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Oat (*Avena sativa* L.) In Vitro Cultures: Prospects and Challenges for Breeding

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Oat (*Avena sativa* L.) In Vitro Cultures: Prospects and Challenges for Breeding

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Abstract: Plant cell and tissue culture has been a crucial component of efforts to enhance crops and advance plant biotechnology ever since the beginning of the XX century when Hildebrand discovered the theory of totipotency – a somatic plant cell still has the capacity to develop into every cell that makes up a whole plant. Further standardization of the techniques for use in crop improvement has resulted from the original interest with the creation of the numerous plant tissue culture techniques through the various advancement. The most important approach used in plant tissue culture is the cultivation of differentiated tissue that results in the development of an undifferentiated mass of parenchymatous cells and their subsequent differentiation by organogenesis or somatic embryogenesis. This has been playing a significant role in the creation of virus-free seedlings and fast clonal multiplication, and it is also a crucial component in transgenic' regeneration. Additionally, cell cultures with a variety of mutations emerging in it offers chances to enhance crops. For the purpose of identifying homozygous recombinants in a single generation, haploid cell culture offers an additional benefit. Wide hybridization and the embryo rescue procedure have helped to produce haploid embryos and homozygous lines of doubled haploids which are difficult and time consuming to produce using traditional methods. Developed haploid embryos from the male gametophyte, carried out in cultures of immature anthers or isolated microspores, are referred to as androgenesis and could be the most efficient way to create lines of doubled haploids. The cell cultures also offer the chance to extract a variety of beneficial biochemicals. Induction of mutations in cultured cells and tissue in vitro to produce beneficial cultivars and manipulation of the centromere-specific histone CENH3 also become possible in recent years. Compared to traditional methods, in vitro cultures offer a number of both scientific and practical advantageous for scientists and plant breeders. However, the usefulness of individual methods should be examined by analyzing their advantages and disadvantages. When it comes to oat (*Avena sativa* L.) the efficient method of plant regeneration is still missing compared to the most common cereals, possibly because this cereal is known as recalcitrant for in vitro culture. An effort has been made to provide a succinct overview of the various in vitro techniques utilized or potentially involved in breeding of oat (*Avena sativa* L.) in this review. The present work aims to summarize the crucial methods of *A. sativa* cultivation under tissue culture conditions with a focus on the progress that has been made in biotechnological techniques which are used in breeding of this species.

Keywords: androgenesis; callus; doubled haploids; embryogenesis; organogenesis; wide crossing

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1. Introduction

In breeding practice, the development of new cereal cultivars takes from several to several dozen years and is mainly based on generating plants with a high degree of homozygosity through inbreeding crosses, followed by the selection of individuals with desirable traits. The use of biotechnological methods allows to shorten this procedure by up to several years and involves obtaining haploid plants, followed by the generation of doubled haploid (DH) lines through *in vitro* culture methods. The obtained homozygous lines guarantee that subsequent generations will be genetically and phenotypically identical. Therefore, they are increasingly utilized in breeding programs. It is also worth noting that the proportion of plants carrying genes of interest to breeders is higher in DH lines compared to F2 generation and subsequent generations obtained using traditional methods [1]. Additionally, DH lines find applications in studies involving molecular markers, by accelerating the derivation of mapping populations and genetic transformations, estimating recombination fractions, and detecting recessive mutants. They are also an effective means of genetic enrichment in plants, introducing more favorable alleles into the genome, carrying traits such as resistance to biotic and abiotic stresses [2].

The genus *Avenae* L. includes more than 20 species occurring at three ploidy levels, i.e., diploid, tetraploid and hexaploid. The most commonly cultivated species, common oat (*Avena sativa* L.), is a hexaploid ($2n = 6x = 42$) with three genomes: AA, CC, and DD. Common oat is grown worldwide in agricultural regions with a temperate climate, and its grains are primarily used for feed and food production [3]. Despite having lesser economic and commercial significance compared to other grains, scientifically proven health benefits of oat grains make it an interesting subject of both breeding and genetic research. However, the size and complexity of the hexaploid oat genome is a significant limitation in the application of conventional research tools, resulting in unsatisfactory progress in oat research [4].

Haploids are plants in the sporophytic development stage, but they have the gametic number of chromosomes (n). They are produced without the involvement of fertilization by male gametophyte cells from *in vitro* cultures of anthers or isolated microspores (androgenesis), or female gametophytic cells from *in vitro* cultures of ovaries or ovules (gynogenesis). Both methods involve the action of various factors leading to the reprogramming of the developmental pathway of haploid male and female cells, resulting in the formation of haploid androgenic or gynogenic embryos rather than gametes. Methods based on gynogenesis also include wide crosses, or wide hybridization, involving forced pollination of plants belonging to different species or genera. The pioneering work on cereal haploidization was carried out by Kasha and Kao [5], who pollinated *Hordeum vulgare* L. with the pollen of the wild species *Hordeum bulbosum* L. This technique, known as the "bulbosum method," proved to be highly efficient and found application in generating haploids not only of barley or common wheat, but also of other plant species. Currently, the most common pollen donor in wide crosses of cereals is maize (*Zea mays* L.), followed by pearl millet (*Pennisetum glaucum* (L.) R. Br.), sorghum (*Sorghum bicolor* (L.) Moench), Job's tears (*Coix lacryma-jobi* L.) or cogon grass (*Imperata cylindrica*) (Ishii et al., 2013). These crosses result in hybrid embryos of wheat, oats, triticale or barley, in which paternal chromosomes are eliminated during successive nuclear divisions, resulting in haploid embryos containing only maternal genetic material. Sometimes, however, elimination does not occur properly and fragments or whole donor chromosomes are incorporated into the recipient's genome. This most commonly occurs in crosses between plants belonging to the two subfamilies, *Pooideae* and *Panicoideae*, within the family Poaceae. Additional chromosomes from maize or pearl millet have been observed in both wheat haploids [6,7], and oat haploids [8]. Investigating the causes of this phenomenon, Mochida et al. [9] found incomplete attachment of the spindle apparatus to maize centromeres, while Ishii et al. [10] reported chromosome breaks in pearl millet. The presence of stable maize chromosomes in the oat genome was first described by Riera-Lizarazu et al. [8], and they were referred to as oat × maize addition (OMA) lines by Kynast et al. [11]. Since then, these lines have been used for physical mapping of the maize genome [12], studies on CENH3 centromere-specific histones [13] or gene expression analysis in the C_4 photosynthetic pathway [14].

2. Callus culture, organogenesis, and somatic embryogenesis of oat (*Avena sativa* L.)

The development of in vitro regeneration techniques is crucial for improving cereals biotechnologically. Similar to other Poaceae species, hexaploid oat (*Avena sativa* L.) can be regenerated from tissue culture by either organogenesis or somatic embryogenesis. Limited reports have been published on the development of effective plant regeneration systems from various tissues and organs in oat, compared to the most common cereals, e.g., maize, rice, wheat, and barley.

Oat callus cultures might be induced from seeds, immature embryos, germination-stage seedling roots, and germination-stage embryo hypocotyls, as firstly described by Carter et al. [15]. Nine years later Lörz et al. [16] reported successful plant regeneration from nonfriable callus that had structured, green primordia. In the same year Cummings et al. [17] used germinating immature embryos obtained from 25 oat genotypes as explants for starting callus cultures on B5 [18] or MS [19] media containing from 0.5 to 3.0 mg L^{-1} 2,4-Dichlorophenoxyacetic acid (2,4-D). These cultures were maintained by subculturing to B5 medium with 1 mg L^{-1} 2,4-D every 4 to 6 weeks. The callus from the cultivar 'Lodi' has been maintained for the longest period of time and retained the regeneration ability through 18 months. Next, Maddock [20] emphasizes that morphogenetic oat callus appears to arise from the whole embryo or the mesocotyl region, but not from the scutellum which becomes necrotic especially when older embryos are cultured.

The highly efficient regeneration system from leaf tissue of six different oat cultivars named 'Coolabah', 'Cooba', 'Blackbutt', 'Mortlock', 'Victorgrain', and 'HVR' was reported by Chen et al. [21]. Callus was produced using leaf base segments from seedlings on MS medium with 2 mg L^{-1} 2,4-D, however, on medium without 2,4-D callus tissue did not develop. Moreover, explants grown in the light or in the dark responded to callus induction similarly. The age of the seedling had a significant impact on callus development from the leaf explants. The two to five day old and the callus from the first leaf segment demonstrated a comparatively high potential for regeneration. Therefore, seedling age must be taken into account as a key variable for in vitro regeneration from leaf explants in oat. Callus obtained in this way was subcultured on three weeks interval, and it has continued to regenerate plants for more than eight months without significant reductions of regeneration capacity.

Shoot apical meristems of *A. sativa* were also used to establish an effective micropropagation method [22]. Initial explants were isolated from aseptically germinated oat seedlings and were cultured in vitro. After five weeks in culture, on MS medium with various combinations of 2,4-D and N^6-benzyladenine (BA), the enlarged apical meristems and multiple adventitious shoots were produced. All tested oat cultivars formed seedlings at a high efficiency and fertile oat plants were produced. These in vitro multiplied shoots could be an alternative target tissue for oat genetic transformation. Also, Cummings et al. [17] obtained callus from apical meristems capable for plant regeneration on B5 medium containing 2,4-D.

By adjusting the nitrogen composition and the concentrations of the sugar as well as auxins in the culture medium, Nuutila et al. [23] increased the regeneration efficiency from oat leaf-base cultures. The authors tested MS and L3 [24] medium for efficiency of embryogenic callus induction and plant development. Leaf-base segments 1 through 6 were compared for their capacity to produce callus. For all cultivars, the first three leaf-base segments generated embryogenic callus, but segments 4-6 produced either little or no embryogenic callus. For both tested cultivars, 'Aslak' and 'Veli', L3 medium turned out to be more efficient and produced more embryogenic callus and plants, compared to MS medium. Lower concentrations of ammonium (4.9 mM) and nitrate (29 mM), and high organic nitrogen (11 mM) in medium caused the highest regeneration of green plants in 'Aslak'. On the other hand, high ammonium (20.1 mM) and high nitrate (46.8 mM), and low organic nitrogen (0.9 mM) concentrations resulted in the greatest green plant regeneration in 'Veli'. For both cultivars, there were obvious discrepancies between the ideal nitrogen contents and those in the induction media. It is interesting to note that for 'Aslak' less nitrogen is needed, while for 'Veli' more nitrogen was needed than in the original L3 medium. This study additionally has demonstrated that both sugar and auxins have a definite impact on the induction of embryogenesis. For oat leaf-base in vitro culture, sucrose and maltose have been investigated as carbohydrate sources [21,25].

