**N E W S**
OF THE NATIONAL ACADEMY OF SCIENCES OF THE REPUBLIC OF KAZAKHSTAN
**SERIES OF AGRICULTURAL SCIENCES**
ISSN 2224-526X
Volume 1, Number 55 (2020), 31 – 39 **https://doi.org/10.32014/2020.2224-526X.5**

UDC 602.6:58

**G. A. Iskakova, D. O. Baisaparova, D. R. Raimbek, K. Zh. Zhambakin**

Institute of Plant Biology and Biotechnology, Almaty, Kazakhstan.
E-mail: gulek-0883@mail.ru, dina15061976@gmail.com,
draimbek@gmail.com; zhambakin@gmail.com

# CULTURE USE PROBLEMS IN SELECTION OF ISOLATED MICROSPORES IN GRAIN

**Abstract.** Production of haploid plants by culture of isolated microspores is a quick way of obtaining homozygous crop lines. Recessive features of mutant homozygous plants are also possible to determine by this biotechnology. Contrary from anthers culture, in which the presence of anther walls can lead to the development of diploid somatic calli and plants, the microspore culture produces only haploid or dihaploid lines. Isolated microspores culture in addition represents and has a unique identification system for studying the mechanisms of embryogenesis in *in vitro* culture. The usage of haploid technology extends the genetic basis of wheat breeding, since it allows increasing the frequency of new gene combinations. This technology significantly increases the efficiency of breeding new highly productive varieties of crops. On this basis, it becomes possible to quickly assess the prospects of dihaploids, which significantly improves the efficiency of the selection process. DH plants are completely fertile and, if necessary, may be used as parents or processed as a cultivar. DHs have been widely used for cultivar development, genetic mapping, mutagenesis, and the study of gene functions.

**Key words:** wheat, double haploid, haploid, microspore.

**Introduction.** The first haploid plant was obtained for *Datura stramonium* experimentally by A.F. Blacksie in 1922. This was the impetus for subsequent researches in the field of haploids [1]. About 50 years ago first reports of double haploids in barley (Clapham, 1973) and rice (Guha-Mukherjee, 1973) appeared [2]. However, the efficiency was so low then that these procedures could not make a significant contribution to the development of haploid technology.

Over the past fifteen years, in Kazakhstan there has been a restoration work on biotechnology, including the culture of cells and plant tissues. First of all, due to the demand for such works as the development of plant biotechnology, and, above all, the need for breeding practice of crops. It is well-established that using haploid technology hundreds of varieties of almost all economically significant crops are created [3]. In some regions of the world dihaploid varieties become dominant. For instance, in Europe 50% of cultivated barley varieties are obtained using haploid biotechnologies, while in Canada three out of five wheat varieties with the largest areas are doubled haploid varieties [4].

Obtaining haploid plants in an *in vitro* culture of male and female generative structures is one of the sought-after areas of modern biotechnology. Their main advantage is the use in breeding to reduce seven to eight sexual generations needed to stabilize the hybrid genotype. Additionally, the promise of haploids is to use the recombination variability of gametes of the first hybrid generations in practical plant breeding. On this basis, it becomes possible to quickly assess the prospects of dihaploids, which signify-cantly improves the efficiency of the selection process. Another area of use is considered dihaploids rapid stabilization of the hybrid material, which previously passed breeding selection. In this case, the dihaploid line can be a direct precursor to a variety of self-pollinating crops. Obtaining haploids from hybrids of older generations some researchers consider the most preferred way of breeding [1].

Technologies have been developed for the production of haploids in economically significant species - wheat, barley, triticale, rice, rape [5,6].

The production of haploid plants through the culture of isolated microspores is a very important tool for accelerating plant breeding [7,8]. Haploid plants derived from microspores provide the fastest way to produce homozygous and homogeneous lines of important crops. This technology therefore allows the selection of recessive mutant lines in the haploid microspore explants for their study in homozygous plants. The culture of isolated microspores is an excellent system for studying the mechanisms of microspore induction and embryogenesis, providing a platform for an ever-expanding range of molecular studies [9].

Nowadays the culture of isolated microspores is the most reliable and effective method for producing doubled haploids. Contrary from anthers culture, in which the presence of anther walls can lead to the development of diploid somatic calli and plants, the microspore culture produces only haploid or dihaploid lines. Nevertheless, there are no universal and established protocols that would allow using this method for large-scale production of doubled wheat haploids. Development of a protocol of isolated microspore culture to produce doubled haploid Kazakh wheat is based on elaborating procedures of anther stress pretreatment, microspore isolation and purification, induction of division and regeneration of haploid and dihaploid plants for Kazakh wheat. According to literature data, in order to "force" microspores to divide and subsequently obtain haploid and dihaploid plants, it is necessary to create certain conditions for their cultivation. Firstly, it was shown that microspores should be in the stage of late mononuclear or early binuclear development. Secondly, to induce the division of microspores and the further formation of colonies and nucleating structures that are able to regenerate into plants, cultures must be stressed. Precisely from the influence of extreme stressful conditions, such as cold processing of anthers, "starvation", thermal shock, that it is possible to change the genetic program of microspores as germ cells and "turn" them into somatic cells that can divide and produce fertile haploid / homozygous plants [9,10,11].

