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Induced Androgenic Embryogenesis in Cereals

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1. Introduction

Plant breeding, a system of gene pooling through generations of phenotypic selection has long been the only method available for crop improvement. For many years *in vitro* plant breeding has been used in many plants for different traits improvement.

The method of *in vitro* plant breeding has been used for improving different traits in many crops. Gamethophytic embryogenesis is one of the methods for production of haploid plants.

Androgenesis is defined as culture of female gametophytic cells/tissues on a plant tissue culture medium in sterile conditions. Androgenesis can be used as anther culture or isolated microspore culture (IMC).

The production of haploids and doubled haploids (DHs) through androgenic embryogenesis allows a single-step development of complete homozygous lines from heterozygous stuck plants, reduction the time required to produce homozygous plants in comparison with the conventional breeding methods that employ several generations of autogamy.

Androgenic embryogenesis is one the different methods of embryogenesis present in the plant kingdom, and it consists in the capacity of male (microspore or anther) to permanently switch from their gametophytic pathway of development towards a sporophytic one. Differently from somatic embryogenesis, which provides the clonal propagation of the genotype (unless the somaclonal variation), androgenic embryogenesis results in haploid plants (unless spontaneous or induced chromosome duplication occurs), because such plants are derived from the regeneration of male gametes, products of meiotic segregation.

Androgenic embryogenesis (also referred to as androgenesis) is regarded as one of the most striking examples of cellular totipotency, but also as a form of atavism. It is an important survival adaptation mechanism in the plant kingdom that is expressed only under certain circumstances and as a consequence of an environmental stress. In comparison to conventional breeding methods, androgenic embryogenesis makes the production of homozygous lines feasible and shortens the time required to produce such lines, allowing the single-step development of completely homozygous lines from the heterozygous parents. Conventional methods performed to achieve homozygosity consist

of carrying out several backcrosses or selfing; as such, they are time-consuming and labour-intensive procedures.

2. Isolated microspore technique

The technique of isolated microspore culture, performed by removing somatic anther wall, requires better equipment and more skills compared to anther culture, although the earlier provides the better method for investigating cellular, physiological, biochemical and molecular processes involved in androgenic embryogenesis.

The isolated microspore culture is as a powerful tool of *in vitro* plant breeding for haploid and doubled haploid plant production. This technique may allow faster production of new varieties than using conventional breeding methods and has been successfully employed in many crop plants. The microspore is at the centre of a variety of topics in modern plant science and breeding. High frequency regeneration of fertile plants (doubled haploids) from isolated microspores is an important tool for different plant breeding and biotechnological applications.

Microspore culture is a form of androgenesis in which the developing immature pollen grain is stressed into switching pathways to become a sporophytic cell with the potential to regenerate into a green plant. Microspores can be isolated in large numbers providing a relatively uniform population of haploid, single cells capable of developing directly into embryos and plants. Thus, they provide excellent tools for studying embryogenesis, *in vitro* selection in culture and cell cycles relative to transformation.

Successful microspore tissue culture systems require a responsive genotype and a healthy homogenous population of donor plants producing physiologically healthy material**.** The genotype of the donor material can affect ethylene production, endogenous auxin and cytokinin activity**,** androgenic embryogenesis, plant regeneration and albinism.

The regeneration potential of the culture is dependent upon donor plants, staging, pre-treatment, isolation and culture media. Haploid plants must be chromosome doubled to restore fertility for use in plant breeding. Chromosome doubling of microspore-derived from plantlets and calli is a critical step in haploid breeding programs.

In a research an experiment was conducted to determine the responses of five barley genotypes to androgenic embryogenesis and spontaneous chromosome doubling (Kahrizi and Mohammadi, 2009). For study on effect of genotype upon androgenesis, after microspore culture, the number of embryos per 100 used anthers was measured. Results showed that genotype significantly affected the embryoid formation . This result is in agreement with the results of Castillo et al. (2000) but is in disagreement with Li and Devaux, (2003) and Kasha *et al*. (2004) that reported there was no significant difference in embryo induction among genotypes.

3. Chromosome doubling

Chromosome doubling to induce polyploidy has been widely used in plant breeding programs to restore fertility in sterile genotypes and to overcome crossing barriers.

