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# HAPLOIDS AS A TOOL IN BREEDING POLYPLOIDS

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**ABSTRACT.** The terminology used is briefly explained. Origin and induction of haploids *in vivo* are treated along with *in vitro* induction. Monoploids as such are rarely utilized in breeding but are valuable for basic research on phylogeny, genetics, and mutagenesis. Doubled (mono)haploids, coded DH, obtained through colchicine-doubling of haploids from diploids and allopolyploids have been studied in many crops.

In autogamous crops, DH-lines can be released as cultivars or used as parents for further crosses, e.g., in a recurrent selection program to improve populations. They enable evaluation of populations from crosses already from $F_1$ onwards. Time saving and increased efficiency of selection are major advantages because of homozygotization in one generation and a reliable discrimination between genotypes. Non-additive variance is completely eliminated and environmental variance can be minimized in the testing procedure.

In allogamous crops DH-lines may be used as parents of hybrid varieties and of synthetic varieties. Owing to inbreeding depression, the number of useful DH-lines may be reduced. Specific potentials of DH-lines are discussed: all-male asparagus hybrids and homozygous potato lines that bypass all natural barriers to repeated selfing.

DH-lines proved to be useful for analysis of the genetics of qualitative and quantitative characters.

Dihaploids from autotetraploid crops allow breeding at the diploid level and greatly facilitate the use of diploid wild and cultivated species. Dihaploids mostly have a reduced fertility or are sterile particularly on the male side; flowering may be poor, and aberrant plants are frequently found. Dihaploids can be efficiently produced in potato and alfalfa. Apart from the advantages of breeding at the diploid level, dihaploids are effective tools in capturing the genetic diversity of related diploid species. Both in potato and alfalfa first-division-restitution-(FDR)2n-gametes are effective instruments for intact transfer of heterozygosity to the autotetraploid level, thus increasing the level of multi-allelism and the performance of the tetraploid progeny. The potential of the dihaploid and 2n-gametes approach is most obvious in vegetatively propagated autotetraploids.

Index Descriptors: haploids, breeding, early generation testing, recurrent selection, homozygous lines, anther culture, and pseudogamy.

## INTRODUCTION AND TERMINOLOGY

A genome of an organism is a functional entity comprising the smallest possible number of structurally and genetically different chromosomes of that organism. This basic chromosome number, which is symbolized by $x$, is characteristic of each species and in economically important crops is predominantly in the range of 5-20. The number of chromosomes in somatic or diplophase cells is indicated by $2n$, $n$ being the number of chromosomes in gametes or haplophase cells.

Haploids are sporophytic individuals which, owing to their origin from a reduced gametic cell in the embryo-sac or in the pollen grain, have the gametic

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Table 1. Types of crops and derived haploids with genome formulas.

| Crops | Genome formula | Derived haploid | Genome formula |
|---|---|---|---|
| Monoploids | $2n = x$ | --- | --- |
| (Auto)diploids | $2n = 2x$ | Monohaploids | $2n = x$ |
| Autotriploids | $2n = 3x$ | --- | --- |
| Autotetraploids | $2n = 4x$ | (Auto)dihaploids | $2n = 2x$ |
| Autohexaploids | $2n = 6x$ | Autotrihaploids | $2n = 3x$ |
| Allodiploids | $2n = x_A + x_B$ | --- | --- |
| Allotetraploids | $2n = 2x_A + 2x_B$ | Allodihaploids | $2n = x_A + x_B$ |
| Allohexaploids | $2n = 2x_A + 2x_B + 2x_C$ | Allotrihaploids | $2n = x_A + x_B + x_C$ |

chromosome number. The haploid embryo arises either from the egg cell (gynogenesis) or from a gametophyte cell other than the egg cell (apogamy), or from a male gamete (androgenesis). When it originates from the pollen grain, it mostly does so from the vegetative cell.

Parallel to the distinction between autoploid and alloploid crops, haploids can be subdivided into autohaploids and allohaploids, as indicated in Table 1. On the basis of breeding behavior the following categories may be distinguished:

1. Functional monoploids. These include all haploids from diploid and allopolyploid crops. They are characterized by having only unpaired chromosomes (no homologous pairing) and hence by exhibiting complete sterility. On spontaneous or induced doubling of the chromosome number, this sterility is generally converted into complete fertility and the plants become fully homozygous. In the literature they are indicated as "doubled haploids" and coded "DH."
2. Functional diploids. These include autodiploids, autodihaploids, all true allopolyploids, and all colchicine-doubled monoploids or DH-lines. Normal meiosis and fertility and disomic inheritance is the rule in this category. However, autodihaploids are largely male sterile and in addition retain some degree of heterozygosity owing to their origin from heterozygous autotetraploids.
3. Functional polyploids. These include natural and induced autopolyploids. This category of plants has complicated polysomic inheritance of characters, and autotriploids and autopentaploids are largely sterile.

