Evaluation of Spontaneous Genome Doubling Ability in Haploids of Diverse Genetic Background

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Ursula Frei, scientist
Nick Boerman, graduate student
Department of Agronomy

Introduction

Doubled haploid (DH) technology using *in vivo* haploid induction has found wide application in corn breeding in the past decade. Progress has been made to overcome the major bottlenecks of the technology: low haploid induction rates, labor-intensive manual haploid selection, and low doubling rates after chemical treatment for chromosome doubling. However, haploid genome doubling rates remain a major limiting factor in the successful production of DH lines in maize.

Haploid plants are sterile. Therefore, during DH production, the haploid seedlings need to be treated with chemicals that initiate genome doubling, for example, colchicine. Colchicine is a highly toxic alkaloid requiring specific standard operation procedures to avoid exposure and contamination, and it causes–depending on the application method–a waste problem. The efficiency of chemically induced genome doubling reaches up to 25 percent in some field corn accessions, but also can be as low as a few percent in recalcitrant genetic backgrounds. Any chemical treatment of seedlings requires availability of greenhouses to start the haploid seed and special equipment for transplanting the plantlets at the 2-3 leaf stage to the field, if not done by hand.

An alternative strategy to colchicine treatment is a genetic mechanism leading to high levels of spontaneous haploid genome doubling (SHGD). Targeted screening of a larger set of publicly available and exPVP lines for high levels of SHGD resulted in identification of three inbred lines with this property. When induced by a maternal haploid inducer, the resulting haploid lines have a male fertility level without colchicine treatment above 50 percent and up to 80 percent, resulting in increased seed. If this property would be present in elite breeding materials, then haploid plants could be sown directly and self-pollinated without need for colchicine treatment. Clearly, the benchmark is the current success rate based on colchicine treatment (averaging 10-20% in elite germplasm), not the high levels of fertility in regular diploid germplasm.

The objective of this study during summer 2018 was to evaluate rates of SHGD in diverse genetic backgrounds.

Materials and Methods

In summer 2017, 20 breeder lines (provided by five breeding companies) and four GEM (Germplasm Enhancement of Maize) lines were crossed with two different SHGD donor lines.

Both the F1 seed and the parental lines were induced during the winter of 2017-18, and haploid seed was selected based on the expression of the kernel color marker R1-nj.

In summer 2018, the haploids were

a) directly seeded without any chemical treatment for chromosome doubling, and
b) germinated in the greenhouse, treated with colchicine by injection, and transplanted to the field.

During flowering, tassels were scored on a daily basis for anther development (percent

plants developing pollen shedding anthers) and pollen shed (amount of pollen produced on a scale of 1-5), as a sign for restored male fertility.

Results and Discussion

The trait SHGD has incomplete penetrance, meaning that even in donor plants, not all haploids will shed pollen. On average, 40-60 percent of the plants showed pollen shed (Figure 1) with an average pollen score of 1.8-2.0 (Figure 2). The application of colchicine has no clear effect in SHGD genetic backgrounds.

Figure 3 summarizes the results for the GEM lines. All genetic backgrounds show very low spontaneous male fertility restoration *per se* with the exception of line GEMS-0227, whereas in the presence of SHGD the percentage is clearly increased. Male fertility restoration in the F1 using SHGD donors varies around 20 percent overall, which is comparable with the result in the SHGD lines if one takes into consideration that only half of the haploids generated on the F1 donor plants have the SHGD trait.

The introgression of SHGD into the breeding pool of a company would eliminate time-consuming germination of haploid seeds in greenhouses, the need for handling toxic chemicals for chromosome doubling and their disposal, and finally allow the direct seeding of haploids to the field without need for transplanting equipment. This would result in a more streamlined and more efficient DH process.

Acknowledgements

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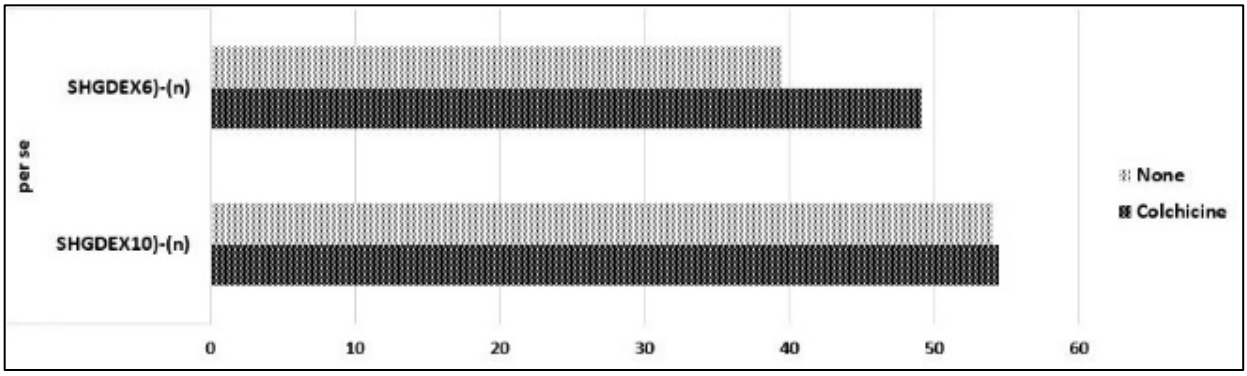

Figure 1. Percentage of plants with pollen shed in SHGD donor lines *per se* without colchicine.

