Doubled Haploid Technology: Achieving Homozygosity in Years to Single Generation

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ABSTRACT

Plant breeders now consider doubled haploid (DH) technology as one of the most exciting advances in their field. Generally, self-pollination takes many generations to develop true-breeding lines, but DH technique makes it possible to get completely homozygous lines in just single step. By producing completely homozygous lines in a one generation, reduces the time required for varietal development and boosts breeding efficiency. Different methods such as anther culture, microspore culture, and distant hybridization followed by chromosome elimination are used for DH plant development. These techniques have been successfully adopted in several crops, wheat, maize, rice, barley, and brassica. In this article, we aim to explain how DH works, the main methods used, and some real examples of its success.

INTRODUCTION

The conventional development of new crop varieties typically requires 8–10 generations of selfing to achieve homozygosity, a state where both alleles at every gene locus are identical. Homozygous plants are true breeding, meaning they consistently express uniform traits such as yield, maturity, and disease resistance across generations. This genetic uniformity is essential for the stability and predictability of crop performance, which is a cornerstone in the release of different varieties. Once

homozygosity is achieved, the traits are fixed, and no hidden variability arises from genetic segregation, allowing breeders to identify and enhance high-performing genotypes with greater precision and reliability. Chemicals like colchicine are traditionally used to double the chromosome number of haploid cells, converting them into fertile doubled haploids.

A haploid plant carries only a single set of chromosomes (n). When these chromosomes are artificially doubled, the plant becomes diploid (2n) and fully homozygous. This shortcut bypasses the lengthy process of repeated selfing, enabling breeders to develop pure lines in just one generation. The concept of haploidy was first demonstrated in *Datura* by Blakeslee and Belling in 1922 (Blakeslee and Belling 1922) marking a pivotal moment in plant genetics. Since then, DH technology has been successfully applied to a wide range of crops including rice, wheat, maize, barley, *Brassica*, and onion, revolutionizing plant breeding. Over the decades, DH technology has evolved from a theoretical concept to a mainstream breeding tool, especially in cereals (Forster *et al*., 2007)**.** In maize, for instance, the discovery of spontaneous haploids in 1929 laid the foundation for the development of haploid inducer lines like Stock 6, which are now genetically enhanced using CRISPR-Cas to improve induction efficiency. DH systems have become integral to genomic selection, QTL mapping, and trait introgression, offering unmatched speed and accuracy in fixing desirable traits.

Archaeological evidence, including remnants of barley grains discovered at ancient sites, indicates that barley was domesticated nearly 10,000 years ago from its wild ancestor Hordeum spontaneum (Bishnoi *et al*., 2022). Since then, extensive research has been carried out to enhance grain yield by exploring the genetic variability among diverse barley accessions and analyzing the correlations between key agronomic traits (Patial *et al*., 2016; 2018; 2021; 2023; 2024). The development and release of improved barley cultivars remain a critical goal in crop breeding. To accelerate this process and ensure precision, modern biotechnological tools such as doubled haploidy, marker-assisted breeding, TILLING (Targeting Induced Local Lesions IN Genomes), and gene editing technologies like CRISPR are being increasingly integrated into breeding programs (Patial *et al*., 2021; Kumar *et al*., 2020). These innovations hold immense potential to significantly advance barley improvement by enabling rapid fixation of desirable traits and enhancing genetic gains.

Methods of DH Production

1. **Anther and Microspore Culture:** This method involves culturing immature anthers or isolated microspores (pollen precursors) in vitro to induce embryogenesis. The wheat × maize system has proven highly efficient for haploid induction, offering an alternative to anther culture (Inagaki & Tahir, 1990). Under controlled laboratory conditions—such as specific temperature, light, and nutrient media—these male gametophytes are triggered to develop into haploid embryos rather than following their usual path of forming pollen. This technique is especially effective in crops like rice, barley, and *Brassica*. For instance, in China, anther culture has been extensively used in rice breeding programs to produce several commercial hybrids, significantly accelerating the development of pure lines.