The doubled haploids represent useful tools for applied breeding and genetic analysis.

For androgenesis experiments, colchicine is used in doubling the chromosomes. It has been applied to regenerated plants after transfer to soil or *in vitro* either initially in the microspore culture substrate. Colchicines however, is toxic carcinogenic and expensive.

For androgenesis experiments, colchicine, (S)-N-(5,6,7,9-tetrahydro-1,2,3,10-tetramethoxy-9-oxobenzo[a]heptalen-7-yl) acetamide, is the most commonly used antimitotic agent in doubling the chromosomes of seedling from such experiments (Inagaki, 1985), or *in vitro* either initially in the microspore culture substrate (Hansen and Anderson, 1998a). Colchicine, however, has a relatively low efficiency for plant microtubules and has carcinogenic effects for human and is expensive (Hansen & Anderson, 1998b).

In cytogenetic section of Kahrizi and Mohammadi (2009) research focused on chromosome doubling of haploids without applying any antimitotic agent as well as effect of genotype upon chromosome doubling was studied.

Spontaneous chromosome doubling rates among microspore-derived from wheat plants are 15-25%. It has been revealed that spontaneous chromosome doubling in barley constituted 70-80% of regenerated population and only 15-20% plantlets were haploids.

Barley is important as a global crop and as a leading mode plant for isolated microspore culture and cereal transformation studies.

The utility of doubled haploid lines in barley breeding programs have been demonstrated, and a number of cultivars have been developed using this system. Microspore culture in barley has been improved more than in other cereals and thus is preferred for investigations such as microspore transformation.

4. Factors influencing androgenic embryogenesis

The success of androgenic embryogenesis is influenced with numerous factors such as: (Bajaj, 1990)

1. The growth and development of stock (donor) plants. Including:
   - 1.1 Photo period
   - 1.2 Light quality and intensity
   - 1.3 Temperature
   - 1.4 Nutrition and fertilizers
   - 1.5 CO_2 concentration
   - 1.6 Biotic and abiotic stresses

   The variation in androgenetic response is dependent upon the donor plants' growth environment. This has been reported in many of crops, including wheat (Picard & De Buyer 1975), rice (Chaleff & Stolarz 1981) and maize (Genovesi, 1990). The donor plants' physiology and vigor were found to greatly influence androgenic response frequency (Nitsch et al. 1982). Genovesi (1990) approved that anthers of weak donor plants produced only a few embryos or calli *in vitro*. This is probably indicative of the importance of endogenous factors for androgenic response. He also mentioned the importance of environmental factors, such as the influence of photoperiod, light intensity and quality, temperature and nutrition on the donor plant vigor. Field grown donor plants gave better embryo induction frequencies (Dieu & Beckert 1986). Nitsch *et al*. (1982) suggested that the stock plants be grown under optimal conditions and that pesticide treatment be avoided. androgenesis also respond differentially to incubation conditions such as temperature, light quality and quantity, CO_2 concentration. Abiotic stresses play a very important role in androgenic induction.

2. Microspore developmental stage

   Many investigators, who have reported success in maize anther culture, agreed that the uninucleate microspore is the most responsive stage for culture. However, some have shown a preference for the mid-uninuleate stage, while others preferred the late-uninuleate stage.

   The best time for harvesting wheat spikes is when the majority of microspores are at the mid- to late-uninucleate stage. During this period, microspores are most susceptible to androgenic induction treatment.

3. Microspore density

   Various microspore densities ranging from 2×10^4 to 2×10^5 / ml had been reported effective for embryo induction, a high density is not necessary for success. In fact, a density of $7–8 \times 10^3$ / ml is quite effective for embryoid development (Zheng et al., 2002b). The effective density ranges from 5×10^3 to 2×10^4 / ml. Relatively low but adequate microspore density eases the competition for nutrients, oxygen, and space for cell divisions and embryoid formation, hence improves both the number and quality of embryoids. The co-culture of microspores with ovaries and/or ovary-conditioned medium (OVCM) makes it possible to employ a lower density. In addition, microspores of high purity in culture also contribute to the success of using lower microspore densities.

