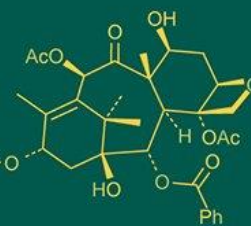
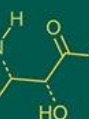
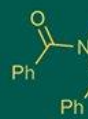


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Recent advances in doubled haploid production in cereals: Efficiency, applications and future prospects

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Abstract

Doubled haploid technology has revolutionized modern Plant Breeding by enabling the rapid development of completely homozygous lines in a single generation. Conventional breeding in cereals is often constrained by long generation cycles, genetic segregation, and environmental influences, which delay the fixation of desirable traits. Doubled haploid (DH) technology overcomes these limitations by producing uniform and stable lines directly from haploid cells followed by chromosome doubling.

This review highlights the concept, genetic basis, and major methods of DH production, including androgenesis, microspore culture, gynogenesis, and wide hybridization systems such as wheat × maize crosses. The technology has been successfully applied in major cereals like *Triticum aestivum*, *Oryza sativa*, *Zea mays*, and *Hordeum vulgare*, contributing to rapid varietal development, hybrid breeding, and genetic studies including QTL mapping and marker-assisted selection.

Despite its advantages, DH technology faces challenges such as genotype dependency, low efficiency in wheat, albinism in regenerants, and high technical requirements. Recent advances integrating CRISPR gene editing, haploid inducer lines, automation, and speed breeding have significantly improved its efficiency. Future prospects focus on enhancing induction systems, integrating artificial intelligence and genomics, and expanding its application for climate-resilient crop improvement, particularly in developing regions.

Keywords: Doubled haploid technology, plant breeding, haploid induction, androgenesis, microspore culture, wide hybridization, marker-assisted selection, quantitative trait loci, *Triticum aestivum*, *Oryza sativa*, *Zea mays*, CRISPR gene editing

Introduction

The development of improved crop varieties depends on the rapid fixation of desirable traits into stable, homozygous lines. In classical Plant Breeding, achieving complete homozygosity typically requires several generations of selfing, which is time-consuming and resource-intensive. This limitation becomes more critical in cereals, where breeding cycles are already long and the demand for improved varieties is continuously increasing due to population growth and climate variability. The need for rapid homozygosity has therefore become a central objective in modern breeding programs, as it enables faster varietal development, uniformity, and enhanced selection efficiency (Dwivedi *et al.*, 2015) [10]. In this context, Doubled haploid technology has emerged as a transformative approach, allowing the production of completely homozygous lines in a single generation, thereby significantly shortening the breeding cycle.

Conventional breeding methods rely on repeated self-pollination and selection over multiple generations to stabilize traits. While effective, these approaches are constrained by several limitations, including the long time required to achieve homozygosity, the influence of environmental variation on phenotypic selection, and the difficulty of fixing complex quantitative traits. Moreover, the segregation of alleles across generations can lead to the loss of desirable gene combinations, reducing breeding efficiency (Forster *et al.*, 2007) [13, 14]. These challenges are particularly pronounced in cereal crops, where traits such as yield, stress tolerance, and quality are controlled by multiple genes. As a result, traditional breeding often fails to meet the urgent demand for improved cultivars under rapidly changing agro-climatic conditions (Hickey *et al.*, 2019) [18].

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The introduction of doubled haploid (DH) technology has addressed many of these constraints by enabling the direct production of homozygous lines from haploid cells. This eliminates the need for multiple generations of inbreeding and allows breeders to fix traits rapidly and accurately. DH lines provide a uniform genetic background, which enhances the efficiency of selection and facilitates precise evaluation of traits. Additionally, DH populations are highly valuable for genetic studies, including quantitative trait locus (QTL) mapping and marker-assisted selection, as they reduce genetic noise and improve the reliability of phenotypic data (Collard *et al.*, 2005) ^[6]. The integration of DH technology into breeding programs has thus revolutionized cereal improvement by accelerating genetic gain and increasing breeding precision.

Among cereal crops, *Triticum aestivum* has been one of the primary beneficiaries of DH technology. Wheat breeding traditionally involves long generation cycles and complex genome interactions, making rapid homozygosity particularly valuable. The wheat × maize wide hybridization system, which induces haploidy through chromosome elimination, has become a widely adopted method for DH production in wheat. This approach has significantly improved the efficiency of breeding programs by enabling the rapid development of pure lines and facilitating the incorporation of desirable traits such as disease resistance and abiotic stress tolerance (Laurie & Bennett, 1988) ^[27]. The use of DH lines in wheat has also enhanced the accuracy of genetic mapping and accelerated the release of improved cultivars.