A very important point is the doubling of chromosomes in microspores. It is proved, using the example of barley, that up to 70% of microspores can, during cultivation, spontaneously double the number of chromosomes. For wheat, the percentage of spontaneous doubling of chromosomes is lower [12,13].

**The processes that occur during the cultivation of microspores.** Microspore or pollen embryogenesis is one of the most striking examples of plant cell totipotency [14]. The first reports of the induction of sporophytic development of microspores appeared in the second half of the 20th century [2]. Successful induction of microspore embryogenesis has been established in more than 250 plant species [15]. Nevertheless, there are still limiting factors that hinder the widespread use of haploid biotechnologies. The main ones are genotypic dependence and low frequency of plant regeneration. For many types of cereals, the most important problem of haploproduction in anther culture *in vitro* remains a high proportion of albino regenerants [1,4,13]. Given problem hinders the development of effective protocols for the production of haploid plants and doubled haploids, which reduce the time and cost of creating varieties compared to traditional breeding. Universal technologies for producing haploid plants in an *in vitro* culture of anthers (microspores) for different species do not exist, but their main stages remain unchanged. They include: growing and selecting donor plants, pretreatment of inflorescences or anthers with various stress factors, isolating anthers (microspores) and their cultivation *in vitro*, inducing embryogenesis, plant regeneration, doubling the number of chromosomes of plant regenerants. Numerous endogenous and exogenous factors influence the responsiveness of anthers in *in vitro* cultivation: conditions for growing donor plants, genotype, methods and duration of pretreatment of inflorescences or anthers, anther development stage, nutrient composition [4, 14, 15, 16]. The discovery that stress is the main signal responsible for changing the genetic program for the development of microspores and their transition to a sporophytic way of development made it possible to unite the induction model of microspore embryogenesis and optimize many technologies for producing haploid plants [17].

Gametic embryogenesis is an embryoid that has formed from a male or female gametophyte cell. When male gametes are involved, the process is called "androgenesis", while "gynogenesis" describes the process when female gametes are used [4]. Double haploid production technologies give possibilities to create homozygotes from heterozygous plants. The development of effective haploid protocols is of great importance for breeding; their use reduces the time and cost of creating new varieties. The culture of isolated microspores is used more widely in comparison with other methods for producing haploid plants. Switching cultured *in vitro* microspores from the gametophytic to the sporophytic developmental pathway

is usually induced by various stresses applied to donor plants, inflorescences, isolated anthers, or microspores in both *in vivo* and *in vitro* conditions. Physical and chemical pretreatments (cold and heat shock, colchicine) act as triggers that induce a sporophytic pathway of development, and prevent gametophyte development of microspores. The accumulated literature data suggests that cold shock actually acts as an anti-stress factor mitigating the effect of real stress caused by starvation of anthers or microspores isolated from plants. Under the influence of stress, the microspore transforms into a depolarized and dedifferentiated cell, which is a prerequisite for reprogramming its development into an embryoid [18].

**Induction of embryogenesis under stress.** The term stress was proposed by Canadian physiologist Hans Selye in 1936 to describe the body's response to any strong adverse effect.

According to F. Bonet with co-authors (1998), microspore embryogenesis is an important adaptive mechanism of plants, which is found only in certain conditions as a result of stressful effects.

The switching of microspores from the gametophytic to the sporophytic developmental pathway is induced by various stresses used *in vivo* and *in vitro* [4, 19]. Regardless of the applied stress, the formation of embryogenic microspores is accompanied by the following general occasions:

1) an increase in the volume of microspores;

2) passing through DNA replication with a delay in the cell cycle;

3) autophagy of the cytoplasm;

4) the transformation of the cytoskeleton, leading to the movement of the nucleus from the peripheral to the central position;

5) the formation of a new cell wall;

6) chromatin compaction;

7) changes in gene expression [20].

Changes in gene expression can be summarized in three fundamental groups: cell responsiveness to stress; gametophyte suppression and expression of sporophytic development [21].

Thereby, stresses not only irreversibly block the gametophytic program for the development of microspores, but also switch their development to the sporophytic pathway. The discovery that stress serves as a general signal for the embryogenic development of microspores has allowed the development of a universal model for the induction of microspore embryogenesis, which includes three main stages:

- irreversible blocking of the gametophytic developmental programs in usage of stressful effects. This is a necessary, but not the only condition for the subsequent development of embryoids;

- formation of a population of embryogenic microspores due to changes at the molecular level;

- implementation of a sporophytic development program on a nutrient medium containing carbohydrates (sucrose) [18].

**Applied stress on donor plants.** For receiving embryogenic callus, stressful effects on donor plants in vivo are possible, while the exposure time can be different: short-term (for one stage of plant development) or long. To a large extent it depends on the impact factor, as well as on the type of plant. A local *in vitro* effect on the anther or inflorescence, on an isolated sporophytic complex is also used [20].