4. Pretreatments. It may be treat on stock plant, spike, anther or microspores that including:
   4.1 Cold pretreatment
   4.2 Warm pretreatment
   4.3 Chemical pretreatment

Low or high temperature shocks are applied as a pre-treatment or at the early stages of induction in most protocols developed for both, mono and dicotyledonous plants. Temperature pretreatments are believed to improve androgenesis by diverting normal gametophytic development into a sporophytic pathway leading to the production of haploid embryo like structure (Nitsch et al. 1982). Genovesi (1990) reported highly significant effects of post-treatment with high temperature on embryoid formation. The role of temperature in androgenic induction is now better understood. It is described as one of many stress factors influencing microspore transition from gametophytic to sporophytic development.

Osmotic and starvation stress are nowadays frequently applied to cereals in combination with a relatively short, 3–5 day treatment with low temperature.

Different stress pretreatments including cold shock (Gustafson et al., 1995; Hu and Kasha, 1999), sugar starvation alone and in combination with cold shock or heat shock (Mejza et al., 1993; Touraev et al., 1996; Hu and Kasha, 1997, 1999) and inducer chemicals alone or in combination with heat shock (Zheng et al., 2001; Liu et al., 2002).

5. Inductive media for embryogenesis

   Improvements in the formulation of culture media have also contributed to the progress of androgenic methods. The composition of basic salts and micro-elements is wide and

varied. The most often modified components are: (1) the source of organic nitrogen, (2) carbohydrates, and (3) growth regulators. Several media are applied for androgenic embryogenesis in different cereal plants that is shown in table 1.

6. Compositions of Media

Improvements in the composition of culture media have also contributed to the progress of androgenic methods. The composition of basic salts and micro-elements is wide and varied. The most often modified components are: (1) the source of organic nitrogen, (2) carbohydrates, and (3) growth regulators.

The first significant step towards better efficiency in barley androgenesis was achieved by lowering the ammonium nitrate content and enriching the glutamine level as a source of organic nitrogen.

Carbohydrates provide as a source of energy, building material and a component that regulates the osmotic properties of the culture media. The most spectacular success in protocols efficiency was achieved by the replacement of sucrose by maltose in numerous versions of induction media. In wheat, triticale, rye and rice the concentration of maltose ranges from 60 to 90 g/l of induction media.

In the examples cited above for cereals, the concentration of sucrose in the regeneration media is in the order of 20–30 g/l, which is a standard amount in many other protocols. Sucrose and maltose are the main sugar components of the media throughout the literature with few examples of other carbohydrates tested.

Several substances are active as growth regulators *in vitro*, many of them are synthetic analogous of plant hormones. The kind of substance, its concentration and the proportions in which several components are composed remain of substantial importance in regulating cell division and morphogenesis. In many protocols for isolated microspore culture, growth regulators are omitted in the induction medium.

In barley androgenic cultures, BAP, IAA, NAA and PAA are added to the induction media alone or in combination at various concentrations. The improved protocol contains 1 mg/l of BAP with 10 mg/l of PAA. On the other hand, in anther culture of wheat, triticale and rye 2, 4-D and kinetin are used in the induction media and NAA with kinetin to stimulate regeneration. Abscisic acid (ABA) was applied to improve regeneration of induced embryos.

Microspore suspensions are often cultured without the addition of growth regulators although the most successful media are conditioned with ovaries. Conditioning with an actively growing suspension culture was also successfully applied to induce in vitro development of isolated zygotes. It can be presumed that the ovaries provide a source of active ingredients, phytohormones or other signaling molecules important for androgenic induction or embryo maturation. However, the data from detailed analysis of conditioned media have not yet been published.

Maize microspore culture was used recently as a model to study androgenic processes. Among others, the latter authors showed that arabinogalactan proteins added to the medium improved regeneration in low responsive genotypes. This discovery opens up new possibilities in improving the regeneration process, and may have beneficial effects

for other species. It is probable, that other molecules that play regulatory role are secreted into the conditioned media however, to prove this hypothesis more detail studies of media during culture are required.

7. Microspore separation method, releasing and purifying microspores

   At least seven different approaches exist for isolating microspores include mechanical separation, blending, maceration, stirring, vortexing, sonication and floating.

