По данным таблицы 2, можно судить о том, что штамм «БГ» вируса инфекционного бурсита кур можно культивировать в течение 2-3 суток. При этом максимальное накопление вируса отмечается при инкубировании в течение 3 суток, биологическая активность вируса составляет 7,91 lgТЦД$_{50}$/см^3. При инкубировании в течение 4 суток биологическая активность получаемого вируссодержащего материала составляет несколько ниже (6,00 lgТЦД $_{50}$/см^3).

Выводы

При определении минимальной инфицирующей дозы вируса наиболее активные вируссодержащие материалы получены при заражении культуры клеток в дозе от 1,0 до 0,01 ТЦД $_{50}$/кл.

При этом необходимо инкубировать инфицированных клеток в течение 3 суток. При соблюдении указанных параметров культивирования биологическая активность вируса составляет свыше 6,5 lgТЦД $_{50}$/см^3.

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Түйін

КФ жасуша культураларында, минималды заралдау мөлшері мен вирусты өсіру уақыты келтірілген. КФ жасуша культураларында, минималды заралдау мөлшері 0,1-0,01ТЦД$_{50}$/см^3 және вирусты өсіру уақыты 2-3 күн екендігі анықталған.

Summary

Minimal infecting doze and period virus cultivation in vitro cell culture are denermineited. Minimal infecting doze is 0,1-0,01 TCD/ cm^3 at culture of virus in during 2-3 deys.

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THE PHENOMENON OF ANDROGENESIS AND GYNOGENESIS FOR HAPLOID PRODUCTION

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Haploid Production and Uses

Haploids are defined as saprophytes with gametophytic chromosome number and have been produced in a variety of plant species using a variety of methods. Although, the significance of haploids in genetics and plant breeding has been recognized for long time, with the advent of new biotechnology it has received renewed emphasis, so that the production of haploids has become an important component of biotechnology programs in different countries. A haploids could be produced following delayed pollination, irradiation of pollen, temperature shocks, colchicine treatment and distant hybridization, the most important methods currently being utilized under biotechnology programs include anther and pollen culture, ovary and ovule culture [1-3].

As a result of haploid induction followed by chromosome doubling, homozygosity can be achieved in the quickest possible way making genetic and breeding research much easier. The genetic segregation is simplified in homozygotes, recessive genes not being masked by dominant

ones. Homozygosity is still more important for those plants which have a very long juvenile phase, such as fruit trees, bulbous plants, and forest trees. Even though, homozygosity can be achieved through self pollination, it is a long process in such plants. As a result of homozygosity, lines which produce pure F1 hybrids are made possible. When working with plants that are normally polyploid, it is very useful by haploid induction to work at a low ploidy level. Monoploids have an advantage for the mutation breeder in that recessive mutations are immediately discernible.

By haploid induction followed by chromosome doubling it is possible to obtain exclusively male plants. An important example of this is Asparagus officianalis in which male plants have a higher productivity and yield earlier in the season than female plants. If haploids are produced from anthers of male Asparagus plants, these are either "X" or "V"; chromosome doubling of V results in supermale plants VV which can subsequently be vegetatively propagated. If XX is crossed with VY, XY plants result. When homozyotes are artificially made into diploids in vivo with the use of colchicine, problems may arise which are not usually found in vitro. In vitro haploids often double spontaneously, giving rise directly to homozygotes. It is much easier to work with haploid protoplasts rather than diploid ones for somatic hybridization.

Among the different uses of haploids, their use in crop improvement is considered to be the most significant and stands out as the most important reason for emphasis on haploid research. Several extensive reviews have appeared during the last two decades, outlining the theory and the application of haploid breeding in general and also in particular crops.

