Available online at www.agrifarming.org

DOI: http://dx.doi.org/10.18782/2582-7146.138

ISSN: 2582 – 7146

Curr. Rese. Agri. Far. (2021) *2*(3), 1-7

Current Research in Agriculture and Farming

Review Article

Peer-Reviewed, Refereed, Open Access Journal

A Review on Haploid and Double Haploids in Ornamental Plants

Monika Karnawat*, S.K. Trivedi, Rakesh Kumar Meena and Deepak Nagar

Career Point University, Alaniya, Kota

*Corresponding Author E-mail: monika.karnawat@cpu.edu.in

Received: 14.04.2021 | Revised: 25.05.2021 | Accepted: 12.06.2021

ABSTRACT

Sporophyte plants with many gametophytic chromosomes are called haploid plants. These plants can be produced naturally or through in vitro or in vivo induction techniques. Double haploid (DH) can be obtained by doubling the number of haploid chromosomes spontaneously or artificially. They are homozygous, and this homozygosity will be realized in the life cycle of a generation using the DH production system. This production system is used to correct heterosis. Easy to interact with the DH population. DH can be used as parental inbreds of new varieties or self-pollinated plants or cross-pollinated plants. Haploids can be used to isolate mutants, especially if the mutant allele is not diploid. If the haploid is transformed immediately after the chromosome is copied, the plant can be obtained step by step. By combining biotechnological means with conventional methods, the important goal of improving cultivated plants can be achieved in a short time. This article analyzes the various developments in the field of haploid species related to economically important ornamental species.

Keywords: *Double haploid, Sporophyte, Gametophyte, Haploids.*

INTRODUCTION

Haploid is a sporophytic plant with many gamete chromosomes. If chromosomal duplication occurs in any of these plants, the resulting plant will become double haploid (DH). Haploid can originate spontaneously in nature as well as by various induction techniques. A haploid derived from a tetraploid (4x) is called a diploid (2n = 2x), and a haploid derived from a diploid species (2n = 2x) is called a haploid. In vivo isolation method (radiation pollen pollination and extensive hybridization) and in vitro isolation method (immature gametophyte culture). Haploid is sterile, and their fertility is restored by doubling the chromosomes. The DH produced is homozygous in all positions. These homozygous or pure lines can represent new varieties or parental inbreds for the production of self-pollinated or cross-pollinated hybrids (Murovec & Bohanec, 2012; & Forster et al., 2007).

Haploid History

Dorothy Bergner described the first natural haploid in Datura in 1921 (Blakeslee et al., 1922), and subsequently reported natural haploids in tobacco, wheat and some other plant species.

Cite this article: Karnawat, M., Trivedi, S. K., Meena, R. K., & Nagar, D. (2021). A Review on Haploid and Double Haploids in Ornamental Plants, *Curr. Rese. Agri. Far. 2*(3), 1-7. doi: http://dx.doi.org/10.18782/2582-7146.138

Guha and Maheshvari (1964) reported for the first time the hypoxic immature anthers of Datura, which represented a breakthrough in haploid development. Plant species (Maluszynski et al., 2003). Recently, some researchers have begun to pay attention to using this technology to improve ornamental plants.

Uses of Haploids

Using Double haploid systems, homozygosity is attained in a single generation. The time saving is sizeable while in comparison with traditional approach of selfing especially in biennial and lengthy juvenile length crops. For dioecious species, self-incompatible species, and species that be afflicted by inbreeding depression, haploidy can be the only method to expand inbred strains. Haploids have cost in permitting the isolation of mutants, especially in which the mutant allele is non-purposeful in diploid. True breeding diploid transgenic flora may be produced in one step, if haploids are converted directly following doubling of chromosomes. Doubled haploid flora produce feasible seed and the preferred man or woman is handed directly to successive generations. DH manufacturing method is also used for fixation of heterosis. DH strains do not segregate after self-pollination and may be propagated indefinitely with the aid of using seeds. This enables green bodily and genetic mapping and genetic dissection of quantitative traits. DH populace is used as an everlasting mapping populace. Moreover, in latest years, haploids are great interest for structural genomics (Aleza et al., 2009).

Monoploid Production Methods of Ornamental Plants

In interspecific and intergenus pollination, the haploid of the female gamete line is used. In most cases, the paternal chromosomes are removed from the hybrid embryo after the egg is fertilized. Embryo rescue and in vitro culture of these hybrid embryos are used as methods to obtain haploids in many crops including ornamentals.

Some of the ornamental crops where haploid production through this method has been tried are reviewed here.