   Shedding is a technique first developed by Sunderland and Roberts (1977) in which cultured tobacco anthers shed their microspores into a liquid medium. These microspores were then collected and cultured for callus development and plant regeneration. The shedding technique was later adopted in wheat. More recently, a 6-7 day pretreatment in 0.3 M mannitol plus macronutrients was recommended for shedding microspores or for a step preceding mechanical isolation of microspores (Kasha et al., 1990). Magnetic-bar stirring is a derivative of shedding in that a stirring force is added to help release the microspores still enclosed within the anther wall. In effect, magneticbar stirring serves to increase microspore yields from the natural shedding. The shedding and stirring procedures, however, are not effective means in wheat microspore cultures due to the low yields of microspores and plants subsequently recovered.

8. Genotype of stock plants.

   The androgenic embryogenesis is highly dependent to stock plant genotype. It may be as intraspecies or interspecies variations (Table 2).

Media	References
N6	Chu, 1978
Yu-Pei (YP)	Ku et al., 1981
MS	Murashige and Skoog, 1962
FHG	Hunter, 1988
CHB-2	Chu et al., 1990
B5	Gamborg et al. 1968
FMN6	Mejza *et al*., 1993
A2	Touraev et al., 1996
MMS3	Hu and Kasha, 1997
NPB-99	Liu et al., 2002

Table 1. The media that are used for androgenic embryogenesis (Kahrizi et al., 2007)

5. Spontaneous chromosome doubling in androgenic embryogenesis

Haploid induction during anther or microspore culture begins with some form of stress applied at a critical stage before or during the culture of the microspores.

Chromosome doubling of microspore-derived from plantlets and embryos is a critical step in haploid breeding programs. In many plants microspores are doubled spontaneously. Spontaneous chromosome doubling rates among microspore-derived from wheat plants are 15-25%. (Kahrizi et al., 2009). It has been revealed that spontaneous chromosome doubling in barley constituted 70-80% of regenerated population and only 15-20% plantlets were haploids (Kahrizi & Mohammadi, 2009; Kahrizi, 2009). (Figures 1 and 2).

Plant	Number of studied genotypes	Responded genotypes[1] (%)	Genotypes with superior response[2] (%)	Regenerated Genotypes (%)	Reference
Barley	11	100	100	100	Logue *et al.*, 1993
	16	100	100	100	Hou *et al.*,1994
Wheat	31	97	77	77	Masojc *et al.*, 1993
	60	98	23	35	Orlav *et al.*,1993
Corn	40	60	3.0	19	Potelino and Jones, 1986
	55	47	4.0	7.0	Hangchang *et al.*, 1991

Table 2. The comparison of androgenic capacity for embryogenesis in barley, wheat and corn (Kahrizi et al., 2007)

The mechanism of chromosome doubling has been one of much speculation and the relationship to the influence of pretreatments is obscure, with endoreduplication and nuclear fusion as the most likely methods. A C-mitosis, such as occurs during colchicine treatment, may result in a simple restitution nucleus with a doubled chromosome number. In *Datura*, it was proposed that both endoreduplication and nuclear fusion were involved in chromosome doubling and that the combination of both methods could explain the ploidy levels obtained that were higher than diploid. Nuclear fusion was described as occurring when two nuclei synchronously entered into division, formed a common metaphase plate and spindle and resulted in two nuclei, each with more than one set of chromosomes. If one or both of the nuclei had undergone endoreduplication prior to nuclear fusion, triploid or higher ploidy level plants could be formed. Sunderland also showed clear evidence of endoreduplication from the generative nucleus and chromosomes from different nuclei on a common metaphase plate.

