

## Importance of doubled haploids technology in plant breeding

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### Abstract

To produce fully homozygous lines from heterozygous donor plants in a single step, doubled haploidy (DH) is a very effective method. However, a number of variables, including media content and genotype, influence it. In order to employ this technique each genotype in the breeding program, it must be standardized. In every species for which the methodology is adequately developed, the approach is currently the method of choice and has been utilized in breeding programs for several decades. The application of doubled haploidy for genomics research and innovative breeding techniques has recently attracted more attention. Doubled haploids are becoming a more popular method of increasing genetic gain per cycle in horticultural crops that are perennial in nature or have high economic value when breeding time is crucial. The very effective microspore culture systems have received a lot of attention, which has advanced the understanding of the molecular aspects of microspore embryogenesis.

### Introduction

Doubled haploidy has been extensively employed in breeding programs worldwide for both cross-pollinating species like maize and self-pollinating species like wheat, barley, and rapeseed. These methods enable the creation of fully homozygous lines from heterozygous parents in a single generation. The ultimate result of self-pollinating species breeding programs is advanced homozygous lines, while the primary objective of open pollinating species breeding programs is to select superior hybrids and improve testcross performance (Li et al. 2013). If the original plant was diploid, the haploid cells are monoploid, and the doubled haploids can be called doubled monoploids. Tetraploids and hexaploids are terms commonly used to describe dihaploid creatures that evolved from tetraploids or hexaploids (Forster et al. 2007).

In self-pollinated organisms, crosses of desired genotypes initiate the basic breeding mechanism, resulting in hybrids with the chromosomal sets of both parents. Recombinations enable the formation of novel gene combinations during gamete development, which are fixed throughout the process of double

haploid generation (Yali, 2022). If deemed appropriate, doubled haploid lines can be employed as parental lines for hybrids or as cultivars in their own right. The doubled haploid technology offers numerous useful applications in agricultural biotechnology, including gene mapping, genomics, mutation induction, cytological research, and inbred development in the smallest amount of time. Different ploidy levels are caused by aberrant polyploidization and spontaneous doubling during the culture and subsequent growth stages (Ferrie et al. 1995).

The species, genotypes, physiology of the donor plants, microspore/pollen development stage, culture medium composition, carbon source, sucrose levels, plant growth hormones, and pre-treatment temperatures are all important factors in the successful production of androgenic plantlets (Chaudhary et al. 2019). A variety of pre-treatments, such as cold or heat shock, water stress, high humidity, anaerobic treatment, starvation of sucrose and nitrogen, gamma radiation, ethanol microtubule disruptive agents, electrostimulation, heavy metal pre-treatments, etc., are applied to anthers in order to improve embryogenesis (Ferrie et al. 1995; Olmedilla, 2010; Shariatpanahi et al. 2006). It is thought that the pre-treatments cause the microspores' gametophytic development pathway to change to a sporophytic one. Temperature is the most efficient pre-treatment to induce embryogenesis out of all of them (Singh et al. 2022).

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Isolated microspores can be switched from the typical gametophytic developmental pathway to a sporophytic pathway by offering the right mix of culture conditions and stressors. This will result in the production of embryos and haploid or doubled haploid (DH) plants (Ferrie and Caswell, 2011). The process of creating DH plants from microspores is a crucial one for plant breeding and genetic investigations (Bhatia, 2016). Because it can quickly produce haploid or DH plants, create genetic maps, find genes of agronomic and economic importance, identify markers for trait selection, expedite crop improvement programs, and increase plant breeding efficiency, isolated microspore culture technique is likely to remain a popular method in Brassica breeding



programs (Forster and Thomas, 2005; Chan and Pauls, 2007; Ferrie and Caswell, 2011).

It is advantageous that a homozygous line develops in one generation as opposed to numerous generations because it speeds up the breeding process for desired variations. For annual self-pollinating crops, a normal breeding program can take up to 10 years to establish a cultivar; however, with the use of DH lines, this time can be shortened by 3–4 years. In a traditional plant breeding program, homozygosity can only be reached at 98% even after 6 years of self-pollination; using two-fold haploidy procedures, homozygosity can be reached at 100% (Ferrie and Möllers, 2011). Reverse breeding is a relatively new breeding technique that makes use of DHs. Breeders can create parental lines from any heterozygous plant using this crop enhancement method (Dirks et al. 2009).

### Development of haploids

The anther wall tissues are eliminated during microspore isolation, preventing maternal sporophytic tissue from interfering with pollen development and somatic tissue regeneration. Androgenetic plants can be regenerated from microsporial callus cells (organogenesis) or from embryo-like structures (direct microspore embryogenesis) under ideal in vitro culturing conditions. Direct embryogenesis is the recommended method because regeneration during the callus stage may result in albinism and unwanted gametoclonal variation (Murovec and Bohanec, 2012). Applying the right physiochemical conditions triggers a stress response that stops juvenile pollen grains or microspores in their gametophytic route. Through the process of embryogenesis, they are made to divide into several cells and create multicellular structures that are encased in an exine wall. Ultimately, the exine wall releases the structures that resemble embryos (Maraschin et al. 2005). According to Murovec and Bohanec (2012), the most often utilized triggering conditions include osmotic stress, starvation of sucrose and nitrogen and temperature pre-treatment.

### Identification of doubled haploidy

It is consequently imperative to choose regenerants quickly and reliably before using putative haploids and doubled haploids in any further applications. Unwanted adventitious regeneration from somatic cells or hybrid embryo germination are not the exclusive causes of regeneration in diploid plants. There is also a chance of spontaneously doubled haploids, which avoids the requirement for chromosome doubling. Depending on which markers are available for a certain plant species, a number of

markers can be used to determine the origin of diploids. In the past, isozyme analysis, progeny testing following self-pollination and phenotypic markers were the primary methods used to evaluate regenerants. These days, homozygosity tests and analyses of plant origin are frequently conducted using DNA molecular markers (Murovec and Bohanec, 2012). More research has been done on the spontaneous diploidization of embryos produced from microspores in barley. The results of the study showed that nuclear fusion following the initial nuclear division causes chromosomal doubling in microspores pre-treated with mannitol (Kasha et al. 2001).

### Conclusion

Double haploids have significantly reduced the cost and time involved in plant breeding operations. Haploid cells can double their DNA at any time during growth to become doubled haploids, which are diploid cells that don't need extra treatment. These days, the haploid induction technique can be effectively integrated with a number of other plant biotechnology techniques to enable a number of innovative breeding accomplishments, including enhanced genetic modifications, hybrid breeding, and mutation breeding.

### Declaration

The authors declare no conflict of interest.

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