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Haploid and doubled haploid technology: Fast-track breeding in vegetables

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Abstract

Haploid and doubled haploid (DH) technologies provide powerful tools to accelerate vegetable breeding by creating completely homozygous lines in a single generation. Haploids are plants with a single set of chromosomes (gametophytic or monoploid number), and when their chromosomes are doubled, fully homozygous diploids (doubled haploids) are obtained. These methods, first demonstrated nearly a century ago by plant scientists like A.F. Blakeslee (*Datura* haploids, 1922) and S. Guha and S.C. Maheshwari (anther-culture haploids, 1964), significantly reduce the breeding cycle. Conventional inbreeding often requires 6-8 generations to achieve similar homozygosity, whereas DH techniques accomplish this in one step. In vegetables, haploid production can be achieved by several approaches (e.g. pollen or ovule culture, wide crosses and irradiated pollen) and is followed by chromosome doubling (commonly using agents like colchicine). This fast-track approach has been applied to many vegetable crops worldwide (tomato, pepper, onion, Brassicas, cucurbits, carrot, potato, etc.), enabling rapid development of uniform inbred lines, hybrid parents, and mapping populations. DH technology brings advantages such as time savings, uniform progeny, and ease of fixing recessive traits, but also faces challenges (genotype dependence, specialized lab requirements, occasional aneuploidy). This comprehensive overview covers the definitions, historical milestones, various induction methods, chromosome doubling techniques, applications in vegetable breeding, and the relative merits of haploid breeding. It highlights how haploid and doubled haploid approaches offer a fast-track to developing improved vegetable cultivars globally.

Keywords: Haploid, doubled haploid, vegetable breeding, haploid induction, chromosome doubling, homozygous lines, accelerated breeding

Introduction

Haploids are plants that contain a single set of chromosomes (n), representing the gametophytic chromosome number of a species [1]. When a haploid plant undergoes chromosome doubling, it produces a doubled haploid (DH) plant with a complete diploid set (2n) that is homozygous at all genetic loci [2]. In diploid crops (2n=2x), haploids are also called monpoids (x) because they carry just one genome copy. For example, a haploid from a diploid species with 12 chromosomes would have 6. In polyploids (e.g. tetraploid potato with 4x=48), a haploid has 2x (24 chromosomes) and a doubled haploid restores 4x. This extreme homozygosity in a single step is the key feature of DH technology [3, 4].

The idea of using haploids in breeding dates back nearly a century. The first naturally occurring haploid plant was reported by Alfred Blakeslee and colleagues in 1922 in jimson weed (*Datura stramonium*), although that was an odd genetic accident [5, 6, 7]. More importantly, mid-20th century research established practical methods to induce haploids. S. Guha and S.C. Maheshwari (India, 1964) pioneered *in vitro* pollen culture (anther culture) to generate haploid embryos in *Datura* [8]. Around the same time,

geneticists like Kasha and Kao (1970) used wide crosses (e.g. barley × *Hordeum bulbosum*) to eliminate one parental genome and rescue haploid embryos [9]. The concept was further developed by S. Jensen (1974) and others who formulated reliable chromosome-doubling protocols. These milestones by scientists such as Blakeslee, Guha, Maheshwari, Kasha, Kao, Jensen and more established the foundation of haploid breeding [10, 11].

Haploid and DH technologies are particularly valuable because they “fast-track” the breeding process. Conventional breeding through repeated selfing or sib-mating typically requires 6-8 generations to approach full homozygosity and develop an inbred line [12]. In contrast, haploid induction followed by chromosome doubling produces instantly homozygous plants (since all alleles are fixed), compressing years of breeding into a single generation [13]. This capability allows breeders to rapidly generate pure lines for cultivar development or parental lines for hybrids, make genetic mapping populations, and fix desirable recessive mutations [15]. For example, a tomato hybrid breeding program can use DH lines to capture traits quickly, whereas a leafy vegetable like cabbage can achieve

uniform resistant cultivars without the uncertainty of multiple generations of selfing^[14].

Haploid induction methods have been developed for hundreds of plant species, including over two hundred vegetable taxa^[16]. Common vegetable crops such as tomato, pepper, eggplant, onion, carrot, cucumbers, melons, cabbage, broccoli, lettuce and many others have been successfully subjected to haploid techniques^[17]. The methods exploit either female or male gametophyte cells. *In vitro* approaches include androgenesis (from pollen; e.g. anther or microspore culture) and gynogenesis (from ovules or ovaries). *In vivo* approaches include wide hybridization (crossing with a related species and eliminating one parental genome) and pollen irradiation (using irradiated pollen to stimulate an unfertilized egg)^[18]. Once haploid plants are obtained, chromosome doubling (e.g. with colchicine) produces fertile doubled haploid lines^[19]. This article explains these methods in detail, reviews the current status of DH technology in vegetable breeding, and examines its advantages and challenges.

Haploid Induction Methods

Haploid induction in vegetables can be broadly categorized into *in vivo* methods (performed on whole plants or crosses) and *in vitro* methods (using cultured tissues)^[20]. Each approach varies by crop, but all share the goal of obtaining a plant with only one set of parental chromosomes^[21].