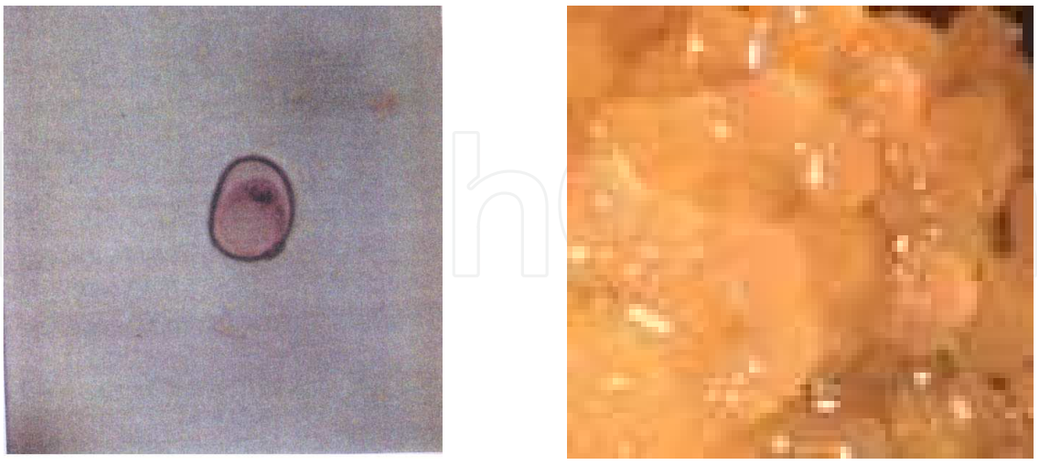


Fig. 1. Isolated microspore culture of barley. Cultured microspore after 4 days (left), Embryoid formation from cultured microspore in liquid medium (right).

[1] Formation at least one embryoid in 100 cultured anthers
[2] Formation at least 10 embryoids in 100 cultured anthers

Both the stage of the microspore when collected for pretreatment and the pathway of nuclear development have also been considered to influence the frequency of doubling. He concluded that microspores collected at uninucleate stages 1–3 (early, mid and late, respectively) resulted in mostly haploid and doubled haploid plants while those collected at later stages (4–6, mitosis and binucleate) resulted in mostly doubled haploids as well as some triploid and tetraploid plants. It has also been demonstrated in wheat that the pretreatment method will influence the pathway along which the nuclei will develop.

Fig. 2. Cytogenetic test for androgenetic plantlets in barley. The majority of plantlets were spontaneous doubled haploid (A) and Low percents of them were haploid (B) . Kahrizi D (2009).

Development from the normal gametophytic to an embryogenic (sporophytic) switch can be induced by the pretreatment of anthers or spikes. Pretreatments also influence the stage of microspores. Hu and Kasha found that uninucleate microspores of wheat completed the first mitotic division during both the 28 d cold pretreatment and the 6–7 d 0.4 M mannitol pretreatment at 28 °C (Hu and Kasha, 1999). It was also reported that a spike pretreatment combining 0.4 M mannitol solution and cold pretreatment for 4 d in wheat essentially blocked the mitotic division of the nucleus, keeping all microspores at the same stage during pretreatment, and also resulted in the formation of large numbers of true embryo-like structures (ELS) (Hu & Kasha, 1999).

6. Genetic control of microspore embryogenesis

Both environmental and genetic factors contribute significantly to the androgenic responses. The influence of environmental factors has been widely reviewed elsewhere. All three components in androgenesis, embryoid induction, total plant regeneration and green / albino plant ratio have been determined to be independently inherited traits. The inheritable nature of androgenic traits provides the basis for introducing these traits into non-responsive genotypes. In most cases, the genetic component of culturability is attributed to additive gene effects, although epistatic and dominant effects have also been observed. The dominant and additive gene effects provide opportunity to improve androgenic response through cross breeding and recurrent selection. In addition, significant interactions exist between nuclear genes and cytoplasm type for all three components of the androgenic response.

7. References

Bajaj,Y.P.S. (1990). In vitro production of haploids and their use in cell genetics and plant breeding, Biotechnology in Agriculture and Forestry, vol. 12, Haploids in Crop Improvement I. 372–380.

Barnabás, B., Pfahler, P. L. & Kovács, G. (1991): Direct effect of colchicine on the microspore embryogenesis to produce dihaploid plants in wheat (*Triticum aestivum* L.). Theor. Appl. Genet., 81, 675–678.

Burton, G. W. & de Vane, R. W. (1953): Estimating heritability in tall fescue (*Festuca arundinaceia*) from replicated clonal material. Agron. J., 45, 478–481.

