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Haploid-Tissue Culture and Biotechnology

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ABSTRACT. Haploid-tissue culture has arisen during the 1960's. Since then haploid plants or calluses have been obtained in a number of families of Angiosperms. Many investigators found that anther culture can be affected by many factors. The particular stage of development of the anthers at the time of culture, and plant-growth environment are two important factors. Chilling anthers or buds, soaking detached flowers in cold water for several days and incorporating activated charcoal to the media help in increasing embryonic potential of pollens in culture.

There are two approaches for doubling chromosome number in haploids: (1) Inducing doubling chemically by colchicine and (2) Selection of diploids among plants regenerated from haploid callus in tissue culture.

Haploids are required for two main reasons: the presence of one set of chromosomes and obtaining homozygous plants from diploidization. These two advantages can help in applying haploids in breeding programs. Some of its uses are: obtaining mutated cells, tissues or flowering plants easily and as fast as possible, obtaining new combinations of characters which otherwise cannot be detected until F_2 or F_3, introducing new characters from wild type of cultivars, to produce heterosis effect and can also be employed for transformation study.

Introduction

Tissue culture technique was developed in the later part of the nineteenth century. The first successful organ culture was achieved by White using tomato root-tips[1]. Later on Gautheret was able to culture cambial tissue isolated from carrot *in vitro*[2].

In 1941, Van Overbeek *et al.* were able to isolate and culture embryos from Datura on medium containing coconut milk, the liquid endosperm extracted from

coconut[3]. Later on many investigators were able to culture embryos of different plants successfully, *in vitro* [4-10].

One method for increasing the propagation of plant is shoot apex culture[11-13]. The growth of bacteria free crown gall tissue was reported by White and Braun[14]. A summary in this field has been outlined by Butcher[15].

The first cell culture that developed into whole plant was by Steward in carrot[16]. Different techniques for culturing single cell were developed[17-19]. Several studies on cell suspensions were also conducted by Torrey & Reinert[20] and Torrey *et al.*[21].

During the 1960s, the technique of protoplast isolation and culture was developed[22]. Later on, several investigators reported on culturing protoplasts[23-25].

Another development during 1960s was culturing the angiosperm anthers, haploid tissues, to produce haploid embryos and plants[26-29].

Applying tissue culture technique was always useful in the field of agriculture. In this paper, haploids produced from anther culture and its application in biotechnology will be outlined.

Haploid Plants from Anther Culture

Haploid plants possess one set of chromosomes. The phenotype, in these plants, is the result of a single-copy genetic information. Haploids have many uses in plant breeding especially in studies on the induction of mutations and also for the production of homozygous plants. In 1964, Guha and Maheshwari discovered that the pollen of Angiosperm, *Datura innoxia*, could be triggered into active growth by culturing it within the anther (anther culture)[26]. Growth was organized and led to the formation of haploid plants[28]. Since then anther culture technique have been applied in many Angiosperms. *Datura innoxia* and *Nicotiana tabacum* have played major roles in the development of anther culture [30]. The families in which haploid plants or calli have been obtained by anther culture are: Liliaceae, Solanaceae, Rubiaceae, Rosaceae, Iridaceae, Gramineae, Ranunculaceae, Geraniaceae, Salicaceae, Primulaceae, Gesheriaceae and Vitaceae[31].

Factors Affecting Anther Culture

1. *Anther Stage of Development.* The particular stage of development of the anthers at the time of culture is the most important factor in achieving success in the production of embryoids. In culture, although androgenesis can be induced in anthers at the tetrad stage or at the binucleate pollen stage[32]. However, microspores just before or at the time of first mitosis are more suitable for the induction of androgenesis[33]. Sunderland reported that in flowers of many plant anthers fall into one of three categories, premitotic, mitotic, and post mitotic[34]. In premitotic category, the best response is obtained by using anthers in which the microspores have completed meiosis but have not yet started the first pollen division (e.g. *Hordeum vulgare*). Anthers of plants belonging to the mitotic group respond successfully at the time of the first pollen division (e.g. *Datura innoxia*). The early bicellular stage of pollen development is best in the post mitotic plants (e.g. *Nicotiana* sp.).