- Wide Hybridization (Uniparental Genome Elimination):** This classical method uses interspecific or intergeneric crosses where one parent's chromosomes are selectively lost^[22]. The best-known example is the barley-*H. bulbosum* cross: pollinating *Hordeum vulgare* with wild *H. bulbosum* results in hybrid embryos that initially contain both parental genomes, but the chromosomes of *H. bulbosum* are eliminated early, leaving a haploid barley embryo^[23]. The embryo is then rescued in tissue culture and grown into a haploid plant^[24]. This "bulbosum technique" has been exploited to produce haploids in cereals and even adapted to potato. In potato (*Solanum tuberosum*), crosses with diploid *S. phureja* lines can induce haploid embryos because of selective fertilization and subsequent parthenogenesis. Haploids obtained via wide crosses typically require embryo rescue and special culture to develop^[25].
- Irradiated Pollen Pollination:** In this *in vivo* approach, pollen is exposed to high-energy radiation (e.g. gamma rays) before pollinating the female parent^[26]. The irradiated pollen is damaged and fails to contribute its chromosomes to the fertilized egg, but it can stimulate the egg cell to begin development^[27]. The result is a maternal haploid embryo. This technique is widely used in cucurbit breeding. For instance, crossing cucumber (*Cucumis sativus*) or melon (*C. melo*) with irradiated pollen from related species (or irradiated pollen of the same species) produces haploid embryos that can be cultured. Similarly, in onion and some *Allium* crops, irradiated pollen from onion cultivars can induce haploids. The haploid embryos are typically tiny and require ovule culture or embryo rescue to grow^[28].
- Androgenesis (Anther Culture):** This *in vitro* method involves culturing immature anthers from flower buds on nutrient media. The anther wall contains numerous microspores (immature pollen grains), which can be induced to switch from gametophytic development to embryogenic development^[29]. Under the right conditions (appropriate pretreatment, culture medium, temperature, etc.), these microspores form haploid embryos. The embryos develop into haploid plantlets. Androgenesis has been achieved in many vegetables: Brassica vegetables (cauliflower, broccoli, cabbage, Chinese cabbage), Solanaceae (tomato, pepper, eggplant, potato), carrot, lettuce and others have protocols for anther culture^[30]. However, the response is highly genotype-dependent; some varieties respond well while others are recalcitrant. The technique is relatively simple and can produce many haploids per anther, but contamination by somatic (diploid) tissue or albino plants can occur^[31].
- Isolated Microspore Culture:** A variation of androgenesis, microspore culture involves first isolating microspores (pollen precursors) from anthers and then culturing the naked microspores directly. This removes inhibiting anther tissues and can improve response^[32]. It has become the preferred method in some crops. For instance, isolated microspore culture is widely used in Brassica vegetables and has been successful in broccoli, cauliflower, cabbage and Chinese cabbage breeding^[33]. In pepper and tomato, both anther culture and isolated microspore culture have been experimented with. Techniques for isolating microspores typically involve macerating flower buds or using centrifugal washes^[34]. This method also often requires specific stress pretreatments (cold shock, heat shock, starvation) to induce embryogenesis. Once induced, microspores develop into haploid embryos similarly to anther culture^[35].
- Gynogenesis (Ovule/Ovary Culture):** When male-derived techniques fail (for example in crops with very recalcitrant pollen), the female gametophyte can be cultured. Gynogenesis refers to culturing unfertilized ovules, ovaries or flower buds to obtain haploid embryos from the egg cell^[36]. This method is essential in *Allium* crops like onion, where androgenesis has not yielded haploids^[37]. For onions (*Allium cepa*), ovules from unfertilized ovaries are excised and cultured on special media; haploid plants can be regenerated. Gynogenesis also works in some cucurbits (squash, pumpkin) where ovary culture can produce haploids, although with low efficiency^[38]. Generally, gynogenesis yields fewer haploids than androgenesis and is more technically demanding, but it is the only route for species like onion or some lilies^[39].
- In Planta Haploid Inducer Lines:** In maize breeding, special "haploid inducer" lines carry mutations (e.g. in the gene *MTL*) that cause a percentage of seeds to be maternal haploids when crossed as pollen donors. Such genetic haploid inducers have revolutionized maize DH production^[40]. In vegetables, this approach is at an early stage: researchers are trying to develop or engineer similar inducer lines in crops like tomato and rapeseed using gene editing, but routine haploid inducer varieties for vegetable species are not yet established^[41]. The promise of this method is that it would simplify haploid production by seed, but currently it is mainly a cereal technology^[42].

In practice, the choice of haploid induction method depends on the crop and its genetics. Some vegetables respond strongly to one approach and poorly to others. For example, onion relies almost entirely on gynogenesis, brassicas respond well to microspore culture, and cucurbits to irradiated pollen^[43]. Within each method, the efficiency can vary widely by genotype and protocol. Extensive research continues to optimize media, physical treatments and culture techniques to improve haploid yields. Once a haploid embryo or plant is obtained, it is usually sterile (having only one chromosome set). To create a fertile doubled haploid, chromosome doubling is performed^[44].