## Origin and induction of haploids

Haploids of higher plants arise mainly through gynogenesis, apogamy, and androgenesis and are often associated with polyembryony (Lacadena, 1974). They are rare in nature, although spontaneous haploids occur in many plant families. Approximate frequencies are 1.0% parthenogenetic and 0.1% androgenetic haploids, but workable frequencies are found in oilseed rape (*Brassica napus*) and flax (*Linum usitatissimum*) (Pressers, 1963; Thompson, 1969, 1974, 1977; and Rajhathy, 1976).

Considerable research efforts during the last four decades have led to a better understanding of the processes involved in haploid induction, to various techniques for producing haploids, and to the utilization of haploids in different crops (Kasha, 1974; Nitszche and Wenzel, 1977; Davies and Hopwood, 1980; and Hermsen and Ramanna, 1981).

### *In vivo* induction

*In vivo* induction occurs in the embryo sac. The modes of origin vary, but in any case either cross or self pollination is required. The haploids then arise following abnormal events during or just after fertilization. Pollinations usually give rise to hybrid haploid seeds, which can most efficiently be separated by a dominant seed or seedling marker. The frequency of haploids is controlled by the genotype of the female parent and of the male parent (or rather the pollinator) and may be influenced by environment, chemicals, delayed pollination, and alien cytoplasm (Lacadena, 1974). The following processes may give rise to haploids *in vivo*.

1. Pseudogamy: Development of an unfertilized reduced female gamete or egg occurs after stimulation by the male nucleus or hybrid endosperm (Chase, 1969; and Rowe, 1974). Examples of pseudogamy are: intervarietal crosses in maize (*Zea mays*) (Chase, 1952), alloplasmic Salmon wheat (*Triticum aestivum*) (Tsunewaki et al., 1968), hap/hap mutants in barley (*Hordeum vulgare*) (Hagberg and Hagberg, 1980), and interspecific crosses in potato (*Solanum tuberosum*) (Hougas et al., 1958), alfalfa (*Medicago sativa*) (Bingham, 1969), poplar (*Populus* ssp.) (Stettler et al., 1969), and tobacco (*Nicotiana tabacum*) (Burk et al., 1979).
2. Preferential elimination of chromosomes of a specific genome during early embryo development following normal double fertilization (Kasha, 1974b; and Jensen, 1975). Main examples: barley and wheat crossed by *Hordeum bulbosum* (Kasha and Kao, 1970; and Barclay, 1975).
3. Semigamy: Reduced male and female gametes do not fuse, but both participate in embryogenesis, resulting in chimeral haploid plants with

sectors of maternal and paternal origin (Turcotte and Feaster, 1974; and Chaudhari, 1978). Example: tetraploid cotton species.

4. Androgenesis: The maternal nucleus is eliminated or inactivated before fertilization and the haploid originates from the male nucleus in the egg cell (Chase, 1963). The frequency of androgenetic haploids is extremely low except in the recessive maize mutant "indeterminate gametophyte" (Kermicle, 1969). In order to select androgenetic haploids, a marker gene, which is dominant in the female and recessive in the male parent, is required.

### *In vitro* induction

*In vitro* induction using anthers or microspores (Sunderland, 1974, 1980; and Maheshwari et al., 1980) and ovules (San Noeum, 1978) is being applied on a large scale. Success requires use of the correct growth media and knowledge of the optimal microspore stage at the onset of the experiment. Environmental conditions, especially temperature shock, may be effective. The chance of obtaining haploids is greater where differentiation can be induced without intermediate callus formation; when a callus phase intervenes, aneuploidy and higher ploidy levels are commonly found. Progress with *in vitro* induction has been rapid, and haploids have been reported in more than 150 species from 23 plant families, mainly *Solanaceae* and *Gramineae* (Maheshwari et al., 1980). Among the *Gramineae*, the common occurrence of albino plantlets from microspores greatly reduces the number of useful haploids. Both anther culture and *in vivo* induction may give rise to heterozygous plants originating directly from 2n-gametes.

