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Review
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AN OVERVIEW OF HAPLOID AND DOUBLE HAPLOID PRODUCTION METHODS IN WHEAT

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Abstract: For about a century, researchers have been working on haploidy approach. Progressively, they discovered the importance and usefulness of haploids in various research fields. On the other hand, it is suggested that climate change impacts on crop production, especially wheat, requires rapid and efficient methods of plant breeding to produce new cultivars with a sufficient level of biotic and abiotic tolerance to avoid significant production loss. Haploid plants are the source for producing homozygous pure lines and genetic variability for breeding programs. It reduces the time for producing pure and stable forms of new recombination by half in plant breeding. Furthermore, haploids are source for exclusive male plants generation, induction of mutations, stress resistance and tolerance cultivars, cytogenetic studies, and doubled haploid crops. This review presents a brief overview of the haploid wheat production methods and previous successful experiments on producing haploid wheat.

Keywords: Anther culture, Gynogenesis, Microspore culture, Wheat haploid

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1. Introduction

A plant with gametic chromosomal number is referred as haploid plant (from the Greek word *haplous*, which means single). They might arise naturally or as a result of different induction procedures (Watts et al., 2018). This phenomenon can occur spontaneously in the nature but it is rare or can be induced by *in vitro* or *in vivo* methods (Dwivedi et al., 2015). Haploid plants were discovered for the first time in 1922 by Blakeslee and his colleagues when studying *Datura stramonium*. In 1964, Guha and Maheshwari produced *in vitro Datura* haploid plants from anthers which increased the potential of haploidy in plant breeding. Many reports were published since then on many other plant species and for producing new cultivars. Many characteristics separate haploid plants from their diploid equivalents. Haploid plants have small size, narrow leaves, and relatively slow growth, which is partially due to their decreased cell size; in general, cell volume in plants is directly related to their ploidy level (Dunwell, 2010).

In wheat, haploid plants can be obtained from diploid or polyploid species. Haploid plants originated from *Triticum monococcum* L. (Einkorn wheat, diploid) have 2n = x = 7 chromosomes, whereas a haploid derived from a polyploid such as *Triticum turgidum* L., (durum wheat) (2n = 4x = 28; AABB) or *Triticum aestivum* L., (bread wheat) (2n = 6x = 42; AABBDD) called polyhaploid. Thus, their polyhaploid has 2n = 2x = 14 chromosomes with the genomic composition of AB, and 2n = 3x = 21 chromosomes with the genomic composition of ABD, for *Triticum turgidum* L. and *Triticum aestivum* L. respectively (Basu et al., 2011).

Haploid plants provide valuable information regarding recombination and genetic control of chromosomal pairing. Perhaps the most significant use of haploid plants was for crop improvement and doubled haploid production in plant breeding, which significantly shorten breeding cycles through the simultaneous genetic fixation at every locus within a single generational step (Kalinowska et al., 2019).

2. Methods of Haploid Plant Production

For many years, haploids have been presented for genetic studies. They were mostly obtained spontaneously from interspecific hybridization or by irradiated pollen, but usually only infrequently and in very small numbers. However, this situation changed after Guha and Maheshwari (1964) discovered that haploid plants could be obtained on a regular basis and in relatively large numbers by placing immature anthers of Datura into *in vitro* culture. The ability to produce haploid plants is a tremendous asset in genetic and plant

breeding studies.

Haploids can occur spontaneously or be induced by *in vivo* (chromosome elimination, intra specific hybridization, and inter specific hybridization) or *in vitro* (male or female gametes, or from unfertilized eggs). Currently, the main methods applicable for haploid production are androgenesis (cultured anthers or isolated microspores undergo embryogenesis/organogenesis directly or through intermediate callus), gynogenesis (cultured unfertilized isolated ovules, ovaries of flower buds, develop embryos from cells of the embryo sac), parthenogenesis (development of an embryo by pseudogamy, semigamy or apogamy), and wide hybridization followed by chromosome elimination from one of the parents. Generally, these methods require several main steps; starting with the induction of microspore derived embryos or calluses, then regenerate the plant either by embryogenesis (from embryos) or organogenesis (initiation and growth of shoots then root develop) and the final step is chromosome doubling of regenerated plants (Kim and Baenziger, 2005).