Widely used stresses include temperature shock, carbohydrate starvation, and colchicine exposure. The most widespread in experiments on the production of haploids in various species was the treatment of donor plants with low positive temperatures (2–4°C) for 2–7 days, and sometimes 3-4 weeks [6, 16]. "Cold processing" has become a routine haploproduction procedure in many laboratories around the world. Exposure to lower positive temperatures was used to create haploids of barley, wheat, rice, triticale, rapeseed, clementine. Shoots, inflorescences, and isolated anthers that are introduced into the culture are maintained at low temperatures [4, 19, 20]. The frequency of embryoid formation increases significantly. Cold stress is often used in combination with osmotic stress or starvation (carbohydrate or nitrogen) [22]. However, the effect of temperature on cultured anthers or microspores is not always unambiguous. For example, in Greece, in experiments with wheat varieties Acheloos and Vergina and their hybrids, was shown that cold pretreatments are not necessary for haploproduction in anther culture. The main role is played by the genotype of the donor plant and the temperature of the anther cultivation. The initiation of sporophytic development of microspores without stress was achieved in the culture of anthers of barley and wheat. These experiments indicate that isolation of inflorescences and anthers from a donor plant, as well as *in vitro* cultivation conditions, can act by themselves as stresses that, without the use of any other stresses, can reprogram the further development of microspores *in vitro* [23].

According to Sv. Zoriniants et al. with co-authorship (2005), cold shock does not act as stress, but as "anti-stress". Cold pretreatment acts as a hardening factor and induces a whole complex of cytological and physiological changes that activate the cellular defense system against other stresses. After exposure to temperature, various proteins involved in the competence of microspores, responsiveness to stress, and the induction of microspore embryogenesis were found in the triticale anther culture [18,19].

Heat shock or high temperature stress is also used *in vitro*. Elevated cultivation temperatures of wheat anthers (up to 32–34°C for four days) increase the productivity of microspore embryogenesis. Short-term exposure to high temperature stress is the most effective method of inducing microspore embryogenesis in species of the genus *Brassica* L. [8]. The positive role of using elevated temperatures has also been established in combination with other stress factors, such as starvation [22]. Heat shock causes a different spectrum of changes in the cell, in particular the induction of heat shock protein synthesis (HSPs), especially HSP70, which block the pollen differentiation program. According to the temperature differences between the growth conditions of donor plants *in vivo* and *in vitro* culturing conditions, the "more stringent" the HSP signal is. At temperatures below 25°C, HSPs do not form - temperatures are too low to show response to stress. Thus, the synthesis of HSPs can serve as a molecular marker of the reaction of microspores to stress and their ability to initiate androgenesis *in vitro* [18].

**Duplicating haploid chromosome set.** One of the ways of duplicating the chromosome set in haploid regenerants is to use colchicine. Colchicine is applied widely in anther and isolated microspore culture of barley, wheat, corn, triticale, and rapeseed [1, 11, 16, 24]. Adding colchicine to induction medium for wheat anther culture in 0.02 and 0.04% concentrations during first several hours of cultivation leads to asymmetric cell division due to the suppression of microtube formation and consequent increase in the number of symmetrically dividing microspores [18]. In corn anther culture, the most successful microspore embryogenesis induction was achieved in co-exposure to low positive temperatures (on donor material) and colchicine in a combination with TIBA used as growth regulator. In a study done by Tadesse, he immersed haploid plants for 4 h in a solution containing 0.2% colchicine with DMSO and few drops of Tween-20 at room temperature. After that, he washed them overnight under running tap water and replanted in pots with a mixture of soil, sand, peat and moss in 2:1:1 ratio [25,26].

Various ways of wheat chromosome doubling *in vitro* were proposed. First way is to add colchicine directly to the induction medium for anther culture in a concentration of 0.2 g/l (500 мМ). After 72 h the anthers were transferred to a colchicine-free medium. As a result, 70% of them were doubled haploids. In 1994, Ouyang with colleagues cultivated calli for regeneration in colchicine-containing medium and that resulted in yield of 54% doubled haploids (17% in control group). However, it was proven that toxicity of high colchicine concentrations decreased the number of embryoids derived from microspore culture. In addition, early chromosome doubling may lead to a possible increase in the frequency of aneuploidy in *in vitro* wheat haploids with duplicated chromosomes [27,28].

The effect of colchicine exposure on embryoid green plant formation depends on genotype [29]. Colchicine binds α- and β-heterodimers of tubulin, thereby inhibiting their further binding to microtubes, which leads to the depolymerization and movement of the nucleus from periphery to the center of the microspore. Reorganization of the cytoskeleton leads to a loss of asymmetry of the microspore and blocks gametophytic development. A connection between microtubes and cyclin-dependent kinases (Cdc2) involved in changes in cell cycle phase was also demonstrated. Cdc2 protein accumulation levels depend on cell proliferation activity. It is assumed that colchicine inhibition of spindle fiber formation may affect Cdc2 protein biosynthesis in microspores and switch to embryogenic pathway [19].