Chrysanthemum

Chrysanthemum is a very heterogeneous culture. Due to its very heterozygous state, reproduction and molecular analysis are very difficult in this kind of culture. Therefore, the development of haploid forms in chrysanthemums to produce haploids or true double breeding lines has attracted great interest. Watanabe (1977) developed a program for planting pseudo-fertilized chrysanthemum ovaries and successfully produced androgen haploids and F1 hybrids from Japanese chrysanthemum species. Wang et al. (2014) 2579 unfertilized egg cells were cultured in vitro from chrysanthemums and pollinated to isolate haploid offspring. As a result of this experiment, a true haploid was obtained. The haplotype was confirmed by cytological research and microsatellite fingerprinting.

Use ionizing radiation to produce haploids

Ionizing and non-ionizing radiation have been used to irradiate pollen to induce haploid plants in situ. Radiation destroys the function of pollen production without affecting the ability of pollen to stimulate egg cells, thereby promoting the development of parthenogenetic embryos. Radiation dose is a key factor to control the in situ generation of haploids. When the radiation dose is low, the pollen-producing nucleus maintains its ability to fertilize eggs. Therefore, the resulting embryo has a heterozygous phenotype and is mutated. The radiation dose tends to reduce the number of developing embryos, but these embryos are usually of haploid origin. Most plant species require in vitro embryo rescue to regenerate haploid plants. Haploid is obtained from apple, cocoa, melon, barley, onion (Sestili & Ficcadenti, 1996), petunia, rose, sunflower and carnation using this method.

Petunia

Raquin (1985) used irradiated pollen (gamma dose 6-100 kR) to pollinate three different F1 genotypes of hybrid petunia. The ovaries were collected 9-14 days after pollination and cultured in vitro. The maximum dose for hybrid plants is 30 kR, and the maximum dose for estrogen haploids is 60 kR or higher.

Raquin et al. use petunia or petunia. Reported the process of inducing androgen production during the in vitro culture of ovaries irradiated with γ-rays at a dose of 50-1000 Gy before pollination. Their results showed that gamma radiation doses in the range of 200 to 1000 Gy led to the development of two kinds of plants: haploid 2n = x = 7 and hyper diploid 2n> 2x = 14, which was confirmed by genetic data The source of androgens for haploids. Marking: Androgen haploid contains irradiated female parent chloroplasts, and there is no visible change in the structure of CP-DNA after irradiation.

Carnation

In carnation (Dianthus caryophyllus L.), most commercially important varieties are vegetative propagated, and are not F1 hybrids. From the perspective of commercial production of rooted cuttings and seeds, there exist some disadvantages with clonal propagation compared with seed propagation. First, the production cost per plantlet is much higher in clonal propagation. Second perfect control of diseases in the nursery is essential. Third, the shelf life of cuttings is far shorter than that of seeds. To breed F1 varieties, producing inbred lines as the parental lines is necessary. Inbreeding depression is a problem in carnation and it is almost impossible to produce S4 seeds. Production of doubled haploids is another way to produce pure lines. Sato et al. (2000) tried the pseudo fertilized ovule culture of carnation and succeeded in DH production. In this research emasculated flower buds were pollinated with pollen inactivated by X ray irradiation (100 kR or 200 kR). After 2±3 weeks, the ovaries were cultured on solid MS medium having 2 mM NAA, 2 mM BAP and 6% sucrose. Regenerated plants (R0) were morphologically different from the mother plants. Root tip cells contained both 2n = 30 cells and 2n = 15 cells. The R1 plants of each R0 plant were identical to their respective R0 plants. From these observations they concluded that the R0 plants were doubled haploids. Dolcet-Sanjuan et al. (2001) summarize the protocol for the production of DHLs in carnation, by in situ induced parthenogenesis and in vitro embryo rescue. They proved the homozygosity of some DHLs by determining the resistance of the progeny to F. oxysporum obtained with a susceptible variety.

Sunflower

After pollinating with irradiated pollen and culturing immature embryos in vitro, sunflowers were obtained from sunflower hybrids Albena and Viki (Todorova et al., 1994). Todorova et al. (1997) found that the effectiveness of this method is affected by the interaction between the pollen-derived genotype, the intensity of gamma radiation and the genotype of the original form of parthenogenetic induction. Todorova and Ivanov (1999) reported that the use of mixed pollen from the best source of pollen can partially overcome the genotype specificity of pollen donors.