As doubled haploids become essential in plant breeding, considerable efforts to improve haploid embryogenesis and increase the frequency of recovery has been made. Puolimatka and Pauk (2000) explored different induction medium, induction period, and physical state of medium for improving spring wheat anther culture efficiency. For this purpose, two W 14 modified medium (double layer medium W14dl and Ficoll supplement W14f,), four different carbohydrates (sorbitol, maltose, trehalose, and cellobiose), and four glutamine concentrations (2.5, 5, 7.5, and or 10 mmol l^{-1}) were used for studying the androgenic response of two wheat cultivars. These were tested for induction durations of 6, 7, 8 and 12 weeks after isolation of the anthers. Based on the findings, embryo-like structures should be transferred to regeneration medium after 7 weeks of induction which was the optimum duration for obtaining maximum regeneration. However, medium supplemented with Ficoll, trehalose, cellobiose, maltose glutamine had no significant effect on studied parameters.

Xynias et al. (2001) cultured anthers of two spring wheat cultivars (Vergina and Acheloos) for haploid production. Spikes with microspore at mid to late uninucleate developmental stage were collected, half of these were pre-treated with cold (4 °C) for one week, then all samples were transferred to W14 induction medium and incubated at 28 °C and 32 °C. Experiment results suggested that genotype affects haploid production of spring wheat rather than temperature of incubation, also cold pre-treatment is not always required. In contrast, Sibi et al. (2001) claimed from findings of two seasons conducted experiment that most of the durum wheat genotypes studied had a significant interaction between genotype, induction medium and the duration of cold pre-treatment. They cultured unpollinated ovaries of six durum wheat genotypes (Sarif, Isly, Cham1, Jori, Oued, Cocorit, and Zénati) in two different induction media after cold pre-treatment at 4 °C in a cold chamber for 7, 11 or 15 days under two photoperiods (12 and 16 hours). Results showed that cold pre-treatment was necessary for tillers before anthesis for 7 or 15 days.

Zheng et al. (2001) used freshly isolated microspores as a method for producing wheat haploid and doubled haploid plants. In the experiment, six wheat cultivars (Pavon 76, WPB 926, Chris, Waldron, WED 202, and Calorwa) were grown at controlled greenhouse. From fresh tillers, microspores at mid to late uninucleate stage were isolated and then treated with four concentrations (0.06, 0.12, 0.18, and 0.30 mM) of inducer chemical solution (2-hydroxynicotinic acid, 2,3-pyridine carboxylic acid, 2,4-dihydroxy pyrimidine 5-carboxylic acid, 3-butanedione monoxime, benzotriazole-5-carboxylic acid, sulfanilamide, anthranilic acid, DL-histidine, benzotriazole, violuric acid monohydrate) for a short time. These chemicals were used to improve plant regeneration through triggering microspore embryogenesis and maintaining viability. The suggested method was easy and direct in determining the potential of the used chemicals for inducing embryogenesis. The method increased the survival rate of the fresh microspores when treated with 0.18 mM 2-hydroxynicotinic acid.

In durum wheat, genotypic variations in anther culture are well recognized and the medium for anther culture seems to be critical for haploid plant regeneration as Jauhar (2003) claimed. Furthermore, interactions between genotypes and growth conditions, as well as genotypes and medium, were also significant. Immature spikes were harvested and treated with cold, then sterilized and placed in water to prevent drying. Spikelets located at the middle of the collected spikes were inspected under light microscope to determine microspores developmental stage. Only those at mid uninucleate stage were cultured in different media at 25 °C. Comparing four different culturing media (BAC-1, BAD-I, BAD-3 and M-42), which were developed by previous scholars, showed that anther response was higher to the media BAC-1.