In species having a determinate number of anthers per bud, the stage in pollen development can be determined by removing one anther from each bud and examining the pollen after treatment with suitable stain. Acetocarmine (4% W/V carmine refluxed for 24 hours in 50% V/V acetic acid and filtered) is a useful general stain, but with species possessing a low DNA content, it is recommended that the pollen should first be stained with Feulgen reagent[30]. Whole anthers are fixed in acetic acid: ethanol, 1:3 V/V, for several hours at 4°C, taken down to water through a graded series of ethanol, hydrolysed in 5N HCl for 1 hour at room temperature and stained in Feulgen reagent for at least 2 hours. The anthers are then squashed in acetocarmine. Staging based on one anther is acceptable for most species and gives a fairly accurate assessment of the situation in the other anthers of the bud.

2. Plant genotype is one of the most important factors affecting the success of *in vitro* induction of haploids. The induction frequency of callus from anthers as well as the frequency of differentiation were higher in hybrid rice than commercial varieties[35]. Foroughi-Wehr in 1982 stated that culture responsiveness in wheat is a heritable complex character involving at least two different and separately inherited mechanisms:

i) the ability of microspores within anthers to divide and give rise to calluses and, subsequently,

ii) the ability of calluses for morphogenesis to yield green or albino plants[36].

It is highly desirable that a general survey of various cultivars be undertaken[37].

3. Plant-growth environment is very important in the formation of pollen embryos and plantlets in anther culture. It includes, temperature, photoperiod, light intensity, and age of plant. It has been found in *Nicotiana knightiana* that the number of anthers responding is doubled by an increase in plant growth temperature from 14°C to 20°C[30]. Higher yields of embryos have been reported by Dunwell (1976) in *Nicotiana tabacum* using donor plants grown under short days and high light intensities[38]. The effect of the age of donor plant on number of anthers-producing plants and on number of plants produced have been reported by Sunderland and Dunwell in *Nicotiana tabacum*[30]. They illustrated extent of the decline in both factors throughout the flowering period.

4. Several treatments of anthers, flower buds or plants before culturing help in increasing the embryonic potential of pollens in culture. Nitsch and Norreel (1973) found an apparent increase in response of anthers of *Datura innoxia* taken from detached buds that had been kept in refrigerator for 48 hours[30]. Low temperature and pretreatment of anthers for periods of 2-30 days at 3-10°C may stimulate embryogenesis. It was found that incase of tobacco, best results will be obtained by chilling the buds approximately 12 days (7-8°C) prior to culture[39].

The effect of cold treatment is indirect. The increase in androgenesis is mainly attributed to the fact that low temperature (3-5°C) retains the pollen viability longer, delays senescence, and prevents the abortion of pollen and thereby increases the number of available viable pollens which are destined to form embryos[37]. Brief ex-

posure to higher temperature have been reported to stimulate repeated division of pollen, for example in brassica pollens, embryogenesis is stimulated in anthers subjected to 30°C for 24 hours or 40°C for just 1 hour[40].

Other type of pretreatment is soaking the detached flowers in water for several days[41]. In case of species like *D. innoxia* which have large buds, individual buds at the critical stage are placed with their stalks in water and kept in a refrigerator at 4-5°C. Species with small buds are best treated as whole or part inflorescence, and grasses as whole tillers[30]. An alternative and easier method is to enclose the excised parts in polytene bags containing a few drops of water. The bags are sealed and placed in refrigerator in darkness. In general 3-4 days are satisfactory.

Sangwan-Norreel found that centrifugation of anther at 3-5°C for approximately 30 min. gave good results in *Datura innoxia* Mill[42].

Anther Media

Basal media of White[1], Murashige and Skoog[43] and Nitsch and Nitsch[33], with slight modifications and the additions of growth regulators have been used for the culture of excised anthers[31]. The normal level of sucrose is 2-4%. Sunderland and Dunwell grouped species into two categories with respect to medium-requirements[30]. The first category includes species that are hormone independent. Only a simple basal medium is required for producing plantlets or calli from pollens in anthers. From these species, *Datura innoxia* and *Nicotiahat abacum* are two examples. Hormones utilized in this class must come from the anther wall or the pollen itself. As a basal medium, they suggested the formulation of Murashige & Skoog (MS)[43] or Bourgin and Nitsch (H medium)[29] for these species. Incorporation of activated charcoal into the medium has stimulated the induction of androgenesis in tobacco anthers[44, 45]. The charcoal is thought to adsorb inhibitory substances and thereby reduce the number of potential pollen embryos that would normally have aborted. However, it is more probable that the level of growth substances (both endogenous and exogenous) is regulated by absorption into charcoal[37].