Chromosome Doubling (Diploidization)

- Haploid plants are valuable as genetic tools, but most vegetable species require two chromosome sets for normal fertility^[45]. Therefore, artificially doubling the chromosomes of a haploid cell produces a fully fertile doubled haploid plant. Diploidization is typically achieved by disrupting the normal mitotic spindle so that sister chromatids do not separate, leading to chromosome duplication within a single nucleus. This is accomplished using antimitotic agents or physical treatments^[46].
- The most commonly used chemical for chromosome doubling is colchicine, a mitotic inhibitor extracted from *Colchicum autumnale*. Colchicine binds tubulin and prevents spindle formation, causing a cell to end up with twice the normal number of chromosomes^[47]. There are many application methods for colchicine in plants: treating germinating seeds, immersing growing root or shoot tips, or culturing tissues on media containing colchicine. For example, seedling apices can be immersed in a 0.1-0.5% colchicine solution for several hours, or the shoot meristem can be painted with a colchicine paste^[48]. Other methods include adding colchicine directly to tissue culture media during haploid regeneration. While effective, colchicine is toxic and must be handled carefully; it can also kill delicate tissues if overused^[49].
- Because colchicine can be harsh, alternative agents are often tested. Oryzalin and trifluralin are newer herbicides that likewise disrupt microtubules but are effective at much lower concentrations and reportedly cause less tissue damage^[50]. Oryzalin has been used for *in vitro* chromosome doubling in haploids of various plants. Aminoprophos-methyl (APM) and anti-microtubule chemicals (e.g. amiprofos-methyl) are also used in laboratory cultures to double cells. Another approach is exposing haploid plants or buds to nitrous oxide (N₂O) gas, which can induce chromosome doubling in treated tissues^[51].
- In addition to chemicals, spontaneous doubling can occur when a haploid plant self-pollinates; sometimes haploid embryos partially double on their own. However, this is unreliable. Therefore, deliberate diploidization is standard in breeding programs^[52]. After treatment, the resulting plants must be screened (e.g. by flow cytometry or chromosome counting) to confirm successful doubling. Only those plants with the doubled (diploid) genome and full fertility are advanced as true DH lines^[53].

Applications in Vegetable Breeding

Haploid and DH technologies have found a wide range of applications in vegetable crop improvement. By producing completely homozygous lines rapidly, breeders gain tools for both basic genetics and cultivar development^[54].

- **Rapid Development of Pure Lines:** A primary use of DH is creating pure inbred lines quickly. Instead of selfing a hybrid for many generations, a breeder can induce haploids from the F1 or other cross and then double them. The resulting DH lines are fixed and identical through seed, suitable as pure-breeding lines^[55]. In self-pollinated vegetables like tomato or pepper, these DH lines can become new varieties. In cross-pollinated vegetables like onion (where hybrid vigor is exploited), DH lines serve as parental lines with guaranteed uniformity. For example, onion breeders have used gynogenetic DH lines to form hybrid parent lines that consistently segregate 50:50 for flesh color or pungency^[56].
- **Hybrid Breeding and Parental Lines:** Doubled haploids play an important role in hybrid cultivar programs, especially for vegetables that benefit from F1 vigor^[57]. The DH approach allows breeders to fix a desirable genotype immediately and then use it as a parent. In brassica crops (cabbage, broccoli, cauliflower), DH lines have been used extensively to create uniform hybrid parents. Similarly, stable DH inbred lines in pepper or eggplant are valuable for creating F1 hybrids with improved yield and disease resistance^[58].
- **Accelerated Breeding of Complex Traits:** Rapid homozygosity makes it easier to stack genes and select for multiple traits. For instance, breeding for combined disease resistance and quality traits in a vegetable can be accelerated by combining genetic elements into one DH line through crossing and immediate fixation^[59]. Marker-assisted backcrossing is also sped up: after one backcross, a breeder can double the selected plant and recover the desired gene in a homozygous state, saving several generations. In mutation breeding, novel variants are stabilized as DH lines to evaluate their effects quickly^[60].
- **Genetic Mapping and Trait Analysis:** Because DH lines are completely homozygous, they are ideal for genetic studies^[61]. A population of DH lines derived from a cross serves as a permanent mapping population: each line has a fixed genome from one F1. This allows construction of high-resolution genetic maps and easy QTL mapping, since segregation is uniform and can be replicated by seed^[62]. In vegetables, DH mapping populations have been used to identify genes for disease resistance, quality, and stress tolerance. For example, DH populations in cauliflower and cabbage have facilitated mapping of head size and disease resistance traits. The uniformity of DH lines also means that phenotypic evaluations can be repeated on identical genotypes, increasing accuracy^[63].
- **Self-Incompatibility and Reproductive Barriers:** Many vegetable crops (onion, brassica vegetables, some Cucurbitaceae) exhibit self-incompatibility or suffer inbreeding depression, making conventional inbreeding difficult. DH technology bypasses these issues^[64]. For

example, onion has a strong self-incompatibility system and suffers from inbreeding depression; by using gynogenesis to produce DH lines, breeders can create fully homozygous onion varieties or maintain lines that would never survive many generations of selfing. Similarly, DH techniques allow fixation of favorable alleles in self-incompatible or highly heterozygous crops^[65].