García-Llamas et al. (2004) examined the influence of different applications of hormone combinations and concentrations on caryopses, embryos and haploid production of durum wheat and maize intergeneric hybridization. Before flowering stage, tillers carrying emasculated spikes were cut off, sprayed with fungicide and insecticide, and placed in 40 g/l sucrose, 100 mg/l silver nitrate and 8 ml/l sulphurous acid solution then transferred to controlled growth chamber. Emasculated spikes were pollinated and placed in hormone containing medium for 48 hours. The hormone treatments were; (1) 100 mg l^{-1} 2,4-D (2) 5 mg^{-1} or 50 mg^{-1} dicamba (3) 95 mg^{-1} 2,4-D with 5 mg^{-1} dicamba (4) 50 mg^{-1} 2,4-D + 50 mg^{-1} dicamba. Later they were transferred to a hormone free medium, and caryopses number, embryos and haploid plants were counted. The authors stated that applying

dicamba with 2,4-D or only dicamba enhanced haploid plant production using the crosses of durum wheat with maize.

An enhanced wheat haploid production method using anther culture was suggested by Kim and Baenziger (2005) in which plants were grown from microspore derived embryos in a single medium and culture environment. For this reason, several experiments were carried out comparing different auxins, phenylacetic acid levels, various cytokinin types and different incubation conditions. In the first experiment, anthers were cultured in a modified C17 medium with five different auxins (9 mM 2,4-D, 9 mM dicamba, 8.3 mM picloram, 11.4 mM IAA, and 14.7 mM PAA). In the second experiment, anthers were cultured in a modified 85D12 basal medium with four different PAA concentrations (7.3, 14.7, 29.3, and 58.7 mM). Three types of cytokinin (kinetin 4.6 mM, zeatin 4.6 mM, and 6-benzylaminopurine 4.4 mM) were compared in experiment three. In experiment four, different incubation conditions were evaluated at 27°C and 30°C in the dark, and 30°C/12 hours dayligth. Finally, the haploid production protocol was improved by pre-treating the tillers at 4°C for 7 to 14 days, then placing the anthers on a modified 85D12 basal medium supplemented with zeatin and phenylacetic acid. The embryos from microspores were obtained in 2–3 weeks and after 3-4 weeks after culturing plants were produced.

Letarte et al. (2006) examined the effect of gum arabic (arabinogalactan protein) and Larcoll (arabinogalactan) to enhance wheat embryogenesis induction using microspore culture. The spikes containing anthers of two wheat varieties (Pavon 79 and Chris) were pre-treated at 4°C with cold mannitol (0.4 M), then microspores were isolated and culture in modified MS medium. The medium contained maltose (90 g l^{-1}), U2.5 amino acid mixture (355 mg l^{-1}), glutamine (975 mg l^{-1}), kinetin (0.5 mg l^{-1}), and PAA (2 g l^{-1}) with different concentrations of Larcoll or gum Arabic (1, 5, 10, 25, 50, or 100 mg l^{-1}). When the size of the embryo like structures were near 2 mm, they were placed on MMS5 medium containing maltose (30 g l^{-1}), U2.5 amino acid mixture (355 mg l^{-1}), GA3 (0.5 mg l^{-1}), kinetin (0.2 mg l^{-1}), PAA (0.5 mg l^{-1}), and $CuSO_4$ (10 mM) for differentiation. The researchers found positive effects of both compounds on the microspore viability and in embryo quantity and quality as well. Also, wheat plants from microspore cultures were generated by using gum arabic without ovaries presence.