In *Oryza sativa*, DH technology has played a crucial role in hybrid breeding and varietal development. Rice is a staple food for a large proportion of the global population, and the demand for high-yielding and stress-tolerant varieties is continuously increasing. Anther culture and microspore culture are commonly used methods for DH production in rice, enabling the rapid development of homozygous lines for hybrid seed production. The application of DH technology in rice has improved breeding efficiency by reducing the time required for line development and enhancing the precision of selection (Chen *et al.*, 2011) ^[5].

The use of DH lines in maize has accelerated hybrid development and improved the accuracy of selection, contributing to substantial gains in yield and stress tolerance (Prigge & Melchinger, 2012) ^[34, 35]. The integration of molecular tools with DH technology has further enhanced its effectiveness, enabling the rapid identification and incorporation of desirable traits.

The use of DH lines in barley breeding has facilitated the rapid development of uniform varieties and improved the efficiency of selection for traits such as malting quality and disease resistance (Ferrie & Caswell, 2011) ^[11]. Additionally, DH populations have been widely used in genetic research to study trait inheritance and identify molecular markers associated with important characteristics. Overall, the need for rapid homozygosity in cereal breeding has driven the adoption of doubled haploid technology as a key tool for crop improvement. By overcoming the limitations of conventional breeding, DH technology has enabled the efficient development of stable, high-performing varieties. Its application across major cereals such as wheat, rice, maize, and barley highlights its versatility and importance in modern agriculture. As breeding programs continue to evolve, the integration of DH

technology with genomic and biotechnological approaches is expected to further enhance its impact, paving the way for the development of climate-resilient and high-yielding cereal crops.

Concept and Genetic Basis of Doubled Haploids

Doubled haploid technology is a specialized approach in Plant Breeding that enables the production of completely homozygous lines from haploid cells in a single generation. The fundamental principle involves the induction of haploid embryos possessing a single set of chromosomes (n), followed by artificial or spontaneous chromosome doubling to restore the diploid state ($2n$). This process bypasses multiple generations of selfing traditionally required to achieve homozygosity, thereby significantly shortening the breeding cycle (Dwivedi *et al.*, 2015) ^[10]. The concept relies on redirecting the normal developmental pathway of gametophytic cells—such as microspores or ovules—towards sporophytic embryogenesis, resulting in haploid plant formation (Germanà, 2011) ^[15].

In contrast, doubled haploids are produced by chromosome duplication of haploids, leading to complete homozygosity at all loci and restoring fertility (Forster *et al.*, 2007) ^[13, 14]. This absolute homozygosity ensures that traits are fixed and can be reliably expressed across generations, which is particularly advantageous for the development of pure lines and parental lines in hybrid breeding (Seguí-Simarro, 2010) ^[37]. Consequently, doubled haploids serve as ideal genetic materials for precise phenotyping and genotyping studies.

Chromosome doubling is a critical step in doubled haploid production and can occur either spontaneously or be induced artificially. Spontaneous doubling may result from endoreduplication or nuclear fusion events during early embryogenesis, although its frequency is often low and genotype-dependent (Testillano, 2019) ^[40]. Artificial chromosome doubling is commonly achieved using antimitotic agents such as colchicine, which disrupt spindle fiber formation during mitosis, leading to the duplication of chromosome sets (Niu *et al.*, 2014) ^[32]. Alternative chemicals such as oryzalin and trifluralin have also been explored due to their lower toxicity and higher efficiency in some species. The effectiveness of chromosome doubling depends on several factors, including treatment concentration, duration, and the developmental stage of the plant material. Proper optimization of these factors is essential to maximize doubling efficiency while minimizing phytotoxic effects.

At the genetic level, doubled haploids exhibit complete homozygosity because the chromosome sets are identical copies derived from a single gamete. This genetic fixation eliminates heterozygosity and allows breeders to capture and stabilize desirable allelic combinations rapidly (Ferrie & Möllers, 2011) ^[12]. The uniformity achieved through doubled haploid technology is particularly valuable in cereals such as *Triticum aestivum* and *Hordeum vulgare*, where consistent performance across environments is essential for cultivar release. Moreover, doubled haploid lines facilitate the identification of recessive traits, which are often masked in heterozygous populations, thereby enhancing selection efficiency (Collard *et al.*, 2017) ^[7].