Establishing tissue cultures in oat has frequently run into complications because the strong dependency of the donor plant cultivar. The variations in cultivars' susceptibilities to genetic programming and the external reprogramming of embryogenically competent cells might be the cause of the discrepancies. The findings suggest that medium should be tailored specifically for each cultivar in order to produce plantlets during embryogenesis to their full potential. Although it may be reasonable, for practical reasons, to choose a medium in routine systems that elicits an average response from the majority of cultivars, these responses do not always reflect how well or badly the cultivars respond in vitro generally. This genotypic dependency was described by Cummings et al. [17]. In their investigation, from 25 tested genotypes, two failed to initiate callus and nine of them were able to produce callus, but had no regeneration ability. Next, Rines and McCoy [26] described that the frequency of callus formation varied between oat cultivars and ranged from 5% to 75%. Studies of Chen et al. [21] showed that it is possible to obtain callus form of all tested genotypes in 100% or almost 100% frequency. However, the frequency of regenerable callus formation obtained from leaf base segments was considerably higher than immature embryos [17,26]. This indicates that leaf explants are less genotype dependent and are suitable donor material for the establishment of regenerable callus cultures of oats.

In comparison to callus cultures only capable for organogenesis, those capable for somatic embryogenesis are more likely to show fast growth rates, and very high levels of plant regeneration. Due of these, the production of friable and embryogenic callus has received most of the attention in the quest to create tissue cultures of monocotyledonous plants. As with all cereals, immature tissues must be used to initiate oat in vitro cultures since these differentiated tissues are typically unable to induce cell division and proliferation. Two different callus types, some of which have the capacity to regenerate plants, are regularly generated during indirect somatic embryogenesis. Typically, the embryogenic callus is friable, compact, and yellowish-white and this non-embryogenic is rough-looking, moist, non-friable, and transparent [27].

Formation of embryogenic oat callus similar like in another species is dependent on the source of the explant, its physiological stage, genotype, and the composition of the culture initiation medium. The majority of work has utilized immature zygotic embryos as explants to initiate embryogenic oat cultures [20, 26, 28-31], cultured on MS medium and supplemented most commonly with 2,4-D in different concentrations. Other explants exhibiting callus formation include mature seeds [15,16], and mesocotyl of germinated seedlings [29]. Embryogenic callus from immature and mature embryos [32,33] and from leaf segments [25] has also been used for gene transfer.

Avena sativa L., *Avena sterilis* L., and *Avena fatua* L. are three hexaploid oat species from which tissue cultures were started and plants were regenerated [26]. Immature embryos were used to start a variety of tissue cultures, with regenerable-type cultures distinguished by the presence of organized chlorophyllous primordia in a compact, yellowish white, strongly lobed callus. The frequency of regenerable-type cultures was depended on the size of the embryo, the species, the genotype, the conditions in which the donor plants were grown, and the concentrations of 2,4-D in the culture induction media. The highest rates of regenerable-type of cultures were consistently produced by the 'Lodi' cultivar and two 'Lodi'-related lines out of the 23 investigated *A. sativa* cultivars. For 'Lodi', this frequency reached 80% in one test. Only three of the 16 *A. sterilis* lines produced regenerable-type cultures, but than 20%. In seven out of the 32 investigated *A. fatua* lines, regenerable-type cultures were generated at rates higher than 45%. The tissue cultures of all 3 species could regenerate plants after 9–10 subcultures and being more than a year in in vitro culture.

Since genotype affects culture initiation frequency and culture type, genotype screening and selection as well as developmental stage of embryos ought to be a successful strategy for enhancing oats' capacity for cell culture. The aim of King et al. [34] investigation was to determine the optimum size of immature embryos of 10 oat cultivars for callus induction and plant regeneration under in vitro culture. One hundred immature embryos per cultivar were cultured on MS medium with 2 mg L^{-1} 2,4-D. Plant regeneration was assessed after three months of culturing and then they were transferred on MS medium with 0.5 mg L^{-1} 2,4-D and consequently to MS medium without 2,4-D. No differences between cultivars were observed in the amount of callus produced, but rate of regeneration from the different cultivars extended from 3 to 42% suggesting that there are genotypic differences in the ability to regenerate plants from calli. Scanning electron microscopy and light microscopy were used by Bregitzer et al. [29, 30] to characterize the stages of development of somatic embryos in friable embryogenic callus. According to Bregitzer et al. [29] culture of nonfriable oat callus derived from immature embryos on MS medium containing 20 mg L^{-1} sucrose and no hormones resulted in the development of distinct somatic embryos that germinated to form complete plants. Embryogenic sectors isolated from nonfriable callus were visually selected during repeated subculture on a modified MS medium containing 2 mg L^{-1}, 2,4-D and 20 g L^{-1} sucrose to produce friable callus. Transfer of friable callus to a modified MS medium containing 60 g L^{-1} sucrose and no hormones induced the maturation of somatic embryos. Subsequent transfer of this friable callus to modified MS medium containing 20 g L^{-1} sucrose and no hormones allowed the germination of some of these embryos. Plants were regenerated from friable callus lines for more than 78 weeks after friable callus establishment. Friable embryogenic callus also was initiated directly from immature embryos of three genotypes and seedling mesocotyls of two genotypes. Genotypic variation in this response was also noted. In Chen et al. [35] study was shown for the first time that callus induced from oat seed developed root primordia and the meristems of these primordia were sites of somatic embryo formation. After one month of culture, callus maintained on MS medium with 1, 2, or 4 mg L^{-1} 2,4-D with underlying root or shoot pieces was transferred to a fresh supply of the same medium. Among the five cultivars tested, cv. 'Risto' showed a callus induction frequency of 93%, while 'Victory', 'Sang', 'Sanna' and 'Vital' showed a frequency of 76%, 63%, 24% and 18%, respectively. The fresh weight of the callus produced ranged from 30 to 200 mg per seed. During callus induction, seedling roots became swollen from proliferating callus cells. Epidermal and cortical cells of the roots tended to be expelled and fall off. Differentiated pericycle cells became meristematic. When these roots were sectioned longitudinally or transversely, numerous lateral root primordia were seen originating from the pericycle cells along the vascular strand. Callus induction occurred also in the basal portions of the shoot. Parenchymatic cells developed a mixture of meristematic cells and single xylem cells. The meristematic cells had prominent nuclei and could directly form root primordia. Groups of root primordia were also formed indirectly after callusing in the vicinity of the shoot bases. Somatic embryos associated with underlying callus cells were germinated on different media. MS medium containing 0.01 mg L^{-1} of abscisic acid (ABA) and 6% sucrose was most suitable for germination. After 30 to 40 days on this medium single plantlets or clumps with 2–5 mm green leaves, with or without roots were obtained. Embryogenic tissue 10-month-old produced up to 200 shoots or plantlets per gram. When seedlings were transferred to a medium containing cytokinin's the growth was improved after 20–30 days. More robust plantlets with well-developed roots were achieved upon transfer to medium without hormones. More than 90% of the green plantlets transferred to soil survived and reached maturity. Embryogenic tissue has been maintained for more than 30 months.

In the next study young seedlings' basal regions were cultivated on auxin-containing media, which led to callus induction and shoot regeneration [25]. The developmental stage and genotype of the explants were associated with callus induction efficiency and regeneration frequencies. The experiment was conducted on five oat cultivars: 'Fuchs', 'Jumbo', 'Gramena', 'Bonus', and 'Alfred', which leaf segments were cultured on two different basal media – MS medium and L3 medium [24]. Media enriched with different phytohormones in different concentrations for callus induction, shoot proliferation and regeneration of plants were tested. Four-week-old culture produced calli were transferred to shoot induction medium and one week later, somatic embryos began to germinate. On the L3 medium with maltose more than 15% regenerants per explant could be produced than with the sucrose-containing MS medium. For several months, the proliferable callus from the leaf explants was kept up without losing its ability to regenerate. In order to develop further, shoots were placed in hormone-free media, and developed plants were morphologically healthy and fertile. The effect of various concentrations of phytohormones on generation of callus and shoots was also evaluated. From the base of the oat leaves callus was induced on all the tested media. However, certain phytohormones had better effects in plant regeneration. With callus induction, the highest regeneration frequencies were attained on medium with 2.5 mg L^{-1} 2,4-D, whereas 6 mg L^{-1} 3,6-dichloro-2-methoxybenzoic acid (dicamba) did not produce a significant amount of regenerants per explant. In five oat genotypes, 25 plants on average could be grown per explant, and for the most responsive Jumbo, more than 50 regenerants could be produced per explant. Hence, the oat leaf bases are very promising for micropropagation as primary explants due to their strong capacity for regeneration.

In order to investigate the effects of polyamines on somatic embryogenesis and plant regeneration oat genotypes Tibor (*Avena nuda* L. with low regeneration factor), GP-1 (*Avena sativa* L. with high regeneration factor) and their crosses, GP-1 × Tibor and Tibor × GP-1, were grown in *in vitro* cultures [36]. Somatic embryos were produced in large amounts from mature embryos of Tibor and Tibor × GP-1 on MS medium supplemented with 2.0 mg L^{-1} 2,4-D and 0.5 mM putrescin. Putrescin treatments induced plant regeneration from other genotypes in most cases, comparing with the results of Somers et al. [32] obtained with the same regeneration media. This suggests that putrescin enriching media can be used to screening other oat lines for regeneration efficiency. Moreover, the shoot proliferation medium containing low concentration of putrescin induced significant numbers of plants from usually recalcitrant cultivars.