The second category includes species that are hormone dependent. Auxins are the main hormones concerned. The need for cytokinins and other hormones is less certain. Examples of species belonging to this class are: *Hordeum, Oryza* and *Triticum.* In this class, sucrose must be supplied to ensure induction and is most effective when supplied in concentrations from 5-15%. MS medium is one of the principal basal media used in this class. Also the modified Miller medium (MM medium) has been used successfully[46]. The basal medium can be supplemented by growth factors in the form of yeast extract, coconut milk, potato extract, hydrolysates of both protein and nucleic acids and amides such as glutamine and asparagine[30]. The formulations of media that have been mentioned in this paragraph are presented in Table 1.

Techniques

The flower buds are excised at the suitable stage, surface-sterilized with a 5% solution of commercially available bleach or a 1% calcium hypochlorite solution for 10

TABLE Formulations of basal medium commonly used in anther culture (mg/1).

	White (1943)	Murashige and Skoog (MS) (1962)	Bourgin & Nitsch (H) (1967)	Nitsch & Nitsch (1969)	Modified Miller (MM) (Chu *et al.* 1975)
Macronutrients					
$Ca(NO_3) 4H_2O$	288	–	–	–	–
KhO_3	80	1900	950	950	2830
NH_3NO_3	–	1650	720	720	–
KCl	65	–	–	–	–
KH_2PO_4	–	170	68	68	400
$NaH_2PO_4 4H_2O$	19	–	–	–	–
$CaCl_2 2H_2O$	–	440	166	166	166
$MgSO_4 7H_2O$	737	370	185	185	185
Na_2SO_4	200	–	–	–	–
NH_4SO_4	–	–	–	–	463
Micronutrients					
$Fe(SO_4)_3$	2.5	–	–	–	–
$FeSO_4 7H_2O$	–	27.8	–	27.8	27.8
Na_2EDTA	–	37.3	37.5	37.3	37.5
$MnSO_4 4H_2O$	6.7	22.3	25	25	4.4
H_3BO_3	1.5	6.2	10	10	1.6
$ZnSO_4 4H_2O$	2.2	8.6	.10	10	1.5
KI	0.75	0.83	–	–	0.8
$Na, MoO_4 2H_2O$	–	0.25	.25	0.25	–
$CuSO_4 5H_2O$	–	0.025	.025	.025	–
$CoCl_2 6H_2O$	–	0.025	27.8	–	–
Organic					
Biotin	–	–	.05	0.05	–
Glycine	3.0	2.0	2.0	2.0	2.0
Inositol	–	100	100	100	–
Nicotinic acid	0.5	0.5	5.0	5	0.5
Pyridoxine–HCl	0.1	0.5	0.5	0.5	0.5
Thiamine–HCl	0.1	0.1	0.5	0.5	1.0
Folic acid	–	–	0.5	5	–
Sucrose	20000	20000	20000	20000	50000

minutes and then washed twice with sterile, distilled water. However, the flower buds obtained from plants grown in green house, if dissected carefully, need no sterilization[31]. Anthers can be cultured either on agar-solidified culture media or by floating them on the surface of a liquid medium. The anthers are cultured at 25°C in light or darkness. Light is essential after plantlet formation has been initiated for the production of chlorophyll and normal plant growth. Continuous illumination from cool-white fluorescent lamp (300 lux) gives satisfactory results[47]. Depending upon the plant species, it takes about 3-8 weeks for pollen plantlets to emerge from the anthers. The plantlets when approximately 5 cm tall can be removed from the culture and freed from agar by gently washing with running tap water. The plantlets are then

transferred to small pots containing autoclaved soil. To reduce sudden shock and to prevent desiccation, it is advisable to cover these plantlets with glass beakers and to keep them in a well-lit humid green house. After one week, the beakers are removed and after further two weeks, they are again transferred into larger pots where they develop to maturity[31].