- **Hybrid Seed Production (CMS Systems):** DH technology can produce cytoplasmic male-sterile (CMS) lines and maintainers more efficiently. In crops like cabbage and carrot, breeders exploit DH to create uniform CMS lines by doubling haploids that carry the CMS cytoplasm^[66]. This allows stable production of hybrid seed without the complexity of traditional backcrossing. The use of DH lines ensures complete homozygosity of the line with the CMS factor intact^[67].
- **Crop Diversification and Novel Crops:** Beyond major staples, haploid/DH methods enable breeding of minor or specialty vegetables. For example, doubled haploids have been developed in medicinal or specialty vegetable crops (a recent study reported DH in valerian for phytopharmaceutical use). Novel or underutilized vegetables can benefit from the rapid-line development that DH provides, accelerating their improvement^[68].
- **Seed Propagation of Clonally Propagated Crops:** In vegetatively propagated vegetables (e.g. potato, sweetpotato, banana, though banana and sweetpotato are tropical starch crops), DH technology can convert clonal material into seed-propagated lines^[69]. For instance, potato breeders generate dihaploids (via haploid induction from tetraploid potato) to quickly introgress traits and then convert them to stable tetraploids again. This creates true potato seed varieties that are easier to distribute^[70].

Advantages and Limitations of Haploid Technology

1. **Advantages:** The principal benefit of DH breeding is the drastic reduction in time and generations needed to obtain homozygous lines. A breeder can obtain a completely homozygous inbred line in 1-2 generations via haploid induction and chromosome doubling, versus 6-8 generations by selfing. This accelerates release of new varieties and shortens breeding cycles^[71]. DH lines provide 100% homozygosity, enabling consistent trait expression and uniform phenotypes, which is useful for variety certification and hybrid seed purity. Generating many DH lines from the same F1 cross also allows rapid selection of superior inbreds out of a large pool^[72, 73].
2. For genetic analysis, DH populations eliminate background genetic noise: since each line is fixed, any phenotypic variation in a DH population is due to genotype differences without within-line variability. This clarity improves mapping of quantitative traits^[74, 75]. DH breeding also enables direct exploitation of recessive alleles: a valuable recessive mutant will become immediately homozygous in a DH line and can be evaluated or combined without additional crossing^[76].
3. In crops with self-incompatibility or severe inbreeding depression, DH technology provides an alternative

route to pure lines that conventional methods cannot achieve. For hybrid breeding, DH lines ensure highly uniform parental lines, improving hybrid consistency. DH also enables seed-propagated alternatives to clonally propagated crops (as mentioned for potato)^[77].

4. **Limitations and Challenges:** Despite these advantages, DH techniques have several limitations. A major challenge is genotype dependency: not all varieties respond to haploid induction methods. For example, anther culture success often varies by cultivar and year. Some species are recalcitrant to tissue culture altogether. Thus, effort is required to develop and optimize protocols for each crop and genotype^[78].
5. Efficiency is another issue. Haploid induction rates can be low in many vegetables. It may take hundreds of explants to obtain a few haploid plants. Techniques like anther culture can also produce abnormal plants (aneuploids, albinos, or mixoploids) that must be discarded. Chromosome doubling is an additional step that is not 100% efficient and can introduce somaclonal variation or damage plants if not carefully managed^[79].
6. DH methods require specialized laboratory facilities and expertise in tissue culture, cytology and ploidy analysis. This raises costs compared to field-based breeding^[80]. Some traditional breeders view DH as expensive and only justify it when it clearly speeds progress. There is also a concern that over-reliance on DH lines could reduce genetic diversity if breeders focus on a small number of lines. Finally, the fixed nature of DH lines means they cannot be further improved by simple selection; any improvement requires new crosses and haploid induction again^[81].
7. In summary, while haploid/DH technology is a powerful fast-track tool, it complements rather than replaces conventional breeding. It is most effective when integrated strategically for example, using DH lines for initial inbred development and mapping, then using traditional crossing for combining traits or expanding diversity^[82, 83].

