Review Article

Doubled Haploids in Crop Improvement: Unraveling Strategies, Advancements and Prospects for Enhanced Genetics

ABSTRACT

Doubled haploids (DH) have emerged as a powerful tool in crop improvement programs, enabling rapid generation of homozygous lines for accelerated genetic enhancement. This review explores the strategies, advancements, and prospects associated with doubled haploids in the context of crop improvement. The first section provides an overview of the principles behind doubled haploidy, including the induction methods and techniques used to obtain doubled haploid plants. Different approaches such as anther culture, microspore culture and in vitro fertilization techniques are discussed, highlighting their advantages, limitations, and applicability across various crop species. The second section delves into the recent advancements in doubled haploid technology. It examines novel techniques for haploid induction and chromosome doubling, including genetic and molecular approaches, biotechnological interventions, and the use of chemical agents. The role of innovative technologies such as genomics, transcriptomics, and marker-assisted selection in enhancing the efficiency and precision of doubled haploid production is also explored. The third section focuses on the utilization of doubled haploids in crop improvement. It discusses the potential applications of doubled haploids in various breeding objectives, such as the development of superior varieties, acceleration of breeding cycles, trait introgression and elucidation of genetic mechanisms. The role of doubled haploids in facilitating the incorporation of desirable genes, promoting genetic diversity, and enhancing crop adaptation to changing environmental conditions is highlighted. Lastly, the review addresses the prospects and future directions of doubled haploids in crop improvement. It outlines emerging technologies, such as genome editing and gene stacking, and their potential integration with doubled haploid systems. In conclusion, doubled haploids have revolutionized crop improvement by offering an efficient means to obtain homozygous lines in a single generation.

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Keywords: *Anther Culture, Microspore Culture, Vitro Fertilization, Chromosome Doubling, Doubled Haploids, Genomics, Transcriptomics and Marker-Assisted Selection.*

1. INTRODUCTION

Genetic improvement forms the foundation of plant breeding, encompassing a range of conventional and non-conventional methods to introduce desirable variability in plant traits. Polyploidy, particularly in perennial crops, has proven to be an effective approach to create genetic diversity with favorable traits [1]. Various types of polyploids, including haploids, diploids and triploids are distinguished by variations in the number of chromosomes [4]. Among these, doubled haploids (DHs) hold significant promise in plant breeding as they possess homozygosity at all loci, making them ideal for the development of new varieties in self-pollinated crops or as parental inbred lines for hybrid varieties in cross-pollinated crops [5]. However, the induction of doubled haploids in cross-pollinated species often leads to inbreeding depression due to the presence of heterozygosity [2].

The process of inducing doubled haploidy not only serves as a means to rapidly obtain homozygous lines but also offers a valuable selection tool to eliminate genotypes that exhibit high levels of inbreeding depression [6]. Through the induction of doubled haploids, selection can be targeted towards traits influenced by recessive deleterious genes associated with vegetative development [7]. This aspect adds an additional advantage to the doubled haploid technique, making it a useful tool for plant breeding.

In this review, we will discuss the induction of doubled haploidy and its significance in plant breeding [3]. We will explore the methodologies employed for haploid induction, such as anther culture, microspore culture, and in vitro fertilization techniques, along with the process of chromosome doubling [8]. The importance of doubled haploids as a means to introduce genetic diversity and accelerate breeding cycles will be emphasized. Furthermore, we will delve into the role of doubled haploids in addressing inbreeding depression, facilitating trait introgression, and understanding genetic mechanisms in various crop species.

By unraveling the strategies and advancements associated with doubled haploids, this review aims to shed light on the potential of this technique for enhanced genetic enhancement in crop improvement programs. Additionally, we will explore the prospects and future directions of doubled haploids, including their integration with emerging technologies such as genome editing and gene stacking. The challenges associated with large-scale implementation, cost-effectiveness and regulatory considerations will also be addressed, along with potential strategies to overcome these obstacles.

In conclusion, the induction of doubled haploids presents a valuable approach in plant breeding, allowing for the rapid production of homozygous lines and serving as a selection tool to eliminate genotypes expressing inbreeding depression. This review will provide a comprehensive understanding of the induction of doubled haploidy and its significance in crop improvement, offering insights into its potential applications and prospects for enhanced genetic enhancement in agriculture.