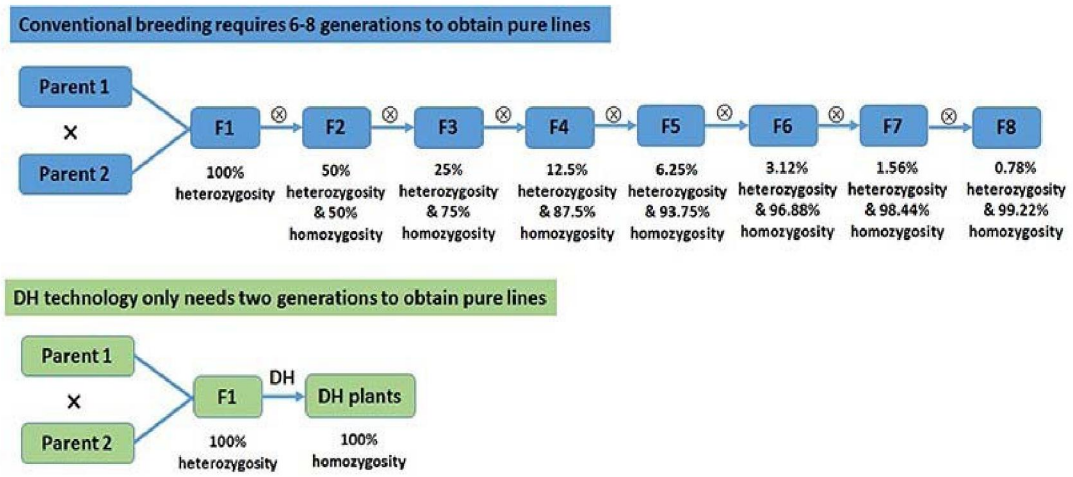
Haploid regeneration from embryoids and further chromosome duplication leads to a production of doubled haploids or DH-lines. Due to their origin, DHs are 100% homozygous, i.e. their genetic fixation may be achieved directly in F1 generation. Thus, DH production technology allows to shorten the propagation cycle (from one hybridization to the next) by several years [1].

**Advantages of haploidy in cereal breeding.** Using methods of doubled haploidy is the most effective way to accelerate the breeding process and obtain new homozygous forms. DH methods are based on cultivating plant reproductive tissues in order to obtain haploid plants. Using haploid technologies widens genetic diversity in wheat breeding, as long as it allows increasing the frequency of genetic combinations. This technology allows to rapidly produce homozygous lines in isolated anther and microspore culture in short period of time, which significantly reduces the amount of time required for creating new highly productive cultivars when used in breeding programs. However, many issues of

experimental haploidy are still relevant, thus limiting the practical use of doubled haploids in breeding programs and their regulation in different stages of the breeding process.

DH generates homozygous lines by doubling chromosomes of haploid plants, derived from egg-cells or sperms. There is a variety of reviews on DH technology in plants, which were improved and modified [3,15,16,22].

The main purposes in crop breeding are high harvest and quality with resistance to biotic and abiotic stresses. Crop growing programs are often based on pure lines. Traditional breeding requires 6-8 generations after crossing for getting the pure lines. From the beginning of the crossing and until the receiving of pure lines it takes 11-13 years (figure).




Comparison between conventional breeding and DH technology [30]

In figure you can see the advantage of DH technology in plant breeding. The advantage of DH technology in comparison with conventional reproduction methods is that DH achieves complete homozygosity in one generation. It allows significantly reducing the production time of pure lines. Complete homozygosity provides more precise phenotyping and gives an opportunity to precisely bind gene features in genetic mapping and studies on gene functions. They also can be used as target for studying cell biology and genetic engineering. DH technology was successfully worked out and improved many crops in which barley and rapeseed are the most responsive, and cotton and many types of legumes are much less responsive [30]. Genotype dependence, high proportion of albinism, high frequency of clones in the result of androgenesis and instability of genome, such as aneuploidy due to somaclonal variation are the main factors affecting to the efficiency of DH production [22].

DH lines are completely homozygous and contain two identical chromosome/gene set. They are ideal for evaluating quantitative trait (QT) × medium (M) interactions, whereas complete homozygosity allows better estimating of average features and allows a more accurate selection by location and year. The expected ratio in genotype segregation is 1:1 independent if a marker is dominant or co-dominant [26]. DH plants are completely fertile and, if necessary, may be used as parents or processed as a cultivar. DHs have been widely used for cultivar development, genetic mapping, mutagenesis, and the study of gene functions. However, distorted segregation coefficients may be observed, which reduces the accuracy of genetic maps. It can be caused by several reason:

1) genetic factors due to gametic or zygotic selection for pollen tube contention, preferential fertilization, chromosome translocation, etc.;

2) the dependence of DH on the genotype, that is, different reactions of cross-parents to the DH method;

3) somaclonal variations that spring up during the production of DH, leading to the production of aneuploids;

4) a high frequency of clones through androgenesis.

Long time ago it was proved that haploids are invaluable material for basic genetic research. It also can be used for quick generation of combinations of 4-fold, 5-fold, 6-fold or higher orders of multiple

mutants, obtaining homozygous maternal gametophytic lethal mutants and identifying cases of gene conversion during meiosis [18].

The first successful results of biotechnological production of doubled haploids were encouraging and offered great prospect of using cultivated crops in breeding. It was noted that the main advantage of dihaploid lines is the possibility of obtaining a homozygous line on their basis in just one generation, whereas in traditional selection several generations of inbreeding are carried out for these purposes. Therefore, the use of dihaploid lines in breeding practice can increase the efficiency and reliability of selection [1].