Although Bregitzer et al. [29] reported that immature embryos provided the highest and most reproducible callus initiation frequency, it was found out that, in generall, the frequency of embryogenic callus initiation of some the elite germplasm lines is still very low [30]. A significant contribution to the formation of embryogenic callus in oat and the subsequent demonstration of plant regeneration by somatic embryogenesis, as well as variables controlling plant regeneration, were reported by Somers et al. [32]. This paper includes the methods used to manipulate oat cells and tissues in tissue culture, the constraints on their usage, and both planned and actual uses for improving oats. The genotype of the oats, the explant utilized to start the development process, and the tissue choice made during subculture can all affect the oat callus structure and its ability for regeneration. However, according to these authors, by seeing and picking out extremely transparent to opaque, compact, highly lobed tissues within early cultures, oat cultures with long-term preservation of plant regeneration potential can be produced. Borji et al. [37] used mature caryopses as initial material for somatic embryogenesis from oat cultivar ‘Meliane’. Longitudinal sections of caryopses were plated on MS medium supplemented with 3 mg L^{-1} 2,4-D. Primary calli were removed from explants after four weeks of growth and placed into the proliferation medium (MS medium with 1.0 mg L^{-1} 2,4-D and 0.5 mg L^{-1} benzyl aminopurine (BAP)). For germination, somatic embryos were transferred to MS medium without plant growth regulators and then to MS medium containing 0.5 mg L^{-1} indole-3-acetic acid (IAA) in order to promote root system. The regenerated seedlings were acclimated to *ex vitro* conditions and were grown until maturity in greenhouse.

The goals of the Gana et al. [38] investigation was to assess the relative adaptability and plant regeneration of four oat genotypes in suspension cultures, as well as to analyze plant regeneration in 19 genotypes from three different oat species in three sequential callus subcultures. In this study which describes the plant regeneration capacity of '88Ab3073' in suspension culture as well as the highly regenerable ability of 'GAF/Park' in both agar and suspension culture systems, highly significant differences were observed among 19 *Avena* genotypes for callus initiation, germination and rhizogenesis. The analysis of variance for plant numbers for genotypes in three subcultures showed highly significant genotype (32.1% of variance) and genotype × subculture interactions (9.9% of variance) effects, and a non-significant subculture (0.3% of variance) effect. Genotypes with the highest callus production were selected to initiate liquid cultures. Two month-old calli from 'GAF/Park', 'Tibor', '88Ab3073', and '87Ab5932' genotypes were used for the suspension culture initiation. In order to better assess the regeneration potential of small and big cell clusters the suspensions were divided into fractions of < 3 mm or > 3 mm. The 'GAF/Park' and '88Ab3073' clusters that were 3 mm and bigger, generated yellow friable callus. Clusters of '87Ab5932' developed slowly and finally ceased multiply, whereas 'Tibor' clusters began rhizomorphic callus, a propensity that was also seen in its suspension cultures. Within two weeks, clusters of 'GAF/Park' had quadrupled in size on the solidified medium. Plant regeneration from clusters larger than 3 mm was observed after three weeks in three of the four tested genotypes. Additionally, on the 'GAF/Park' callus, many globular somatic embryos were observed. The 42 plants were regenerated from suspension clusters larger than 3 mm and 50 plants that were derived from the agar-based callus culture.

Wise et al. [39] used suspension cultures of *Avena sativa* L. cv. 'Belle' for the biosynthesis of avenanthramides. Callus of oat was initiated from the shoot apical meristem which were placed on solid MS medium in the dark conditions, and subcultured until the appropriate amount was obtained. Liquid cultures were established from 1.0 g callus and 25 ml of MS media containing 2 mg L^{-1} 2,4-D. In order to stimulate avenanthramides production chitin (poly-N-acetyl glucosamine) was added as elicitor, and two unique callus phenotypes named "aggregate" and "friable" were identified. The more brittle aggregate tissue easily shed off and was easily separated from the friable tissue,

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which remained evenly scattered in the culture medium. Separate isolations and cultures were made of these scattered cell types. Visual observation revealed a reduction in the relative quantity of friable tissue with subsequent subcultures. Because the suspension cultures produced relatively large quantities of avenanthramides, these results point to the potential of oat suspension culture as a tool for future in-depth research into the processes that initiate their production as well as the variables that determine the specific kinds of avenanthramides that are produced.

Selection for toxin-insensitivity in tissue cultures of Vb Vb homozygous and Vb vb heterozygous oat sensitive to *Helminthosporium victoriae* toxin described Rines and Luke [28]. Oat tissue cultures were initiated from ten-day-old embryos on MS medium with 2 mg L^{-1} 2,4-D. After 5 months began selection by transferring cultures on MS medium with 1 mg L^{-1} 2,4-D and a predetermined "marginally lethal" level (5 units toxin per ml) of victorin. After 45 days, shoots were transferred to regeneration medium with 2 mg L^{-1} naphthaleneacetic acid (NAA) and 0.2 mg L^{-1} BAP. Two of the 21 immature embryos of the cultivar 'Victoria' and twelve of the 65 immature embryos of the cultivar 'Victorgrain' over a period of five months both produced enough regenerable-type calli to try selection. There were around six callus components per line produced from an embryo. In any of these calli, there were no discernible areas of tissue development or survival 30 days following transfer to a victorin-containing media. Regenerated plants from two fines and nine toxin-selected culture lines were found to be toxin insensitive, respectively. The sole remaining tissue in these two toxin-sensitive regenerant cultures had differentiated into tiny shoots. These shoots could have been toxins that escaped the toxin choice. The more distinct tissues in the morphologically mixed cultures tended to withstand toxin selection for longer, maybe because of the toxin's decreased absorption or penetration. According to the authors, presented tissue culture selection scheme can be used to test effects of chemical agents and culture condition on induction of mutations, somatic recombination, and chromosome loss – all of them mostly uncharacterized aspects with prospects major effects on the use of tissue cultures in plant somatic cell genetic manipulations.

The summary of research on *A. sativa* callus induction, organogenesis, and somatic embryogenesis with the improved biotechnological potential of named species is presented in Table 1.

Table 1. Callus culture, organogenesis, and somatic embryogenesis of oat (*Avena sativa* L.).

***A. sativa* genotype**	**Explant used**	**Media/PGRs**	**Experimental outcomes**	**References**
cv. 'Lodi', cv. 'Moore', cv. 'Lyon', cv. 'Benson', cv. 'Marathon', cv. 'Dal', cv. 'Stout', cv. 'Tippecanoe', cv. 'Lang', cv. 'Victorgrain', cv. 'Garry', cv. 'Hudson', cv. 'Terra', cv. '0A338', cv. 'Victory', cv. 'Black', cv. 'Mesdag', cv. 'Victoria', cv. 'Selma', cv. 'AJ10915', cv. 'NP3/4', cv. 'Karin', cv. 'Rallus', cv. 'Coolabah'	immature embryos	MS/B5 medium with 2 mg L^{-1} 2,4-D for initiation; MS/B5 medium with 1.0, 2.0 and 5.0 mg L^{-1} 2, 4-D for embryo regeneration	tissue cultures capable of plant regeneration after more than 12 months in culture	[26]
cv. 'Victorgrain', cv. 'Victoria' GAF (*A. sativa* cv. 'Garland' × *A. fatua*) × *A. sativa* cv. 'Victoria'	10- to 12-days old embryos	MS medium with 2 mg L^{-1} 2,4-D; MS medium with 1 mg L^{-1} 2,4-D and 5 units mL^{-1} victorin; MS medium with 2 mg L^{-1} NAA, and 0.2 mg L^{-1} BAP for regeneration	12 of 65 immature embryos of the cv. 'Victorgrain' and 2 of 21 embryos of cv. 'Victoria' developed regenerable callus;	[28]

			without tissue growth or survival on a victorin-containing medium	
cv. 'Trafalgar', cv. 'Rollo', cv. '07408 in 111/2', cv. ‘Rhiannon’, cv. ‘Dula’, cv. ‘Avalanche’, cv. ‘Caron’, cv. ‘Pennal’, cv. ‘Cabanna’, cv. ‘Margam’	embryos	MS medium with 2 mg L^{-1} 2,4-D for callus initiation and growth; MS medium with 0.5 mg L^{-1} 2,4-D followed by PGRs free MS medium for regeneration	the highest level of regeneration from 4 – 4.5 mm long embryos with the genotyping differences of plant regeneration	[34]
GAF (*A. sativa* cv. ‘Garland’ × *A. fatua*) × *A. sativa* cv. 'Victoria' lines GAF-18, GAF-30, GAF-30, GAF-30/’Park’ and GAF-30/Park//GAF-30	immature embryos	MS medium with 4 mg L^{-1} 2,4-D for callus initiation; MS medium with 2 mg L^{-1} 2,4-D for callus maintenance; MS medium with 2 mg L^{-1} NAA and 0.2 mg L^{-1} BAP for shoot differentiation; MS medium free of PGRs for rooting	embryogenic cultures maintained the ability to regenerate plants for more than 78 weeks	[29]
cv. ‘Risto’, cv. ‘Sang’, cv. ‘Sanna’, cv. ‘Vital’, cv. ‘Sol’	embryos	MS medium with 2 mg L^{-1} 2,4-D for embryos; MS medium with 0.01 mg L^{-1} ABA and 6% sucrose for germination	suppressed root elongation, promoted secondary root initiation and proliferation of embriogenic cells with 2,4-D in the medium	[35]
cv. ‘Coolabah’, cv. ‘Cooba’, cv. ‘Blackbutt’, cv. ‘Mortlock’, cv. ’Victorgrain’, cv. ‘HVR’	immature embryos, leaf segments	MS medium with 2 mg L^{-1} 2,4-D for callus induction and growth; N6 medium (Chu et al. 1975) with 2 mg L^{-1} KIN, and 2 mg L^{-1} NAA for shoot regeneration; MS medium with 0.3 mg L^{-1} KIN for root regeneration	callus formation from the leaf segments and plant regeneration are comparable to that of the immature embryos; plants were grown to maturity	[21]
line GAF, line GAF/Park	immature zygotic embryos	MS medium with 2 mg L^{-1} 2,4-D for embryos; MS medium with 6% sucrose for embryo maturation, and sucrose reduction for bipolar plant development	friable embryogenic callus inoculated into liquid medium will produce rapidly growing dedifferentiated suspension cultures	[30]
cv. ‘Corbit’, cv. ‘Dark Husk’, cv. ‘Winter Turf’, cv. ‘Monida’, cv. ‘SO87213’, cv. ‘Dal’	embryos	MS medium with 2 mg L^{-1} 2,4-D for callus initiation/proliferation; CIP medium with 0.5 mg L^{-1} picloram, and 5 mg L^{-1} KIN for plant regeneration	high level of plant regeneration	[38]
cv. ‘Prairie’, cv. ‘Porter’, cv. ‘Pacen’, cv. ‘Ogle’	apical meristems, leaf primordia, leaf bases	MS medium with 2,4-D (0 and 0.5 mg L^{-1}) and BA (0, 1.0, 2.0, 4.0, and 8.0 mg L^{-1})	multiple shoot differentiation from shoot apical meristems on medium with 0.5 mg L^{-1} 2,4-D, and 2.0 or 4.0 L^{-1} BA	[22]
cv. ‘Fuchs’, cv. ‘Jumbo’, cv. ‘Gramena’, cv. ‘Bonus’, cv. ‘Alfred’	leaf bases of young seedlings	L3 medium for callus induction; 2.5 mg L^{-1} 2,4-D for plant regeneration	for cv. ‘Jumbo’ average of 50 regenerants per explant could be regenerated, whereas for cv. ‘Gramena’, only 3–4 plants per explant could be regenerated	[25]