2. THE SOURCE OF DOUBLED HAPLOIDS

The source of doubled haploids (DH) can be traced back to monoploids, which are plants with a single set of chromosomes in their haploid phase (2n=x) [9]. These monoploids are derived from diploid species with a chromosomal number of 2x [10]. The doubled haploid technique serves as a valuable tool for genetic mapping of complex traits and expediting breeding technologies.

The induction of haploid lines is an efficient approach to accelerate plant breeding. Haploid plants have widespread use in the study and development of various agricultural crops [12]. Haplotypes, which are unique plants with distinct genetic compositions, offer researchers access to genetic information that is not easily attainable with typical diploid individuals [12]. Haplotypes can be categorized into three groups based on their genetic makeup.

Maternal haplotypes consist of the nucleus and cytoplasm solely from the maternal parent. In the case of in vitro androgenic haploids generated through anther or microspore culture, both the cytoplasm and nucleus of the developing micro sporophyte are present [13]. Haploids produced through microspore culture are considered superior and more precise. On the other hand, in vivo androgenic haploids can arise from the development of the embryo sac or any other type of cell, resulting in the loss of the maternal parent's chromosomes during development [14,15].

Overall, doubled haploids serve as a valuable resource for plant breeders, enabling the rapid production of homozygous lines and offering unique genetic compositions for studying and improving agricultural crops. The different sources of haplotypes, including maternal haplotypes, in vitro androgenic haploids, and in vivo androgenic haploids, provide

researchers with diverse materials to explore and enhance genetic variation in crop improvement programs.

3. THE HISTORY OF DOUBLED HAPLOIDS (DH)

The history of doubled haploids (DH) can be traced back to the early exploration of haplotypes, which are divided into three main groups based on their genetic makeup [16]. Maternal haplotypes consist solely of the nucleus and cytoplasm from the maternal parent, while in vitro androgenic haploids, generated through anther or microspore culture, possess both the cytoplasm and nucleus of the growing micro-sporophyte [17]. Haploids produced by microspore culture are considered superior and more precise. Additionally, in vivo androgenic haploids can arise from the development of the embryo sac or other types of cells, resulting in the presence of only the male parent's chromosomes and the maternal plant's cytoplasm [18,19].

The possibility of obtaining androgenic haploids in maize was first mentioned by Kermicle in 1969, where 1-3% of seeds from plants with the homozygous gene IG1 (indeterminate gametophyte 1) were found to be androgenic haploids [20]. This discovery highlighted the potential of in vitro androgenesis, the process of inducing and regenerating haploids and doubled haploids from male gametic cells. Due to its effectiveness, wide application across various plant species, and exceptional potential for plant breeding and economic exploitation, in vitro androgenesis gained significant attention [21].

Several plant species, including barley, wheat, maize, rice, triticale, rye, tobacco, rapeseed and cabbage, have a long history of utilizing haploid embryogenesis in plant breeding, genetic research and intentionally induced mutations [23]. The induction of haploids through the manipulation of the female gametophyte alone, such as pollination with pollen from the same species or irradiated pollen, has been employed to produce maternal haploids in situ [24]. Pollination using pollen from unrelated or wild relatives has been successful in crops like wheat. In such cases, after pollination, the egg cell can be fertilized and develop into a hybrid embryo with elimination of the paternal chromosome [25].

Naturally occurring maternal haploids in maize are limited in frequency, typically occurring at a rate of one haploid maize plant for every 1,000-2,000 regular diploid plants [26]. However, an alternate method for acquiring and studying maternal haploids in maize was reported following the discovery of a line called "Stock 6" [27]. This alternative approach demonstrated high induction rates, ranging from 8 to 10%, for current haploid-inducing lines [28]. In cases where haploid embryos-containing kernels germinate normally and produce healthy haploid seedlings, in vitro culture is not required. The selection of haploid embryos based on morphological and physiological criteria can be done early in the breeding process. Another method for inducing maternal haploids is gynogenesis, which involves in vitro culture of unpollinated floral parts such as placenta connected to ovules, ovaries, or complete flower buds [29]. Regenerants obtained through gynogenesis are genetically more stable and exhibit fewer diseases compared to androgenetic plants. Successful gynogenesis has been achieved in several species, including onions, sugar beets, cucumber, squash, gerbera, sunflower, wheat, and barley, although the success is influenced by genotype and environmental factors [29,30].