Production of Homozygous Diploid Plants

There are two approaches for doubling chromosome number of haploid plants: (1) Selection of diploids from among plants regenerated from haploid callus in tissue culture. (2) Inducing doubling chemically by colchicine. The first method includes the production of callus from stem segments or other parts of the pollen plant and then transferring the callus to a regeneration medium[30]. A technique for inducing plantlets formation starting with leaf explants from haploids has been given by Kasperbauer and Wilson[48]. One method of chromosome doubling employs aged leaf tissue from haploid plant because older leaves have the potential to regenerate both haploid and diploid plants. The diploids result from chromosome endoreduplication, which frequently occurs in cultured plant tissue[47].

In the second method, colchicine may be applied to either embryos or haploid plants. A simple procedure is to immerse the anthers with the newly formed plantlets in anaquous solution of colchicine 0.5% W/v) for 24-48 hrs[47]. Alternatively, mature plants may be treated with the drug either in the form of lanoline paste (0.4%) applied to auxiliary buds of decapitated plants or as a 0.1% solution applied to whole inflorescence for 1-2 days. Inagaki showed that in wheat, the haploid plants treated at the 2-3 tiller stage with 0.1% colchicine solution produced seeds at a frequency of 95.6% treated plants[49].

However, spontaneous doubling of chromosomes in anther culture is highly desirable because the time consuming and tedious procedure of artificially doubling can be avoided. Bajaj reported that the diploid plants, in general, appear to rise from the somatic anther tissue[37]. Some seem to arise from fusion of pollen nuclei. The embryos and plants produced from such pollen would be completely homozygous as well as being diploid. Metz *et al.* produced high frequency of spontaneous doubling (more than 60%) in two lines of wheat. Centurk and NB88 using longer time in culture and high culture temperature conditions[50]. The frequency of diploidy might be high enough to eliminate colchicine treatment step.

Haploids and Its Biotechnological Applications

Significance and uses of haploids are summarized by Reinert and Bajaj[31] and by Straub[51]. Haploids are required for two main reasons: the presence of one set of chromosomes, and obtaining homozygous plants from diploidization. These two advantages of haploid plants can help in applying haploids in breeding programs. Some of its uses are briefly outlined in the following points :

1. Haploid is the way to obtain, as fast as possible, mutated cells, tissues, or flowering crop plants since the second unchanged genome is absent, and the gene activity

can express itself freely. In most cases haploids are sterilize, because meiosis leads to germ cells that lack chromosomes. If mutants are made by colchicine treatment, these diploid mutants should be fully fertile.

2. It is known that chromosomes exchange parts not only during meiosis but also during mitosis. When this exchange of parts takes place in diploid plants, it will remain genetically unnoticed, because it occurs between two homologous chromosomes and it is an exchange of identical parts. In mitosis of haploids, the exchange takes place between non-homologous chromosomes. Many haploids apparently form a source and many genetically changed cells are produced. These changes which first occur in parts of tissues can also be turned into mutated plants. In the haploid *Petunia* Straub showed that parts of haploid genome very regular undergo such dislocation[52]. The transferred parts are involved in the genetic basis of anthocyanin formation in the flowers. After exchange the gene for anthocyanin in haploid *Petunia* is located in position where it remains inactive. This is why normally blue-flowering haploid *Petunia* shows white sectors of various sizes and a great degree of variegation.

3. A feature of anther culture is that the plant which are obtained exhibit various levels of ploidy. Desirable plants can be obtained and incorporated into the breeding program.

Badea and Raicu obtained plants from culture of anthers of diploid, tetraploids and hexaploids of *Datura innoxia*[53]. The produced plants are haploid, diploid, triploid, tetraploid, hexaploid and aneuploid. Lanee *et al.* were able to obtain secondary embryoids from the epidermal or subepidermal zones of the primary embryoids of *Datura innoxia*[54]. 12% of the secondary embryoids contained a few aneuploid cells or were mixoploids.

Shimada obtained variation of chromosome number in 2 cultivars of wheat: Orofen and Dansheng 15. The variation of chromosome number in both cultivars was maintained after eighteen months of subculturing[55]. Wang produced 62 plantlets from anthers of F_1 hybrid between hexaploid triticale Rosner and common wheat Kdong 58 through anther culture[56]. The cytological studies showed that the chromosome compositions of pollen plants were various including different aneuploids. Metz *et al.* reported that doubled haploid population was obtained from two hard red winter wheats, Centurk and NB88. Most regenerates were either haploid or diploid. Among the regenerates haploid aneuploids and diploid aneuploids were observed[50].