cv. 'GP-1'	mature embryos	MS medium with 2 mg L^{-1} 2,4-D for callus induction and shoot proliferation; after 6 weeks, 0.5 or 1.0 mM of putrescine was applied	significant regeneration of plants in presence of 0.5 mM putrescine	[36]
cv. 'Aslak', cv. 'Velik'	leafe-based segments from 3- to 4-days old seedlings	L3 or MS medium for callus induction; L3 or MS medium with 0.2 mg L^{-1} for regeneration	optimization of nitrogen, sugar, and auxin in media	[23]
cv. 'Belle'	shoot apical meristem	MS medium with 2 mg L^{-1} 2,4-D for liquid cultures	suspension cultures produced large quantities of aventhramides A and aventramides G in response to 0.25 mg mL^{-1} chitin (poly-N-acetyl glucosamine) elicitation	[39]
cv. 'Meliane'	mature caryopses	MS medium with 3 mg L^{-1} 2,4-D for callus induction; MS medium with 1 mg L^{-1} 2,4-D and 0.5 mg L^{-1} BAP for embryogenic callus induction and somatic embryos differentiation; MS medium with 0.5 mg L^{-1} IAA for rooting	ultrastructural changes and cytological modifications of oat somatic embryogenesis	[37]

3. Androgenesis of oat (*Avena sativa* L.)

In recent years, the production of doubled haploid (DH) lines using methods involving male gametic lines for developing haploid plants has proven efficient for species belonging to the families Solanaceae, Brassicaceae and Graminae. Consequently, *in vitro*-induced androgenesis has become the most promising biotechnological method applied in breeding practice [40]. However, not all species respond equally to the induction of this process. There are model species that respond with high efficiency to the application of this method, but other species are more resistant to it. The largest group consists of species in which induction of microspore embryogenesis is possible but not very efficient from a practical standpoint. Although about 250 protocols related to androgenesis have been described so far, only in a few species, such as barley (*Hordeum vulgare* L.), oilseed rape (*Brassica napus* L.), tobacco (*Nicotiana* spp.), wheat (*Triticum aestivum* L.), pepper (*Capsicum annum* L.) or rice (*Oryza sativa* L.) this method have been applied in breeding programs due to the high regenerative efficiency of the obtained plants [41].

The process of androgenesis is defined as an alternative developmental pathway of microspores, involving redirecting their natural gametophytic development that leads to pollen grain formation towards a sporophytic pathway, along with their reprogramming and initiation of embryo development [42]. By inducing zygotic embryo-like structures (ELS), followed by their regeneration, androgenic embryos with a haploid number of chromosomes (n) are obtained. The literature indicates that microspores in the late uninucleate or early binucleate stage, directly after division, are most susceptible to androgenesis induction, and the process of microspore differentiation occurs under the influence of abiotic stress in the period preceding culture initiation [43]. Among the most used stress-inducing factors are exposure to low or high temperatures, application of sugar- or nitrogen-free media, and treatments with colchicine, heavy metal ions, or mannitol [43]. In cereals, storing spikes at low temperature can disrupt cytoplasm polarity and impair the direction of spindle formation, leading to a change in the developmental pathway of microspores towards embryo formation [44]. Additionally, subjecting spikes to cold treatment prolongs the viability of anthers, which promotes synchronization of nuclear divisions and inactivates substances that inhibit androgenesis. In practice, a combination of two or three of these factors is applied, and depending on the method, they are used on whole donor plants, cut shoots with spikes, isolated spikes from leaf sheaths, or anthers alone [45]. The main factors determining the androgenic response in *in vitro* cultures include the genotype of the donor plants, the physiological state and growth conditions of the plants, *in vitro* medium composition, as well as physical factors during tissue culture and their interactions [46].

In cereals, the process of androgenesis is a more commonly used method for obtaining homozygous plants, and *in vitro* production of androgenic embryos is more efficient than methods based on gynogenesis [2]. *In vitro* anther cultures enable the rapid and efficient production of haploid plants, primarily due to the abundance of male reproductive cells. Thousands of microspores present in each anther can potentially give rise to androgenic embryos, and subsequently haploid plants [47]. However, the main challenge associated with the anther culture technique is the strong dependence of androgenesis not only on the species but also the genotype of the donor plant [46]. In addition, albinism, i.e., the formation of plants with disrupted chlorophyll production is a serious problem in anther and isolated microspore cultures. Such plants significantly lower the efficiency of the applied method, expressed in the number of regenerated, green plants with doubled chromosome number [48].

Despite the progress that has been made in improving the effectiveness of methods based on microspore embryogenesis in cereals, common oat is still considered a species recalcitrant to this process. The first oat regenerants using androgenesis were obtained by Rines [49], who acquired 1 haploid (n = 3x = 21) and 2 diploid (2n = 6x = 42) plants of the cultivar 'Stout' from around 65,000 isolated anthers. Prior to usual incubation at 22 °C and immediately following plating, these anthers were also heat-shocked at 35 °C for 24 hours. Sucrose concentrations were decreased by cold shocks, and the addition of various growth agents either had no impact on callus initiation rates or had negative consequences. Additionally, plant differentiation was not aided by these additions. MS medium without hormone, supplemented with 10% of sucrose, had the highest anther callus initiation frequencies among all media tested, but only from anthers which have been plated on a modified potato extract medium containing 2.0 mg L^{-1} 2,4-D and 0.5 mg L^{-1} kinetin (KIN), seedlings were produced. Subsequently, Kiviharju and Pehu [45] reported unsuccessful regeneration of androgenic embryos in *Avena sativa* L. and the production of haploid plants in *Avena sterilis* L. Five days heat pretreatment (32 °C) radically increased the embryos induction of *A. sterilis* (27.5 embryos/100 anthers), compared to 3 days (3.8 embryos/100 anthers) and 1 day (0.6 embryos/100 anthers) treatments. Embryo production of *Avena sterilis* was better on high maltose concentrations than that of *Avena sativa* L. The highest number of embryos was obtained on the medium with 14% maltose under both temperature pretreatments. During 10 weeks, 230 embryo-like structures were transferred onto differentiation media. Two haploid green plants survived transfer to the greenhouse, one from medium with 10% maltose, and another one from medium with 14% maltose. These plants formed panicles in the greenhouse, but they did not produce seeds. An attempt to induce androgenesis in Polish oat cultivars was made by Ślusarkiewicz-Jarzina and Ponitka [51], who tested the androgenic response of 15 genotypes on solid, liquid, and two-layer media. Oat panicles were harvested and cold-treated at 4 °C for a few days in N6 mineral salt medium [52] with 2.0 mg L^{-1} 2,4-D. Of the 45,000 anthers plated in this experiment, 637 ELS (1.4%; in all three physical states) were generated on W14 media. Genotype had a significant impact on the frequencies of ELS and green plants production. Eight genotypes yielded ELS (average 1.4/100 anthers). The plants were obtained only from two tested genotypes: CHD1780/05 (2.2%) and CHD1989/05 (1.3%), what is more plantlets were regenerated only from ELS obtained on solid medium, and not on liquid or double-layer media. Successful induction of ELS on W14 [53] and C17 media [54] from F3 generation of nine hexaploid oat hybrids was described by Ponitka and Ślusarkiewicz-Jarzina [55]. When compared to medium W14, which generated 137 ELS (from 0.6 to 3.3/100 anthers), medium C17 produced 409 ELS (from 0.6 to 12.1/100 anthers), achieving a greater induction efficiency for all genotypes. Crossing of Bohun × Deresz gave the best ELS induction rates on both media.

In the same year Skrzypek et al. [56] analyzed the possibility of inducing androgenic ELSs depending on the genotype, the length of the panicle cooling period, the density of anthers in a Petri dish, and the type and physical properties of the media. These studies have shown that pretreatment of oat panicles with low temperature (4 °C) for 1-2 weeks stimulated induction of ELS the most on W14 and C17 media. Even then, the authors found that the formation of ELS strongly depends on the plant genotype, and in a lesser extent on anther culture density or the type of media. Thus far, the highest efficiency of this method has been achieved by Kiviharju et al. [57], resulting in 30 green plants per 100 anthers from the crossing of Aslak × Lisbeth. In this study the cut tillers were pretreated for 7 days at 4 °C, the isolated anthers followed by treatment for 5 days at 32 °C on double layer induction medium. The effects of cytokinins, amino acids, reducing and ethylene-increasing factors, light and temperature conditions were tested with naked-type oat cv. 'Lisbeth'. Induction medium containing 2,4-D, BAP, ethephon, cysteine and myo-inositol, yielded significantly better green plant regeneration rates than the medium containing only 2,4-D and KIN, under the dark induction conditions for cv. 'Aslak' (2.1/100 anthers) and 'Lisbeth' (5.3/100 anthers). The conversion rate of ELS to green plants was also significantly better for cv. 'Lisbeth' (13%), and markedly higher (33%) for cv. 'Aslak' compared with other treatments, suggesting that the 2,4-D and KIN used together improve the quality of ELS. When dim light was used for induction, regeneration rates between these two induction media did not differ significantly from each other, probably due to a lower overall response.