Pratap et al. (2006) found that haploid induction of triticale x wheat hybrids and intergenotypic triticale was more efficient and economically valuable using chromosome elimination technique rather than anther culture. They conducted crosses under field conditions among 8 wheat cultivars (Raj 3702, HS 396, PW 565, VL 798, RL 14-1, VL 802, UP 2418, and HPW 42) with 10 triticale genotypes (TL 2919, ITSN 109, TL 2920, DT 126, ITSN 163, TL 2900, DT 123, ITSN 105 #58, ITSN 65, and DT 123,). The selection of the crossed cultivars was based on their parental diversity, yield traits, quality traits of grain, and resistance to disease. From each cross, main spike was selected and cut at the mid- to late uninucleate stage of the pollen development and pre-treated with cold at 4 ◦C for 48 hours before culturing. Then the excised anthers were cultured on Potato-II medium containing glutamine (0.5 mg l^{-1}) and supplemented with 2,4-D (2 mg l^{-1}) and kinetin (0.5 mg l^{-1}). The results indicated that the frequency of haploid embryo formation for the first-generation genotypes was 20.4% and 17.0% for triticale x wheat and triticale x triticale crosses respectively using chromosome elimination technique through crossing with maize. Furthermore, the frequencies of haploid plantlet regeneration were significantly higher, 42.7% and 49.4% for both triticale x wheat and triticale x triticale for the first-generation genotypes, while these values were 8.2 and 4.0% using anther culture method.

An efficient system for producing haploid/doubled haploid wheat using microspore culture without stress was reported by Shariatpanahi et al. (2006). In this system, tillers with late-unicellular to the pre-mitotic stage microspores were harvested and cold treatment was not applied to the tillers. The microspores were isolated using Shed microspore culture (SMC) (culturing in starvation medium), and the culture of freshly isolated microspores without stress (WM), and the same regeneration condition was used for both systems. A significant increase was recorded in the frequency of regeneration and green plants percentage using culture of freshly isolated microspores without stress (WM) method comparing to Shed microspore culture (SMC).

Ovary co-culture method was demonstrated by Broughton (2008) for Australian spring wheat as protocol for anther culture. Anthers at mid to late and late uninucleate microspores development stage of the two spring wheat varieties were incubated in liquid induction medium containing ovaries. The medium was supplemented with 0.5 mg l^{-1} Kinetin and 2 mg l^{-1} 2,4-D or 1 mg l^{-1} 6-Benzyladenine (BA) and 1 mg l^{-1} Indole-3-acetic acid (IAA). Results specified that using ovary co-culture method had a significant effect on the embryo-like structures and green plants production in Australian spring wheat varieties. Mean number raised from 7.6 to 50.1 and from 0.6 to 8.9 for embryo-like structures and green plants respectively in induction medium containing five ovaries. Furthermore, these findings lead to the development of a protocol for Australian spring wheat anther culture which is used in small scale breeding program.

Using butanol alcohol was claimed to enhance anther culture production in wheat. Soriano et al. (2008) tested the effect of *n*-butanol, sec-butanol and tert-butanol on microspore embryogenesis of two *Triticum aestivum* varieties in two experiments. After determining microspores development stage, pre-treated microspores at the mid to late uninucleate stage were inoculated in 2

ml liquid MS3M with 0.1% or 0.2% *n*-butanol (experiment 1), and with 0.2% tert-butanol, secbutanol or *n*-butanol (experiment 2). For each experiment, the number of responsive anthers, callus, embryo, green and albino plants per 100 anthers, and chromosome doubling percentage were recorded. Statistical analysis showed a strong genotypic and treatment effect on the responsive anthers number, divisions, embryos and green plants. Treatment with 0.1 and 0.2 % *n*-butanol triggers microspore division and embryogenesis. Thus, strong embryos and green plants can be produced.

Broughton (2011) reported similar effect of *n*-butanol with adding macronutrients and calcium to the mannitol pre-treatment medium, improving embryo and green plant production. In the experiment, anthers of Australian spring wheat cultivars were placed on two different medium; the first medium contained 182 g l^{-1} mannitol and 10 g l^{-1} agar, and the second medium contained 5.9 g l^{-1} CaCl2.2H2O, macronutrients, 182 g l^{-1} mannitol and 10 g l^{-1} agar. Then, anthers were divided between 0.2% *n*-butanol and without n-butanol (control) and both treatments were directly placed on the ovary conditioned in liquid induction medium plus ovaries. A significant increase in number of embryos and green plants was observed when combining *n*-butanol with calcium and macronutrient addition to the mannitol pre-treatment medium.