4. New combinations of characters which otherwise cannot be detected until the F_2, and in the homozygous form until the F_3 or even later generations, may already be selected from haploid plants which develop from the pollen grains of an F_1 hybrid. When these are diploidized, the new combination is already available as a fertile pure line.

5. Using wild forms as plant material for the introduction of certain characters, we are dealing with plants of mixed genetic background. If one obtains haploids from

such wild forms, the haploids will proved to have variability for the production of agronomically useful substances. There is no doubt that all wild types segregate when haploids are produced. To change any character, whether morphological or metabolical, success will come faster by the haploid technique than by the analysis of the F_2 after selfing.

6. Heterosis effect can be achieved by first obtaining homozygous material through inbreeding and second, intercrossing the inbred lines. Cases are known in which haploids behave like inbred lines. A plant that has been analyzed in this respect is the cultivated potato.

7. Haploids can be used to obtain homozygosity for genes in cases where it is difficult to achieve such as self-incompatible alleles in rye, and thus the number of generations of self pollinations are eliminated. This is very important in winter barley because of the time required for vernalization.

8. Haploids can be used to transfer cytoplasm from one line to another in one step. Using *zea mays* monoploids Kernicle successfully transferred the genotypes of inbred lines into cytoplasm that caused male sterility[31].

9. In some plants of horticulture importance which are normally propagated vegetatively by means of corns, it takes 8-10 years to produce a clone which is large enough for commercial purpose. In these cases, anther culture has obvious advantages. Specific desirable gene combinations may be linked in a haploid form and then converted to the fertile homozygous diploid or polyploid forms in relatively short time.

10. Haploids can be employed for transformation studies. Doy *et al.* used haploid tomato *Lycopersicon esculentum* cell cultures and reported their transformation by the incorporation of phage lambda[57].

References

[1] **White, P.R.**, Potentially unlimited growth of excised tomato root tips in liquid medium, *Plant Physiol.* **9:** 585-600 (1934).

[2] **Gautheret, R.J.**, Sur la possibilite de realiser la culture indefinie des kissu de tubercules de carrotte, *C.R. Acad. Sci. (Paris)*, **208:** 118-121 (1939).

[3] **Van Overbeek, J.**, **Conclin, M.E.** and **Blakeslee, A.F.**, Factors in coconut milk essential for growth and development of very young *Datura* embryos, *Science* **94:** 350-351 (1941).

[4] **Nakajima, T.** and **Morishima, H.**, Studies on embryo culture in plants. II. On embryo culture of interspecific rice hybrids, *Jap. J. Breeding* **8:** 105-110 (1958).

[5] **Davies, D.R.**, The embryo culture of interspecific hybrids of *Hordeum*, *New Phytologist* **59:** 9-14 (1960).

[6] **Grant, W.F.**, **Buillen, M.R.** and **De Nattancourt, D.**, Embryo, cultured interspecific diploid hybrids in *Lotus*. *Amer. J. Bot.* **48:** 533-534 (1961).

[7] **Grant, W.F.**, **Buillen, M.R.** and **De Nattancourt, D.**, Cytogenetics of *Lotus*. I. embryo cultured interspecific diploid hybrids closely related to *L. corniculatus* L., *Canad. J. Genet. Cyt.* **4:** 105-128 (1962).

[8] **Norstog, K.J.**, Culture of small barley embryos on defined media, *Science* **142:** 1655-1656 (1963).

[9] **Shahin, S.A.**, *Interspecific hybridization between Zinnia pauciflora through embryo culture*. Ph.D. thesis, Utah State University, Logan, USA (1966).

