

Production dihaploids in Genetics and Plant Breeding

Some Definitions:

Haploid pertains to a condition, a cell, or an organism that has half of the usual complete set of chromosomes in somatic cells.

Doubled haploid (DH) is a genotype formed when haploid cells undergo chromosome doubling.

Dihaploid refers to a haploid cell in which its nucleus contains two copies of the set of chromosomes. For instance, haploids of tetraploid species are called dihaploid.

Androgenesis can be defined as the set of biological processes leading to an individual genetically coming exclusively from a male nucleus. In tissue culture, the term androgenesis refers to plant regeneration directly from microspore culture under in vitro conditions.

In vitro gynogenesis, a parallel term to "androgenesis", means the process of plant regeneration from the unfertilised egg cells (in broad sense also including the other haploid cells of the female gametophyte) in the cultures of unpollinated ovaries or ovules.

Introduction: Haploids are plants (sporophytes) that contain a gametic chromosome number (n). They can originate spontaneously in nature or as a result of various induction techniques. Spontaneous development of haploid plants has been known since 1922, when Blakeslee first described this phenomenon in *Datura stramonium* (Blakeslee et al., 1922); this was subsequently followed by similar reports in tobacco (*Nicotiana tabacum*), wheat (*Triticum aestivum*) and several other species (Forster et al., 2007). However, spontaneous occurrence is a rare event and therefore of limited practical value. The potential of haploidy for plant breeding arose in 1964 with the achievement of haploid embryo formation from in vitro culture of *Datura* anthers (Guha and Maheshwari, 1964, 1966), which was followed by successful in vitro haploid production in tobacco (Nitsch and Nitsch, 1969).

Diploidization of haploid plants (doubled haploid production):

Doubled Haploid production: Haploids produced from diploid species ($2n=2x$), known as monoploids, contain only one set of chromosomes in the sporophytic phase ($2n=x$). They are smaller and exhibit a lower plant vigor compared to donor plants and are sterile due to the inability of their chromosomes to pair during meiosis. In order to propagate them through seed and to include them in breeding programs, their fertility has to be restored with spontaneous or induced chromosome doubling. The obtained doubled haploids (DHs) are homozygous at all loci and can represent a new variety (in self-pollinated crops) or parental inbred line for the production of hybrid varieties (in a cross-pollinated crop). In fact, cross pollinated species often express a high degree of inbreeding depression. For these species, the induction process per se can serve not only as a fast method for

the production of homozygous lines but also as a selection tool for the elimination of genotypes expressing strong inbreeding depression. Using DH production systems, homozygosity is achieved in one generation, eliminating the need for several generations of self-pollination. The time saving is substantial, particularly in biennial crops and in crops with a long juvenile period. For self incompatible species, dioecious species and species that suffer from inbreeding depression due to self-pollination, haploidy may be the only way to develop inbred lines. The induction of DH lines in dioecious plants, in which sex is determined by a regulating gene, has an additional advantage. Such a case is well studied in asparagus, in which sex dimorphism is determined by a dominant gene M. Female plants are homozygous for the recessive alleles (mm), while male plants are heterozygous (Mm). Androgenically produced DH lines are therefore female (mm) or 'supermale' (MM). An advantage of supermales is that, when used as the pollinating line, all hybrid progeny are male. Haploids from polyploid species have more than one set of chromosomes and are polyhaploids; for example dihaploids ($2n=2x$) from tetraploid potato (*Solanum tuberosum* ssp. *tuberosum*, $2n=4x$), trihaploids ($2n=3x$) from hexaploid kiwifruit (*Actinidia deliciosa*, $2n=6x$) etc. Dihaploids and trihaploids are not homozygous like doubled haploids, because they contain more than one set of chromosomes. They cannot be used as true-breeding lines but they enable the breeding of polyploid species at the diploid level and crossings with related cultivated or wild diploid species carrying genes of interest.

Haploids plants are sterile as these plants contain only one set of chromosomes. By doubling their chromosomes number, the plants can be made fertile and resultant plants will be homozygous diploid or isogenic diploid. The fertile homozygous diploid plants are more important than the sterile haploid plants and can be used as pure lines in breeding programme.

Doubled Haploids plants can be obtained by following methods:

- i) **Colchicine Treatment:** Colchicine has been utilised widely as spindle inhibitor to induce chromosome duplication and to produce polyploid plants. The young plantlets are treated with 0.5% colchicine solution for 24-48 hrs. Treated plantlets after thorough washing.
- ii) **Endomitosis:** Haploids cells are unstable in culture and have tendency to undergo endomitosis. i.e chromosome duplication without nuclear division. This property can be used for obtaining homozygous diploid plants.
- iii) **Fusion of Pollen Nuclei:** Homozygous diploid callus or embryoids may arise from the spontaneous fusion of two similar nuclei of the cultured pollen after first division. In Brassica, the frequency of spontaneous nuclear fusion in microspore is high in culture.

Genetics of DH population

In DH method only two types of genotypes occur for a pair of alleles, A and a, with the frequency of $\frac{1}{2}$ AA and $\frac{1}{2}$ aa, while in diploid method three genotypes occur with the frequency of $\frac{1}{4}$ AA, $\frac{1}{2}$ Aa, $\frac{1}{4}$ aa. Thus, if AA is desirable genotype, the probability of obtaining this genotype is higher in haploid method than in diploid method. If n loci are segregating, the probability of getting the desirable genotype is $(1/2)^n$ by the haploid method and $(1/4)^n$ by the diploid method. Hence the efficiency of the haploid method is high when the number of genes concerned is large.

Studies were conducted comparing DH method and other conventional breeding methods and it was concluded that adoption of doubled haploidy does not lead to any bias of genotypes in populations, and random DHs were even found to be compatible to selected line produced by conventional pedigree method. Using DH technology, completely homozygous plants can be established in one generation thus saving several generations of selfing comparing to conventional methods, by which also only partial homozygosity is obtained. Another feature that should be considered is the breeding strategy. Within the breeding process, DH lines can be induced as soon as from F1 generation (note that gametes on F1 plants represent the F2 generation), although some breeders prefer to induce Doubled Haploids lines from later generations. Induction in the F2 generation was proposed as an option because lines originated from F3 generation gametes had passed through another recombination cycle. The role of DH in the breeding process largely depends on the plant mode of reproduction. In self-pollinated species, they can represent final cultivars or they can be used as parental lines in hybrid production or test-crosses of cross-pollinated species.