Sourour et al. (2011) implemented three experiments on different concentrations of $AgNO_3$, 2,4-D and their combination to originate an effective method of haploid production through intergeneric cross. They crossed two landraces and two durum wheat cultivars (as female parent) with a maize genotype under field grown conditions, and these pollinated spikes with maize pollens (male parent) were cultured in a solution supplemented with 8 ml l^{-1} of sulphurous acid and 40 g l^{-1} sucrose. Three different experiments were conducted on 10 spikes from each cross after 12, 14, 16, 18 and 20 days of pollination to examine the effects of applying $AgNO_3$ at different concentrations (0, 25, 50, 75, 100, 125, 150, 175 mg l^{-1}), 2,4-D (0, 25, 50, 75, 100, 125, 150, 175 mg l^{-1}), and a combination of 100 mg l^{-1} of 2,4-D + 75 mg l^{-1} of $AgNO_3$. The frequency of developed ovaries, formed embryos, and haploid plants were recorded for each experiment. The researcher claimed that the method is efficient for plant regeneration from durum wheat × maize crosses as 877 plants were regenerated and all obtained haploid plants were green. In addition, the results indicated that higher numbers of embryo and haploid plants were obtained from the combination of the two compounds.

Microbial contamination is a major concern in microspore culture which affects the success of the whole process. Asif et al. (2013a) examined contamination using two antibiotics, cefotaxime and vancomycin, to enhance anther culture during the induction phase. Spike of two wheat cultivars and one triticale cultivar were sterilized and from each, four ovaries placed in petri dishes with microspores at mid to late uninucleate microspore development stage. These microspores were treated with different concentrations of antibiotics (Van 100 $mg^{-1}l$, Van 500 $mg^{-1}l$, Cef 50 $mg^{-1}l$, Cef 100 $mg^{-1}l$, Van 100 $mg^{-1}l$ + Cef 50 $mg^{-1}l$, and Van 500 mg/l + Cef 100 mg/l). After incubating for 20-30 days at 28 ^{o}C at dark, microspore derived multicellular structures were observed and contamination signs were checked daily. Moreover, the number of embryos or embryolike structures, albino and green plants were recorded. Through fatty acid analysis and 16S ribosomal RNA sequences analysis, contamination with five bacteria species and yeast was recognized. The analyzed data showed that Cefotaxime at 50 and 100 mg/l besides preventing contamination also improved microspore culture, since the number of microspore derived embryo like structures and the ratio of green plants increased.

The importance and the role of antioxidants in green plant production frequency and albinism by isolated microspore culture was investigated by Asif et al. (2013b) on the embryogenesis or embryo development. For this purpose, two antioxidants with different concentrations were supplemented in the NPB99-10F induction medium. Plastid antioxidants treatments were glutathione, ascorbate and salicylic acid at 200 nM (1×) and 2 µM (10×), and mitochondrial antioxidants 10 mM proline, 10 nM MB, 100 µM NtBHA, 100 µM 2iP, and 100 µM of 2iP + 10 mM of proline + 10 nM MB + 100 µM NtBHA. Microspores from spikes of four spring wheat cultivars and one triticale cultivar were isolated and used in each experiment to study the effect of the two antioxidants supplemented in the induction medium. At 10 to 14 days, multicellular structures and embryos development was verified, and the determination of embryos or embryo like structures number was made after 3 to 4 weeks of isolation, green and albino plants number determination was made after the embryo germination. The study concluded that adding glutathione and proline to the culture medium increased the embryo and green plant formation.