**Conclusion.** The method for obtaining haploid and dihaploid lines is associated with significant theoretical and methodological difficulties. Most successfully, the anther cultivation technique is used especially for cereals. This technology became the main one in obtaining the majority of dihaploid lines in Kazakhstan.

The production of haploid plants through the culture of isolated microspores provides the fastest way to produce homozygous and homogeneous lines of important crops. The culture of isolated microspores today is the most reliable and effective method for producing doubled haploids. In contrast to anther culture, in which the presence of anther walls can lead to the development of diploid somatic calli and plants, a microspore culture produces only haploid or dihaploid lines. At the same time, there are no universal and established protocols that would allow using this method for large-scale production of doubled wheat haploids.

**Г. А. Искакова, Д. Байсапарова, Д. Райымбек, К. Ж. Жамбакин**

Өсімдіктер биологиясы және биотехнология институты, Алматы, Қазақстан

## ДӘНДІ ДАҚЫЛДАР СЕЛЕКЦИЯСЫНДА ОҚШАУЛАНҒАН МИКРОСПОРАЛАР КУЛЬТУРАСЫН ҚОЛДАНУДЫҢ ПРОБЛЕМАЛАРЫ

**Аннотация.** Ауылшаруашылығы үшін маңызды гомозиготалы линияларды алудың жылдам әдістерінің бірі – оқшауланған микроспоралар культурасы арқылы гаплоидты өсімдіктерді алу. Сонымен қатар бұл биотехнология мутантты гомозиготалы өсімдіктердегі рецессивтік белгілерді анықтауға мүмкіндік береді. Оқшауланған тозаң культурасына қарағанда, микроспора культурасын дақылдау кезінде, тек гаплоидты және дигаплоидты линиялар шығады. Оқшауланған микроспоралар культурасы – in vitro культурасындағы эмбриогенез механизмдерін зерттеудің тамаша жүйесі. Гаплоидты технологияны қолдану бидай селекциясының генетикалық негізін кеңейтіп, жаңа гендік комбинациялардың жиілігін арттыруға мүмкіндік береді. Бұл технология ауылшаруашылық дақылдарының жоғары өнімді жаңа сорттарын өсірудегі тиімділікті арттырады.

Барлық өсімдіктерге келетін in vitro жағдайында оқшауланған микроспоралар (тозаңдар) культурасы арқылы гаплоидты өсімдіктерді алудың универсалды технологиялары жоқ, бірақ олардың негізгі сатылары өзгеріссіз қалады. Олар: донорлық өсімдіктерді өсіру және іріктеу, әртүрлі стресс факторлары арқылы гүлшоғыр мен тозаңдарды алдын ала өңдеу, тозаңдар мен микроспораларды бөліп алу және оларды in vitro жағдайында өсіру, эмбриогенез индукциясы, өсімдіктердің регенерациясы, өсімдік-регенеранттардың хромосомаларын екі есеге арттыру. Көптеген эндогендік және экзогендік факторлар тозаңдар мен микроспоралардың in vitro-да өсуіне әсер етеді. Олар: донорлық өсімдіктерді өсіру жағдайлары, генотип, гүлшоғырларды немесе тозаңдарды алдын-ала өңдеудің әдістері мен уақыты, тозаңның даму кезеңі, қоректік орта құрамы. Түрлер мен генотипке тәуелділік, альбинизмнің жоғары үлесі, андрогенезге байланысты клондардың жоғары жиілігі және сомаклоналды өзгерулерге байланысты анеуплоидия сияқты геномның тұрақсыздығы – дигаплоид (ДГ) өндірісінің тиімділігіне әсер ететін негізгі факторлар.

Эмбриогендік каллусты алу үшін донорлық өсімдіктерге in vivo жағдайында стресстік сипатта әсер етуі мүмкін, ал әсер ету уақыты әртүрлі болады: қысқа мерзімді (өсімдіктер дамуының бір кезеңінде) немесе ұзақ мерзімді. Бұл көбінесе әсер ету факторына, сондай-ақ өсімдік түріне байланысты. Сонымен қатар тозаңдарға немесе гүлшоғырға да локалды in vitro әсер етуі мүмкін. Гаплоидты технологияда кеңінен таралған стрестік әсер ету әдістерінің бірі – төменгі температурамен (2-4 °С) 2-7 күндей, кейде 3-4 аптадай өңдеу. Осы әдістің көмегімен арпа, бидай, күріш, тритикале, рапс, клементин және т.б. көптеген өсімдіктерден гаплоидттар алынды. Өскіндерді, гүлшоғырларды және оқшауланған тозаңдарды төменгі температурада өңдеу арқылы эмбриоидтардың өсу жиілігі едәуір артады. Төменгі температурада өңдеумен қоса, жылу температурамен (төрт күн ішінде 32-34 °С-қа дейін) өңдеу де микроспора эмбриогенезі өнімділігінің артуына әкеледі.

Гаплоидты регенеранттағы хромосомалардың гаплоидты жиынтығын екі есе көбейту үшін колхицин қолданылады. Ол арпа, бидай, жүгері, тритикале, рапс және т.б. оқшауланған микроспора мен тозаңдар дақылында кеңінен қолданылады.