2. **Wide Hybridization or Chromosome Elimination Technique:** In this approach, a crop species is crossed with a distantly related species, such as wheat with maize or wheat with Imperata cylindrica. The pollen from the distant species fertilizes the ovule of the crop plant, but during the early stages of embryo development, the chromosomes from the pollen donor are

selectively eliminated. This results in the formation of haploid embryos that are genetically derived solely from the female parent. The embryos are then rescued and cultured to develop into viable haploid plants. This method is particularly useful in species like wheat, where conventional in vitro techniques may be less efficient.

3. **Ovary Culture:** Ovary culture is employed in cases where anther or microspore culture proves inefficient due to genetic limitations. In this method, unfertilized ovaries or ovules are isolated and cultured using tissue culture techniques to stimulate haploid development. Specialized media and hormonal treatments are used to support the growth of embryos from these female reproductive tissues. A notable example is the development of doubled haploid lines in onion, where ovary culture has successfully overcome the challenges posed by poor anther response.

4. **Chemical and Genetic Approaches:** This method utilizes chemical agents or genetic tools to induce haploidy. More recently, genetic approaches have gained prominence, especially in crops like maize. Haploid inducer lines such as Stock 6 are bred to trigger haploid embryo formation when crossed with normal lines. Furthermore, genome editing technologies like CRISPR-Cas are being used to enhance the efficiency of haploid induction by modifying specific genes responsible for this trait. These advanced methods offer precision and scalability, making them highly valuable for modern breeding programs.

Success Stories of DH production in Major Crops

Maize: The foundation of modern haploid induction in maize traces back to the discovery of the inducer trait at the University of Missouri, where Stock 6 was identified as the original source capable of triggering maternal haploid embryo formation. This breakthrough laid the genetic groundwork for doubled haploid (DH) breeding systems worldwide. Building on this, China achieved large-scale DH success by incorporating Stock 6-derived inducer traits into elite inbred lines such as Zheng 58, which, although not an inducer itself, served as a robust recipient background due to its superior agronomic performance (Liu *et al*., 2016). In Europe, the University of Hohenheim developed high-efficiency inducer lines like UH400 and RWS—UH400 distinguished by its red root marker for early haploid identification, and RWS recognized for its strong seed set and induction rates (Röber *et al*., 2005; Würschum *et al*., 2012). These lines are now integral to CIMMYT's tropical breeding programs and European maize research, forming a global backbone for rapid and reliable DH production.

Rice: Taipei 309 emerged as the first model japonica rice variety to regenerate doubled haploids through anther culture (Niizeki & Oono, 1968). International Rice Research Institute, Philippines released IR36, IR43, IR54 varieties which showed low anther culture response but using alternative methods like ovary/ovule culture and optimized cold/chemical pre-treatments, doubled haploids were successfully produced for disease resistance breeding and stress tolerance studies (Khush & Virmani, 1996). IR64, a mega-variety, was used in DH production not just by anther culture but also through anther-derived callus regeneration and androgenesis variants. Its DH lines were pivotal in QTL mapping of yield, blast resistance, and grain quality traits (Ni *et al*.,1996). Recent molecular approaches, including the use of OsMATL (*Oryza sativa* MATRILINEAL) together with OsDMP1 or OsDMP3 mutations, have significantly increased the efficiency of haploid induction, particularly in *Indica* rice

(Liang *et al*., 2025). These developments make the production of doubled haploid lines faster, more consistent, and more reliable for breeding programs.

Wheat: Chinese Spring was the first model wheat variety shown to regenerate doubled haploids (DHs) via anther culture, while Pavon 76 is another high-response variety widely used in mapping and disease resistance studies at CIMMYT. The wheat × maize system has proven effective for DH induction in varieties such as Bobwhite, enabling rapid development of homozygous lines for genetic studies. In India, the first DH wheat line, Him Pratham (DH 114), was developed by CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur, using the wheat × maize system demonstrating the potential of this approach for accelerating breeding programs (Chaudhary *et al*., 2015). Additionally, the Imperata cylindrica-mediated chromosome elimination technique has been successfully applied to Indian wheat genotypes, resulting in DH lines such as HS 542 × China 84-40,022 (DH-1), which exhibit resistance to yellow and brown rust (Patial *et al*., 2021). These DH induction techniques offer cost-effective, efficient, and genotype-non-specific approaches, complementing conventional breeding by producing completely homozygous lines within a single generation.