Problems Concerned with Haploid Production

Although haploids have been raised mainly from anther culture of a large number of species, this technique has not proved successful in respect of all genotypes of crop species. A number of problems are encountered with the induction of haploids [1-3]:

1. Anthers often fail to grow in vitro or the initial growth is followed by abortion of the embryos.
2. 2. The tissue or callus developing from the anther an ovary generally comprises a chimera of diploid, tetraploid, and haploid cells.
3. Selective cell division must take place in the haploid micro- and macrospores, con comitantly restricting proliferation of unwanted diploid and polyploid tissues. Selective cell division is often impossible.
4. Formation of albinos in anther cultures, especially with cereals can neither be avoided nor the loss of plants due to albinism reduced.
5. The technique of inducing haploids in vitro is not economically viable due to low success rate.
6. Callus derived from anther or pollen, ovary or embryo sac in a medium supplemented with growth regulators is usually detrimental for haploid production.
7. It is difficult to isolate a haploid from a mixture of haploids and higher ploidy levels since the polyploids outgrow the haploids.
8. Doubling of haploids is time consuming and may not always result in the production of a homozygote. Double haploids sometimes exhibit segregation in their progeny.
9. The anther and ovary culture technique works well on plants belonging to only a few families, of which *Solanaceae* is one.

Factors Affecting Haploid Production

A number of factors influence androgenesis and gynogenesis in vitro [1-3]. The genotype of the donor plant plays a significant role in determining the frequency of pollen and embryo sac plant production. Anther and ovule wall factors also support pollen and embryo sac embryo development. Histological studies support this view. As induction of the pollen and embryo sac into embryoids occurs most easily within the confines of an anther and ovary, the anther and ovule wall seems to provide a nursing effect. There are two schools of thought regarding the role of the anther and ovule wall. One is that it may have a stimulatory effect on the growth of pollen and embryo sac embryos (probably due to the presence of enhanced levels of some amino acids such as glutamine and

serine); the other view holds that it may emanate some inhibitory substances into the culture medium thereby blocking the growth of more pollen and embryo sac into embryos.

The culture medium also plays a vital role since the requirements vary with the genotype and probably the age of the anther and ovary as well as conditions under which donor plants are grown. The medium should contain the correct amount and proportion of inorganic nutrients to satisfy the nutritional as well as physiological needs of the many plant cells in culture. In addition to basal salts and vitamins, hormones in the medium are critical factors for embryo or callus formation. Cytokinins (e.g. kinetin) are necessary for induction of pollen embryos in many species of *Solanaceae* except tobacco. Auxins, in particular 2,4-0, greatly promote the formation of pollen and embryo sac callus in cereals. For regeneration of plants from androgenic and gynogenic calli, a cytokinin and lower concentration of auxin are often necessary. Sucrose has been considered the most effective carbohydrate source which cannot be substituted by other disaccharides. Glucose can be used in anther culture in some cases but fructose is far less effective. The concentration of sucrose also plays an important role in induction of haploid plants. Activated charcoal is also added to the culture medium. The culture medium also plays a vital role since the requirements vary with the genotype and probably the age of the anther as well as conditions under which donor plants are grown. The medium should contain the correct amount and proportion of inorganic nutrients to satisfy the nutritional as well as physiological needs of the many plant cells in culture. It helps in the removal of inhibitors from the agar used for gelling the medium. Another role assigned to activated charcoal is the adsorption of 5hydroxymethylfurfural, a product of sucrose dehydration during autoclaving, assumed to bean inhibitor of growth in anther and ovary cultures. Certain organic supplements added to the culture medium often enhance the growth of anther cultures. Some of these include the hydrolyzed products of proteins such as casein (found in milk), nucleic acids, and others. Coconut milk obtained from tender coconuts is often added to tissue culture media. It contains a complex mixture of nucleic acids, sugars, growth hormones and some vitamins.

The physiological state of the parent plant plays a role in haploid production. Success in haploid induction is in part dependent on knowledge of the physiology of the pollen yielding plant. In various plant species it has been shown that the frequency of androgenesis and gynogenesis is higher in anthers and ovaries harvested at the beginning of the flowering period and declines with plant age. This may be due to deterioration in the general condition of the plants, especially during seed set. The lower frequency of induction of haploids in anthers and ovaries taken from older plants may also be associated with a decline in pollen and female gametophyte viability. Seasonal variations, physical treatment, and application of hormones and salts to the plant also alter its physiological status, which is reflected in a change in anther and ovary response.