Conclusion

Haploid and doubled haploid technologies have opened a fast-track path for vegetable crop improvement worldwide. By enabling the rapid creation of completely homozygous lines, they greatly reduce the time, space and effort needed in breeding programs. Since the early discoveries by scientists such as Blakeslee, Guha, Kasha and Jensen, the methods have evolved to include anther/microspore culture, gynogenesis, wide crosses, and even genetic haploid inducers. Today, many major vegetables (tomato, pepper, onion, Brassica greens, cucurbits, carrot, potato and more) can be improved with DH approaches. These techniques are especially valuable for fixing complex traits, developing hybrid parents, and conducting genetic studies. Nevertheless, successful application of haploid technology requires substantial expertise and optimization. Every crop and even variety can behave differently, demanding tailored protocols. As research continues to improve induction rates and develop new inducers (for example via gene editing), we can expect DH methods to become more robust and widely accessible. Combined with modern tools like marker-assisted selection, DH breeding can drive faster

genetic gains. In the context of global agriculture, where the demand for improved vegetables with higher yields, nutrition and stress resistance is growing, DH technology offers a timely advantage. By fast-tracking breeding cycles, it helps meet production challenges more quickly. For students and researchers in plant science, understanding and applying haploid/DH methods is a key part of the toolkit for modern vegetable breeding. Through continued innovation, haploid and doubled haploid technology will remain at the forefront of efforts to develop superior vegetable cultivars that benefit farmers and consumers around the world.

References

- Kalinowska K, Chamasb S, Unkel K, Demidov D, Lermontova I, Dresselhaus T, *et al.* State-of-the-art and novel developments of *in vivo* haploid technologies. *Theor Appl Genet.* 2019;132:593-605.
- Nair SK, Molenaar W, Melchinger AE, Boddupalli PM, Martinez L, Lopez LA, *et al.* Dissection of a major QTL *qhir1* conferring maternal haploid induction ability in maize. *Theor Appl Genet.* 2017;130:1113-1122.
- Yan G, Liu H, Wang H, Lu Z, Wang Y, Mullan D, *et al.* Accelerated generation of selfed pure line plants for gene identification and crop breeding. *Front Plant Sci.* 2017;8:1786-1796.
- Melchinger AE, Böhm J, Utz HF, Müller J, Munder S, Mauch FJ. High-throughput precision phenotyping of the oil content of single seeds of various oilseed crops. *Crop Sci.* 2018;58:670-678.
- Meng D, Liu C, Chen S, Jin W. Haploid induction and its application in maize breeding. *Mol Breed.* 2021;41:20-33.
- Chaikam V, Molenaar W, Melchinger A, Boddupalli P. Doubled haploid technology for line development in maize: Technical advances and prospects. *Theor Appl Genet.* 2019;132:3227-3243.
- Marimuthu MPA, Maruthachalam R, Bondada R, Kuppu S, Tan EH, Britt A. Epigenetically mismatched parental centromeres trigger genome elimination in hybrids. *Sci Adv.* 2021;7:1-19.
- Brauner PC, Schipprack W, Utz HF, Bauer E, Mayer M, Schön CC, *et al.* Testcross performance of doubled haploid lines from European flint maize landraces are promising for broadening the genetic base of elite germplasm. *Theor Appl Genet.* 2019;132:1897-1908.
- Ishii T, Karimi-Ashtiyani R, Houben A. Haploidization via chromosome elimination: Means and mechanisms. *Annu Rev Plant Biol.* 2016;67:421-438.
