

Production and Use of haploids, dihaploids and doubled haploids 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).

Production of haploids and doubled haploids Haploids produced from diploid species ($2n=2x$), known as monopluids, 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 (self-pollinated crops) or parental inbred line for the production of hybrid varieties (cross-pollinated crops). 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. Selection can be expected for traits caused by recessive deleterious genes that are associated with vegetative growth. Traits associated with flower fertility might not be related and should be eliminated by recurrent selection among DH lines. The production of pure lines using doubled haploids has several advantages over conventional methods. 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.

Anther and microspore culture (Androgenesis)

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 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: 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 interspecific and inter-generic pollinations. The fertilization 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, fertilization 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 are needed (e.g., barley). In barley, haploid production termed bulbosum technique is the result of wide hybridization between cultivated barley (*Hordeum vulgare*, $2n=2x=14$) as the female and wild *H. bulbosum* ($2n=2x=14$) as the male. After fertilization, a hybrid embryo containing the chromosomes of both parents is produced. During early embryogenesis, chromosomes of the wild relative (*H. bulbosum*) are preferentially eliminated from the cells of developing embryo, leading to the formation of a haploid embryo, which is due to the failure of endosperm development. A haploid embryo is later extracted and grown *in vitro*. The 'bulbosum' method was the first haploid induction method to produce large numbers of haploids across most genotypes and quickly entered into breeding programs. Pollination with maize pollen could also be used for the production of haploid barley plants, but at lower frequencies. Paternal chromosome elimination has also been observed after interspecific crosses between wheat (*Triticum aestivum*) and maize. After pollination, a hybrid embryo between wheat and maize develops but, in the further process, the maize chromosomes are eliminated so that haploid wheat plantlets can be obtained. Such haploid wheat embryos usually cannot develop further when left on the plant, because the endosperm fails to develop in such seeds. By applying growth regulator 2,4-dichlorophenoxyacetic acid in planta, embryo growth is maintained to the stage suitable for embryo isolation and further *in vitro* culture. The maize chromosome elimination system in wheat enables the production of large Haploids and Doubled Haploids in Plant Breeding. Pollination with maize is also effective for inducing haploid embryos in several other cereals, such as barley, triticale (x *Triticosecale*), rye (*Secale cereale*) and oats (*Avena sativa*) (Wędzony, 2009). Similar processes of paternal chromosome elimination occur after the pollination of wheat with wild barley (*H. bulbosum*),

sorghum (*Sorghum bicolor* L. Moench) and pearl millet (*Pennisetum glaucum* L.R.Br.; Inagaki, 2003). Haploid production in cultivated potato can be achieved by inter-generic pollination with selected haploid inducer clones. The tetraploid female *S. tuberosum* (*Solanum tuberosum* L. ssp. *tuberosum*, $2n=4x$) produces an embryo sac containing one egg cell and two endosperm nuclei, all with the genetic constitution $n=2x$, while the diploid pollinator *S. phurea* ($2n=2x$) produces two sperms of the genetic constitution $n=x$ or $2x$. After pollination, dihaploid ($2n=2x$) embryos can develop from un-fertilized egg cells, which are supported by a $6x$ endosperm formed by the fusion of polar nuclei with both reduced sperm cells. The frequency of dihaploid seeds is low. Dihaploid potatoes can be used for breeding purposes, including alien germplasm introgression or selection at the diploid level, but such plants are not homozygous.

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Pollination with irradiated pollen: This is another possibility for inducing the formation of maternal haploids using intra-specific pollination. Embryo development is stimulated by pollen germination on the stigma and growth of the pollen tube within the style, although irradiated pollen is unable to fertilize the egg cell. It has been used successfully in several species.

The production of maternal haploids stimulated by irradiated pollen requires efficient emasculation, which has in some cases been shown to limit its use because the method is too laborious. To overcome such an obstacle in onion, for instance, only male sterile donor plants were used as donor plants, but such lines, possessing cytoplasmically inherited male sterility, are of very limited practical use.

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 diploidized 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.

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 chromosome 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 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 or 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 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 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.

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.

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- 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.

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 chromosome 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 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 or 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 are 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 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.

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.