Microspores competence for androgenesis varies not only among species or cultivars, but is primarily limited temporally, and has been referred to as the "developmental window" by Pechan and Smykal [58]. During this short period, it is possible to redirect microspores differentiation from gametophytic to sporophytic pathway by applying appropriate physicochemical factors known as stress factors. In addition, by manipulating the composition of the induction media, especially the content of auxins or their analogs, it is possible to effectively induce callus formation and subsequently embryogenic structures from microspores [59]. The architecture and morphology of oat panicles contribute to the non-linear maturation of anthers, which significantly complicates the identification of microspores at the appropriate developmental stage and likely accounts for the low efficiency of androgenesis in this species. Research conducted by De Cesaro et al. [43] has confirmed that the developmental stage of microspores depends not only on the genotype and age of the plant, but primarily on the position of the anthers in the inflorescence, which results in their uneven maturation. It has also been observed that microspores within a single anther often differ in their embryogenic competence due to slight differences in their developmental stage. The aim of the experiments presented in the work of Warchoł et al. [60] was to determine which external stimuli should be used to arrest the gametophytic pathway of the microspores and direct their development towards embryo formation. The optimization of media composition for the initiation of embryo-like structures was also performed. In addition, to correlate the developmental stage of microspores with shoot morphology, the distance from the base of the flag leaf to the penultimate leaf of the panicle was measured. In this way, four distances were determined: (i) 0.0 – 4.0 cm, (ii) 4.1 – 8.0 cm, (iii) 8.1 – 12.0 cm, and (iv) 12.1 – 16.0 cm, thereby selecting panicles based on the competence of their microspores for androgenesis. In the first stage of the experiment, the cultivars 'Akt', 'Bingo', 'Bajka', and 'Chwat' were tested for their susceptibility to androgenesis induction. For this purpose, panicles of donor plants were subjected to 4 °C for 14 and 21 days, and subsequently the isolated anthers were plated on C17 medium (Wang and Hu 1984) with the addition of picloram, dicamba, and KIN at a concentration of 0.5 mg L^{-1}. In the latter experiment, a significant impact of oat cultivar and the distance from the base of the flag leaf to the penultimate leaf of the inflorescence on the formation of ELS was observed. ELS formation was observed in all cultivars, but the highest number of structures was recorded in the cultivars 'Chwat' and 'Bingo' (3.6% and 1.6%, respectively). In addition, the highest ELS production was observed on anthers isolated from the youngest panicles, i.e., when the measured distance did not exceed 4.0 cm. This stage of plant development proved to be the most favorable for all tested cultivars not only in terms of the number of ELS obtained, but also haploid plants and DH lines. The second stage of the experiment aimed to increase the efficiency of androgenesis in the cultivars 'Bingo' and 'Chwat' by changing the length and type of thermal stress, as well as modifying the composition of the induction media. For the first time, the induction of oat ELS was carried out using a combination of low temperature (4 °C) followed by high temperature (32

°C). The induction media differed in the composition of micro- and macronutrients and growth regulators. The anthers were plated on C17 [61] and W14 [53] media, which were supplemented with the following auxins: 2,4-D (2.0 or 5.0 mg L^{-1}), picloram (0.5 mg L^{-1}), dicamba (0.5 mg L^{-1}) and NAA (2.0 mg L^{-1}), and cytokinins: KIN (0.5 mg L^{-1}) and BAP (0.5 mg L^{-1}). The experiment showed that the cultivar, pretreatment of panicles and composition of induction media significantly differentiated the number of ELS formed. More ELS were obtained from the anthers of the cultivar 'Chwat' compared to the cultivar 'Bingo'. A strong anther response to the applied combinations of growth regulators in the medium was also observed. Anthers formed ELS on all media, but the highest number of anthers (2.7%) was recorded on W14 medium with the addition of 2.0 mg L^{-1} 2,4-D and 0.5 mg L^{-1} KIN. Differences in androgenesis response depending on the hormones in the induction medium was manifested in the number of obtained haploid plants and DH lines. Based on the results, it was shown that treating oat panicles for 14 days with a low temperature of 4 °C, and a high temperature of 32 °C for 24 hours before anther isolation, increased the efficiency of androgenesis in the cultivar 'Chwat'. The most susceptible to this process were anthers isolated from panicles where the distance from the base of the flag leaf to the penultimate leaf did not exceed 4 cm. The best medium for induction of ELS and haploid plants was W14 with the addition of 2.0 mg L^{-1} 2,4- D and 0.5 mg L^{-1} KIN.

The literature suggests that increasing the concentration of Cu^{2+}, Zn^{2+} or Ag ions in the induction medium not only stimulates haploid embryogenesis of microspores, but also regulates numerous physiological and biochemical cellular processes. Cu^{2+} and Zn^{2+} ions stimulate normal division of chloroplasts, while Ag^{+} ions act as an inhibitor of ethylene biosynthesis in *in vitro* cultures, preventing aging of microspores [62-64]. Warchoł et al. [65] studied the efficiency of induction of embryonic structures in oat anther cultures depending on the concentration of $CuSO_4 \times 5\ H_2O$ (10 and 20 µM), $ZnSO_4 \times 7\ H_2O$ (90 and 180 µM) and $AgNO_3$ (25 and 50 µM). Copper, zinc, and silver ions were added to the media at two stages of androgenesis: during pretreatment of panicles of donor plants and as an addition to the induction medium. The results of the conducted experiment demonstrated a strong interaction between genotype and added ions, resulting in variations in the effectiveness of androgenesis depending on their concentration and application method. Ions added to the medium during the pretreatment of panicles had a significant effect on the formation of embryonic structures. The highest number of ELS was obtained when oat panicles were treated with 50% Hoagland medium supplemented with $CuSO_4 \times 5\ H_2O$ at a concentration of 10 or 20 µM (2.1% and 1.8%, respectively). The introduction of Cu^{2+}, Zn^{2+} or Ag^{+} ions into W14 induction medium had no significant statistical effect on the number of ELS. However, the highest number of ELS (0.8%) and haploid plants (0.2%) was obtained when anthers were cultured on W14 medium with the addition of 25 µM $AgNO_3$. When comparing the cultivars, it was observed that the highest number of ELS (0.7%) was obtained from the cultivar 'Chwat;, resulting in the production of haploid plants only in this cultivar. The present results demonstrated that the treatment of panicles with $CuSO_4 \times 5\ H_2O$ at a concentration of 10 or 20 µM increased the efficiency of androgenesis in the tested cultivars. Table 2 summarizes recent progress in androgenesis in various cultivars of *A. sativa*.

Table 2. Androgenesis of oat (*Avena sativa* L.).

A. sativa genotype	Culture conditions	Experimental outcomes	Reference
cv. 'Clintford', cv. 'Stout'	4 or 8 °C cold pretreatment	the highest anthers callusing initiation on MS medium with 10% saccharose and no hormones	[49]
line WW 18019, cv. 'Stout'	4 °C in the dark cold pretreatment for anthers from the main culm; 4 °C in the dark for cold pre-treatment for tillers, and MS medium with no PGRs; 32 °C heat pre-treatment for anther cultures	the pretreatment of isolated anthers for 5 days at 32 ◦C, before culture at 25 ◦C, is the key point	[50]
44 genotypes	4 °C in the dark cold pretreatment for anthers from the main culm;	callus growth, ELS production rates and plant regeneration	[66]

	MS medium with or without 5 mg L^{-1} 2,4-D for ELS induction;	differed between naked oat, wild oat, and crosses	
line WW 18019, cv. 'Kolbu'	4 °C in the dark cold pretreatment for anthers from the main culm; MS medium with 2,4-D and KIN for anthers; MS medium with 1 mg L^{-1} KIN for embryo structures; 32 °C heat pre-treatment for anther cultures	high 2,4-D concentrations enhanced embryo induction with or without heat pre-treatment	[67]
cv. 'Lisbeth', cv. 'Virma', cv. 'Cascade', cv. 'Kolbu', cv. 'WW 18019', cv. 'OT 257', cv. 'Stout', cv. 'Sisu', cv. 'Katri', cv. 'Yty', cv. 'Sisko', cv. 'Talgai', cv. 'Roope', cv. 'Salo'	tillers pretreated at 4 °C for 7 days; double layer induction medium MS (Murashige and Skoog 1962) or W14 (Ouyang et al. 1989) with 10% maltose and PGRs; 32 °C heat pre-treatment for anther cultures	regenerable-type embryos from heat-pretreated anthers on media containing 2, 3 or 5 mg L^{-1} mg 2,4-D and 0.2 or 0.5 mg L^{-1} KIN	[68]
cv. 'Lisbeth'	4 °C for 7 days for the tillers; 32 °C heat pretreatment; W14 medium with 10% maltose and PGRs for anthers, W14 medium with 2 mg L^{-1} NAA, and 0.5 mg L^{-1} KIN for ELS and regeneration; MS with 0.2 L^{-1} NAA for rooting	improved number of derived plants by application of W14	[57]
oat hybrids 1705/05, 1717/05, 1725/05, 1780/05, 2038/05, 1889/05, 1893/05, 1903/05, 1944/05, 1954/05, 956/05, 1967/05, 1985/05, 1989/05, 1997/05	4 °C for 6-9 days for the tillers in N6 medium with 2 mg L^{-1} 2,4-D; liquid, solid or double-layer W14 salts and vitamins, 5.0 mg L^{-1} 2,4-D, and 0.5 mg L^{-1} BAP for ELS induction;	development of ELS after 6 weeks of culture on liquid medium, and between the 7th and 8th weeks on solid and double-layer medium	[51]
cv. 'UPF 7', cv. 'UPF 18', cv. 'UFRGS 14', cv. 'Stout'	samples were collected when the distance between the flag leaf and the last node was one third of the distance between the last node and flag leaf	the use of anther size for the identification of microspore developmental stage is inefficient selection criterion	[43]
cross combination of hexaploid oat: Lisbeth × Bendicoot, Flämingsprofi × Rajtar, Scorpion × Deresz, Aragon × Deresz, Deresz × POB7219/03, Bohun × Deresz, Krezus × Flämingsprofi, Krezus × POB10440/01, Cwał × Bohun	4 °C for 6-9 days for the tillers in N6 medium with 2 mg L^{-1} 2,4-D; C17 induction medium with W14 salts and vitamins, 5.0 mg L^{-1} 2,4-D, and 0.5 mg L^{-1} BAP for ELS induction; 190-2 regeneration medium (Zhuang and Xu 1983)	the highest number of ELS on C17 medium; incubation at 22 °C in the dark for the first two weeks for the highest rate of green plants per 100 ELS	[55]
genotype 2000QiON43 (LA9326E86)	0.3 M mannitol pretreatment of the tillers for 7 days; W14 medium and continuous incubation at 28 °C; W14 medium for embryos observed; 0.2% colchicine for 4 h for DH	protocol for the production of microspore-derived embryos of oat, 80% of the plants were converted to DH	[45]