[10] **Shahin, S.A., Campbell, W.F., Pollard, L.H.** and **Hamson, A.R.**, Interspecific Hybrids of *Zinnia peruviana* and *Z. elegans* through embryo culture, *J. Amer. Soc. Hort. Sci.* **96**(3): 365-367 (1971).
[11] **Ball, E.**, Development in sterile culture of stem tips and subjacent regions of *Tropaeolum majus* L. and *Lupinus albus* L., *Am. J. Bot.* **33:** 301-318 (1946).
[12] **Wetmore, R.H.** and **Wardlaw, C.W.**, Experimental morphogenesis in vascular plants, *Annu. Rev. Plant Physiol.* **2:** 269-292 (1951).
[13] **Morel, G.**, Producing virus-free Cymbidiums, *Am. Orchid Soc. Bull.* **29:** 495-497 (1960).
[14] **White, R.R.** and **Braun, A.C.**, A cancerous neoplasm of plants. Autonomous bacteria-free crown gall tissue, *Cancer Res.* **2:** 597-617 (1942).
[15] **Butcher, D.N.**, Plant tumour cells. *In:* **Street, H.E. (ed.)** *Plant Tissue and Cell Culture,* Oxford: Blackwell Scientific Publications, pp. 429-461 (1977).
[16] **Steward, F.C.**, Growth and development of cultivated cells. III. Interpretations of the growth from free cell to carrot plant. *Am. J. Bot.* **45:** 709-713 (1958).
[17] **Muir, W.H.**, *Culture conditions favouring the isolation and growth of single cells from higher plants in vitro,* Ph.D. thesis, Univ. of Wisconsin, Madison, USA (1953).
[18] **Torrey, J.G.** and **Shigomura, J.**, Growth and controlled morphogenesis in pea root callus tissue grown in liquid media, *Am. J. Bot.* **44:** 334-344 (1957).
[19] **Jones, L.E., Hildebrandt, A.C., Riker, A.J.** and **Wu, J.H.**, Growth of somatic tobacco cells in microculture, *Am. J. Bot.* **47:** 468-475 (1960).
[20] **Torrey, J.G.** and **Reinert, J.**, Suspension cultures of higher plant cells in synthetic medium, *Plant Physiol.* **36:** 483-491 (1961).
[21] **Torrey, J.G., Reinert, J.** and **Merkel, N.**, Mitosis in suspension cultures of higher plant cells in synthetic medium, *Am. J. Bot.* **49:** 420-425 (1962).
[22] **Cocking, E.C.**, A method for the isolation of plant protoplasts and vacuoles, *Nature,* **187:** 962-963 (1960).
[23] **Takebe, I., Labib, G.** and **Melchers, G.**, Regeneration of whole plants from isolated mesophyl protoplasts of tobacco, *Naturwissenschaften,* **58:** 318-320 (1971).
[24] **Bajaj, Y.P.S.**, Protoplast isolation, culture and sómatic hybridization. *In:* **Reinert, J.** and **Bajaj, Y.P.S. (eds.)** *Applied and Fundamental Aspects of Plant Cell, Tissue and Organ Culture.* pp. 467-496 (1977).
[25] **Mastrangelo, I.A.**, Protoplast fusion and organell transfer. In Nicotiana. Procedures for experimental use, ed. R.D. Durbin, pp. 65-73. Washington, D.C., U.S. Dept. of Agriculture, *Tech. Bull.* No. 1586 (1979).
[26] **Guha, S.** and **Maheshwari, S.C.**, *In vitro* production of embryos from anthers of Datura, *Nature,* 204-497 (1964).
[27] **Guha, S.** and **Maheshwari, S.C.**, Cell division and differentiation of embryos in the pollen grains of Datura *in vitro, Nature.* **212:** 97-98 (1966).
[28] **Guha, S.** and **Maheshwari, S.C.**, Development of embryoids from pollen grains of *Datura in vitro, Phytomorphology,* **17:** 454-461 (1967).
[29] **Bourgin, J.P.** and **Nitsch, J.P.**, Development of embryoids from pollen grains of *Datura in vitro, Phytomorphology* **17:** 454-461 (1967).
[30] **Sunderland, N.** and **Dunwell, J.M.**, Anther and pollen culture. *In:* **Street, H.E. (ed.)** *Plant Tissue and Cell Culture,* Oxford, Blackwell Scientific Publications, pp. 223-265 (1977).
[31] **Reinert, J.** and **Bajaj, Y.P.S.**, Anther culture: Haploid production and its significance. *In:* **Reient J.** and **Bajaj, Y.P.S. (eds.)** *Applied and Fundamental Aspects of Plant Cell, Tissue, and Organ Culture,* Springer-Verlag, pp. 251-267 (1977).
[32] **Kameya, T.** and **Hinata, K.**, Induction of haploid plants from pollen grains of Brassica, *Jpn. J. Breed.* **20:** 82-87 (1970).
[33] **Nitsch, J.P.** and **Nitsch, C.**, Haploid plants from pollen grains, *Science* **163:** 85-87 (1969).
[34] **Sunderland, N.**, Towards more effective anther culture, *Newsletter IAPTC* **27:** 10-12 (1979).
[35] **Hu Han.**, Advances in anther culture investigations in China, *Proc. of Symposium on Plant Tissue Culture,* Pitman Publishing Lit. and The Science Press (1981).
[36] **Foroughi-Wehr, Friedt, W.** and **Wenzel, G.**, On the genetic improvement of androgenetic haploid formation in *Hordium vulgare* L., *Theor. Appl. Genet.* **62:** 233-239 (1982).

