

Production of 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:

Haploid Production:

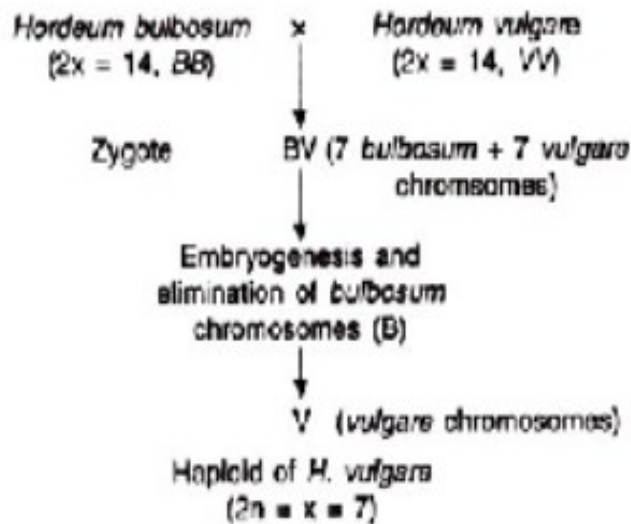
- 1) **Anther and microspore culture (Androgenesis):** The development of numerous pollen plantlets in anther culture of *Datura innoxia*, first reported by two Indian Scientists (Guha and Maheshwari, 1964) was a major breakthrough in haploid breeding of higher plants. This technique of haploid production through anther culture (anther androgenesis or simply androgenesis) has been extended to numerous plant species including cereals, vegetables, oil and tree species.
- 2) **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.

3) **Embryo rescue from wide hybridization/wide crosses:**

Bulbosum method: 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). 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.



- 4) **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.
- 5) **Unpollinated ovary culture.**