Use of haploids, dihaploids and doubled haploids in Genetics

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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.

Haploids are defined as saprophytes with gametophytic chromosome number and have been produced in a variety of plant species using a variety of methods. Although, the significance of haploids in genetics and plant breeding has been recognised for long time, with the advent of biotechnology it received renewed emphasis, so that the production of haploids become an important component of biotechnology programmes in different countries. Although, haploids could be produced following delayed pollination, irradiation of pollen, temperature shocks, colchicine treatment and distant hybridization, the most important methods currently being utilised include in vitro anther culture and bulbosum technique.

Anther and microspore culture

The impact of haploid production in genetics and plant breeding has long been realised. However, their exploitation remained restricted because of the extremely low frequency with which they occur in nature. The artificial production of haploids was attempted through distant hybridization, delayed pollination, application of irradiated pollen, hormone treatments and temperature shocks. However, none of these methods

![Diagram of haploid production process]
are efficient. The development of numerous pollen plantlets in anther culture of *Datura innoxia*, first reported by two Indian Scientists (Guha and Maheswari, 1964) was a major breakthrough in haploid breeding of higher plants.

This technique of haploid production through anther culture (anther androgenesis or simply androgenesis) has been extended to numerous plant species including cereals, vegetables, oil and tree species. The anthers may be taken from plants grown in the field or in pots, but ideally these plants should be grown under controlled temperature, light and humidity. Often the capacity for haploid production declines with age of donor plants. Flower buds of the appropriate developmental stage are collected, surface sterilised and their anthers are excised and placed horizontally on 2 culture medium. Care should be taken to avoid injury to anthers since it may induce callus formation from anther walls. Alternatively, pollen grains can be separated from anthers and cultured on a suitable medium.

**In vitro induction of maternal haploids – gynogenesis**

Spontaneous production of haploids usually occurs through the process of parthenogenesis (embryo development from unfertilised egg). *In vivo* occurrence of androgenic haploids has been reported in *Antirrhinum*, Nicotiana etc. *In vitro* induction of maternal haploids, so-called gynogenesis, is another pathway to the production of haploid embryos exclusively from a female gametophyte. It can be achieved with the *in vitro* culture of various un-pollinated flower parts, such as ovules, placenta attached ovules, ovaries or whole flower buds. Gynogenetic regenerants show higher genetic stability and a lower rate of albino plants compared to androgenetic ones. This technique is used mainly in plants in which other induction techniques, such as androgenesis and the pollination methods above described, have failed. Gynogenic induction using un-pollinated flower parts has been successful in several species, such as onion, cucumber, squash, sunflower, wheat, barley, etc. but its application in breeding is mainly restricted to onion and sugar beet.

Wide hybridization between species has been shown to be a very effective method for haploid induction and has been used successfully in several cultivated species. It exploits haploidy from the female gametic line and involves both inter-specific and inter-generic pollinations. The fertilisation of polar nuclei and production of functional endosperm can trigger the parthenogenetic development of haploid
embryos, which mature normally and are propagated through seeds (e.g., potato). In other cases, fertilisation of ovules is followed by paternal chromosome elimination in hybrid embryos. The endosperms are absent or poorly developed, so embryo rescue and further in vitro culture of embryos is needed (e.g., barley, wheat). This ‘bulbosum’ method developed by Kasha and Kao in 1970 was first haploid induction method to produce large numbers of haploids across most genotypes and quickly adapted into breeding programs.

**Diploidization of haploid plants**

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. Haploids plants can be diplodized 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.

**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 inbreeds 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 fertile homodiploids with all desirable mutations can be obtained through chromosome doubling.

(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. An inbred population produced through 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 to the conventional “50% male + 50% female” populations.

13) Monoploids obtained from dihaploids through parthenogenesis of androgenesis can be used to select and evaluate various genomes to be put together through protoplast fusion in asexually propagated crop like potato.

14) Dihaploids may be used in selection or crossing at diploid level before chromosome doubling (autotetraploid) as suggested by Chase in 1963 in the “analytical breeding” scheme for potato (Solanum tuberosum).

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 haploid 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 and estimating the magnitude of their effects and aid our understanding of genotype/phenotype associations. DH populations have become standard resources in genetic mapping for species in which DHs are readily available. 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) QTL analysis has generated a vast amount of information on gene locations and the magnitude of effects on many traits, the identification of the genes involved has remained elusive. This is due to poor resolution of QTL analysis. The solution for this problem would be production of recombinant chromosome substitution line, or stepped aligned recombinant inbred lines. Here, backcrossing is carried out until a desired level of recombination has occurred and genetic markers are used to detect desired recombinant chromosome substitution lines in the target region, which can be fixed by doubled haploidy. In rice, molecular markers have been found to be linked with major genes and QTLs for resistance to rice blast, bacterial blight, and sheath blight in a map produced from DH population.

(21) 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. 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.

Note: In syllabus only “Use of haploids, dihaploids and doubled haploids in Genetics” is given. Production of haploids and doubled haploids are discussed for better understanding of their uses.