Another important aspect of genetic fixation in doubled haploids is the reduction of genetic variability within lines, resulting in highly uniform populations. This uniformity is crucial for the accurate evaluation of agronomic traits,

disease resistance, and quality parameters, as environmental effects can be assessed without the confounding influence of genetic segregation (Prigge & Melchinger, 2012) [34, 35]. Furthermore, doubled haploid populations are extensively used in quantitative trait locus (QTL) mapping and genomic studies because they provide stable and reproducible genetic material (Khan *et al.*, 2020) [26]. The absence of heterozygosity simplifies genetic analysis and enhances the precision of marker-trait associations.

Despite these advantages, the genetic basis of doubled haploid production is influenced by several factors, including genotype, species, and the method of haploid induction. Some genotypes exhibit higher responsiveness to haploid induction and chromosome doubling, indicating the involvement of specific genetic determinants controlling embryogenesis and regeneration (Dwivedi *et al.*, 2015) [10]. Advances in molecular biology and genomics have begun to uncover the genes and pathways involved in these processes, offering opportunities to improve the efficiency and applicability of doubled haploid technology.

Methods of Doubled Haploid Production

Doubled haploid (DH) production in cereals relies on inducing haploid embryos followed by chromosome doubling to achieve complete homozygosity in a single generation. Several *in vitro* and *in vivo* techniques have been developed, each with distinct biological principles, efficiencies, and limitations. The major approaches include androgenesis, microspore culture, gynogenesis, and wide hybridization.

Androgenesis (Anther Culture)

Androgenesis via anther culture is one of the earliest and most widely used methods for haploid production in cereals. In this approach, immature anthers containing microspores at the appropriate developmental stage are cultured on nutrient media to induce embryogenesis. The process involves several stages, including selection of donor plants, collection of spikes at the uninucleate microspore stage, surface sterilization, and inoculation onto induction media. Under controlled conditions, microspores deviate from their normal gametophytic pathway and instead follow a sporophytic developmental route, forming embryoids that regenerate into haploid plants (Maluszynski *et al.*, 2003) [29]. Several factors influence the success of anther culture. Genotype plays a critical role, as some cereal genotypes respond more favorably than others, reflecting strong genetic control over androgenic competence (Germanà, 2011) [15]. Environmental conditions, including temperature pretreatment and light regimes, significantly affect embryogenesis induction. Media composition, particularly the balance of growth regulators such as auxins and cytokinins, also determines the efficiency of plant regeneration. For instance, cold pretreatment of spikes has been shown to enhance embryogenic response in wheat (Touraev *et al.*, 2001) [42].

Despite its utility, anther culture has notable limitations. One of the major constraints is genotype dependency, which restricts its applicability across diverse breeding materials. Additionally, the occurrence of albino plantlets, especially in cereals like barley and wheat, reduces the recovery of viable green plants. Spontaneous chromosome doubling is often inconsistent, necessitating chemical treatments such as colchicines to achieve stable doubled haploids (Ferrie &

Caswell, 2011) [11]. These challenges have prompted the development of alternative methods with improved efficiency.

Microspore Culture

Microspore culture involves the isolation and culture of individual microspores rather than entire anthers, offering a more direct and controlled approach to haploid production. In this technique, microspores are extracted from anthers at the appropriate developmental stage and cultured in liquid or semi-solid media under optimized conditions to induce embryogenesis. The isolated microspore technique eliminates the influence of surrounding anther tissues, allowing for better manipulation of the cellular environment (Touraev *et al.*, 2001) [42].

One of the primary advantages of microspore culture over anther culture is its higher efficiency and reproducibility in responsive genotypes. The uniform exposure of microspores to induction conditions enhances embryogenic response and reduces variability. Additionally, this method minimizes the risk of somatic tissue contamination, which can lead to diploid regenerants. Microspore culture also facilitates the study of cellular and molecular mechanisms underlying embryogenesis, making it a valuable tool in functional genomics (Ferrie & Caswell, 2011) [11].

