

Advances in *in vitro* doubled haploid technology for grain corn (*Zea mays* L.) breeding: current progress, challenges and perspectives

Abstract

Grain corn (*Zea mays* L.) is an important cereal crop for food, livestock feed and industrial applications. Conventional maize breeding requires repeated self-pollination to develop homozygous inbred lines, resulting in lengthy breeding cycles. Doubled haploid (DH) technology provides an alternative approach by rapidly generating completely homozygous lines. This review summarizes advances in *in vitro* DH production in grain corn, focusing on androgenesis, gynogenesis, chromosome doubling, major limitations and emerging breeding technologies. *In vitro* systems remain strongly influenced by genotype and are constrained by low induction and regeneration efficiency and inconsistent chromosome doubling. Nevertheless, advances in tissue culture, haploid identification, molecular genetics and genomic selection provide opportunities to improve DH production. Integration of DH technology with modern breeding approaches may accelerate the development of improved and climate-resilient grain corn cultivars.

Keywords: androgenesis; gynogenesis; tissue culture; maize breeding

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Introduction

Grain corn is one of the world's major cereal crops and is widely utilized for food, livestock feed and industrial purposes. The development of improved maize cultivars requires efficient breeding systems capable of generating genetically stable parental lines. Homozygosity gradually rises with each generation under conventional self-pollination, reaching roughly 97% to 98%.¹ This progressive increase in homozygosity occurs because heterozygosity is expected to decline by approximately 50% in each successive generation of selfing.² Doubled Haploid (DH) technology substantially shortens this process by reducing 3-4 cycles of self-pollination to generate fully homozygous lines after haploid induction and chromosome doubling.³ Consequently, DH technology has become an important component of modern maize breeding. DH lines provide genetic uniformity and can be generated more rapidly than conventional inbred lines, allowing breeders to accelerate line development and subsequent hybrid evaluation.³

In Vitro doubled haploid production

In vitro DH production is based primarily on androgenesis and gynogenesis. Androgenesis involves the culture of immature male gametophytic tissues, particularly microspores or anthers, and their redirection from normal gametophytic development towards embryogenesis. Regenerated plants are initially haploid and can subsequently undergo chromosome doubling to restore fertility. Androgenesis is an important pathway for *in vitro* DH production because cultured microspores can develop into haploid embryos and subsequently regenerated plants. However, the response is strongly dependent on genotype and culture conditions; therefore, it is essential to optimize the donor material, developmental stage, pretreatment and culture conditions.⁴ Gynogenesis provides an alternative pathway in which unfertilized female gametophytic tissues, such as ovules or ovaries, are cultured to induce haploid embryo development. In maize, however, *in vitro* gynogenesis is considerably less established than androgenesis and haploid inducer-based systems, as shown in Table 1. Consequently, *in vitro* androgenesis and gynogenesis remain more specialized approaches compared with the established *in vivo* DH system.³ The overall DH process can be summarized as follows.

Table 1 Applications of doubled haploid technology in maize breeding: Comparison of *in vitro* pathways

Parameter	Androgenesis	Gynogenesis
Explant source	Anther or microspore	Ovule or ovary
Gametophyte organ	Male	Female
Induction frequency	Moderate	Very low
Genotype dependency	High	High
Commercial application	More advanced	Limited
Regeneration efficiency	Higher	Lower

Haploid induction → haploid identification → chromosome doubling → fertile DH plant → seed multiplication.

In maize, both *in vitro* and *in vivo* approaches can be used. Nevertheless, *in vivo* maternal haploid induction has demonstrated

greater reliability and efficiency for large-scale DH production and is widely adopted in commercial breeding programs.³

Chromosome doubling

Chromosome doubling is a critical step in DH production because haploid plants possess a single set of chromosomes and are generally sterile. Doubling restores the diploid chromosome set and enables the production of fertile, completely homozygous plants. Artificial chromosome doubling can be achieved using antimetabolic treatments, while spontaneous genome doubling may also occur at variable frequencies. Research comparing spontaneous and artificial genome doubling demonstrates that chromosome doubling efficiency remains an important component of DH production.⁴ Colchicine has historically been widely used for chromosome doubling, although its effectiveness depends on genotype, developmental stage and treatment conditions. Recent research has investigated alternative genome-doubling strategies and the genetic basis of spontaneous haploid genome doubling.⁵

Major Challenges

The major limitations of *in vitro* DH production include genotype dependency, low haploid induction, poor plant regeneration, chromosome-doubling inefficiency and tissue-culture-related abnormalities. Genotype is particularly important because different maize germplasm can exhibit substantial differences in tissue-culture response and haploid production efficiency. Consequently, protocols developed for one genotype may not perform similarly in another genetic background.

Compared with *in vivo* DH systems, *in vitro* approaches remain less efficient for large-scale maize breeding. Chaikam et al.³ reported that *in vivo* methods are generally more reliable and efficient for large-scale DH line production, whereas *in vitro* methods remain useful for specific applications and germplasm. Another important limitation is the identification of haploid plants. Accurate discrimination between haploid and diploid individuals is necessary before chromosome doubling and field evaluation. Marker-based systems such as R1-nj, together with molecular and phenotypic approaches, have therefore become important components of DH pipelines.⁶

Molecular advances and perspectives

Recent molecular studies have substantially improved understanding of haploid induction in maize. The genes MATRILINEAL (MTL), ZmPLA1 and NOT LIKE DAD (NLD) were independently identified as the same major genetic factor associated with maternal haploid induction. These genes encode a pollen-associated phospholipase and have become important targets for understanding and engineering haploid induction.⁷⁻⁹ Recent research has expanded the list of genes associated with maize haploid induction, including ZmDMP, ZmPLD3, ZmPOD65 and CENH3, while genome-editing approaches have provided new opportunities for modifying haploid induction systems.⁵ The integration of DH technology with genomic selection represents another important development. DH breeding programs can generate large numbers of homozygous lines that are suitable for genomic prediction. Wang et al.¹⁰ demonstrated that genomic prediction across multiple years can support early-stage testcross selection and potentially reduce the requirement for extensive early-stage phenotyping. Future research should therefore focus on improving haploid induction, regeneration

and chromosome doubling, while reducing genotype dependency. Integration of DH technology with genome editing, molecular markers, genomic selection and emerging omics approaches may provide more efficient breeding pipelines for diverse maize germplasm, including tropical materials.⁵

Conclusion

In vitro DH technology provides an important approach for accelerating maize breeding through haploid production by androgenesis or gynogenesis followed by chromosome doubling. However, its broader application is limited by genotype dependency, regeneration efficiency, haploid identification and chromosome-doubling constraints. Although *in vivo* DH currently provides a more efficient platform for large-scale maize breeding, improvements of *in vitro* systems remain valuable for specific germplasm and research applications. Continued integration of tissue culture with molecular genetics, genome editing and genomic selection may further improve DH production and contribute to the development of improved grain corn cultivars.

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Conflicts of interest

Authors declare that there is no conflicts of interest.

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