The effect of cold pre-treatment and genotypic effect on microspore culture was researched by Khound et al. (2013). Anthers were dissected from spikes containing mid to late uninucleated stage microspores of three spring and three winter wheat cultivars and incubated at 25 ^{o}C - 28 ^{o}C for 4 - 5 days in dark, then for another 5 days at 4 ^{o}C for cold pre-treatment. Those microspores were incubated in the dark at 27-28˚C for 25 to 30 days after co culturing in induction medium (MMS4) containing ovaries (5–7). It was concluded that for both winter and spring wheat cultivars the number of multicellular structures, transferable embryos and green plants raised with cold pre-treatment in comparison to the control treatment.

Scagliusi (2014) setup a protocol for isolated microspore culture for Brazilian bread wheat genotypes suggesting that the method can be used in the Brazilian wheat breeding program. In the experiment, spikes of three

wheat genotypes were pre-treated with cold at 4 °C for 3 weeks in the dark, then obtained uninucleated microspores were cultured in petri dishes containing semi-liquid NPB 99 media, and finally, four ovaries were added to each petri dish. The evaluation of the embryo like structures were made on a daily base for gametic source confirmation, and green and albino plants number per genotype was recorded. The method recommended that induction medium along with ovary co-culture were essential to promote microspore culture. Furthermore, the influence of genotypic variation among wheat genotypes for microspore culture cannot be ignored.

Gupta et al. (2016) conducted various wheat x maize crosses to standardize a haploid production protocol. Five commercial wheat cultivars were used in wheat x maize haploid production and nine F1 lines were used for developing homozygous lines. In the crosses they carried out, spikes were pollinated after emasculation with fresh maize pollen after 3 to 4 days. Then, spikes were treated after 24, 48, 72 hours of pollination with 200 ppm 2,4-D to sustain embryo formation. Dissected embryos from caryopses were transferred to half strength MS medium supplemented with 40 g l^{-1} sucrose and solidified with 3g l^{-1} phytagel having pH 5.8, and treated with cold for 8 hours then incubated in the dark. After germination, regenerated plants were kept at 25˚C with 8-10 hours photoperiod. Finally, the developed haploids were placed for 30 days in hardening medium. The recorded data from the plants were the caryopses formation frequency, embryo formation frequency and plantlet regeneration frequency. Results indicated that in case of cultivars the range of caryopses formation frequency, embryo formation frequency, and plant regeneration frequency ranged between 25.9 to 51.4 %, 4.6 to 22.4 %, and 6.4 to 63.6 % respectively. For the F1 corn lines, the range was 21.4 to 60.5%, 1.3 to 21.2 % and 9.8 to 44.4 % for caryopses formation frequency, embryo formation frequency, and plant regeneration frequency respectively**.** In total 100 haploid plants were regenerated.

Lantos and Pauk (2016) compared anther culture of 10 winter wheat F1 combinations using two media in order to examine the genotypic effect and induction medium on the anther culture efficiency. Tillers were pre-treated for 2 weeks at 2 to 4 °C, anthers collected from tillers at early and mid uninucleated stages and cultured in W14mf and P4mf medium. Anthers within each medium were treated with heat shock (32 °C) for three days, and then incubated for 8 weeks at 28 °C. Every week, observations were made for the microspore derived embryo like structures and for embryo like structures. Green plantlets, albinos, and transplanted plantlets were measured for anther culture efficiency. The results indicated significant effect of the genotypes and induction medium on all measured parameters. Using W14mf medium resulted in increasing green plant regeneration compared to P4mf medium, 16.9% and 9.6% respectively. Comparing both media, the number of produced embryo like structures using P4mf medium was higher than W14mf medium, 48.84 and 28.14 embryo like structures per 100 anthers, respectively. Furthermore, green plantlets production using P4mf medium was 4.82 per 100 anthers which was higher than W14mf medium, 4.59 plantlets per 100 anthers.