Androgenesis

The process of regenerating haploids from male gamete cells is called Androgenesis. It has extraordinary potential in improving the installation and commercial development of DH. This method is based on the ability of immature microspores and pollen grains to convert their gametophytic pathways into sporophytic pathways. Causes cell division at the haploid level. This can be achieved by culturing immature anthers and isolated microspores in vitro. The isolated microspore is an improved technique. In the process of separating microspores, the anther wall tissue is lost, preventing the regeneration of somatic tissues. It is affected by several factors; genotype, developmental stage of male gametes, stress effects (preliminary temperature treatment of flower buds, osmotic stress, lack of nitrogen and sucrose in the plant substrate) and growth conditions of the donor plant. Majority of studies have focused on effect of culture media constituents on androgenesis. The most commonly used carbohydrate is sucrose (13%), particularly in microspore media. Under optimal in vitro culturing conditions, androgenetic plants are regenerated by embryogenesis or organogenesis. Direct embryogenesis is

favored, since regeneration through the callus might induce undesired gametoclonal variation.

Anther culture

Anemone

Laura et al. (2006) cultured anthers of Anemone coronaria on double layer medium; the lower solid layer consisted of NN medium with activated charcoal, whereas the top layer was liquid NN medium without charcoal. Embryos were observed after 12-14 weeks of culture. The regenerated plants had various ploidy levels, including some plants which were haploid. Their androgenetic origin were confirmed by RAPD- based DNA fingerprinting.

Phlox

Phlox drummondii (Phlox drummondii) is used as an annual garden plant and cut flowers. Hoekstra et al. (1993) developed an efficient anther culture program for the production of haploid Beatles. The seedless step was inoculated into basal MS medium containing 9% sucrose and 10 µg. M 2, 4-D + 5 µ M contains BA in the dark to induce callus. The callus tissue (~2mm) was transferred to MS medium containing 3% sucrose + 10µ, and propagated for a 16-hour photoperiod. M BA + 5 µ includes M NAA. 60 plants were produced, of which haploids accounted for 50%, diploids accounted for 30%, and aneuploidies accounted for 20%.

Carnation

Attempts have been made to develop an anther culture protocol in carnation. Callus was produced from anthers cultured on MS medium with 2, 4-D and BA, 2, 4-D and NAA, TDZ and NAA (Mosquera et al., 1999; & Nontaswatsri et al., 2008). All resulting plants were diploid or tetraploid and it was determined that the plants originated from the anther wall (Fu et al., 2008).

Purple coneflower

Zhao et al. (2006) developed 19 haploid plant of Echinacea purpurea by anther culture. They found that, in callus induction cultures, N6 basal medium was more effective than MS media, and a combination of BA at 2.22 µM with NAA at 0.054 µM was more effective than 2, 4-D alone.

Microspore culture

Camalia

Three method of microspore culture were tested for the induction of microspore embryogenesis in Camalia japonica L. cv. Elegans (Pedroso & Pais, 1994). Microspore suspension plated over solid MS medium containing 4.5 µM 2, 4- D and 0.5 µM kinetin with sucrose and glucose were seen as the best culture condition for induction of embryogenesis.

Cow cockle

A microspore culture program was developed for Saponaria vacaria L. (Kernan & Ferrie, 2006), a member of the Caryophyllaceae family. Genotypic differences were observed among the five strains evaluated. The most embryogenic line (White Beauty variety) has produced more than 350 copies. Embryos/100 kidneys and 4-7.9 mm kidneys produce the highest number of embryos/100 kidneys. Among the various medium formulations studied, intact NLN containing 15% sucrose resulted in the largest number of embryos. They also reported that microspores need to be pretreated for 3 days at 32°C to obtain embryos from microspores. In this study, more than 800 DH plants were regenerated.

Ornamental kale

Wang et al. (2011) studied the effects of solid medium, developmental stage, embryo age, cold treatment and medium addition on plant regeneration of ornamental cabbage embryos (*Brassica oleracea var acephala)* obtained from microspores. The best results can be obtained when embryos are cultured in B5 medium solidified with 1% agar. Cold treatment (2 or 5 days at 4°C) significantly improved the plant's regenerative ability, with a frequency of up to 79.0%. They also noticed the addition of 3.0 or 5.0 mg/l silver nitrate can increase the frequency of plant regeneration.