Селекцияда басты мақсаттардың бірі – алынған өнімнің биотикалық және абиотикалық стерстерге төзімді және сапасының жоғары болуы. Ауылшаруашылық дақылдарын өсіру бағдарламалары көбінесе таза линияларға негізделген. Ал бұл таза линияларды дәстүрлі селекция арқылы алатын болсақ, будандастырудан кейін 6-8 ұрпақты қажет етеді. Яғни будандастырудан бастап, таза линияларды алу үшін ең кемінде 11–13 жыл қажет. Сондықтан ДГ технологиясының басқа дәстүрлі әдістерінен негізгі артықшылығы мынада: ДГ бір ұрпақта толық гомозиготалыққа қол жеткізеді. Бұл таза линия алу уақытын едәуір қысқартады. Толық гомозиготалық фенотипті неғұрлым дәл анықтауға және гендік карта мен ген функциясын зерттеуде гендік белгілерді дәл байланыстыруға мүмкіндік береді. Сонымен қатар оларды жасуша биологиясында және гендік инженерия зерттеу жұмыстарында нысан ретінде де қолданады. ДГ технологиясы көптеген дақылдарды жақсартты. Олардың ішінде арпа мен рапс өсімдіктерден ДГ алу оңайырақ болса, ал мақта мен бұршақ дақылдарының көптеген түрлері әлі де ДГ технологияда көптеген қиындықтар туғызады. Алынған ДГ өсімдіктері толығымен ұрпақ бере алады және қажет болған жағдайда оларды ата-ана ретінде пайдалануға немесе асылдандыру бағдарламасының бөлігі ретінде қолдануға болады. Алынған дигаплоид өсімдіктерін алуан түрлілікті дамыту, генетикалық карта жасау, мутагенез және ген функцияларын зерттеу үшін кеңінен қолданады.

**Түйін сөздер:** бидай, екі еселенген гаплоид, гаплоид, микроспора.

**Г. А. Искакова, Д. Байсапарова, Д. Райымбек, К. Ж. Жамбакин**

Институт биологии и биотехнологии растений, Алматы, Казахстан

## ПРОБЛЕМЫ ИСПОЛЬЗОВАНИЯ КУЛЬТУРЫ ИЗОЛИРОВАННЫХ МИКРОСПОР В СЕЛЕКЦИИ ЗЕРНОВЫХ

**Аннотация.** Производство гаплоидных растений посредством культуры изолированных микроспор является быстрым способом получения гомозиготных линий сельскохозяйственных культур. Эта биотехнология позволяет также определять рецессивные признаки у мутантных гомозиготных растений. В отличие от культуры пыльников, в которых присутствие стенок пыльников может привести к развитию диплоидных соматических каллусов и растений, культура микроспор производит только гаплоидные или дигаплоидные линии. Культура изолированных микроспор является, кроме того, и отличной системой для изучения механизмов эмбриогенеза в культуре in vitro. Использование гаплоидной технологии расширяет генетическую основу селекции пшеницы, поскольку она позволяет увеличить частоту новых комбинаций генов. Эта технология значительно повышает эффективность выведения новых высокопродуктивных сортов сельскохозяйственных культур.

Универсальных технологий получения гаплоидных растений в культуре in vitro изолированных микроспор (пыльников) для разных видов не существует, однако основные их этапы остаются неизменными. Они включают: выращивание и отбор донорных растений, предобработку соцветий или пыльников различными стрессовыми факторами, выделение микроспор (пыльников) и их культивирование в условиях in vitro, индуцирование эмбриогенеза, регенерацию растений, удвоение числа хромосом растений регенерантов. На отзывчивость пыльников и микроспор при культивировании in vitro влияют многочис-ленные эндогенные и экзогенные факторы: условия выращивания донорных растений, генотип, способы и продолжительность предобработок соцветий или пыльников, стадия развития пыльника, состав питательных сред. Основными факторами, влияющими на эффективность производства ДГ, являются зависимость от видов и генотипов, высокая доля альбинизма, высокая частота клонов в результате андрогенеза и нестабильность генома, такая как анеуплоидия из-за сомаклональных вариаций.

Для получения эмбриогенного каллуса возможны стрессовые воздействия на донорные растения in vivo, при этом время воздействия может быть различным: кратковременным (на один этап развития растения) или длительным. В значительной степени это зависит от фактора воздействия, а также от вида растения. Применяется и локальное воздействие in vitro на пыльник или соцветие, на изолированный спорофитный комплекс. Наибольшее распространение в опытах по производству гаплоидов у различных видов получила обработка донорных растений пониженными положительными температурами (2–4 °С) в течение 2–7 дней, а иногда и 3–4 недель. «Холодовая обработка» стало рутинной процедурой гаплопродукции во многих лабораториях мира. Воздействие пониженными положительными температурами применялось для создания гаплоидов ячменя, пшеницы, риса, тритикале, рапса, клементина и т.д. При пониженных температурах выдерживаются побеги, соцветия и изолированные пыльники, введенные в культуру. Частота

формирования эмбриоидов существенно повышается. Холодовой стресс часто применяют в комбинации с осмотическим стрессом или голоданием (углеводным или азотным). Используется также тепловой шок или высокотемпературный стресс in vitro. Повышенные температуры культивирования пыль-ников пшеницы (до 32–34 °С в течение четырех дней) приводят к возрастанию продуктивности микро-спорового эмбриогенеза.