- Zhu YC, Sun DX, Deng Y, An GL, Li WH, Si WJ, *et al.* Comparative transcriptome analysis of the effect of different heat shock periods on the unfertilized ovule in watermelon (*Citrullus lanatus*). *J Integr Agric.* 2020;19:528-540.
- Kurtar ES, Balkaya A. Production of *in vitro* haploid plants from in situ induced haploid embryos in winter squash (*Cucurbita maxima* Duchesne ex Lam.) via irradiated pollen. *Plant Cell Tissue Organ Cult.* 2010;102:267-277.
- Burlutsky V, Pronina I, Zavarykina T, Tulinova E, Tsygankova N. Factors of the haploproducer method in the F1 hybrids system *Triticum aestivum* L. × *Zea mays* L. optimization. *IOP Conf Ser Earth Environ Sci.* 2021;723:022087. (In Russian)
- Kanbar OZ, Lantos C, Chege PK, Kiss E, Pauk J. Generation of doubled haploid lines from winter wheat (*Triticum aestivum* L.) breeding material using *in vitro* anther culture. *Czech J Genet Plant Breed.* 2020;56:150-158.
- Chen C, Xiao Z, Zhang J, Li W, Li J, Liu C, *et al.* Development of *in vivo* haploid inducer lines for screening haploid immature embryos in maize. *Plants.* 2020;9:739-747.
- Andorf C, Beavis WD, Hufford M, Smith S, Suza WP, Wang K, *et al.* Technological advances in maize breeding: Past, present and future. *Theor Appl Genet.* 2019;132:817-849.
- Dwivedi SL, Britt AB, Tripathi L, Sharma S, Upadhyaya HD, Ortiz R. Haploids: Constraints and opportunities in plant breeding. *Biotechnol Adv.* 2015;33:812-829.
- Kumlehn J. Haploid technology. In: Kumlehn J, Stein N, editors. *Biotechnological Approaches to Barley Improvement.* Berlin/Heidelberg: Springer; 2014. p. 379-392.
- Shen Y, Pan G, Lubberstedt T. Haploid strategies for functional validation of plant genes. *Trends Biotechnol.* 2015;33:611-620.
- Burlutsky V, Tulinova A, Tsygankova V, Pronina I, Zavarykina T, Zhiltsov AV, *et al.* Haploid albinism *Hordeum vulgare* L. In: Voronova SI, editor. *Modern Trends in Solving the Problems of the Agro-Industrial Complex Based on Innovative Technologies, Proceedings of the International Scientific and Practical Conference Dedicated to the 90th Anniversary of the Federal Research Center "Nemchinovka", Volgograd, Russia, 23 April 2021.* Volgograd: Federal Research Center "Nemchinovka"; 2021. p. 76-80. (In Russian)
- Conner JA, Mookkan M, Huo H, Chae K, Ozias-Akins P. A parthenogenesis gene of apomict origin elicits embryo formation from unfertilized eggs in a sexual plant. *Proc Natl Acad Sci USA.* 2015;112:11205-11210.
- Anderson SN, Johnson CS, Chesnut J, Jones DS, Khanday I, Woodhouse M, *et al.* The zygotic transition is initiated in unicellular plant zygotes with asymmetric activation of parental genomes. *Dev Cell.* 2017;43:349-358.
- Khanday I, Skinner D, Yang B, Mercier R, Sundaresan V. A male-expressed rice embryogenic trigger redirected for asexual propagation through seeds. *Nature.* 2019;565:91-95.
- Chen J, Strieder N, Krohn NG, Cyprys P, Sprunck S, Engelmann JC, *et al.* Zygotic genome activation occurs shortly after fertilization in maize. *Plant Cell.* 2017;29:2106-2125.
- Conner JA, Podio M, Ozias-Akins P. Haploid embryo production in rice and maize induced by *PsASGR-BBML* transgenes. *Plant Reprod.* 2017;30:41-52.
- Boutilier K, Offringa R, Sharma VK, Kieft H, Ouellet T, Zhang L, *et al.* Ectopic expression of *BABY BOOM* triggers a conversion from vegetative to embryonic growth. *Plant Cell.* 2002;14:1737-1749.
- Ghadamkheir M, Vladimirovich KP, Orujov E, Bayat