Gynogenesis

Gynogenesis is the production of haploid embryos from female gametophytes, which can be achieved by culturing various non-pollinating parts of flowers in vitro, such as egg cells, egg cells attached to the placenta,

ovaries, or whole flower buds. A possible alternative source of haploid production in plants, especially in plants that are male sterile, male sterile, and dioecious. The effectiveness of this method is strongly influenced by many biological and non-biological factors. The pretreatment of gametes and flower buds, the composition of the in vitro medium and the culture conditions are some of the factors that affect the reaction of gamete embryos in culture. All of these factors depend on genotype, and there is no universal protocol for in vitro estrogen production. It is usually immature at the time of inoculation and will continue to develop during in vitro culture to form a mature embryo sac (Musial et al., 2005). They contain several theoretical haploid cells (egg cell, synergids, antipodal cells and non-fused polar nuclei) that can form haploid embryos, which can be haploid directly or through intermediate callus stages Body plant.

Marigold

Thaneshwari and Aswath (2018) studied the effects of plant growth regulators and sucrose concentration on the callus induction and bud differentiation of marigold ovary (Tagetes spp) cultures. The maximum callus induction rate in MS medium, supplemented by 4.44 µM BAP and 4.52 µM. When the callus was sub cultured in MS medium supplemented with 4.44 µM BAP and 1.07 µM NAA, 2, 4-D and the maximum bud differentiation rate were reported. It was found that MS medium without plant growth regulators is the best rooting medium. The sucrose concentration is 4%. In the EM environment, it was found to be most suitable for callus induction. Thaneshwari et al. (2018) also studied the effect of pressure on callus initiation and bud regeneration in marigold ovarian culture, and found that 2 hours of flower bud pretreatment at 45°C can better induce callus in MS medium Induction and bud differentiation. 4% sucrose is 4.44. M BAP and 4.52 µ M 2, 4-D, and incubate in the dark at 25°C for 4 weeks. When flower buds were investigated for 24 hours at 4°C, the minimum number of days before callus onset and the maximum bud differentiation rate were also given (Thaneshwari et al., 2018).

The conclusion is that ornamental plants are mainly produced due to their aesthetic value. In order to improve our internal and external environment, consumers continue to demand new varieties of ornamental plants, which must have new colors, flavors, plant structures and resistance to biotic and abiotic stresses. Haploid has been successfully used in breeding programs to create new breeding lines and varieties. Embryos and DH lines derived from microspores can also be used to improve mutagenesis and genetic modification. In some decorations, haploids and double haploids have been formed. They have been used to create repeated haploid and haploid plants for each species, and the similarities between the schemes are small, and the average composition and growth conditions between species vary greatly. Within the species, there are differences in genotype in terms of its implementation. Observed in crop species with repeated haploid protocols. Although the progress is slow compared with major crops, attempts have been made to develop a double haploid method for a variety of ornamental plants. There are many ornamental plants that have not yet been evaluated for their response to microspore culture. Further research is needed to develop and optimize protocols for producing double haploids from ornamental plants. Efficient protocols are important because duplicate haploids must be produced in large quantities to be used in breeding programs. It would be useful to conduct basic research to clarify the factors that control embryogenesis.

REFERENCES

Aleza, P., Juarez, J., Hernandez, M., Pina, J. A., Ollitrault, P., & Navarro, L. (2009). Recovery and characterization of a *Citrus clementina* Hort. ex Tan. 'Clemenules' haploid plant selected to establish the reference whole citrus genome sequence. *BMC Plant Biology*, *9*, 110.

Blakeslee, A. F., Belling, J., Farnham, M. E., & Bergner, A. D. (1922). A haploid mutant in the Jimson weed, *Datura stramonium*. *Science*, *55*, 646-647.

Dolcet-Sanjuan, R., Clavería, E., Llauradó, M., Ortigosa, A., & Arús, P. (2001). Carnation (*Dianthus caryophyllus* L.) di haploid lines resistant to *Fusarium oxysporum* f. sp. dianthi. *Acta Horticulturae*, *560*, 141-144.

Forster, B. P., Heberle-Bors, E., Kasha, K. J., & Touraev, A. (2007). The resurgence of haploids in higher plants. *Trends in Plant Science*, *12*(8), 368-375.

Fu, X., Yang, S., & Bao, M. (2008). Factors affecting somatic embryogenesis in anther cultures of Chinese pink (*Dianthus chinensis* L.). *In Vitro Cellular and Developmental Biology - Plant*, *44*, 194-202.

Guha, S., & Maheshwari, S. C. (1964). *In vitro* production of embryos from anthers of *Datura*. *Nature*, *204*(4957), 497.