However, microspore culture is technically demanding and requires precise control of developmental stages and culture conditions. The success of the technique depends heavily on factors such as donor plant physiology, stress treatments, and media optimization. While highly effective in crops like barley and rapeseed, its application in wheat remains limited due to lower responsiveness and genotype specificity (Germanà, 2011) [15]. Nevertheless, ongoing advancements in culture protocols and molecular interventions are improving its applicability in cereal breeding programs.

Gynogenesis

Gynogenesis involves the development of haploid plants from female gametophytic tissues, such as ovules or ovaries, without fertilization. This method is particularly useful in species or genotypes where androgenesis is inefficient or unsuccessful. In ovule culture, unpollinated ovules are excised and cultured on nutrient media to induce embryogenesis, while ovary culture involves the culture of entire ovaries under similar conditions (Maluszynski *et al.*, 2003) [29].

The process of gynogenesis typically requires specific pretreatments, such as cold or heat shock, to stimulate embryogenic development. The resulting embryos are haploid and can be doubled to produce homozygous lines. This method has been successfully applied in crops like onion and sugar beet, as well as in certain cereal genotypes that are recalcitrant to androgenesis (Germanà, 2011) [15].

One of the key advantages of gynogenesis is its ability to bypass the limitations associated with male gametophyte-based methods. It provides an alternative route for haploid production in cases where microspores fail to respond. However, the efficiency of gynogenesis is generally lower compared to androgenesis and microspore culture. The technique is also labor-intensive and requires precise handling of delicate reproductive tissues. Despite these challenges, gynogenesis remains an important tool for expanding the scope of doubled haploid technology in difficult-to-culture species.

Wide Hybridization (Chromosome Elimination)

Wide hybridization, also known as chromosome elimination, is an *in vivo* method of haploid production that involves crossing two distantly related species. In cereals, the most widely used system is the wheat × maize cross, where *Triticum aestivum* is pollinated with *Zea mays* pollen. Following fertilization, maize chromosomes are selectively eliminated during early embryogenesis, resulting in haploid wheat embryos (Laurie & Bennett, 1988) ^[27].

The success of this method depends on efficient pollination, embryo development, and subsequent embryo rescue. Since the hybrid embryos lack endosperm, they are unable to develop naturally and must be excised and cultured *in vitro*. The embryo rescue technique involves transferring immature embryos to nutrient media, where they develop into haploid plantlets (Kasha & Maluszynski, 2003) ^[23].

The role of maize in this system is crucial, as it acts as a haploid inducer through chromosome elimination. This method is highly efficient and less genotype-dependent compared to *in vitro* techniques, making it particularly valuable for wheat breeding programs. It has been widely adopted due to its reliability and scalability, enabling the rapid production of large numbers of doubled haploid lines (Prigge & Melchinger, 2012) ^[34, 35].

Despite its advantages, wide hybridization also presents challenges. The requirement for controlled pollination and embryo rescue increases labor and technical complexity. Additionally, the efficiency of chromosome elimination can vary depending on environmental conditions and parental genotypes. Nonetheless, this method remains one of the most effective approaches for doubled haploid production in cereals, especially in wheat.

Molecular and Cytological Mechanisms

The success of Doubled haploid technology in cereals is fundamentally governed by a series of molecular and cytological events that ensure the formation of haploid embryos followed by chromosome doubling. One of the most critical processes involved is chromosome elimination, particularly evident in wide hybridization systems such as wheat × maize crosses. In this system, chromosomes of one parental species (maize) are selectively eliminated during early embryogenesis, resulting in haploid embryos carrying only the genome of *Triticum aestivum*. This selective

elimination is not random but is regulated by incompatibility between parental genomes, leading to uniparental chromosome retention (Ishii *et al.*, 2016) ^[19].

The mechanism of chromosome elimination is closely associated with the behavior of centromeres and the mitotic spindle apparatus. Centromeres play a crucial role in chromosome segregation during cell division, and differences in centromeric histone proteins such as CENH3 can lead to genome elimination. Altered or incompatible centromeres fail to attach properly to spindle fibers, resulting in lagging chromosomes that are eventually excluded from daughter nuclei (Karimi-Ashtiyani *et al.*, 2015) ^[22]. Spindle formation further determines the fidelity of chromosome segregation, and abnormalities in spindle organization can accelerate the elimination of specific chromosomes, thereby promoting haploid induction.