- M, Madumarov MM, Avdotyin V, *et al.* Influence of sulfur fertilization on infection of wheat take-all disease caused by the fungus *Gaeumannomyces graminis* var. *tritici*. *Res Crops*. 2020;21:627-633.
27. Tsunewaki K, Mukai Y. Wheat haploids through the Salmon method. In: Bajaj YPS, editor. *Biotechnology in Agriculture and Forestry*. Berlin/Heidelberg: Springer; 1990. Vol. 13. p. 460-478.
 28. Matzk F, Meyer HM, Bäumlein H, Balzer HJ, Schubert I. A novel approach to the analysis of the initiation of embryo development in *Gramineae*. *Sex Plant Reprod*. 1995;8:266-272.
 29. Kumlehn J, Kirik V, Cizhal A, Altschmied L, Matzk F, Lörz H, *et al.* Parthenogenetic egg cells of wheat: Cellular and molecular studies. *Sex Plant Reprod*. 2001;14:239-243.
 30. Kőszegi D, Johnston AJ, Rutten T, Cizhal A, Altschmied L, Kumlehn J, *et al.* Members of the RKD transcription factor family induce an egg cell-like gene expression program. *Plant J*. 2011;67:280-291.
 31. Koi S, Hisanaga T, Sato K, Shimamura M, Yamato KT, Ishizaki K, *et al.* An evolutionarily conserved plant RKD factor controls germ cell differentiation. *Curr Biol*. 2016;26:1-7.
 32. Rövekamp M, Bowman JL, Grossniklaus U. *Marchantia* MpRKD regulates the gametophyte-sporophyte transition by keeping egg cells quiescent in the absence of fertilization. *Curr Biol*. 2016;26:1-8.
 33. Kermicle JL. Androgenesis conditioned by a mutation in maize. *Science*. 1969;166:1422-1424.
 34. Coe EH. A line of maize with high haploid frequency. *Am Nat*. 1959;93:381-382.
 35. Li Y, Lin Z, Yue Y, Zhao H, Fei X, Liu C, *et al.* Loss-of-function alleles of *ZmPLD3* cause haploid induction in maize. *Nat Plants*. 2021;7:1579-1588.
 36. Hu H, Schrag TA, Peis R, Unterseer S, Schipprack W, Chen S, *et al.* The genetic basis of haploid induction in maize identified with a novel genome-wide association method. *Genetics*. 2016;202:1267-1276.
 37. Prigge V, Xu X, Li L, Babu R, Chen S, Atlin GN, *et al.* New insights into the genetics of *in vivo* induction of maternal haploids, the backbone of doubled haploid technology in maize. *Genetics*. 2012;190:781-793.
 38. Barret P, Brinkmann M, Beckert M. A major locus expressed in the male gametophyte with incomplete penetrance is responsible for *in situ* gynogenesis in maize. *Theor Appl Genet*. 2008;117:581-594.
 39. Dong X, Xu X, Miao J, Li L, Zhang D, Mi X, *et al.* Fine mapping of *qhir1* influencing *in vivo* haploid induction in maize. *Theor Appl Genet*. 2013;126:1713-1720.
 40. Gilles LM, Khaled A, Laffaire JB, Chaignon S, Gendrot G, Laplaige J, *et al.* Loss of pollen-specific phospholipase NOT LIKE DAD triggers gynogenesis in maize. *EMBO J*. 2017;36:707-717.
 41. Gilles LM, Martin JP, Rogowsky PM, Widiez T. Haploid induction in plants. *Curr Biol*. 2017;27:1095-1097.
 42. Kelliher T, Starr D, Richbourg L, Chintamanani S, Delzer B, Nuccio ML, *et al.* MATRILINEAL, a sperm-specific phospholipase, triggers maize haploid induction. *Nature*. 2017;542:105-109.
 43. Liu C, Li X, Meng D, Zhong Y, Chen C, Dong X, *et al.* A 4-bp insertion at *ZmPLA1* encoding a putative phospholipase A generates haploid induction in maize. *Mol Plant*. 2017;10:520-522.
 44. Rietz S, Dermendjiev G, Oppermann E, Tafesse FG, Effendi Y, Holk A, *et al.* Roles of *Arabidopsis* patatin-related phospholipases A in root development are related to auxin responses and phosphate deficiency. *Mol Plant*. 2010;3:524-538.
 45. Li L, Xu X, Jin W, Chen S. Morphological and molecular evidences for DNA introgression in haploid induction via a high oil inducer CAUHOI in maize. *Planta*. 2009;230:367-376.
 46. Zhang Z, Qiu F, Liu Y, Ma K, Li Z, Xu S. Chromosome elimination and *in vivo* haploid production induced by Stock6-derived inducer line in maize (*Zea mays* L.). *Plant Cell Rep*. 2008;27:1851-1860.
 47. Zhao X, Xu X, Xie H, Chen S, Jin W. Fertilization and uniparental chromosome elimination during crosses with maize haploid inducers. *Plant Physiol*. 2013;163:721-731.
 48. Tian X, Qin Y, Chen B, Liu C, Wang L, Li X, *et al.* Hetero-fertilization along with failed egg-sperm cell fusion supports single fertilization involved in *in vivo* haploid induction in maize. *J Exp Bot*. 2018;69:4689-4701.
 49. Liu C, Li W, Zhong Y, Dong X, Hu H, Tian X, *et al.* Fine mapping of *qhir8* affecting *in vivo* haploid induction in maize. *Theor Appl Genet*. 2015;128:2507-2515.
 50. Li J, Cheng D, Guo S, Yang Z, Chen M, Chen C, *et al.* Genomic selection to optimize doubled haploid-based hybrid breeding in maize. *bioRxiv*. 2010.
 51. Zhong Y, Liu C, Qi X, Jiao Y, Wang D, Wang Y, *et al.* Mutation of *ZmDMP* enhances haploid induction in maize. *Nat Plants*. 2019;5:575-580.
 52. Jacquier NMA, Gilles LM, Pyott DE, Martinant JP, Rogowsky PM, Widiez T. Puzzling out plant reproduction by haploid induction for innovations in plant breeding. *Nat Plants*. 2020;6:610-619.
 53. Vanous K, Lübberstedt T, Ibrahim R, Frei UK. *MYO*, a candidate gene for haploid induction in maize causes male sterility. *Plants*. 2020;9:773-781.
 54. Yao L, Zhang Y, Liu C, Liu Y, Wang Y, Liang D, *et al.* *OsmATL* mutation induces haploid seed formation in *indica* rice. *Nat Plants*. 2018;4:530-533.
 55. Eder J, Chalyk S. *In vivo* haploid induction in maize. *Theor Appl Genet*. 2002;104:703-708.
 56. Kebede AZ, Dhillon BS, Schipprack W, Araus JL, Bänziger M, Semagn K, *et al.* Effect of source germplasm and season on the *in vivo* haploid induction rate in tropical maize. *Euphytica*. 2011;180:219-226.
 57. Wu P, Li H, Ren J, Chen S. Mapping of maternal QTLs for *in vivo* haploid induction rate in maize (*Zea mays* L.). *Euphytica*. 2014;196:413-421.
 58. De La Fuente GN, Frei UK, Trampe B, Nettleton D, Zhang W, Lübberstedt T. A diallel analysis of a maize donor population response to *in vivo* maternal haploid induction: I. Inducibility. *Crop Sci*. 2018;58:1830-1837.
 59. Wędzony M, Forster BP, Żur I, Golemic E, Szechyńska-Hebda M, Dubas E, *et al.* Progress in