In wheat, a different technique for creating haploids involves chromosome removal after wide hybridization. Maize pollen has proven to be the most effective in inducing the largest frequency of haploids [31]. Hybridization of Hordeum vulgare with Hordeum bulbosum demonstrated that haploid plants of *Hordeum vulgare* could be produced on a large scale. During the hybridization process, the chromosomes from *H. bulbosum* are lost during seed development [32].

4. METHODS FOR THE PRODUCTION OF HAPLOIDS IN PLANT BREEDING

Researchers have employed various methods for the production of haploids in plant breeding. These methods include:

1. Anther Culture: Anther culture involves the in vitro culture of anthers, the male reproductive organs, on a suitable nutrient medium. The anthers contain microspores, which can undergo embryogenesis and develop into haploid plants [33]. This technique has been successfully applied to several plant species, including wheat, rice, maize, and barley [34].

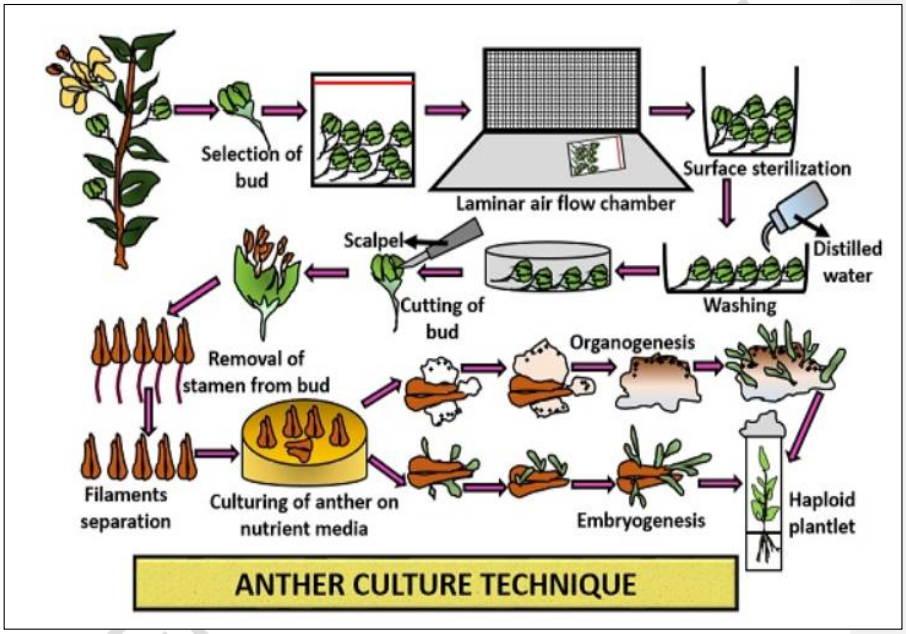


Figure 1. *Diagrammatic presentation of the steps of anther culture. Source: https://biologyreader.com/anther-culture.html*

2. Ovule Culture: Ovule culture is another technique used for haploid production. It involves the isolation and in vitro culture of ovules, the female reproductive structures, under controlled conditions [35]. Haploid embryos can be induced to develop from unfertilized ovules, and these embryos can be further cultured to obtain haploid plantlets.

3. Wide Hybridization: Wide hybridization involves crossing two distantly related species or even genera to induce haploid production. The resulting hybrid embryos may undergo chromosome elimination, resulting in haploid embryos [36]. This method has been employed in crops like wheat, where the hybridization of wheat with maize leads to haploid wheat plants [37].

4. Pollen Irradiation: Pollen irradiation is a technique where pollen grains are exposed to ionizing radiation, such as X-rays or gamma rays. Irradiation can induce chromosomal aberrations and disruptions in the fertilization process, resulting in the production of haploid embryos [38].

5. Chemical Induction: Chemical treatments can be used to induce haploid production. Chemicals such as colchicine, oryzalin, and specific herbicides act as mitotic inhibitors and disrupt cell division. This leads to the doubling of chromosome numbers and the production of doubled haploids [39].

6. Genetic Manipulation: Genetic manipulation techniques, such as the introduction of haploid inducer genes, can enhance the production of haploids. These genes are capable of inducing haploid formation or influencing chromosome elimination during gamete formation, resulting in haploid embryos [10,31].