cv. 'Akt', cv. 'Bingo', cv. 'Bajka', cv. 'Chwat'	for tillers: 2 and 3 weeks at 4 °C, or 2 and 3 weeks at 4 °C followed by 32 °C for 24 h; for ELS induction: C17 medium with 0.5 mg L^{-1} picloram, 0.5 mg L^{-1} dicamba, and 0.5 mg L^{-1} KIN, or W14 medium with different concentrations of 2,4-D, NAA, and BAP	cold pretreatment and high temperature enhanced the technique efficiency; W14 medium with 2 mg L^{-1} and 0.5 mg L^{-1} KIN for the highest number of ELS	[60]
cv. 'Bingo', cv. 'Chwat'	2 weeks at 4 °C for tillers pretreatment in Hoagland and Arnon (1938) liquid medium alone or with Cu^{2+}, Zn^{2+}, or Ag^{+} ions followed by 32 °C for 24 h	ELS formation depended on cold pretreatment combined with Cu^{2+}, Zn^{2+}, or Ag^{+}	[65]

* ELS – embryo-like structures.

4. Wide crossing of oat (*Avena sativa* L.) with chosen species from Poaceae family

Obtaining DH lines of oat is very challenging compared to other cereals, and both breeding and biotechnological research conducted by research groups from Poland, the USA, Finland, or Japan unanimously confirm the recalcitrance of this species to haploidization. Since the techniques used successfully in other plants are still not very effective in oat, a commercially viable and efficient method of obtaining DH lines in this species has not yet been developed. The reasons for the low efficiency of the methods used, which typically yield between 0.5% and 10.0% of haploid embryos per emasculated floret [69], are attributed to the presence of numerous pre- and postzygotic barriers. Prezygotic barriers include all factors that hinder the successful fertilization of the ovum, i.e., the formation of a zygote. The most commonly listed prezygotic barriers include the inability of pollen to germinate on a foreign stigma, inhibition of pollen tube growth or rupture of the pollen tube [70]. On the other hand, postzygotic barriers impede the development of the zygote after fertilization [71] and are often a result of genetic incompatibility between the parental plants in wide crosses. Hence, a thorough understanding and overcoming these barriers can contribute to the development of an effective and universal method for obtaining oat haploids and subsequently DH lines. From a practical standpoint, this opens up new possibilities for improving haploidization methods not only for oat, but also for other plants recalcitrant to this process, such as legumes or woody plants. In addition, the production of new cultivars based on homozygous DH lines is becoming increasingly important in crop breeding programs and represents one of the key opportunities for adapting agriculture to ongoing climate change.

Pioneering work on obtaining DH oats through wide crosses with maize was conducted by Rines and Dahleen [72]. In a series of tests, pollen from maize (*Zea mays* L.) was applied to previously emasculated oat florets. Extracted caryopses and the embryos formed from them were then cultured on a MS medium with 7% sucrose and amino acids supplements. Recovered plantlets were raised in soil-filled pots until they were fully developed. Following the pollination of maize pollen from around 3300 emasculated oat florets, 14 haploid oat seedlings were successfully produced via embryo rescue technique. Subsequently, in studies conducted by Matzk [73], eastern gamagrass (*Tripsacum dactyloides* L.), pearl millet (*Pennisetum americanum* L.), and maize (*Zea mays* L.) were used to pollinate five varieties of oat. Postzygotic obstacles appeared while using early colchicine-mediated chromosomal doubling, exogenous auxins, and embryo rescue media. The embryo frequencies ranged from 0.4% in maize to 9.8% in pearl millet, depending on the type of pollinator. Although many plantlets in the embryo rescue process perished, beginning of growth usually occurred. Four viable plants were formed overall, including hybrids with pearl millet and for the first time using eastern gamagrass. One to four chromosomes from pollinator species were discovered in oat root tip cells during the tillering stage. The authors stated that while the efficiency of haploid formation (0.1%) was too low to use in plant breeding programs, crossings of oat with maize and pearl millet looked promising for the transfer of genes or chromosomes. In 2015, Nowakowska et al. [74] conducted research aimed at developing an effective method for obtaining oat DH lines and demonstrated a significant influence of individual steps of the procedure on the efficiency of haploid production. In these experiments, the optimal timing between emasculation of florets, pollination with maize, treatment of ovaries with auxin, as well as the appropriate timing for the isolation of haploid embryos were determined. The highest number of haploid embryos and plants was obtained by pollinating donor plants with maize pollen 2 days after emasculation and when auxins were applied 2 days after pollination, and embryos were isolated 3 weeks after pollination.

The treatment of oat ovaries after pollination has also been the subject of many experiments. Initially, Rines et al. [75] negated the need for auxin application to oat ovaries in order to increase the efficiency of wide hybridization. However, later on, Sidhu et al. [76] have emphasized that growth regulators not only prevent the degeneration of ovaries but most importantly stimulate and sustain embryo development until its isolation from the ovary. Currently, to facilitate the formation of oat haploid embryos, pollinated flowers are most often treated with the following synthetic auxins: 2,4-D, dicamba, picloram or gibberellic acid (GA_3) [69,74,76,77], or a combination of 2,4-D and GA_3 [11]. Only in a few cases, pollinated oat panicles were cut and placed in a solution containing sucrose and 2,4-D [78]. Research by Smit and Weijers [79] has shown that auxins play a key role in the early stages of embryogenic plant development, mediating the formation of zygotic embryos. Exogenous 2,4-D application alters the levels of endogenous auxins, such as IAA, thereby modifying their intracellular metabolism, which leads to the establishment of proper embryonic symmetry [80]. Warchoł et al. [81] described the process of determining which auxin to apply at 100 mg L^{-1} to the ovary after removal of the anthers and pollination with maize pollen to induce the development of haploid embryos. In this study, the most effective auxin for the production of oat haploid embryos was selected, and the impact of the applied hormones on subsequent stages of the procedure was described. This included the regeneration of haploid plants and the production of fertile DH lines. It was determined that the tested auxins did not have a significant impact on the number of enlarged ovaries (83.4% – dicamba; 83.9% – 2,4-D, calculated based on emasculated flowers), nor did they affect the number of resulting haploid embryos. However, the applied auxins significantly differentiated the capacity of embryos to germinate, thus affecting the production of haploid plants and DH lines. Nearly half of the generated embryos (48%) germinated when placed on 190-2 medium[61], but only 22% of them developed into haploid plants on MS medium. The final number of haploid plants was 45 (0.64%, based on emasculated florets) when using dicamba, and 104 plants (1.37%, based on emasculated florets) when 2,4-D was applied. The same concentration (100 mg L^{-1}) of the auxin analogues 2,4-D, dicamba, and picloram, as well as GA_3, were tested by Sidhu et al [76]. A specific growth regulator was applied to emasculated oat florets of the AK-1 and F1 hybrid genotypes on the 2nd and 3rd post-pollination days after being pollinated by maize pollen. The ability of each growth regulator to promote caryopsis development varied significantly between the two genotypes. The largest proportion of caryopses were produced by the dicamba treatment, 94.5% for AK-1 and 94.1% for 01095, respectively. Following 2,4-D and GA_3, picloram stimulated caryopses development. There was no discernible difference between genotype and growth regulator interaction. Kynast et al. [11] used phytohormone mixture (50 ppm 2,4-D + 50 ppm GA_3) and sprayed them 24 or 48 h after application of freshly pollen grains of maize Mo17, on emasculated oat panicles of Starter and Sun II to stimulate the development of the haploid embryos.

As mentioned above, the application of synthetic auxins to pollinated ovaries is a required step in the process of oat haploidization, because it leads to the proper distribution of endogenous auxins necessary for establishing embryogenic patterns. The studies conducted by Nowakowska et al. [74] and Mahato and Chaudhary [82] have emphasized that the efficiency of this process is influenced not only by the timing and method of hormone application, but primarily by their concentration. However, it is important to remember that using high concentrations of 2,4-D in *in vitro* cultures is toxic to plants and can result in tissue necrosis [83] or inhibition of embryo germination, as observed by Bronsema et al. [84] in maize. Considering that synthetic auxins applied at high concentrations exhibit strong toxic properties, which could consequently result in low survival rates of oat haploid embryos, the aim of the experiments published by Juzoń et al. [85] was to determine how two different 2,4-D concentrations affected the conversion of embryos into haploid plants and the subsequent development of fertile DH lines. Treating the ovaries with 50 mg L^{-1} 2,4-D yielded 27 haploid plants (8.5%, based on emasculated flowers), while using 100 mg L^{-1} of 2,4-D increased their number to 49 (16.3%, based on emasculated flowers). This trend persisted during the subsequent stages of the experiment, i.e., chromosome doubling and acclimatization. The higher concentration of 2,4-D led to the survival of all haploid plants from 17 genotypes after colchicine treatment (approx. 58% of obtained plants), resulting in twice as many DH lines (44 plants) compared to the lower concentration of 2,4-D (22 plants). Oat florets from genotype AK-1, that had been emasculated and pollinated with maize pollen were exposed to four different doses of dicamba (5, 25, 50, and 100 mg L^{-1}) [76]. With increasing dicamba concentration, the proportion of caryopses per floret grew considerably, reaching a maximum at 50 mg L^{-1}. Caryopsis development and embryos formation at 50 and 100 mg L^{-1} did not significantly differ. In Kynast et al. [11] studies the phytohormone combination (50 ppm 2,4-D + 50 ppm GA_3) has been proven to be more effective for embryo formation than the 100 ppm 2,4-D solution without GA_3.