Gene regulation during haploid induction is another key aspect influencing the efficiency of doubled haploid production. Several genes associated with embryogenesis, cell cycle regulation, and stress responses are differentially expressed during the induction process. For instance, genes controlling microspore reprogramming are activated under stress conditions such as temperature shock, which redirects the developmental pathway from gametophytic to sporophytic growth (Seguí-Simarro, 2016) ^[39]. Additionally, mutations in genes like MATRILINEAL (MTL) in maize have been shown to enhance haploid induction rates, highlighting the genetic control underlying this process (Kelliher *et al.*, 2017) ^[24].

Recent advances in Genomics have provided deeper insights into the molecular basis of haploid induction and chromosome doubling. High-throughput sequencing and transcriptomic analyses have enabled the identification of candidate genes and regulatory networks involved in embryo development and genome stability. Epigenetic modifications, including DNA methylation and histone acetylation, have also been implicated in regulating gene expression during haploid formation, suggesting that chromatin remodeling plays a vital role in this process (Zhao *et al.*, 2019) ^[47]. Furthermore, genome editing tools such as CRISPR/Cas systems are being explored to manipulate key genes involved in haploid induction, thereby improving efficiency and applicability across different cereal species (Jacquier *et al.*, 2020) ^[20].

Table 1: Comparison of DH Methods in Cereals

Method	Crops	Efficiency (%)	Advantages	Limitations
Anther culture	Rice, Wheat	5-30%	Simple, widely used	Genotype dependent
Microspore culture	Barley	20-60%	High efficiency	Technical skill required
Wheat × maize	Wheat	10-40%	Widely used in wheat	Requires embryo rescue
In vivo induction	Maize	8-15%	No tissue culture	Limited to maize

Applications in Cereal Breeding

Development of Pure Lines

One of the most significant applications of doubled haploid technology is the rapid development of completely homozygous lines. In conventional breeding, achieving homozygosity requires several generations of selfing, which is both time-consuming and resource-intensive. In contrast, doubled haploid production enables the fixation of alleles in a single generation, drastically reducing the breeding cycle (Forster *et al.*, 2007) ^[13, 14]. This rapid homozygosity is particularly beneficial in crops like *Oryza sativa* and wheat,

where uniformity and genetic stability are essential for cultivar development.

Doubled haploid lines exhibit complete genetic uniformity, making them ideal for selection and evaluation. Since there is no segregation in subsequent generations, breeders can directly assess phenotypic traits and identify superior genotypes with greater accuracy. This not only accelerates the breeding process but also enhances selection efficiency, ultimately leading to the development of improved varieties with desirable agronomic traits (Dwivedi *et al.*, 2015) ^[10].

Hybrid Breeding Programs

Doubled haploid technology plays a crucial role in hybrid breeding programs by facilitating the development of pure and stable parental lines. Hybrid vigor, or heterosis, depends on the genetic divergence of parental lines, and the availability of homozygous inbred lines is essential for producing high-performing hybrids. Doubled haploids provide a rapid and reliable method for generating such inbred lines, thereby enhancing the efficiency of hybrid breeding (Prigge & Melchinger, 2012) ^[34, 35].

In crops like *Zea mays*, doubled haploid lines are extensively used in commercial hybrid seed production. The use of haploid inducer lines has revolutionized maize breeding by enabling large-scale production of doubled haploids with high efficiency. This approach reduces the time required to develop parental lines from several years to just a few seasons, thereby accelerating the release of new hybrids (Chaikam *et al.*, 2019) ^[3]. Additionally, doubled haploid technology ensures genetic purity and consistency in hybrid seeds, which is critical for maintaining yield performance and quality traits.

QTL Mapping and Genetic Studies

Doubled haploid populations are invaluable resources for quantitative trait locus (QTL) mapping and genetic studies. Since doubled haploid lines are completely homozygous, they provide a stable genetic background for the accurate identification of genomic regions associated with important traits. This stability eliminates the complications arising from heterozygosity and segregation, thereby improving the precision of genetic analyses (Collard *et al.*, 2005) ^[6].

In cereal crops, doubled haploid populations have been widely used to dissect complex traits such as yield, drought tolerance, disease resistance, and quality parameters. By combining phenotypic data with molecular marker information, researchers can identify QTLs controlling these traits and utilize them in breeding programs. The use of doubled haploids also facilitates the construction of high-density genetic maps, which are essential for understanding genome organization and gene interactions (Xu *et al.*, 2017) ^[45].

