

Use of haploids, dihaploids and doubled haploids

Haploids may be utilised for various investigations of both fundamental and applied importance as briefly described below:

- (1) Haploids are used to study the chromosome behaviour during meiosis. Study of chromosome pairing in mono-haploids indicates the presence of duplications in the chromosomes.
- (2) Study of chromosome pairing in haploids indicates the origin of different species of a plant. For example, in Brassica, chromosome pairing in haploids indicated that the basic chromosome number in the genus is $x = 6$, and different species originated through dysploidy.
- (3) Information on the ancestry of species can be obtained through the study of homoeologous chromosome pairing in the haploids of different allopolyploid species.
- (4) One of the most important uses of mono-haploids and polyhaploids is the production of homozygous lines in the shortest possible time. This is achieved by extracting haploids from heterozygous plants, followed by chromosomes doubling of such haploids; the resulting plants/lines are called doubled haploids or homodiploids. Chromosome doubling may occur naturally or may be induced using colchicine or some other suitable treatment. Doubled haploids may be used directly as cultivars. Cultivars derived from haploid systems have been produced in various crops such as wheat, rice, rapeseed, barley and tobacco.
- (5) In cross-pollinated species, haploidy is an effective method for selecting viable combinations of genes which are then used as inbreds after chromosome doubling.
- (6) There is no segregation of genes in the homodiploids and therefore, it permits selection for quantitative characters; thus selection efficiency increases.
- (7) In cases of self-incompatibility, inbred lines are readily produced by doubling the chromosome number of haploids.
- (8) Haploid tissues can be maintained in vitro in undifferentiated condition and they provide a source of suspension of haploid cells. Like micro-organisms, these haploid cells of higher plants can also be used to carry out new genetic researches such as mutational studies at physiological levels and biochemical analyses.
- (9) Monoploids can be used efficiently in mutational studies because they possess only a single set of genes. Therefore, both the dominant and recessive mutations are expressed in the M1 generation itself. Desirable mutants can be selected from among haploid cells cultured in vitro or

from haploid plants and, through chromosome doubling fertile homodiploids with all desirable mutations can be obtained.

(10) New genotypes can be incorporated into alien cytoplasm through androgenetic haploidy (androgenetic haploids may be produced by semi gamy and disruption of egg nucleus by irradiation). This method enables the transfer of a new genotype into the cytoplasm possessing factors for male sterility.

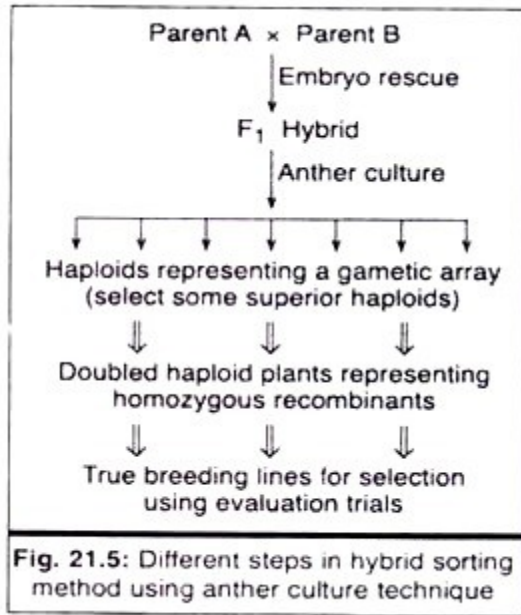
(11) Transfer of genes from wild diploid species to cultivated species can be done through dihaploids of polyploid species.

(12) Haploidy can be used in specific breeding schemes for dioecious plants, such as, *Asparagus officinalis*. *Asparagus* has the XX-XY system of sex determination, the male (XY) being more valuable commercially as they produce spears with a lower fibre content. Sib-mating between pistillate (XX) and staminate (XY) plants consists of 50% males and 50% female plants. Androgenetic haploids, derived especially through anther culture, are used to produce homozygous female (XX) and super-male (YY) lines; crossing of such female and super-male plants yields an all male population, which is commercially superior in yield to the conventional “50% male + 50% female” populations.