Achieving approx. 10% of haploid embryos per emasculated florets in the first stage of wide hybridization does not guarantee a high efficiency of obtaining haploid plants or fertile DH lines. This is because the embryos formed after fertilization have a very low viability, and most of them die in the early developmental stages. Rines [69] has reported that the rate of embryo germination and their regeneration into plants typically falls below 20%. This was also confirmed by other studies, e.g., Warchoł et al. [81] and Juzoń et al. [85] isolated a relatively high number of embryos (683 and 619, respectively), but obtained only 149 and 76 haploid plants, respectively. In addition, as pointed out by Rines [69], the low regenerative capacity of embryos hampers conducting experiments that would allow for a statistical comparison of factors influencing their germination effectiveness, and thus assessing the reproducibility of the applied method. *In vitro* culture of embryos is an essential step in the process of plant haploidization. In oat, the haploid embryos resulting from wide crosses with maize are most often devoid of endosperm or this tissue is rudimentary. In consequence, the lack of access to nutrients leads to their death, and the *in vitro* culture stage where suitable conditions for their growth are provided is referred to as the embryo rescue technique [86,87]. In oat, it is necessary to isolate embryos from the ovary of the mother plant and transfer them directly to the medium approx. 21 days after pollination. The first attempts at cultivating plant embryos outside their maternal tissues for the purpose of obtaining an interspecific cross of *Linum perenne* × *Linum austriacum*, were conducted by Laibach [88]. Analyzing his research it can be seen that the smaller the embryo, the more complex medium was required to continue its growth and development. In practice, this means that the regeneration medium closely mimic the composition of the maternal endosperm, thereby providing the appropriate nutritional components for the specific developmental stage of the embryo. Meanwhile, the appropriate pH and osmotic pressure of the medium ensure the availability of the nutrients it contains. Literature data indicate that the concentration of carbohydrates depends on the developmental stage of the embryo. The younger the embryos, the higher should be the concentration of sugars in the medium (even up to 12%). Sugars added to the medium at the appropriate concentration serve not only as a carbon source for heterotrophic embryos, but also ensure a suitable level of osmotic pressure [89]. The necessity to overcome postzygotic barriers, including the selection of appropriate regeneration medium that serves as an endosperm substitute for developing embryos, makes germination a critical stage in obtaining oat haploids. Therefore, in the subsequent stage of wide hybridization, medium with a carefully selected composition, acting as a functional endosperm, determines the effectiveness of the method for obtaining oat DH lines.

Each species requires the development of a detailed procedure concerning both culture conditions and the appropriate selection of regeneration medium components. Warchoł et al. [90] optimized the composition of the medium for embryo germination under *in vitro* conditions in order to improve the efficiency of obtaining oat haploid plants. This experiment analyzed for the first time the germination capacity of embryos on media with varying maltose concentrations and pH values. The resulting haploid embryos were plated on 190-2 agar medium [91] enriched with KIN and NAA at a concentration of 0.5 mg L^{-1}. Maltose was added to the medium at two concentrations – 6% and 9%, and the pH was set at 5.5 and 6.0. MS medium without growth regulators (pH 5.8) containing 3% sucrose, was used as a control. The medium with a pH of 6.0, compared to pH 5.5, increased the efficiency of embryo germination, similarly to the increased maltose content (9%) in the medium. The previous studies on obtaining oat DH lines have shown a high efficiency in inducing haploid embryos, but unsatisfactory conversion of these embryos into plants [74,76,77]. The most frequently indicated reasons involved not only the lack of endosperm, but also disrupted hormonal balance and a range of deformations visible at various stages of their development [92]. Moreover, the development of haploid oat embryos is not synchronized in time. Despite the same time of ovary pollination with maize and auxin treatment, the embryos transferred onto regeneration media differ in size and level of differentiation. When establishing *in vitro* cultures of embryos isolated from immature seeds, known as pseudo-seeds [93], it is important to remember that the establishment of the axis of symmetry is possible only when the embryo's development, at least up to the early globular stage, occurs in its natural environment, i.e. in the ovary. On the other hand, the initiation of cultures must occur before the critical point of developmental arrest, namely the cotyledon formation stage. Culture of the resulting wide crosses, especially young oat embryos, has low efficiency. It has been observed that in *in vitro* cultures, the majority of these embryos either die immediately or form small seedlings, bypassing normal stages of embryogenesis. Additionally, in immature embryos, a phenomenon called "premature germination" is observed, typically occurring before the embryo axis formation. Since this type of germination is characterized by elongation of cells and low intensity of divisions, the resulting haploid plants are weak and usually die back [94].

The experiments conducted by Noga et al. [95] aimed to increase the conversion efficiency of haploid embryos into haploid plants by analyzing possible correlations between the germination capacity of haploid oat embryos at different developmental stages and the type of growth regulators added to the regeneration medium. Although the isolation was performed at the same time, i.e., 3 weeks after pollination, the embryos plated on the media exhibited differences in morphological structure. As a result, they were divided into four size classes: < 0.5 mm, 0.5 – 0.9 mm, 1.0 – 1.4 mm, and ≥ 1.5 mm. Subsequently, they were cultured on 190-2 regeneration medium [91], containing 9% maltose, 0.6% agar, and the following growth regulators: medium 1 with 0.5 mg L^{-1} KIN and 0.5 mg L^{-1} NAA; medium 2 with 1.0 mg L^{-1} zeatin (ZEA) and 0.5 mg L^{-1} NAA; medium 3 with 1.0 mg L^{-1} dicamba, 1.0 mg L^{-1} picloram, and 0.5 mg L^{-1} KIN. Microscopic observations revealed that embryos smaller than 0.5 mm were spherical, those ranging from 0.5 to 1.4 mm were elongated without distinct basal and apical parts, while embryos larger than 1.5 mm had a visible coleoptile and embryonic root. The conducted analysis of oat embryo germination capacity in relation to their developmental stage showed that the largest embryos germinated at nearly 80%, while the smallest ones lacked regenerative capacity and died after plating on the medium. Furthermore, it was observed that the size of haploid embryos and their germination capacity varied significantly among different oat genotypes. Similar observation was done by Sidhu et al. [76]. Compared to self-pollinated embryos, the white, embryo-like structures (ELS) of the four oat genotypes AK-1, Carrolup, Dumont, Mortlock, and S093658 obtained by crossing oats with maize, differed in size and shape. The type of growth regulators added to the regeneration medium did not exert a significant effect on the regeneration of haploid embryos into plants. Nevertheless, the highest percentage of haploid embryos (19%) germinated on medium with 0.5 mg L^{-1} NAA and 0.5 mg L^{-1} KIN, and the smallest (11%) on medium with 1 mg L^{-1} dicamba, 1 mg L^{-1} picloram and 0.5 mg L^{-1} KIN.

Skrzypek et al. [96] studied the role of light intensity applied *in vivo* to initiate haploid embryos and *in vitro* to regulate their development. The light intensity of 800 µmol m^{-2} s^{-1}, under which the donor plants were grown, more effectively stimulated the formation of haploid embryos (9.4%) compared to the light intensity of 450 µmol m^{-2} s^{-1} (6.1%). Light intensity during *in vitro* cultures of embryos also had an impact on their conversion into plants. Light intensity of 110 µmol m^{-2} s^{-1} during culture most optimally stimulated embryo germination (38.9%) and plant development (36.4%) compared to light intensities of 20, 40, and 70 µmol m^{-2} s^{-1}. In previous study of Sidhu et al. (2006) it was amply shown that temperature has no influence on the caryopsis development. Despite this they observed higher embryos production at 24°C. However, this difference was not statistically significant, most likely as a result of the few repetitions (one donor plant per treatment).

In order to understand the slow rate of oat embryo germination, research was conducted to investigate the phytohormone content in ovaries during embryo development. Additionally, the hormonal profiles of zygotic and haploid embryos were analyzed. Dziurka et al. [97], compared ovules with embryos (OE) and ovules without embryos (OWE). The latter study analyzed the phytohormone content using HPLC-MS/MS (high performance liquid chromatography with tandem mass spectrometry) and found significantly higher concentrations of IAA, trans-zeatin (tZ) and KIN in OE compared to OWE. It was also demonstrated that an excess of cytokinins in OE was detrimental to embryogenesis, while reduced cytokinin levels increased the efficiency of obtaining DH lines. The presence of IAA was detected only in OWE, indicating its role in plant aging processes. Further experiments revealed differences in the morphology and anatomy of the examined embryos, as well as variations in the phytohormone content [92]. Extremely low levels of endogenous auxins, higher levels of cytokinins, as well as a ten-fold higher cytokinin/auxin ratio in haploid oat embryos compared to zygotic ones, may suggest an earlier developmental stage for the former despite the embryo isolation performed at the same time. It was also demonstrated that in addition to hormonal regulation of embryogenesis, weak germination of haploid embryos could result from the overproduction of reactive oxygen species, leading to higher levels of low-molecular-weight osmoprotectants and stress hormones [92]. The summary of progress in wide crossing of *A. sativa* with various species from Poaceae family is presented in Table 3.

Table 3. Wide crossing of oat (*Avena sativa* L.) with chosen species from Poaceae family.