Для того, чтобы удвоить гаплоидный набор хромосом у гаплоидных регенерантов, используется колхицин. Он широко применяется в культуре пыльников и изолированных микроспор ячменя, пшеницы, кукурузы, тритикале, рапса и т.д.

Высокий урожай и качество с устойчивостью к биотическим и абиотическим стрессам являются основными целями в селекции культур. Программы выращивания сельскохозяйственных культур часто основаны на чистых линиях. Для получения чистых линий традиционная селекция требует 6-8 поколений после скрещивание. От начала скрещивания до получения чистых линий уходит 11–13 лет. Преимущество ДГ-технологии по сравнению с обычными методами размножения состоит в том, что ДГ достигает полной гомозиготности в одном поколении. Это позволяет значительно сократить время производства чистых линий. Полная гомозиготность позволяет более точное фенотипирование и позволяет точно связать признаки гена в исследованиях генетического картирования и функции генов. Также они могут быть использованы в качестве мишеней для изучения клеточной биологии и генной инженерии. ДГ-технология была успешно разработана и улучшила многие культуры, в которых ячмень и рапс являются одними из наиболее отзывчивых, а хлопок и многие виды бобовых являются непокорными. Растения ДГ полностью плодородны и, при необходимости, могут быть использованы в качестве родителей или выпущены в качестве сорта в рамках селекционных программ. ДГ широко использовались для развития сорта, генетического картирования, мутагенеза и изучения функций генов.

**Ключевые слова:** пшеница, удвоенный гаплоид, гаплоид, микроспора.

**Information about author:**

Iskakova G.A., Senior Researcher, Institute of Plant Biology and Biotechnology, PhD student, Kazakh National Agrarian University, Almaty, Kazakhstan; gulek-0883@mail.ru; https://orcid.org/0000-0002-1989-9031

Baisaparova D.O., Assistant, Institute of Plant Biology and Biotechnology, Almaty, Kazakhstan; dina15061976@gmail.com; https://orcid.org/0000-0003-4500-5350

Raimbek D.R., Junior Researcher, Institute of Plant Biology and Biotechnology, Almaty, Kazakhstan; draimbek@gmail.com; https://orcid.org/0000-0002-4729-2165

Zhambakin K.Zh., the Dr.Sci.Biol., Professor, Member of NAN RK, Director of Institute of Plant Biology and Biotechnology, Almaty, Kazakhstan; zhambakin@gmail.com; https://orcid.org/0000-0001-5243-145X

**REFERENCES**

[1] Zhambakin K.Zh. Haploid biotechnology of plants // Interligal. 2004. 184 p. (in Russ.).

[2] Guha S.S., Maheshwari S.C. In vitro production of embryos from anthers of Datura. Nature. 1964; 204: 497-498.

[3] Dunwell J.M. Haploids in flowering plants: origins and exploitation // Plant Biotechnol J. 2010. Vol. 8. P. 377-424.

[4] Germana M.A. Anther culture for haploid and doubled haploid production. Plant Cell Tissue Organ Cult. 2011; 104: 283-300.

[5] Pauk J., Jancsó M., Simon-Kiss I. Rice doubled haploids and breeding. In: Touraev A., Forster B.P., Jain S.M. (Eds.). Advances in Haploid Production in Higher Plants. Springer Science + Business Media, 2009; 189-197.

[6] Ignatova S.A. Cellular technologies in crop production, genetics and breeding of cultivated plants: tasks, opportunities, development of in vitro systems. Astroprint, Odessa. 2011.

[7] Patel M., Darvey N.L., Marshall D.R., Berry J.O. Optimization of culture conditions for improved plant regeneration efficiency from wheat microspore culture // Euphytica. 2004. Vol. 140. P. 197-204.

[8] Ferrie A.M.R, Caswell K.L. Isolated microspore culture techniques and recent progress for haploid and doubled haploid plant production // Plant Cell Tiss Organ Cult. 2011. Vol. 104. P. 301–309.

[9] Ismagul A., Iskakova G., Eliby S.S., Bashabayeva B., Abugaliyeva A.I. Analysis of plant homozygotization methods in breeding and development of isolated microspore culture protocols for wheat cultivars in Kazakhstan. Vestnik KazNU. Serija biologicheskaja. N2(54). 2012. P. 21-28 (in Russ.).

[10] Touraev A., Vicente O., Heberle-Bors E. Initiation of microspore embryogenesis by stress // Trends Plant Sci. 1997. Vol. 2. P. 297-302.

[11] Ismagul A., Eliby S., Bashabayeva B.M., Abugaliyeva A.I. Analysis of homozygotization methods in breeding and development of new protocols for isolated microspore culture of Kazakhstan wheat cultivars // Vestnik KazNU. Serija biologicheskaja. 2012. N2(54). P. 17-23 (in Russ.).

[12] Bashabaeva B.M., Ismagul A.Zh., Abugalieva A.I. Method of homozygotization material in tissue cukture of barley // Eurasian journal of applied biotechnology. 2013. N 1 (in Russ.) http://biotechlink.org/1-2013/article5

[13] Davies P.A., Charles Oti-Boateng, Cate Schmerl, Sheridan Morton. Barley Isolated Microspore Culture // Workshop Proceedings. ACPFG. SARDI, Waite Campus, 1997. 13 p.