## HAPLOIDS AS TOOLS IN PLANT BREEDING AND RESEARCH

The potential use of haploids in research and plant breeding depends upon the nature of the species: allogamous, autogamous, functionally diploid, or autopolyploid.

### Monohaploids

Monohaploids are haploids from functional diploids. A monohaploid has unpaired chromosomes at meiosis in nearly all spore mother cells and thus produces aneuploid sterile gametes. To be of direct use, the somatic chromosome number must be stable, and vegetative propagation would have to be possible. Monohaploids are of great value for the study of phylogenetic relationships in alloploid species, for genetic studies (simplified ratios), and for mutation research (mutations directly visible). However, their direct use by breeders and growers will rarely be possible, except in some ornamentals.

### Doubled monohaploids: the DH-breeding procedure

The production of monohaploids and subsequent chromosome doubling to obtain doubled haploids (coded DH) allows the development of completely homozygous lines from heterozygous parents in one single generation, whereas the conventional procedure of homozygotization requires five to seven generations of selfing. Snape (1982) mentions three criteria for successful exploitation of the DH-system:

1. Easy and consistent production of large numbers of DH-plants of all genotypes used in order to capture all genetic variation needed for selection. The required number of DH-lines is dependent upon the genotypic difference between the parents in the initial cross and upon the filial generation of selfing.
2. The DH-plants should be cytologically stable and phenotypically normal. Monohaploids obtained through anther culture, especially when an intermediate callus phase is involved, may be unstable and undesirable recessives may appear (e.g., albinos in *Gramineae*). Diploids obtained directly from the test tube may be, but need not be, homozygous (2n-gametes).
3. The DH-plants should be a random gametic sample from the parent plant when the DH-approach is being used for genetic analysis. Randomness has been reported for several major genes (Kasha and Reinbergs, 1980; and Collins and Legg, 1980). For polygenic characters it is difficult to establish whether gametal selection has occurred. Park et al. (1976) did not observe differences between DH-lines and conventionally derived homozygous lines in barley as to means, variances, and stability of performance. Thus, complete homozygosity as such does not appear to have adverse effects in barley.

Results from comparable experiments in allotetraploid tobacco are equivocal and in addition unexpected. Several authors observed variation and a reduced performance among and even within DH-lines from homozygous tobacco cultivars (Burk and Matzinger, 1976; and Arcia et al., 1978). Schnell et al. (1980) crossed two tobacco lines and from the $F_1$ produced 50 lines obtained via the DH-method and 50 $S_8$ lines obtained via single-seed descent (SSD). Contrary to the results in barley (Park et al., 1976), the tobacco DH-lines on an average yielded 10.6% less than the SSD-lines. These results were tentatively explained on the basis of remnant heterozygosity in the homozygous parents, mutagenic effect of colchicine, and loss of cytoplasmic factors during haploidization through anther culture (Collins and Legg, 1980).

On the other hand, Nakamura et al. (1974) and Chinese workers (Chinese Acad. of Agric. Sci., 1978) report genetic stability of DH-lines of tobacco

during several generations and no reduced vigour as compared to the parental lines. These different results may in part be a consequence of the techniques used in monoploid production via anther culture in tobacco and the *H. bulbosum* technique in barley. In this connection it would be of interest to compare tobacco DH-lines derived from maternal haploids (Burk et al., 1979) with those derived from pollen haploids.

## The DH-procedure in autogamous crops

In autogamous crops stable DH-lines with high-level performance can be released as cultivars. The Canadian barley variety Mingo has been produced this way (Kasha and Reinbergs, 1980). Chase (1952) proposed the use of DH-lines in conjunction with recurrent selection in maize. Collins and Legg (1974) proposed a similar procedure for autogamous crops: DH-lines selected in one cycle are used to initiate another cycle. In spring barley, each cycle of production and evaluation could be performed in two years (Kasha and Reinbergs, 1981). A gradual improvement in population performance is expected and at any generation, varieties may be selected.

