pollinated, independent if it had restored male fertility or not. It was obvious plants that had restored male fertility also showed increased female fertility and resulted in better seed set. This might be one of the reasons why self-pollinations yielded more seed, as these represent haploids with both male and female fertility restored. F1 seed amounts of 20 seeds and more are sufficient to generate at least 100 haploids for the next cycle.

The scoring for the SHGD trait focuses on the levels of restored male fertility, as this is the major bottleneck in DH production, while female fertility restoration usually is sufficient for self-pollinations. With the goal to use haploid × haploid crosses for rapid cycling, female fertility restoration and sufficient seed set becomes more important. The F1 generated in 2022 between haploids serve as donor populations for inductions during the winter, for another cycle of haploid × haploid crosses in the coming season, and a more thorough evaluation of the female side of the equation.

## Acknowledgements

This research is funded by USDA NIFA Project 2020-51300-32180.



**Figure 2. Percent ears with seed sets below or above 20 seeds in self- and cross-pollinations of haploids.**