Moreover, doubled haploid lines serve as permanent mapping populations that can be evaluated across multiple environments and years. This allows for the identification of stable QTLs and genotype $\times$ environment interactions, which are critical for developing resilient crop varieties. The integration of advanced genomic tools further enhances the utility of doubled haploid populations in genetic research (He *et al.*, 2014) ^[16].

Marker-Assisted Selection

The integration of doubled haploid technology with Marker-assisted selection has significantly improved the efficiency and precision of plant breeding. Marker-assisted selection

(MAS) enables the identification and selection of desirable traits at the DNA level, reducing reliance on phenotypic screening. When combined with doubled haploids, MAS allows for the rapid fixation and selection of favorable alleles in a single generation.

This integration is particularly useful for traits that are difficult to measure phenotypically, such as disease resistance or abiotic stress tolerance. By using molecular markers linked to these traits, breeders can screen doubled haploid lines at an early stage and select superior genotypes with high accuracy. This not only saves time and resources but also enhances the effectiveness of breeding programs (Xu & Crouch, 2008) ^[44].

In addition, the combination of MAS and doubled haploid technology facilitates the pyramiding of multiple genes controlling important traits. This is especially relevant in cereal crops, where resistance to multiple diseases or tolerance to various stresses is required for stable production. The use of doubled haploids ensures that selected gene combinations are fixed and maintained in subsequent generations, thereby improving the durability and performance of new varieties (Dwivedi *et al.*, 2015) ^[10].

Factors Affecting DH Efficiency

The efficiency of Doubled haploid technology is influenced by a complex interaction of genetic, physiological, and technical factors. Among these, genotype dependence remains one of the most critical constraints. For instance, in *Triticum aestivum*, significant variation exists among cultivars in their response to androgenesis and chromosome elimination systems, limiting the universal applicability of protocols (Ferrie & Möllers, 2011) ^[12].

The composition of culture media and environmental conditions also plays a decisive role. Factors such as nutrient composition, temperature pretreatment, light intensity, and osmotic conditions directly affect microspore viability and embryogenesis. Optimized media formulations enriched with appropriate carbon sources and micronutrients have been shown to enhance callus formation and plant regeneration (Germanà, 2011) ^[15].

Hormonal balance is equally important, as the ratio of auxins to cytokinins determines the developmental pathway of cultured cells. Imbalances can lead to callus formation without regeneration or abnormal embryo development. Fine-tuning hormonal regimes has been a key area of improvement in tissue culture-based haploid production systems (Touraev *et al.*, 2009) ^[41].

Finally, technical constraints, including laboratory infrastructure, skilled personnel, and precise handling of explants, significantly influence success rates. Variability in protocol execution often leads to inconsistent results across laboratories, emphasizing the need for standardization and capacity building (Dwivedi *et al.*, 2015) ^[10].

Table 2: Applications of Doubled Haploids in Cereals (2015-2025)

Crop	Method Used	Application Area	Key Outcome	Reference
Wheat (<i>Triticum aestivum</i>)	Wheat × maize	Rapid development of pure lines	Reduced breeding cycle by 4-5 generations	Niu Z. (2014) ^[32] , updated in Khan M.A. (2020) ^[26]
Wheat	Anther culture	Genetic variability studies	Efficient selection of homozygous lines	Ferrie A.M.R. (2011) ^[11] , extended by Murovec J. (2018) ^[31]
Barley (<i>Hordeum vulgare</i>)	Microspore culture	QTL mapping	Stable mapping populations	Prigge V. (2012) ^[34, 35] , applied in Herzog E. (2016) ^[17]
Maize (<i>Zea mays</i>)	In vivo haploid induction	Hybrid breeding	Fast inbred line development	Chaikam V. (2019) ^[3]
Rice (<i>Oryza sativa</i>)	Anther culture	Stress tolerance breeding	Improved salinity tolerance lines	Mishra R. (2016) ^[30]
Wheat	Wheat × maize	Disease resistance breeding	Fixation of rust resistance genes	Broughton S. (2016) ^[2]
Barley	Microspore culture	Malting quality improvement	Uniform quality traits	Lantos C. (2019) ^[28]
Rice	Anther culture	Hybrid rice breeding	Development of parental lines	Zhang Z. (2018) ^[46]
Maize	Haploid inducer lines	Genomic selection	Increased selection accuracy	Prasanna B.M. (2017) ^[33]
Wheat	Wheat × maize	Climate resilience breeding	Development of drought-tolerant DH lines	Alahmad S. (2018) ^[1]

Challenges and Limitations

Despite its transformative potential, doubled haploid technology faces several persistent challenges. One of the most prominent issues is the low efficiency in wheat, particularly in androgenesis-based methods. Compared to crops like barley, wheat exhibits poor embryogenic response and regeneration capacity, making haploid production less reliable.