The principal potential advantages of the DH-procedure are time saving and increased efficiency of selection. Saving of time is obvious because of rapid homozygotization. The saving in time is larger in winter than in spring cereals (and even more striking in allogamous biennial, dioecious, and self-incompatible crops as well as in crops with a long juvenile period). Selection efficiency may be greatly increased because of a more reliable discrimination between genotypes (especially in early segregating generations), and therefore a better response to selection is achieved. Early-generation testing in autogamous crops is hampered by the considerable nonadditive genetic variance. This variance is eliminated in DH-lines, so all genetic variance is additive and thus selectable. In addition, environmental variance can be minimized in the DH-system, because replication can be introduced in the testing procedure. Griffing (1975) has demonstrated for different genetic models that, with recurrent selection, the DH-procedure yields a larger change in population mean per generation of selection than conventional methods, even when the populations are relatively small.

## Optimum generation for DH-production in autogamous crops

Basically, every generation from $F_1$ onwards can be used as parental material for DH-production. The $F_1$ generation up to now has most commonly been used because time saving is maximal. This advantage is offset by the fact that (1) large populations need to be developed to obtain transgressive segregants and desired combinations of characters, (2) $F_1$-DH lines constitute an unselected sample of genotypes: there is no opportunity for selection of

simply inherited traits or for characters with high heritabilities (short stature, earliness), and (3) recombination is restricted owing to linkage disequilibrium as demonstrated by Snape and Simpson (1981a). These authors, on the basis of theoretical calculations and experimental evidence—albeit from one barley cross only—reached the conclusion that the $F_2$ would appear to be the best generation for producing DH-lines. The $F_2$ allows some selection between individuals both positive and negative. A time delay until $F_3$ would only produce a small change in variance, whereas an $S_3$ generation (from intermated $F_2$ plants) would require extra labor for extensive crosses in addition to a generation delay. In this experiment, the generation delay ($F_2$ instead of $F_1$) increased variation for ear emergence time, height, grain number per ear and ear number per plant but resulted in a decrease in the variance for grain weight per plant and 250 grain weight. The authors hypothesize that, if linkage is important, this would most likely require the breakup of repulsion linkages, but the delay can also break down desirable coupling linkages built up by previous recombination and selection. As usual in plant breeding, the breeder has to find the best compromise.

### The DH-procedure in allogamous crops

DH-lines from allogamous crops may be used as parents of hybrid varieties and of synthetic varieties. Such lines mostly suffer from inbreeding depression and may thus display low fertility or lethality, reducing the number of useful DH-lines. On the other hand, when DH-lines can be produced at a low cost and in large numbers, relatively vigorous homozygous lines might be selected. Vigorous and fertile inbred lines allow the production of large amounts of single-cross seed, although it has to be admitted that vigor need not imply a good combining ability. In maize, the laborious testing for combining ability is a limiting factor rather than the production of inbred lines.

Haploids may be a tool for creating specific and novel material. Examples are the production of homozygous diploid and tetraploid potatoes via gynogenetic monoploids (Van Breukelen et al., 1977) and supermale (YY) lines of asparagus (*Asparagus officinalis*) (via anther culture), thus allowing the production of all-male hybrids which are generally superior to female ones (Hondelmann and Wilberg, 1973). Homozygous tetraploid potato lines have been proposed as a tool for estimating the average coefficient of double reduction $\propto$, the additive genetic variance, and the dominance variance of quaduplex, triplex, duplex, simplex, and nulliplex genotypes in potato populations, a system of mating and data processing designed and developed by Tai (1983).

When inbred lines are developed via haploids in allogamous crops, all natural barriers to repeated selfing are bypassed, e.g., dioecy, self-incompatibility, and long juvenile periods. An additional advantage for self-incompatible crops is that selection is avoided for weak incompatibility alleles or genetic

backgrounds inhibiting the activity of such alleles (Hermsen and Ramanna, 1981).

## DH-lines and genetic analysis in functional diploids

In addition to their use in breeding, DH-lines may be useful for analysis of the genetics of qualitative and quantitative characters. A few examples may be mentioned. Snape and Simpson (1981b), working with barley, demonstrated the use of the DH-procedure for selecting and estimating linkage values of major genes and for determining pleiotropic effects of specific major genes on other agronomic characters. The use of DH-lines for genetic analysis of quantitative characters has been investigated by Choo et al. (1979), Choo (1981), Choo and Reinbergs (1982), and Snape and Simpson (1981a, 1981b). The components of genetic variation and the number of effective segregating factors could be estimated and linkage disequilibrium detected. Collins and Legg (1980) reported genetic applications of haploids in allotetraploid *Nicotiana*. Dublin (1974) discussed the possible utilization of haploids for studying the genetics of the cacao (*Theobroma cacao*) tree.