[37] **Bajaj, Y.P.S.**, *In vitro* Production of Haploids. *In:* **David, A.E., William, R.S., Philip, V.A.** and **Vasuyuki, Y. (eds.)** *Handbook of Plant Cell Culture.* McMillan Publishing Company, pp. 228-287 (1983).

[38] **Dunwell, J.M.**, A comparative study of environmental and developmental factors which influence embryo induction and growth in cultured anthers of *Nicotiana tabacum, Environ. Exp. Bot.* **16:** 109-118 (1976).

[39] **Sunderland, N.** and **Roberts, M.**, New approaches to pollen culture, *Nature* **270:** 236-238 (1977).

[40] **Keller, W.A., Armstrong, K.C.** and **dela Roche, I.A.**, The production and utilization of microspore-derived haploids in cruciferous crop species, *Proc. Int. Symp. Plant Cell Culture in Crop Improvement,* Calcutta (1981).

[41] **Wilson, H.M., Mix, G.** and **Foroughi-Wehr, B.**, Early microspore divisions and subsequent formation of microspore calluses at high frequency in anthers of *Hordeum vulgare* L., *J. Exp. Bot.* **29:** 227-238 (1978).

[42] **Sangawan-Norreel, B.S.**, Androgenic stimulating factors in the anther and isolated pollen grain cultures of *Datura innoxia* Mill, *J. Exp. Bot.* **28:** 843-852 (1977).

[43] **Murashige, T.** and **Skoog, F.**, A revised medium for rapid growth and bioassays with tobacco tissue cultures, *Physiol. Plant.* **15:** 473-497 (1962).

[44] **Weatherhead, M.A., Bordon, J.** and **Henshaw, G.G.**, Some effects of activated charcoal as an additive to plant tissue culture media, *Z. Pflanzenphysiol.* **89:** 141-147 (1978).

[45] ————. Effects of activated charcoal as an additive to plant tissue culture media, Part 2, *Z. Pflanzenphysiol.* **94:** 399-405 (1979).

[46] **Chu, C.C., Wang, C.C., Sun, C.S., Hsu, C., Yin, K.C.** and **Chu, C.Y.**, Establishment of an efficient medium for anther culture of rice through comparative experiments on the nitrogen sources, *Sci. Sinica* **18:** 659-668 (1975).

[47] **Dodds, J.H.** and **Roberts, L.W.**, *Experiments in Plant Tissue Culture,* Cambridge University Press, Cambridge (1985).

[48] **Kasperbauer, M.J.** and **Wilson, H.M.**, Haploid plant production and use. In *Nicotiana.* Procedures for Experimental Use. ed. R.D. Durbin. Washington, D.C., U.S. Department of Agriculture, *Tech. Bull.* No. 1586, pp. 33-39 (1979).

[49] **Inagaki, M.**, Chromosome doubling of the wheat haploids obtained from crosses with *Hordeum bulbosum* L., *Japan J. Breed.* **35:** 193-195 (1985).

[50] **Metz, S.G., Sharma, H.C., Armstrong, T.A.** and **Mascia, P.N.**, Chromosome doubling and aneuploidy in anther-derived plants from two winter wheat lines. *Genome* **30:** 177-181 (1988).

[51] **Straub, J.**, Theoretical Aspects of Haploid Techniques, *Proceedings of the First International Congress on Medicinal Plant Research, Section B, University of Munich, Germany,* 336-413 (1977).