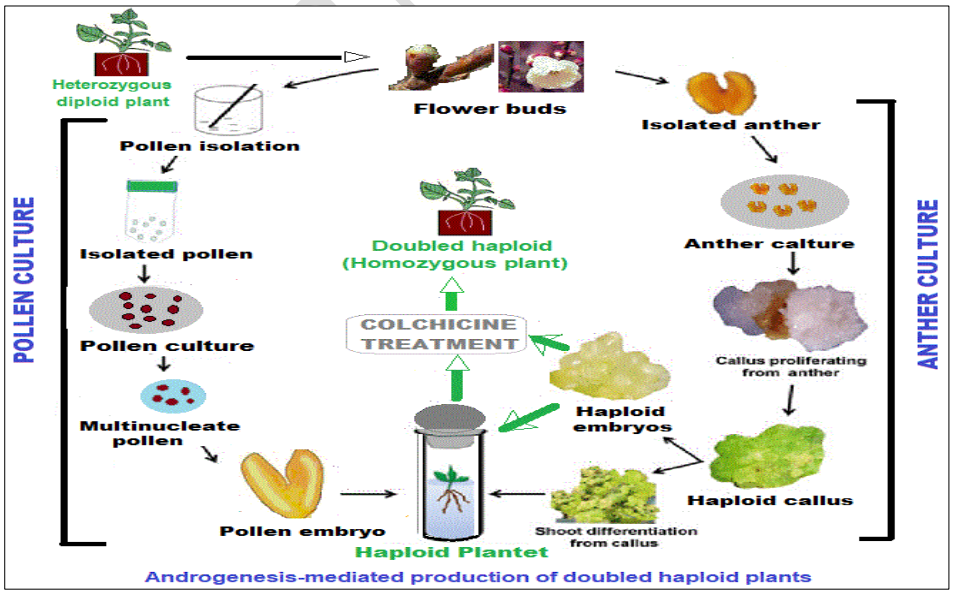
7. Haploid Inducer Genes: In some plant species, specific genes known as haploid inducer genes have been identified and utilized to induce haploid production. These genes promote the development of haploid embryos or affect the formation of gametes with reduced chromosome numbers [40]. By introducing these inducer genes into breeding programs, the frequency of haploid production can be increased, facilitating the development of pure-line cultivars.

8. Interspecific/Intergeneric Hybridization: Interspecific or intergeneric hybridization involves the crossing of two different species or genera to induce haploid production. One commonly used example is the hybridization of wheat (*Triticum aestivum*) with maize (*Zea mays*) [41]. During the hybridization process, a hybrid embryo is formed, which later undergoes the elimination of maize chromosomes to produce haploid wheat plantlets. This technique has proven successful in generating haploid wheat embryos, although further development beyond the haploid stage is limited [32, 42].

The choice of method depends on the specific plant species, breeding goals, and available resources. Each method has its advantages and limitations, and researchers select the most suitable approach based on the target crop and the desired outcomes in terms of haploid production for genetic improvement and breeding programs.

Figure 2. In vitro cultures by androgenesis and gynogenesis. Haplo-dipoidization techniques.

Source: https://www.biotech-ecolo.net/androgenesis-in-vitro-haploid-plants.html



5. APPLICATIONS OF DOUBLE HAPLOIDS IN PLANT BREEDING RESEARCH

Double haploid (DH) technology plays a significant role in plant breeding research, offering various applications that contribute to the development of improved plant varieties. Here are some key applications:

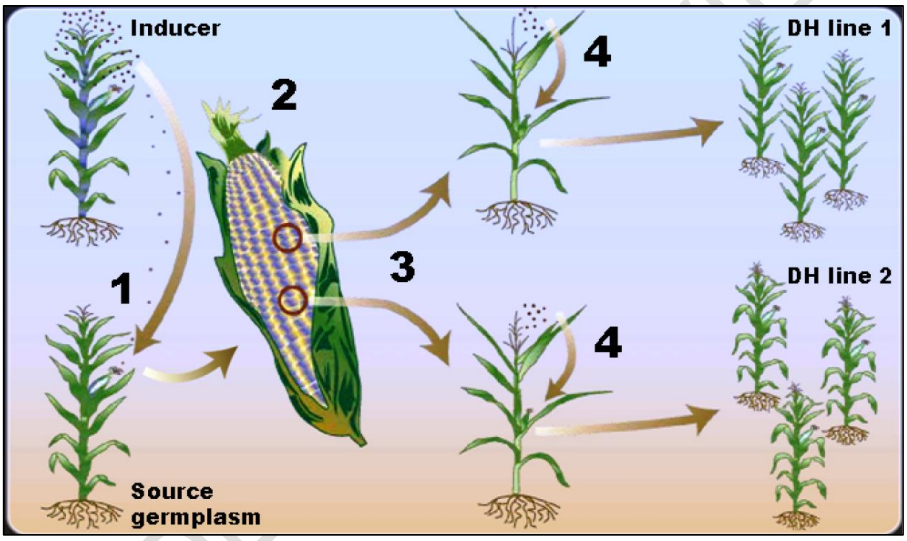
- ***Accelerated Breeding:*** Double haploids allow for the rapid production of homozygous plants in a single generation. By bypassing several generations of inbreeding, DHs significantly speed up the breeding process, reducing the time required to develop new varieties [43]. This acceleration enables breeders to release improved cultivars more quickly, addressing the demands of the agricultural industry.
- ***Trait Mapping:*** Double haploids are valuable for trait mapping and genetic analysis. Since DHs are homozygous, they exhibit less genetic variation, making it easier to identify and map the genes responsible for specific traits of interest. DH populations can be used for quantitative trait loci (QTL) analysis, association mapping, and marker-assisted selection, aiding in the identification and incorporation of desirable traits in breeding programs [44].
- ***Hybrid Development:*** DH technology enables the production of homozygous lines for both parents involved in hybrid crosses. This allows for the efficient development of hybrid varieties with improved traits such as yield, disease resistance, and quality. DHs can also be used in the production of parental lines for hybrid seed production, enhancing the efficiency and effectiveness of hybrid breeding programs [45].
- ***Genetic Manipulation:*** Double haploids are useful for introducing and studying genetic modifications in plants. They provide a platform for gene editing techniques like CRISPR/Cas9 to create targeted mutations and evaluate their effects. DHs can facilitate the analysis of gene function, gene expression studies, and the validation of candidate genes, enabling the development of genetically modified plants with desired traits [46].
- ***Genetic Diversity and Germplasm Conservation:*** DH technology can be employed to create a permanent collection of homozygous lines representing diverse genetic resources. These DH lines can serve as a valuable germplasm collection for future breeding programs, genetic studies, and conservation efforts. Preserving genetic diversity is crucial for sustainable agriculture and ensuring the availability of genetic resources for future generations [47].
- ***Studying Heterosis:*** Double haploids are advantageous in studying heterosis or hybrid vigor. By comparing the performance of DHs with their corresponding parental lines, breeders can evaluate the degree of heterosis and select the most promising hybrids for commercial production. Understanding and harnessing heterosis can lead to the development of high-performing hybrid varieties with superior traits [48].
- ***Genetic Stability:*** Double haploids offer genetic stability since they are homozygous and exhibit fixed genetic traits. This stability allows for consistent evaluation of plant performance, simplifies the selection process, and increases the reliability of breeding programs. Genetic stability is essential for ensuring the consistent expression of desirable traits in subsequent generations [49].

6. PRODUCTION OF DOUBLED HAPLOID LINES IN MAIZE

The production of doubled haploid (DH) lines by in vivo haploid induction in maize is initiated by pollinating source germplasm, from which DH lines are to be developed, with a specific genotype called “inducer” [50]. For maternal haploids, the inducer is used as male parent to induce the production of seeds with haploid embryo on the ears of the female parent [18].

1. Haploidy is induced by pollinating the source germplasm with pollen from a haploid inducer genotype.
2. After shelling, putative haploid seeds are identified based on the expression of seed coloration.
3. These seeds are treated with mitotic inhibitors to artificially double their chromosomes and produce DH plants.
4. DH plants are self-pollinated to produce seeds for maintenance and multiplication of the DH line.

Figure 3: *Schematic description of doubled haploid (DH) line development with the in vivo haploid induction approach.*



Source: *https://www.unihohenheim.de/fileadmin/einrichtungen/plant-breeding*

7. FUTURE ASPECTS OF DOUBLE HAPLOIDS IN PLANT BREEDING

The future of double haploids (DHs) in plant breeding holds promising potential for further advancements and applications. Here are some future aspects of double haploids in plant breeding:

1. Enhanced Trait Discovery: Double haploids can serve as a valuable resource for identifying and characterizing new traits. By studying the genetic makeup of DH populations, breeders can uncover novel genetic variations that contribute to important agronomic traits such as yield, disease resistance, stress tolerance, and nutritional quality. This knowledge can be leveraged to develop improved crop varieties with enhanced traits [51].