Temperature and light are two physical factors which play an important role in culture of anthers and ovaries. Higher temperatures (30°C) yield better results for some plants. Temperature shocks also enhance the induction frequency of microspore androgenesis and female gametophyte gynogenesis. Frequency of haploid formation and growth of plantlets are generally better in light.

The developmental stage of pollen and female gametophyte greatly influences the fate of the microspore, Androgenesis occurs when a microspore or pollen is induced to shift from a gametophytic pathway to a sporophytic pathway of embryo formation.

Anthers of some species (datura, tobacco) give the best response if pollen is cultured at first. mitosis or later stages (postmitotic), whereas in most others (barley, wheat, rice) anthers are most productive when cultured at the uninucleate microspore stage (premitotic). Anthers at a very young stage (containing microspore mother cells m tetrads) or a late stage (containing binucleate, starch filled pollen) of development are generally ineffective, albeit some exceptions are known.

Female gametophyte can developed from a wide range of developmental stages whereas the male gametophyte uninuclear stage is optimal. During the cultivation of ovaries of rice and barley containing 1-4 nuclear embryo sacs, indiction of haploid embryogenesis was possible when the in vitro conditions allowed the formed mature female gametophyte to form. These dates suggest that induction of haploid plants often cultivation of young ovaries is possible. At the same time, it appears that the late stages of development of the embryo sac is the optimal time for cultivation of

ovaries for majority species of plants. Certain physical and chemical treatments given to flower, buds or anthers ovaries prior to culture, can be highly conducive to the development of pollen and embryo sac structures into plants. The most significant is cold treatment.

Male gametophyte culture

One of the very popular methods for production of haploids is through culturing anthers or microspores on artificial culture medium [4]. This leads to the growth of microspores into saprophytes. After the initial reports of successful production of haploids from anther culture in *Datura*, haploids have been obtained in more than 150 species belonging to 23 families of angiosperms. These include a wide variety of economically important species.

More often, anthers rather than microspores are cultured, since the extraction and culture methods for microspores differ and have been successful only in a few species (*Datura inoxia, Nicotiana sylvestris, N. tabacum, Oryza sativa*, etc.). The following parameters have been recognized as particularly important for successful anther and microscope culture: conditions of growth of donor plant; genotype of donor plant; the pretreatment; the developmental stage of anther/microspore; the culture medium and the conditions during culture growth.

More often, anthers rather than microspores are cultured, since the extraction and culture methods for microspores differ and have been successful only in a few species (Datura inoxia, Nicotiana sylvestris, N. tabacum, Oryza sativa, etc.).

In either case, flower buds are brought to a laminar flow chamber and sterilized using appropriate chemical treatment before their dissection. While removing anthers from the flower buds, care is exercised to avoid injury, because injury leads to development of callus, giving a mixture of diploids, haploids and aneuploids. The anthers are generally cultured on solid agar medium, where they may directly give rise to embryoids or may lead to callus formation, before differentiation. The embryoids develop into haploid plantlets, which are colchicine treated to get diploid homozygous plants to be field tested for selection. Microspore culture may be preferred over anther culture, even though the degree of success is low in this case. Microspores are collected first using the following steps: anthers, dissected as above, are taken in a sterile beaker containing liquid medium, and are pressed with a glass rod or syringe piston to allow the microspores squeeze out; the suspension with anthers and microspores is filtered through a nylon sieve, which allows only the microspores to pass through; the filtrate is centrifuged thrice, for 5 minutes each, at 500-800 rpm (the pellet resuspended in a fresh medium each time); the microspores are inoculated on a solid or in a liquid medium maintained at 25 °C and 16/8 hr photoperiod. The microspores may develop directly into embryoids within 15 days or follow one of the several indirect paths to produce haploid plantlets. In anther culture as well as in micros pore culture, spontaneous doubled haploids (SDH) are also obtained, so that no colchicine treatment will be needed in such cases. Efforts are being made to increase the frequency of SDH, so that these can be multiplied and directly field tested.