Plant material	Culture conditions	Experimental outcomes	Reference
Oat × maize oat: cv. 'Stout', cv. 'Starter', cv. 'Steele', cv. 'Black Mesdag' maize: A188, B73, Honeycomb, A619 × W64A	single haploid plants recovered by embryo rescue following field-grown maize pollen application to emasculated florets of growth chamber-grown oat	recovered haploids were from a different oat cultivar and different source of maize pollen – the process is not genotype unique	[72]
Oat × maize oat: genotypes AK-1, S093658, Carrolup, Dumont, Mortlock maize: early extra sweet F1, and Kelvedon Glory F1 varieties	100 mg L^{-1} GA$_3$, 2,4-D, 3,6-dichloro-o-anisic acid (dicamba) or 4-amino-3,5,6,-trichloro-picolinic acid (picloram) applied after pollination; four different temperature regimes (32/24, 24/20, 21/17 and 17/14 °C day/night) applied before flowering	the highest number of caryopses produced with dicamba, but without effects on embryo production; genotype dependent temperature effects	[76]
Oat × pearl millet Oat: cv. 'Best Enbaku' pearl millet: *Pennisetum glaucum* cv. 'Ugandi'	100 ppm 2,4-D dropped onto each floret 12 h after pollination; 100 ppm 2,4-D and 4% sucrose for the spike culture	retention of all seven pearl millet chromosomes in embryos from the crosses with oat; oat haploid developed to a fertile adult plant	[10, 78]
Oat × maize oat: lines Black Mesdag, GAF- Park, Kanota, MN97201-1, Preakness, Starter, Steele, Stout, Sun II, and F1 (MN97201- 1 9 MN841801-1) oat hybrid maize: lines Seneca60, *bz1-mum9*, A188, B73, Mo17, and the F1 (A188 9 W64A) maize hybrid	50 ppm 2,4-D and 50 ppm GA$_3$ for embryo formation delay of endosperm collapse	euhaploid plants with complete oat chromosome complements without maize chromosomes; aneuhaploid plants with complete oat chromosome complements and different numbers of retained individual maize chromosomes; uniparental genome loss during early steps of embryogenesis causing the elimination of maize chromosomes in the hybrid embryo	[98]

Oat × maize Oat: genotypes 80022, 80031, 81711, 81350, 81384, 81524, 81559, 82072, 82091, 82230, 82266, 83200, 83207, 83213, 83421, 83430, 85924, and 85931 Maize: Waza, Dobosz, and Wania	oat florets pollinated with maize pollen after 0, 1 or 2 days; 100 mg L^{-1} 2,4-D or 100 mg L^{-1} dicamba placed on the floret pistils 1, 2-, 3-, 4-, and 5-days following pollination	genotype-dependent haploid embryo formation and plant regeneration; 2nd day pollination together with auxin treatment was the most effective	[77]
Oat × maize Oat: 80031 - Deresz × Szakal, 81350 - Krezus × STH 454, 82072 - Bajka × STH 454, 82091 - Bajka × STH 7706, 83213 - Flamingstern × Chwat. Maize: *Zea mays* L. var. *saccharata*, **oat × sorghum** sorghum: *Sorghum bicolor* (L.) Moench **oat × common millet** Common millet: *Panicum miliaceum* L.	100 mg L^{-1} dicamba one day after pollination; enlarged ovaries collected at 2, 3 and 4 weeks after pollination cultivated on 6 or 9% of maltose	2.5 – 6.9% of HE for genotypes pollinated with maize, 1.3% for sorghum, and 1.2% for millet; the highest frequency of HE germination and number of plants 3 weeks after pollination; 9% maltose for embryo formation, germination, and haploid plants development	[74]
Oat × maize oat: STH 4.8456/1, STH 4.8456/2, STH 4.8457/1, STH 4.8457/2, STH 5.8421, STH 5.8422, STH 5.8423, STH 5.8424, STH 5.8425, STH 5.8426, STH 5.8427, STH 5.8428, STH 5.8429, STH 5.8430, STH 5.8432, STH 5.8436, STH 5.8440, STH 5.8449, STH 5.8450, STH 5.8458, STH 5.8460 maize: Waza	< 0.5 mm HE, 0.5–0.9 mm HE, 1.0–1.4 mm HE, and ≥ 1.5 mm HE on 0.5 mg L^{-1} KIN and 0.5 mg L^{-1} NAA, or 1 mg L^{-1} ZEA and 0.5 mg L^{-1} NAA, or 1 mg L^{-1} dicamba, 1 mg L^{-1} picloram, and 0.5 mg L^{-1} KIN	germination of HE ≥ 1.5 mm on medium with 0.5 mg L^{-1} NAA and 0.5 mg L^{-1} KIN	[95]
oat × maize 32 oat genotypes were pollinated with *Zea mays* L. var. *saccharata* (maize) genotypes: MPC4, Dobosz and Wania	different light intensity during the growing period of donor plants and *in vitro* cultures	9.4% HE formed in a greenhouse, 6.1% in a growth chamber; 38.9% of embryo germination, 36.4% conversion into plants, and 9.2% DH line production with 110 µmol m^{-2} s^{-1} light intensity	[96]
Oat × maize Oat: F1 progeny of thirty-three oat genotypes maize: *Zea mays* L. var. *saccharata* (maize) genotypes MPC4, Dobosz and Wania	immersion of haploid plants for 7.5 h in a 0.1% colchicine, 40 g L^{-1} DMSO, 0.025 g L^{-1} GA^3 at 25 °C and 80–100 µmol m^{-2} s^{-1} light intensity for chromosome doubling procedure	From 149 haploid plants 61 survived chromosome doubling procedure, 52 (85%) were fertile and produced seeds	[81]
Not specified	colchicine solution with DMSO for chromosome doubling	detailed description of a method for DHs generation	[1]

Oat × maize 80 oat genotypes pollinated with maize cv. 'Waza'	colchicine solution applied on HP roots for chromosome doubling	from 138 oat lines, the presence of maize chromatin was indicated in 66 lines from which 27 OMA lines were fertile and produced seeds	[99]
Oat × maize oat: F1 progeny of twenty-two oat genotypes pollinated with *Zea mays* L. var. *saccharata* (maize) genotypes MPC4, Dobosz and Wania	for chromosome doubling HP roots were immersed in a 0.1% colchicine with 4% DMSO, 0.025 g L^{-1} GA^3, and 20 µl of Tween 20, left for 7.5 h at 25 °C and 80–100 µmol m^{-2} s^{-1} light intensity	591 HE formed, 48 fertile DH plants producing in all 4878 seeds	[90]
Oat × maize oat: F1 progeny of twenty-nine oat genotypes pollinated with *Zea mays* L. var. *saccharata* (maize) genotypes MPC4, Dobosz and Wania	9465 florets were pollinated with maize pollen 2 days after emasculation and treated with 2,4-D at 50 mg L^{-1} and 100 mg L^{-1}; colchicine solution applied on HP roots for chromosome doubling	higher 2,4-D concentration is more efficient in obtaining haploid/DH plants with better vitality and fertility	[85]

* HE – haploid embryos; DH – doubled haploids.

5. Summary

A variety of variables, including screening practices, tolerance sources, and mechanisms, gene action and inheritance, and their linkages to agronomic traits, all have an impact on the choice of an appropriate breeding strategy for the creation of cultivars that are of interest to us. As new cultivars have been created mostly using conventional breeding methods, the typical approach involves recombining DNA by distinct chromosomal assortment and crossing-over employing both the sexual cycle and the genetic diversity already present. Conventional breeding methods are focusing mainly on: (1) yield rather than particular agronomical traits; (2) the tolerance traits, which include issues caused by genotype, by environment, or genotype × environment interactions; (3) employing fundamental physiological characteristics as indications of tolerance to environmental stresses. Traditional breeding has been slow to produce high-yielding plants that are resistant to biotic stress conditions due to difficulties in accurately defining the target environment, complex interactions between e.g., drought tolerance and surroundings, and a lack of adequate screening methods.

In conventional breeding, the number of generations is needed to produce stable variations by natural segregation from the heterozygous progeny of the original crosses. The in vitro methods could eliminate the necessity for back-crosses or selfing repeatedly. However, plant tissue culture techniques have developed significantly since the first publication in 1967 on effective plant regeneration from oat callus are still low and strong genotype dependent. The variation in the establishment of embryogenic callus from the different oat genotypes tested on a few types of initiation media suggests that genotype × media interactions are still an important factor in further improving tissue culture methodologies. Oat plants must be grown year-round for the isolation of immature embryos, which is costly, requires elaborate equipment, and may subject donor plants to physiological fluctuation that could influence how frequently tissue cultures are started. Furthermore, it takes a lot of effort to isolate immature embryos from oats since the panicle's fertilization is not as synchronized as it is for example in wheat or barley. As a substitute, mass-producing mature seeds is affordable and provides a reliable source of explant for starting tissue cultures, potentially removing the variability in culture initiation from immature embryos. On the other hand, by manipulating gamete development, doubled haploid (DH) approaches can regenerate fully homozygous plants in just one generation. For oat species, the generation of doubled haploids has become an essential tool in advanced plant breeding. However, doubled haploids are now being used in less sophisticated breeding programs due to the development of new, more cost-effective, and efficient large-scale techniques. Because of the oat genome's size and complexity as well as the fact that DNA sequence information is just now becoming available, oat molecular genetics research lags far behind that of other cereal crops. Since the first linkage-based QTL map in oat was published there have been ongoing efforts to improve the density of the map using various kinds and quantities of markers [100]. The main goals of oat breeding as well as genetic modification are directed at improving disease resistance, abiotic stresses, yield and agronomic traits, quality for milling, feed, and end uses, and obtaining naked types of grains [101]. However, hexaploid oat genome sequencing (*Avena sativa* L.) has recently been completed, and its genome size is to be over 11 Gb composed of two different ploidy species as *Avena longiglumis* (AA) (3.7Gb) and *Avena insularis* (CCDD) (7.3Gb) which aids in quickening the process of improving the oat for several qualities [100].

These reviewed findings may encourage the spread of this technology's application for accelerating and creating new oat breeding opportunities. We expect that this review will also assist molecular scientists in the production of doubled haploid segregating populations in oat species, which are necessary for the production of genetic maps employing molecular markers.

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