[52] ————. Die genetische variabilitat haploider *Petunien, Z. Pflanzenzuchtg.* **70:** 265-274 (1973).

[53] **Badea, E.** and **Raicu, P.**, Experimental androgenesis in *Datura innoxia* of plants with different levels of ploidy (2n, 4n, hn), Revue Roumaine de Biologie, *Biologic Vegetab.* **28**(1): 59-61 (1983).

[54] **Lanee, O., Sangwan-Norreel, B.S.** and **Sangwan, R.S.**, Nuclear DNA contents and ploidy in somatic embryos derived from androgenic plantlets of *Datura innoxia, J. Plat. Physiol.* **130**(1): 37-48 (1987).

[55] **Shimada, T.**, Diploid and haploid cultures of common wheat, *Triticum Aestivum* L. I. Chromosome stability of haploid cell culture, *Proc. 6th International Wheat Genetics Symposium, Kyoto, Japan,* 583-586 (1983).

[56] **Wang, X.**, The application of anther culture technique for improving primary distant hybrids of wheat, *Proc. 6th International Wheat Genetics Symposium. Kyoto, Japan,* 587-590 (1983).

[57] **Doy, C.H., Gresshoff, P.M.** and **Rolfe, B.G.**, Biological and molecular evidence for the transgenosis of genes from bacteria to plant cells, *Proc. Natl. Acad. Sci. U.S.* **70:** 723-726 (1973).

زراعـــة الأنسجـــة الأحاديـــة (المتـــوك) ودورها في التكنولوجيـــا الحيويـــة

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قسم النبات ، كلية التربية للبنات ، جـــدة ، المملكة العربية السعودية

المستخلص . بدأت زراعة الأنسجة الأحادية (المتوك) منذ عام ١٩٦٤م وقد نجحت هذه الطريقة في عدد لابأس به من عائلات النباتات مغطاة البذور فأنتجت في بعض الحالات نبـاتـات أحـادية العـدد الكروموسومي وفي البعض الآخر انتجت أنسجة أحادية العدد الكروموسومي . وهناك العديد من العوامل التي تؤثر على نجاح زراعة المتوك أهمها الدور الذي تؤخذ عنده المتوك لزراعتها ، وقد وجد أن ذلك يختلف بإختلاف النباتات المستخدمة كما يتـأثر نجاحها أيضًا باختلاف الظروف البيئية للنبات تحت الدراسة مثل شدة الضوء وطـول فترة الإضـاءة ودرجـة الحـرارة وعمـر النبات ، كما وجدت أن هناك أيضًا بعض المعاملات التي تزيد من إنتاج الأجنة الأحادية والناتجة من المتوك ومن هذه المعاملات إضافة الفحم للبيئات المستخدمة ومعاملة المتوك أو البراعم بدرجات الحرارة المنخفضة أو نقعها في ماء بارد لعدة أيام .

وتعتـبر النبـاتات الأحادية المتحصل عليها نباتات عقيمة إلا أنه يمكن مضاعفة عدد كرومـوسـومـاتها بالمعاملة بمادة الكولشيسين أو بزراعة بعض الأنسجة الأحادية وانتخاب النباتات الثنائية من النباتات المتكونة .

وللنباتات الأحادية أهمية كبيرة في مجالات الزراعة فهي تحتوي على ميزتين : احتوائها على مجموعة كروموسومية واحدة وإمكانية الحصول على نباتات ثنائية أصيلة . والميزة الأولى هامة بالنسبة لاستحداث الطفرات والتي يكون معظمها متنحي وأيضًا بالنسبة لانتخاب صفات جديدة نادرة نتيجة الانعزال في الأصناف الخليطة والأصناف البرية . أما الميزة الثانية فهي تسهل إمكانية استخدام الطفرات المتنحية والسلالات المحتوية على صفات جديدة ، كما ثبت أيضًـا إمكـانية استخـدام هذه النبـاتات لإعطاء ظاهرة قوة الهجن وظاهرة التحول الوراثي . كما تستخدم في مجالات الزراعة للإسراع من إنتاج سلالات محتوية على جينات مرغوبة في النباتات التي تتكاثر خضريا بالكورمات .