- doubled haploid technology in higher plants. In: Touraev A, Forster BP, Jain SM, editors. *Advances in Haploid Production in Higher Plants*. Dordrecht: Springer; 2009. p.1-33.
60. John KN, Valentin V, Abdullah B, Bayat M, Kargar MH, Zargar M. Weed mapping technologies in discerning and managing weed infestation levels of farming systems. *Res Crops*. 2020;21:93-98.
 61. Davies DR. Cytogenetic studies in wild and cultivated species of *Hordeum* [PhD thesis]. Cardiff (UK): University of Wales; 1956. p.145.
 62. Symko S. Haploid barley from crosses of *Hordeum bulbosum* (2x) by *Hordeum vulgare* (2x). *Can J Genet Cytol*. 1969;11:602-608.
 63. Lange W. Cytogenetical and embryological research on crosses between *Hordeum vulgare* and *H. bulbosum*. *Versl Landbouwk Onderz*. 1969;719:162-178.
 64. Lange W. Crosses between *Hordeum vulgare* L. and *H. bulbosum* L. II. Elimination of chromosomes in hybrid tissue. *Euphytica*. 1971;20:181-194.
 65. Laurie DA, Bennett MD. Wheat × maize hybridization. *Can J Genet Cytol*. 1986;28:313-316.
 66. Laurie DA. The frequency of fertilization in wheat × pearl millet crosses. *Genome*. 1989;32:1063-1067.
 67. Liu D, Zhang H, Zhang L, Yuan Z, Hao M, Zheng Y. Distant hybridization: a tool for interspecific manipulation of chromosomes. In: Pratap A, Kumar J, editors. *Alien Gene Transfer in Crop Plants*. New York (NY): Springer; 2014. p.25-42.
 68. Inoue E, Sakuma F, Kasumi M, Hara H, Tsukihashi T. Maternal haploidization of Japanese pear through intergeneric hybridization with apple. *Acta Hortic*. 2004;663:815-818.
 69. Bayat M, Zargar M, Murtazova KM-S, Nakhaev MR, Shkurkin SI. Ameliorating seed germination and seedling growth of nano-primed wheat and flax seeds using seven biogenic metal-based nanoparticles. *Agronomy*. 2022;12:811-823.
 70. Kavhiza NJ, Zargar M, Prikhodko SI, Pakina EN, Murtazova KM-S, Nakhaev MR. Improving crop productivity and ensuring food security through the adoption of genetically modified crops in Sub-Saharan Africa. *Agronomy*. 2022;12:439-455.
 71. Bitsch C, Gröger S, Lelley T. Effect of parental genotypes on haploid embryo and plantlet formation in wheat-maize crosses. *Euphytica*. 2000;103:319-323.
 72. Campbell AW, Griffin WB, Burritt DJ, Conner AJ. The importance of light intensity for pollen tube growth and embryo survival in wheat × maize crosses. *Ann Bot*. 2001;87:517-522.
 73. Garcia-Llamas C, Ramirez MC, Ballesteros J. Effect of pollinator on haploid production in durum wheat crossed with maize and pearl millet. *Plant Breed*. 2004;123:201-203.
 74. Sanei M, Pickering R, Kumke K, Nasuda S, Houben A. Loss of centromeric histone H3 (*CENH3*) from centromeres precedes uniparental chromosome elimination in interspecific barley hybrids. *Proc Natl Acad Sci USA*. 2011;108:E498-E505.
 75. Geiger HH, Gordillo GA. Doubled haploids in hybrid maize breeding. *Maydica*. 2011;54:485-499.
 76. Liu C, Zhong Y, Qi X, Chen M, Liu Z, Chen C, *et al*. Extension of the *in vivo* haploid induction system from diploid maize to hexaploid wheat. *Plant Biotechnol J*. 2020;18:316-318.
 77. Fu S, Yin L, Xu M, Li Y, Wang M, Yang J, *et al*. Maternal doubled haploid production in interploidy hybridization between *Brassica napus* and *Brassica allooctaploids*. *Planta*. 2018;247:113-125.
 78. Laurie DA, Bennett MD. The timing of chromosome elimination in hexaploid wheat × maize crosses. *Genome*. 1989;32:953-961.
 79. Ravi M, Chan SW. Haploid plants produced by centromere-mediated genome elimination. *Nature*. 2010;464:615-618.
 80. Burlutsky V, Zavarykina T, Pronina I, Tulinova E, Tsygankova N, Zhiltsov A. Technologies for creating haploids *in vivo*. In: *Modern Trends in Solving Problems of the Agro-Industrial Complex Based on Innovative Technologies*. IOP Conf Ser Earth Environ Sci. 2021;81-88. (In Russian)
 81. Ingouff M, Rademacher S, Holec S, Šoljić L, Xin N, Readshaw A, *et al*. Zygotic resetting of the histone 3 variant repertoire participates in epigenetic reprogramming in *Arabidopsis*. *Curr Biol*. 2010;20:2137-2143.
 82. Gohard FH, Zhiteneva AA, Earnshaw WC. Centromeres. In: *eLS*. Chichester (UK): Wiley; 2014.
 83. Lermontova I, Kuhlmann M, Friedel S, Rutten T, Heckmann S, Sandmann M, *et al*. *Arabidopsis* KINETOCHORE NULL2 is an upstream component for centromeric histone H3 variant *CENH3* deposition at centromeres. *Plant Cell*. 2013;25:3389-3404.