## Dihaploids from autotetraploid crops

Although the following discussion, in principle, applies to all autotetraploid crops, the requirements for an efficient use of dihaploids in breeding are fulfilled only in alfalfa and in potato.

The advantages of dihaploids relate to the complicated tetrasomic inheritance in autotetraploids. Combination of and selection for favorable characters at the tetraploid level are much more laborious and require larger populations than at the diploid level. Furthermore in related wild and cultivated species, there is a wealth of genetic variation for specific desirable traits, such as resistances to diseases, pests, and abiotic factors, as well as abundant allelic diversity for obtaining maximum heterozygosity. Most of these relatives are diploid species, which in general can easily be hybridized with dihaploids but are difficult to cross with autotetraploid cultivars. Hybrids between dihaploids and diploid relatives have normal chromosome pairing and gene recombination at meiosis. Therefore, desirable genes can easily be transferred from wild species to the cultivated autotetraploids via dihaploids.

However, a high proportion of dihaploids is completely male sterile. Also, female fertility is greatly reduced in most dihaploids, and flowering is predominantly poor or even lacking. Deleterious recessives and otherwise aberrant plants occur frequently. On the other hand, these drawbacks may differ greatly depending on the cultivar used. In addition, large numbers of dihaploids can efficiently be produced from nearly every autotetraploid genotype in potato, and, though to a lesser extent, also in alfalfa. Finally, about one-third of the

dihaploids can be used as females in crosses with wild and cultivated diploids, which mostly results in increased fertility, flowering, vegetative vigor, and (in potato) tuberization.

### Breeding of autotetraploids at the diploid level

Owing to the ease with which large gametic samples can be produced in potato and alfalfa, sufficient numbers of dihaploids can be obtained that can be used for further crosses. Van Suchtelen (unpubl. results), working within *Solanum tuberosum*, has succeeded in breeding agronomically well-performing diploid material. However, Peloquin and his associates have clearly shown that including cultivated related diploids and even wild species greatly increases the potential of breeding at the diploid level. The overall breeding strategy as adopted and defined by Peloquin (1982) involves three main components: (1) the wealth of species as sources of genetic diversity, (2) dihaploids from autotetraploid potatoes as effective tools in capturing the genetic diversity of the species, and (3) first-division-restitution-(FDR)2n gametes as instruments for intact transfer of genetic diversity to the autotetraploid level. The rationale of this breeding strategy is based on the following experimentally acquired views:

1. The allelic diversity within the autotetraploids is too small and has to be supplemented by new alleles from relatives (Glendinning, 1979).
2. Performance of autotetraploids is directly related to the degree of multi-allelism in a genotype or population (Busbice and Wilsie, 1966; and Mendoza and Haynes, 1974).
3. Breeding at the diploid level is most efficient; however, owing to their inherent di-allelism, the varietal potential of diploids is inferior to that of autotetraploids.
4. Sexual polyploidization through FDR gametes warrants a nearly intact transfer of diploid superior genotypes to the tetraploid progeny (Mendiburu and Peloquin, 1977; Mok and Peloquin, 1975).

The views on which the aforementioned breeding strategy is based hold true for alfalfa as well (Demarly, 1963; Busbice and Wilsie, 1966; Bingham, 1971; Dunbier and Bingham, 1975; Bingham and McCoy, 1979; and Bingham, 1979). However, alfalfa is a seed-propagated crop. Consequently, once an excellent variety would be produced from this technique, repeated sexual propagation through random mating would result in a decrease of tetra-allelic loci until a constant, relatively low level of tetra-allelism is attained at equilibrium. Performance would decrease at the same rate.

Dunbier and Bingham (1975) used colchicine-doubled dihaploid x dihaploid hybrids for producing autotetraploid parents with a defined genotypic structure viz. only duplex-diallelism at the heterozygous loci. Using the diploid

and corresponding autotetraploid parents they produced diploid and corresponding autotetraploid single and double crosses in order to study their performance. The tetraploid double crosses performed far better than single crosses and parents. Although double crosses have a higher potential performance than synthetic varieties, problems arise with the production of sufficient amounts of commercial seed.

In vegetatively reproducing crops where the cultivated form is autotetraploid and a wealth of diploid relatives and dihaploids is available, breeding at the diploid level in association with bilateral sexual polyploidization and possibly somatic hybridization of divergent superior diploid hybrids holds great promise.

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