[14] Cistue L., Soriano M., Castillo A.M., Valles M.P., Sanz and B. Echàvarri J.M. Production of doubled haploids in durum wheat (Triticum turgidum L.) through isolated microspore. Plant Cell. Rep., 2006; 25: 257–264.

[15] ] Kasha K.J., Simion E., Miner M., Letarte J., Hu T.C. (2003) Haploid wheat isolated microspore culture protocol. In: Maluszynski, Kasha KJ, Forster BP, Szarejko I, editors. Doubled haploid production in crop plants. Kluwer Academic Publishers, The Netherlands. P. 77–82.

[16] Zheng M.Y., Liu W., Weng Y., Polle E., Konzak C.F. (2003) Production of doubled haploids in wheat (Triticum aestivum L.) through microspore embryogenesis triggered by inducer chemicals. In: Maluszynski M, Kasha KJ, Froster BP, Szarejko I, editors. Doubled haploid production in crop plants, Kluwer Academic Publishers. P. 83-94. 12.

[17] Ismagul A., Bashabayeva B., Iskakova G., Abugaliyeva A., Eliby S., Kenenbayev S. Methodological instructions. Kazakh Research Institute of Agriculture and Plant Growing, Almaty. 2013. 19 p.

[18] Aionesei T., Touraev A., Heberle-Bors E. Pathways to Microspore Embryogenesis. In: Palmer C.E., Keller W.A., Kasha K. (Eds.). Haploids in Crop Improvement II (Ser. Biotech. in Agricultural and Forestry). Berlin; Heidelberg: Springer-Verlag, 2005; 56: 11-34.

[19] Touraev A., Indrianto A., Wratschko I., Vicente O., Heberle-Bors E. Efficient microspore embryogenesis in wheat (Triticum aestivum L.) induced by starvation at high temperatures. Sex Plant Rep. 1996. 9: 209-215.

[20] Dubas E., Wedzony M., Petrovska B., Salaj J., Zur I. Cell structural reorganization during induction of androgenesis in isolated microspore cultures of Triticale (×Triticosecale Wittm.). Acta Biologica Cracoviensia. Ser. Botanica. 2010; 52: 73-86.

[21] Zoriniants Sv., Tashpulatov A., Heberle-Bors E., Touraev A. The Role of Stress in the Induction of Haploid Microspore Embryogenesis. In: Palmer C.E., Keller W.A., Kasha K. (Eds.). Haploids in Crop Improvement II (Ser. Biotechnology in Agricultural and Forestry). Berlin; Heidelberg: Springer-Verlag, 2005; 56: 35-51.

[22] Kruglova N.N., Seldimirova O.A., Zinatullina A.E. Morphogenic microspore as an initial cell for androgenesis in vitro: review of the problem. Scientific result. Physiology. 2017. N 3(1). P. 3-7. DOI 10.18413/2409-0298-2017-3-1-3-7.

[23] Pauls K.P., Chan J., Woronuk G., Schulze D., Brazolot J. When microspores decide to become embryos – cellular and molecular changes. Can. J. Bot. 2006; 84: 668-678.

[24] Tian Q.Q., Lu C.M., Li X., Fang X.W. Low temperature treatments of rice (Oryza sativa L.) anthers changes polysaccharide and protein composition of the anther walls and increases pollen fertility and callus induction. Plant Cell Tissue Organ Cult. 2015; 120: 89-98. DOI 10.1007/s11240-014-0582-5.

[25] Amangeldikyzy Z., Kochorov A.S., Karakaya Aziz. Immune-phytopathological assessment of resistance of spring wheat varieties to stem rust in the northern, western and south-eastern regions of Qazaqstan. News of the Natioal Academy of Sciences of the Republic of Kazakhstan. Series of Agricultural Sciences, ISSN 2224-526X Vol. 5, N 47 (2018), 27–34, https://doi.org/10.32014/2018. 2224-526X.4

[26] Barnabas B., Phaler P.L. and Kovacs G. Direct effect of colchicine on the microspore embryogenesis to produce dihaploid plants in wheat (Triticum aestivum L.). Theor Appl Genet, 1991; 81: 675–678.

[27] Smykal P., Pechan P.M. Stress as assessed by the appearance of smHSPs transcripts, is required but not sufficient to initiate androgenesis. Physiol. Plant. 2000;110: 135-143.

[28] Tadesse W., Tawkaz S., Inagaki M.N., Picard E., and Baum M. Methods and Applications of Doubled Haploid Technology in Wheat Breeding. ICARDA, Aleppo, Syria. 2013, 36 p.

[29] Navarro Alvarez W., Baenziger P.S., Eskridge K.M., Hugo M. and Gustafson V.D. Addition of colchicine to wheat anther culture media to increase doubled haploid plant production. Plant Breeding, 1994; 112: 192-198.

[30] Yan G., Liu H., Wang H., Lu Z., Wang Y., Mullan D., Hamblin J. and Liu C. Accelerated Generation of selfed pure line plants for gene identification and crop breeding. Front. Plant Sci., 2017 | https://doi.org/10.3389/fpls.2017.01786