Carlson, A. R. (1998): Visual selection of transgenic barley (*Hordeum vulgare*) structures and their regeneration into green plants. Thesis, University of Guelph.

Castillo, A. M., Valles, M. P. & Cistue, L. (2000): Comparison of anther and isolated microspore cultures in barley. Effects of culture density and regeneration medium. Euphytica, 113, 1–8.

Chaleff, R.S. & Stolarz, A. (1981) Factors influencing the frequency of callus formation among cultured rice (*Oryza sativa* L.) anthers. Physiol. Plant. 51 : 201 - 206.

Chu, C. (1978) The N6 medium and its applications to anther culture of cereal crops. In: Proc Symp Plant Tissue Culture. Science Press, Peking, pp 43–50

Chu, C.C., Hill, R.D. & Brûlí-Babel, A.L. (1990) . High frequency of pollen embryoid formation and plant regeneration in *Triticum aestivum* L. on monosaccharide containing media. Plant Science, 66: 255-262.

Chu, C.C., Hill, R.D. & Brûlí-Babel, A.L. (1990) . High frequency of pollen embryoid formation and plant regeneration in *Triticum aestivum* L. on monosaccharide containing media. Plant Science, 66: 255-262.

Dieu P & Beckert M (1986) Further studies of androgenetic embryo production and plant regeneration from *in vitro* cultured anthers in maize (*Zea mays* L.). Maydica 31: 245-259.

Gamborg OL, Miller RA. & Ojima K (1968) Nutrient requirement suspension cultures of soybean root cells. Exp Cell Res 50:151–158.

Genovesi AD & Collins GB (1982) *In vitro* production of haploid plant of corn via anther culture. Crop Sci. 22: 1137 - 1144.

Gustafson VD, Baenziger PS, Wright MS, Stroup W.W. & Yen Y (1995). Isolated wheat microspore culture. Plant Cell Tissue Organ Cult. 42: 207-213.

Hagio, T., Hirabayashi, T., Machii, H. & Tomotsune, H. (1995): Production of fertile transgenic barley (*Hordeum vulgare* L.) plants using the hygomycin-resistance marker. Plant Cell Rep., 14, 329–334.

Hansen, N. J. P. & Anderson, S. B. (1998a): In vitro chromosome doubling with colchicine during microspore culture in wheat (*Triticum aestivum* L.). Euphytica, 102, 101–108.

Hansen, N. J. P. & Anderson, S. B. (1998b): *In vitro* chromosome doubling potential of colchicine, oryzaline, trifuralin and APM in *Brassica napus* microspore culture. Euphytica, 88, 159–164.

Hoekstra, S., van Zijderwold, M. H., Louwerse, J. D., Heidekamp, F. & Vandermark, F. (1992): Anther and microspore culture of *Hordeum vulgare* L. cv. Igri. Plant Sci., 86, 89–96.

Hoekstra, S., Van Zijderwold, M. H.,Heidekamap, F. L. & Roue, C. (1993): Microspore culture of *Hordeum vulgare* L.: the influence of density and osmolality. Plant Cell Rep., 12, 661–665.

Hu T. & Kasha KJ (1997). Improvement of isolated microspore culture of wheat (*Triticum aestivum* L.) through ovary co-culture. Plant Cell Rep. 16: 520-525.

Hu, T. C., Kasha, K. J. (1999): A cytological study of pretreatment effects on isolated microspore culture of wheat *Triticum aestivum* cv. Chris. Genome, 42, 432–441.

Hunter CP (1988). Plant regeneration from microspores of baley (*Hordeum vulgare*). Ashford, Kent: University of London. 1988. PhD Thesis. Wye College , UK, 110 P.

Inagaki, M. (1985): Chromosome doubling of wheat haploids obtained from crosses with *Hordeum bulbosum* L. Jpn. J. Breed., 35, 193–195.

Jähne, A., Lörz, H. (1995): Cereal microspore culture (Review). Plant Sci., 109, 1–12. Johnson, H. W.,

Kahrizi D (2009). Study of Androgenesis and Spontaneous Chromosome Doubling in Barley (*Hordeum vulgare* L.) Advanced Line Using Isolated Microspore Culture. International Journal of Plant Breeding. (3) 2: 111-114.