Female gametophyte culture

Production of a haploid individual by development of an unfertilized egg cell as a result of delayed pollination is referred to as gynogenesis [5]. Gynogenesis is found in the interspecific cross Solanum tuberosum (2n = 4x) x S. phureja (2n = 2x), resulting in the production of a dihaploid (2n = 2x) potato. San Noeum (1976) was the first to demonstrate that gynogenesis, an essentially in vivo phenomenon, can be induced under in vitro conditions. She obtained gynogenic haploids using an ovary culture of Hordeum vulgare. Subsequently, gynogenic haploids have been obtained from unpollinated ovaries and ovules. Induction of haploids from female gametophytes may not be inaccessible. Subsequently, gynogenic haploids have been obtained from unpollinated ovaries and ovules. Induction of haploids from female gametophytes may not be inaccessible. Haploid have also been successfully produced from cultured female gametophytes. Success in this connection was first achieved with some Gymnosperms like Zamia, Ephedra and some Cycads.

Although initial attempts for the production of haploids from cultured ovules in an giosperms were unsuccessful, recently haploids could be obtained from cultured ovaries in a few crop species, like barley, wheat and tobacco. With more efforts in the future, haploids from ovule culture will be

produced in a number of plant species. Further research is, therefore, necessary to improve our understanding about the following: the events leading to induction of haploidy in female gametophytes; the factors which control the in vitro development of proembryo into the fully organized plants; the differences in the growth patterns of in vitro development of unfertilized female gametophytes (in ovules) and the male gametophytes (in pollen).

Development of Androgenic Haploids

Under normal conditions the microspores mature into pollen grains in situ but in culture their morphogenesis is altered. Depending on the composition of the medium, development may lead either to the formation of embryoids and/or plantlets or a mass of parenchymatous callus. Four pathways based on the few initial divisions in the microspores have been identified as leading to in vitro androgenesis [1, 4]. In pathway the microspores divide by an equal division and two identical daughter cells contribute to sporophyte development. Vegetative and generative cells are not distinctly formed in this pathway (e.g. Datura in noxia). In pathway 2 the division of uninucleate microspores is unequal, resulting in the formation of a vegetative and a generative cell.

The sporophyte arises through further divisions in the vegetative cell while the generative cell either does not divide or does so once or twice before degenerating (e.g. Nicotiana tabacum, Hordeum vulgare, Triticum aestivum, Triticale spp., and Capsicum annuum). In pathway 3 the uninucleate microspore undergoes a normal unequal division but the pollen embryos are predominantly formed from the generative cell alone (e.g. Hyoscyamus niger).

The generative cell either does not divide at all or does so only to a limited extent. In pathway 4 the division of microspore is asymmetrical as in pathway 2. Both vegetative and generative cells divide further and contribute to development of the sporophyte (e.g. Datura metel, Atropa belladonna). Irrespective of the above early pattern of microspore divisions, the embryogenic pollen grains ultimately become multicellular and burst open, gradually assuming the form of a globular embryo. This is followed by the normal stages of postglobular embryogeny until development of a plant. Alternatively, the multicellular mass liberated from the bursting pollen grain proliferates to form callus which may later differentiate whole plants either on the same medium or on a modified medium. It is sometimes possible to obtain haploids via embryo formation or through callusing within the same species (Oryza sativa) by manipulating constituents of a medium. The generative cell either does not divide at all or does so only to a limited extent. In pathway 4 the division of microspore is asymmetrical as in pathway 2. Both vegetative and generative cells divide further and contribute to development of the sporophyte (e.g. Datura metel, Atropa belladonna).

Development of Gynogenic Haploids

Attempts have been made to elucidate what elements of the embryo sac are able to develop on in vitro conditions. Results of some experiments indicate that this structures can have mono- or polyepigenetic origins that arise from one or a few cells [5-7].