(13) Dihaploids may be used in crossing at diploid level before chromosome doubling (autotetraploid) as suggested by Chase in 1963 in the “analytical breeding” scheme for potato (*Solatum tuberosum*). Analytical breeding schemes rely mainly on ploidy manipulations to “capture” diversity from exotic (wild or non-adapted germplasm) and use $2n$ gametes to incorporate this genetic diversity through unilateral (USP; $n \times 2n$ or $2n \times n$) or bilateral (BSP; $2n \times 2n$) polyploidization. Most interesting examples of analytical breeding in crop improvement are in vegetatively propagated species such as potato, sweetpotato, cassava, among roots and tubers, and plantain/banana among fruit crops. This breeding approach appears promising in blackberry, blueberry, strawberry and other fruits.

15. Hybrid Sorting in Haploid Breeding:

Haploid breeding involves the hybrid sorting and it is considered superior to pedigree and bulk method as the superior haploid plants are selected and hybridized. Haploids derived from anther culture in less time and with less effort give the advantage of selection of recombinant superior gametes to be used for haploid breeding (Fig. 21.5).



(15) Through chromosome doubling in haploids, homozygous lines can be produced for various climatic regions in one laboratory.

(16) Haploids may be used to produce translocation stocks and aneuploid stocks which are of cytogenetic importance and can be used in improvement of crop plants.

(17) Most of the economic traits are controlled by genes with small but cumulative effects. Although the potential of DH populations in quantitative genetics has been understood for some time, it was the advent of molecular marker maps that provided the impetus for their use in identifying loci controlling quantitative traits. As the quantitative trait loci (QTL) effects are small and highly influenced by environmental factors, accurate replicated trials is needed. This is possible with doubled haploidy organisms because of their true breeding nature and because they can conveniently be produced in large numbers. Using DH populations, 130 quantitative traits have been mapped in nine crop species.[5] In total, 56 DH populations were used for QTL detection.

(18) Genetic maps are very important to understand the structure and organisation of genomes from which evolution patterns and syntenic relationships between species can be deduced. Genetic maps also provide a framework for the mapping of genes of interest. Doubled haploid populations are ideal for genetic mapping. It is possible to produce a genetic map within two years of the initial cross regardless of the species. Map construction is relatively easy using a DH

population derived from a hybrid of two homozygous parents as the expected segregation ratio is simple, i.e. 1:1. DH populations have now been used to produce genetic maps of barley, rapeseed, rice, wheat, and pepper. DH populations played a major role in facilitating the generation of the molecular marker maps in eight crop species.

(19) In bulked segregant analysis, a population is screened for a trait of interest and the genotypes at the two extreme ends form two bulks. Then the two bulks are tested for the presence or absence of molecular markers. Since the bulks are supposed to contrast in the alleles that contribute positive and negative effects, any marker polymorphism between the two bulks indicates the linkage between the marker and trait of interest. BSA is dependent on accurate phenotyping and the DH population has particular advantage in that they are true breeding and can be tested repeatedly. DH populations are commonly used in bulked segregant analysis, which is a popular method in marker assisted breeding. This method has been applied mostly to rapeseed and barley.

(20) Genetic transformation at haploid level has been studied in several ways. The most common approach has been for haploid plants to be transformed using established transformation methods. To give just one example, transformed haploid bread wheat with an HVA1 gene to obtain drought tolerance. Chromosome doubling thus enabled stable fixation of the integrated gene, and this feature can be tested for 14 generations. Another approach has been for haploid cells themselves, mainly microspores, to be targets of transformation prior to haploid induction. It should again be mentioned that, in addition to breeding, haploids and doubled haploids have been extensively used in genetic studies, such as gene mapping, marker/trait association studies, location of QTLs, genomics and as targets for transformations.

22) In cross-pollinated crops, the pure lines derived from heterozygous material can be used as parents in single cross or double cross.

Conclusion Doubled haploidy is and will continue to be a very efficient tool for the production of completely homozygous lines from heterozygous donor plants in a single step. Since the first discovery of haploid plants in 1920 and in particular after the discovery of in vitro androgenesis in 1964, techniques have been gradually developed and constantly improved. The method has already been used in breeding programs for several decades and is currently the method of choice in all species for which the technique is sufficiently elaborated. Species for which well-established protocols exist predominantly belong to field crops or vegetables, but the technique is

gradually also being developed for other plant species, including fruit and ornamental plants and other perennials. It should be mentioned that, in addition to breeding, haploids and doubled haploids have been extensively used in genetic studies, such as gene mapping, marker/trait association studies, location of QTLs, genomics and as targets for transformations. Furthermore the haploid induction technique can nowadays be efficiently combined with several other plant biotechnological techniques, enabling several novel breeding achievements, such as improved mutation breeding, backcrossing, hybrid breeding and genetic transformation.