In order to compare and determine the most efficient methods to produce durum wheat haploids, Slama-Ayed et al. (2019) started a study on two durum wheat cultivars, one landrace, and a maize genotype. They tested gynogenesis, isolated microspores culture, and intergeneric wheat x maize crosses for selecting the best haploid production method. In the isolated microspore culture method, tillers were pre-treated for 5 weeks at 4 °C then microspores were cultured in CHB3 medium, and before incubation at 24 °C immature ovaries were added to the culture. Then obtained embryos were transferred to growth regulators free MS medium and incubated, and regenerated plantlets number was recorded 2 weeks after transfer. For the gynogenesis method, tillers were pre-treated for 2 weeks at 4 °C and 1 to 1.5 mm ovaries were cultured for 5 to 6 weeks at 27 °C. Then calli were transferred to a differentiation medium. In the final method, three wheat cultivars were crossed with a maize genotype, and tillers with pollinated spikes were collected and cultured in 8 ml l^{-1} H_2SO_3, 40 g l^{-1} sucrose, 75 mg $^{-1}$l $AgNO_3$ and 100 mg l^{-1} 2,4-D. The grown embryos were cultured in B5 medium in growth chamber until germination. Based on the findings, it was suggested that induction, embryogenesis and microspore regeneration were the most important steps using isolated microspore culture method. Whereas for gynogenesis and interspecific crosses embryo or callus forming, and regeneration were critical. They claimed that gynogenesis as a method for producing durum wheat haploid was promising.

Wang et al. (2019) believed that the existing methods for microspore culture in wheat, especially winter types, are still insufficient as a routine application. Therefore, providing an effective procedure for microspore culture is essential. Through testing pre-treatments, maltose gradients, and histone deacetylase inhibitors there might be a chance to raise the frequency of microspore embryogenesis and improve the generation of fertile green plants. Findings of evaluating a number of wheat genotypes showed that spike cold pre-treatment for 21 and 28 days was optimum for spring and winter wheat respectively. Moreover, embryogenesis and/or green plant regeneration improved when trichostatin A was applied. Previously, Jiang et al. (2017) reported similar effect when using trichostatin A. In the experiment, different concentrations of trichostatin A (0, 0.1, 0.3, 0.5, and 10 μM) was compared on microspores culture of spring wheat. It was found that exposing microspores to 0.1μM of trichostatin A raised the yield of microspore-derived green haploid plants.

Orłowska et al. (2020) mentioned that regeneration of green plants from anther culture is affected by three

factors, the length of induction step, the concentration of silver nitrate, and the concentration of copper sulphate. They cultured anthers in a semi-solid induction medium with 2 mg l^{-1} 2,4-D and 0.5 mg l^{-1} 190–2 medium kinetin supplemented, and the regeneration medium (90–2 medium) supplemented with 0.5 mg l^{-1} NAA and 1.5 mg l^{-1} kinetin. For examining these three factors they used the Taguchi method in nine trials. They indicate that wheat prefers low concentration of copper sulphate (0.1µM) and high concentration of silver nitrate (60 µM) to produce higher number of green regenerants.

3. Conclusion

Studies have proved the importance of haploid wheat, and attempts to establish a simple haploid production procedure have been made in the past. In addition, the role of haploids in breeding programs for wheat improvement and double haploid production has been defined. However, additional research into the factors that influence the effectiveness of haploid production methods is needed. Studies on determining the optimum period for selecting ovaries and microspores is important, as well as studying cold pre-treatment duration and temperature. Adding supplemental compounds to the culturing medium, such as antibiotics or hormones, affects the plant regeneration. Finally, genotypic variation should be considered, since it has been confirmed that the generation of haploid plants is more genotype dependent than the incubation conditions for a certain technique.

Author Contributions

N.M.B. (%25), S.S. (%25), M.Y. (%25) and S.L. (%25) review and editing. N.M.B. (%25), S.S. (%25), M.Y. (%25) and S.L. (%25) original draft preparation. N.M.B. (%25), S.S. (%25), M.Y. (%25) and S.L. (%25) writing up. N.M.B. (%25), S.S. (%25), M.Y. (%25) and S.L. (%25) submission and revision. All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

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