In barley, the ability for in vitro morphogenesis is possessed by the individual cells of embryo sac (egg cell, synergids, antipodals). Synergids formed only callus but antipodals gave rise to embryo. During embryogenesis, cells first differentiated into two proembryo cells, which developed into a polycellular globular embryo, that was morphologically similar to the zygotic embryo. In rice, callus and embryo are formed from synergids. Antipodals are viable but do not develop. The egg cell development was abnormal. During cultivation ovaries both barley and rice the unfertilized central cell formed an endosperm like structure. Autonomous division of the endosperm was induced by in vitro culture of unpollinated ovaries of *Lupinus, Helleborous, Melandrium*. The endosperms usually contained up to 20 free nuclei, only a few ovules with 80-240 nuclei were found. In beet, gynogenic embryous developed from egg cells or antipodals. Synergids usually degenerated. In tobacco, embryo were formed as a consequence of cell division of the egg cell. In experiments with *Crepis*, embryogenesis was induced in ovaries first from the antipodals. In sunflower, induction of morphogenesis was observed from egg cell, synergids and antipodals.

Two types of callus were formed in vitro from ovules of sage. The first type of callus formed from somatic tissue and second type formed from egg cell. Regeneration of diploid plants from cells of the nucellus and integuments was described in ovary culture of grapes, rice, tobacco.

Two patterns of gynogenesis have been observed in ovary culture: embryo – plant (direct embryogenesis) and embryo – callus – plant (indirect embryogenesis). Observations suggest that embryogenesis and callus formation do not take place simultaneously in the same ovule. Direct embryogenesis is the most desirable pattern because, in this case, photosynthetizing plants, while plants from callus may be albino or demonstrate different levels of ploidy.

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Түйін

Шолу мақалада ауылшаруашылығы дақылдарының аталық және аналық гаметофит клеткаларын жасанды қоректік ортада өсіру арқылы гаплоидты өсімдіктер алудың жолдары қарастырылған.

Резюме

В настоящем обзоре представлены основные пути получения гаплоидных растений сельскохозяйственных культур в культуре клеток мужского и женского гаметофитов.

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ВЛИЯНИЕ КУЛЬТУРАЛЬНОЙ СУСПЕНЗИИ МИКРОВОДОРОСЛИ *CHLORELLA VULGARIS BEIJR., УА-1-20* НА ПОСЕВНЫЕ КАЧЕСТВА, НА ПОВЫШЕНИЕ ИНДУКЦИИ УСТОЙЧИВОСТИ К ФИТОПАТОГЕНАМ И УРОЖАЙНОСТЬ ЛУКА

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В результате проведенных исследований установлено, что предпосевная обработка семян лука культуральной суспензией *Chlorella vulgaris Beijr., УА-1-20* оказывает положительное влияние на их посевные качества, а также индуцирует устойчивость растении к болезням, биологическая эффективность при этом против фузариоза составляет 50,8%, против бактериоза - 41,2%, урожайность лука повышается на 26,8%.

Как известно лук является ценной овощной культурой и используется в течении круглого года в рационе питания населения. Для удовлетворения потребности населения республики в данной витаминной продукции необходимо постоянное увеличение его урожайности. Однако в последние годы в республике наблюдается существенное снижение урожая и ухудшение качества лука. Одной из серьезных причин недобора урожая данной культуры являются потери от болезней. Так, по данным А.А. Джаймурзиной и др., [1] наиболее распространенными болезнями на Юго-Востоке Казахстана являются фузариоз и бактериоз, поражение которыми достигает 72 и 46% соответственно. Особенно на этой культуре вредоносны факультативные паразиты-грибы Botrytis allii, Fusarium oxysporum, бактерия - Erwinia carotovora. В период хранения луковиц, они вызывают массовую гниль продукции, ухудшают их качество. К тому же, повсеместное применение химических обработок приводит к развитию устойчивых форм фитопатогенов к применяемым фунгицидам.