This phenomenon is often linked to plastid genome instability and improper chloroplast development under *in vitro* conditions (Jähne & Lörz, 1995) ^[21]. The high cost and technical skill requirement further restrict widespread adoption. Establishing and maintaining tissue culture facilities, along with the need for trained personnel, increases operational costs. This is particularly challenging for breeding programs in resource-limited settings, where access to advanced infrastructure is limited (Forster *et al.*, 2007) ^[13, 14].

Additionally, limited adaptability across genotypes continues to hinder the scalability of DH technology. Protocols optimized for specific genotypes may not perform well in others, necessitating repeated optimization and reducing efficiency. This genotype specificity remains a bottleneck in integrating DH technology into routine breeding pipelines (Ferrie & Caswell, 2011) ^[11].

Recent Advances

Recent years have witnessed significant progress in enhancing the efficiency and applicability of doubled haploid technology. One notable advancement is the integration of CRISPR gene editing with haploid induction systems. Gene editing tools have been used to manipulate key genes involved in haploid induction, thereby improving induction rates and enabling precise genetic modifications in doubled haploid lines (Kelliher *et al.*, 2019) ^[25].

The development of haploid inducer lines in maize has revolutionized DH production. Specialized inducer lines carrying genetic factors that promote haploid embryo formation have enabled large-scale, efficient haploid production in *Zea mays*. These systems have also been adapted for use in other cereals, expanding their applicability (Prigge *et al.*, 2012) ^[34, 35].

Advances in automation and high-throughput systems have further improved efficiency. Robotic handling, automated embryo rescue, and digital monitoring of culture conditions have reduced human error and increased scalability. Such

innovations are particularly valuable for large breeding programs aiming to generate thousands of DH lines annually (Rasheed *et al.*, 2018) ^[36]. This approach is especially relevant under changing climatic conditions, where rapid adaptation is essential (Watson *et al.*, 2018) ^[43].

Future Prospects

The future of doubled haploid technology lies in addressing current limitations while leveraging emerging scientific tools. A key priority is improving induction efficiency, particularly in recalcitrant crops like wheat. Advances in molecular biology and genomics are expected to identify genes controlling embryogenesis and chromosome doubling, enabling targeted interventions (Dwivedi *et al.*, 2015) ^[10].

The application of DH technology in climate-resilient breeding is another promising area. Rapid development of homozygous lines allows breeders to quickly fix desirable traits such as drought tolerance, heat resistance, and disease resistance. This is crucial for ensuring food security in the face of climate change (Rasheed *et al.*, 2018) ^[36].

The integration of artificial intelligence (AI) and genomics is expected to further enhance DH systems. AI-driven models can optimize culture conditions, predict genotype responsiveness, and streamline selection processes, thereby improving efficiency and reducing costs. Coupled with genomic selection, this approach can accelerate genetic gain in breeding programs (Crossa *et al.*, 2017).

There is also significant scope in developing countries, where the adoption of DH technology can transform breeding programs. With appropriate investment in infrastructure and training, DH systems can enable faster development of improved varieties tailored to local conditions, benefiting smallholder farmers and enhancing agricultural productivity (Dwivedi *et al.*, 2015) ^[10].

Conclusion

Doubled haploid technology has emerged as a cornerstone of modern Plant Breeding, offering unparalleled advantages in achieving rapid homozygosity and genetic uniformity. Its application in cereals has significantly enhanced the efficiency of breeding programs, enabling faster development of improved varieties. Despite challenges such as genotype dependence, albinism, and high operational costs, continuous advancements in molecular biology, tissue culture, and automation are addressing these limitations.

The integration of DH technology with emerging tools such as genome editing, speed breeding, and artificial intelligence underscores its central role in next-generation breeding strategies. Moving forward, interdisciplinary approaches combining genetics, biotechnology, computational tools, and agronomy will be essential to fully realize the potential of this technology. Strengthening research capacity and ensuring accessibility in developing regions will further expand its impact, making doubled haploid technology a key driver of sustainable and climate-resilient agriculture.

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