Onion (*Allium cepa*): Modern onion DH technology relies on reproducible gynogenesis and a few well-characterized DH lines used as breeding tools. Notably, the USDA release Onion Haploid-1 (OH-1) distributed to breeding programs worldwide — functions as a standard, highly-responsive control for extracting gynogenic haploids while in-breds B8667 A/B and the synthetic Sapporo-Ki-1 (SKI-1 A/B) (Havey & Bohanec, 2007) have been released for use in hybrid seed systems. Early research produced DH lines from cultivars such as “Dorata di Parma” and “Senshyu Yellow”, and DH lines developed at public programs (Cornell, Wisconsin, Texas Agriculture and Mechanical university and collaborating European institutes) have developed.

Pepper (*Capsicum annuum*): Advances in pepper DH breeding have involved genotypes and DH lines with specific trait value, especially for disease resistance and fruit quality. Multidisciplinary Digital Publishing Institute in Iran, several DH lines such as DH55, DH64, DH57, DH53, DH217, DH202, and DH90 have been evaluated in performance cross, with DH55 showing markedly high fruit yield (≈ 8.58 kg/plant) when crossed to ‘California Wonder’ (Zarebayati *et al*., 2022).. Also, in Poland, eleven anther-derived DH-R_2 lines from hybrids (‘ATZ1 × PO’, ‘ATZ1 × CDT’, ‘ATZ1 × TG’) have been assessed for uniformity of fruit shape, color and enzyme isozyme markers, demonstrating that certain crosses (e.g. ATZ1 × PO) produce DH lines with high phenotypic uniformity (Olszewska *et al*., 2011). These named DH lines now serve as parental stocks in breeding programs targeting hybrid quality, resistance, and uniformity.

Cucumber (*Cucumis sativus*): Latest Bulgarian study developed DH lines (named DH2, DH6, DH7, DH9, DH14, DH19, DH21) from germplasm resistant to Cucumber Mosaic Virus (CMV). Among these, DH7 and DH9 were highly resistant to multiple CMV isolates (Ivanova *et al*., 2025).

Triticale (×*Triticosecale Wittmack*): Modern triticale DH technology uses wide hybridization with wheat and rye and chromosome doubling to produce homozygous lines with enhanced yield, disease resistance, and stress tolerance. Reported DH lines such as ‘Hewo × Magnat’ DH89 and spring triticale T225 have been used for genetic mapping and breeding (Randhawa *et al*., 2015; Lagunovskaya *et al*., 2020; Tyrka *et al*., 2015).

Challenges and Limitations of Doubled Haploid Technology

Doubled haploid technology encounters several challenges regardless of its revolutionary potential in crop breeding. One major limitation is variable efficiency across crops and genotype dependency. For example, wheat anther culture usually shows lower and inconsistent outcomes because all cultivars do not respond equally to haploid induction or tissue culture techniques. As a result the range of varieties that can be efficiently converted into DH lines is restricted. Moreover, DH technology requires technical expertise and robust infrastructure, including accurate embryo rescue techniques, tissue culture conditions, and controlled growth environments. The need for crop- and variety-specific optimization introduces additional complexity because the protocol that work well for one genotype may not respond for another, resulting in extensive trial-and-error to identify the ideal conditions. Therefore, these challenges highlight the importance of continued research to improve reproducibility, enhance applicability across genotypes, and optimize technical requirements.

CONCLUSION

Doubled haploid (DH) technology has become a vital tool in modern crop breeding. It allows scientists to develop fully genetically stable lines rapidly and efficiently. Its successful application in crops like wheat, maize, rice, barley, and *Brassica* shows how it can accelerate the development of improved varieties alongside maintaining homogygosity. With ongoing advances in molecular breeding and genome editing, DH technology is likely to become even more precise and widely used.

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