2. Genomic Selection: Genomic selection is a breeding approach that utilizes genomic data to predict the breeding value of plants. Double haploids provide a unique opportunity for capturing the full genetic potential of individuals, as they are homozygous and exhibit fixed genetic traits. By combining genomic selection with DH technology, breeders can expedite the breeding process by accurately predicting the performance of individuals early on, leading to more efficient selection and improved breeding outcomes.

3. Accelerated Breeding for Climate Resilience: With the challenges posed by climate change, there is a pressing need to develop crop varieties that are resilient to changing environmental conditions [53]. Double haploids can play a crucial role in accelerating the breeding of climate-resilient crops. By rapidly generating homozygous lines and utilizing techniques such as marker-assisted selection, breeders can introduce and stack multiple favorable traits associated with climate resilience, such as drought tolerance, heat tolerance, and disease resistance [53, 54].

4. Integration with Genomic Editing Technologies: The advent of genomic editing technologies, such as CRISPR/Cas9, provides precise tools for targeted modification of specific genes [55]. Double haploids offer a platform for efficient gene editing, as they are amenable to genetic transformation and provide a uniform genetic background. Combining DH technology with genomic editing can enable the rapid development of crop varieties with desired traits, including improved nutritional content, enhanced pest resistance, and increased productivity [27, 56].

5. Expanding Crop Species: While double haploids have been extensively used in major crop species such as wheat, maize and rice, there is potential for expanding its application to other important crops. Research efforts can focus on developing efficient protocols and optimizing the DH production process for diverse plant species. By broadening the scope of double haploid technology, breeders can unlock its benefits for a wider range of crops, ultimately contributing to global food security and agricultural sustainability.

6. Integration of Omics Approaches: Integrating omics approaches, such as genomics, transcriptomics, proteomics, and metabolomics, with double haploid technology can provide a comprehensive understanding of the genetic and molecular basis of complex traits [57]. By elucidating the underlying mechanisms and pathways associated with important traits, breeders can make more informed decisions in selecting and breeding superior varieties. This integration of omics data with DH technology can further enhance the precision and efficiency of breeding programs.

In summary, the future of double haploids in plant breeding is characterized by the continued exploration and integration of cutting-edge technologies, enhanced trait discovery, accelerated breeding for climate resilience, expansion to new crop species, and the integration of omics approaches. These advancements will contribute to the development of improved crop varieties that address global challenges and pave the way for sustainable agriculture.

8. CONCLUSION

In conclusion, the applications of double haploids in plant breeding research, including accelerated breeding, trait mapping, hybrid development, genetic manipulation, genetic diversity conservation, studying heterosis, and genetic stability, contribute to the development of improved plant varieties with desired traits. These applications enable breeders to efficiently and effectively enhance crop productivity, disease resistance, quality, and other important agronomic traits, benefiting the agricultural industry and global food

security. Development of double haploids have proven to be crucial in accelerating wheat breeding programs and improving crop productivity. The techniques of anther culture and wheat x maize crossing have emerged as popular methods for producing double haploid wheat. However, further research is needed to understand the factors that affect the efficiency of haploid production methods. Important considerations for future research include determining the optimal timing for egg and microspore selection, as well as the duration and temperature of cold pre-treatment. The addition of substances such as hormones or medications to the growing medium can significantly influence plant growth and regeneration. Furthermore, genotypic variation should be taken into account, as it has been demonstrated that the genotype plays a more significant role than incubation conditions in haploid plant production. Looking ahead, it is essential to focus on improving the wheat x maize crossing system, particularly in terms of regeneration under different environmental conditions. This research will contribute to the advancement of double haploid breeding by facilitating cultivar development, genetic improvement, and a deeper understanding of genetic phenomena. The applications of double haploids in plant breeding research are wide-ranging and impactful. They offer opportunities for accelerated breeding, trait mapping, hybrid development, genetic manipulation, genetic diversity conservation, studying heterosis, and ensuring genetic stability. Through these applications, double haploid technology can contribute to the development of improved plant varieties with desirable traits, reducing the cultivar development period and addressing the challenges of conventional breeding. Overall, the study and implementation of haploid wheat and double haploids have revolutionized plant breeding research, and ongoing efforts in research and technology refinement will continue to drive advancements in cultivar development, genetic improvement, and our understanding of genetic phenomena in the field of agriculture.

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