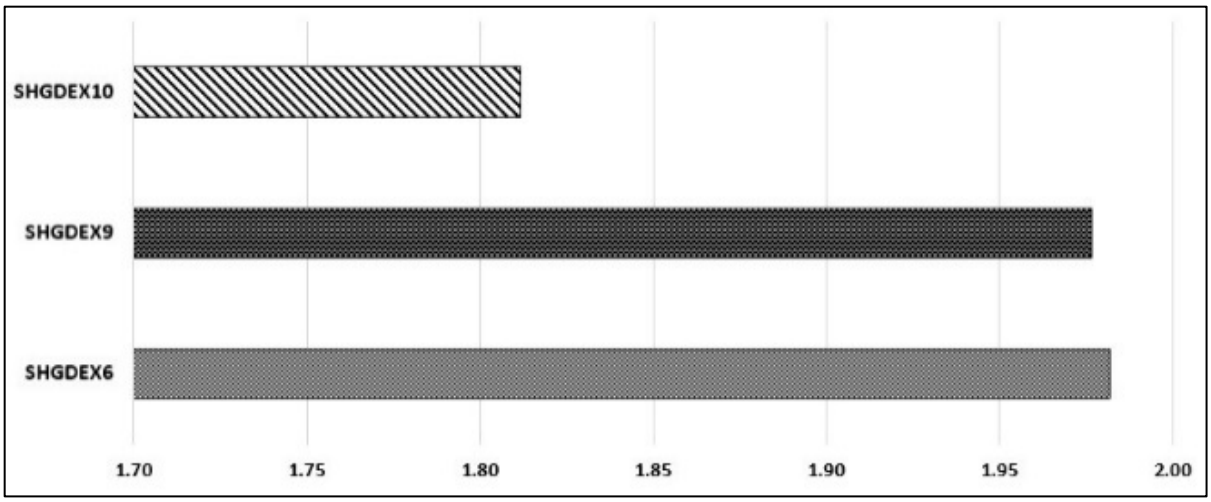

Figure 2. Average pollen shed score in haploids of SHGD donor lines *per se* without colchicine.

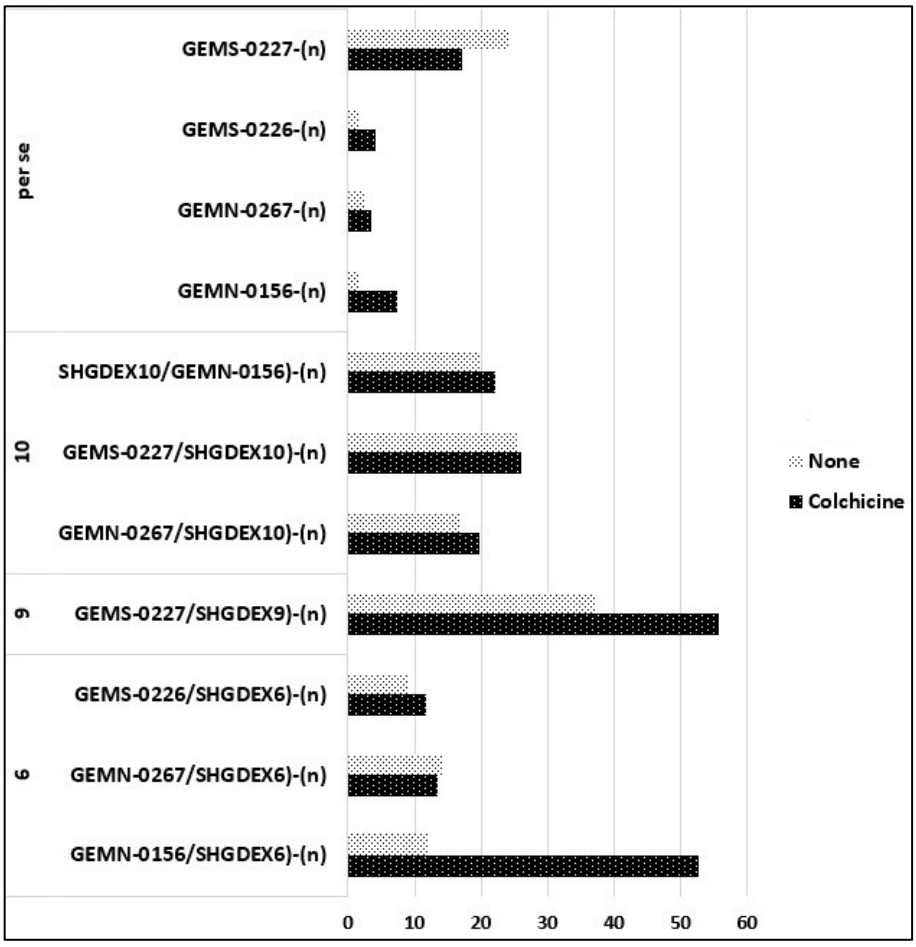

Figure 3. Percentage of plants with pollen shed in haploids of the GEM lines and F1 with different SHGD donor lines. Results show plots with and without colchicine treatment.