Hoekstra, S., Van Zijder Veld, M. H., Heide kamp, F., & Vander Mark, F. (1993). Microspore culture of *Hordeum vulgare* L: The influence of density and osmolalily. *Plant Cell Reports*, *12*, 661-665.

Kernan, Z., & Ferrie, A. M. R. (2006). Microspore embryogenesis and the development of a double haploidy protocol for cow cockle (*Saponaria vaccaria*). *Plant Cell Reports*, *25*, 274–280.

Laura, M., Safaverdi, G., & Allavena, A. (2006). Androgenetic plants of *Anemone coronaria* derived through anther culture. *Plant Breeding*, *125*, 629-634.

Maluszynski, M., Kasha, K. J., & Szarejko, I. (2003). Published doubled haploid protocols in plant species. In: *Doubled Haploid Production in Crop Plants: A Manual*, Maluszynski, M., Kasha, K. J., Forster, B. P., & Szarejko, I. Kluwer Academic Publishers, Dordrecht. pp.309–335.

Mosquera, T., Rodríguez, L. E., Parra, A., & Rodríguez, M. (1999). *In vitro* adventive regeneration from carnation (*Dianthus caryophyllus*) anthers. *Acta Horticulturae*, *482*, 305-308.

Musial, K., Bohanec, B., Jakse, M., & Przywara, L. (2005). The development of onion (*Allium cepa* L.) embryo sacs *in vitro* and gynogenesis induction in relation to flower size. *In Vitro Cellular and Developmental Biology-Plant*, *41*(4), 446-452.

Nontaswatsri, C., Ruamrungsri, S., & Fukai S. (2008). Callus induction and plant regeneration of *Dianthus chinensis* L. and *Dianthus barbatus* L. *via* anther culture. *Acta Horticulturae*, *788*, 109-114.

Pedroso, M. C., & Pais, M. S. (1994). Induction of microspore embryogenesis in *Camellia japonica* L., cv. Elegans. *Plant Cell Tissue and Organ Culture*,*37*, 129-136.

Raquin, C. (1985). Induction of haploid plants by *in vitro* culture of *Petunia* ovaries pollinated with irradiated pollen. *Zeitschrift Fur Pflanzenzuchtung*, *94*, 166-169.

Sato, S., Katoh, N., Yoshida, H., Iwai, S., & Hagimori, M. (2000). Production of doubled haploid plants of carnation (Dianthus caryophyllus L.) by pseudofertilized ovule culture. *Scientia Horticulturae*, *83*, 301- 310.

Sestile, S., & Ficcadenti, N. (1996). Irradiated pollen for haploid production. In: Jain, S. N., Sopory, S. K., & Veilleux, R. E. (*eds*). *In vitro* production in higher plants. *1*, Kluwer Academic Publishers, Dordrecht. pp 263-274.

Thaneshwari, C., & Aswath (2018). Effect of plant growth regulators and sucrose concentration on callus induction and shoot differentiation from ovary culture of marigold (*Tagetes* spp). *International Journal of Chemical Studies*, *6*(1), 618-623.

Todorova, M., & Ivanov, P. (1999). Induced parthenogenesis in sunflower: Effect of pollen donor. *Helia*, *22*(31), 49-56.

Todorova, M., Ivanov, P., & Nenova, N. (1994). Induced parthenogenesis in sunflower (*H. annuus* L.) using irradiated pollen. Eucarpia, *Oil and Protein Crops Section*, 22.09–24.09.94, Albena, Bulgaria

Todorova, M., Ivanov, P., Shindrova, P., Christov, M., & Ivanova, I. (1997). Dihaploid plant production of sunflower (*Helianthus annuus* L.) through irradiated pollen-induced. parthenogenesis *Euphytica*, *97*, 249-254.

Wang, H., Dong, B., Jiang, J., Fang, W., Guan, Z., Liao, Y., Chen, S., & Chen, F. (2014). Characterization of *in vitro* haploid and doubled haploid *Chrysanthemum morifolium* plants *via* unfertilized ovule culture for phenotypical traits and DNA methylation pattern. *Frontiers in plant science*, *5*, 738.

Watanabe, K. (1977). Successful ovary culture and production of F hybrids and androgenic haploids in Japanese chrysanthemum species. *The Journal of Heredity*, *68*, pp. 317-320.

Wang, Y., Tong, Y., Li, Y., Zhang, Y., Zhang, Y., Feng, J., & Feng, H. (2011). High frequency plant regeneration from microspore-derived embryos of ornamental kale (*Brassica oleracea* L. var. acephala). *Scientia Horticulturae*, *130*, 296–302.