Kahrizi D & Mohammadi, R (2009). Study of Androgenesis and Spontaneous Chromosome Doubling in Barley (*Hordeum vulgare* L.) genotypes using Isolated Microspore Culture. Acta Agronomica Hungarica, 57(2): 155–164.

Kahrizi, D., Arminian, A., Masumi Asl, A. (2007): *In vitro* Plant Breeding. Razi University Press, Razi, Iran (in Persian).

Kahrizi, D., Moieni, A. and Bozorgipour, R. (2000): Effect of genotype and carbohydrate source on androgenesis of hexaploid wheat (*Triticum aestivum* L.). Seed Plant, 16, 41–51 (in Persian).

Kahrizi, D., Zebarjadi, A. R., Jalali Honarmand, S., Khah, E. M. & Motamedi J. (2009) . Study of Spontaneous Chromosome Doubling in Wheat (*Triticum aestivum* L.) Genotypes Using Androgenesis . World Academy of Science , Engineering and Technology 57: 1162-1166.

Kahrizi, D.,Mahmoodi, S. Bakhshi Khaniki G.R. & Mirzaei, M. (2011). .Effect of genotype on androgenesis in barley (*Hordeum vulgare* L.). Biharean Biologist 5(2): 132-134.

Kahrizi, D., Mahmoodi, S.,Bakhshi Khaniki, B.K. & Mirzaei, M. (2011). Effect of genotype on androgenesis in barley (*Hordeum vulgare* L.). Biharean Biologist. 5(2): 132-134.

Kasha KJ, Ziauddin A & Cho UH (1990) Haploids in Cereal improvement: anther and microspore culture. In: Gustafson JP (ed) Gene Manipulation in Plant Improvement II (pp. 213-235). Plenum Press, New York.

Kasha, K. J., Hu, T. C., Oro, R., Simion, E. & Shim, Y. S. (2001): Nuclear fusion leads to chromosome doubling during mannitol pretreatment of barley (*Hordeum vulgare* L.) microspores. J. Exp. Bot., 52, 1227–1238.

Liu W, Zheng MY, Polle EA. & Konzak C.F. (2002). Highly efficient doubled-haploid production in wheat (*Triticum aestivum* L.) via induced microspore embryogenesis. Crop Sci. 42: 686-692.

Mejza SJ, Morgant V, DiBona DE & Wong JR (1993). Plant regeneration from isolated microspores of *Triticum aestivum* L. Plant Cell Rep. 12: 149-153.

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Phisiol Plant. 15: 473-497.

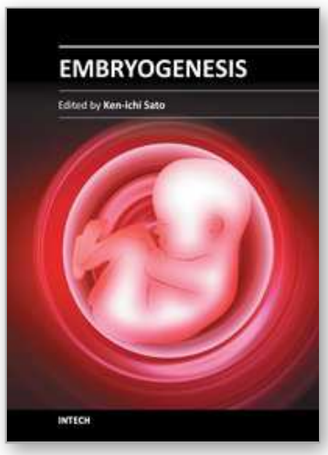
Nitsch C, Andersen S, Godrad M, Neutter MG & Sheridan WF (1982) Production of haploid plants of *Zea mays* L. and *Pennisetum* through androgenesis. In: Earle ED & Demarly Y (eds.) Variability in plant regeneration from tissue culture. Praeger, New York. pp. 69 - 91.

Picard EJ & De Buyser (1975) Nouveaux resultants concernant la culture d'antheres in vitro de ble tendure (Triticum aestivum L.). Effects d'un choe thermique at de la position de I'anthere dans I'epis. C.R. Acad. Sci. (Paris), 280D : 127 - 130.

Sunderland N & Roberts M (1977) New approach to pollen culture. Nature 270: 236-238

Touraev A, Indrianto A, Wratschko I, Vicente O. & Heberle-Bors E (1996). Efficient microspore embryogenesis in wheat (*Triticum aestivum* L.) induced by starvation at high temperature. Sex. Plant Reprod. 9: 209-215.

Zheng MY, Liu W, Weng Y, Polle E & Konzak CF (2001). Culture of freshly isolated wheat (*Triticum aestivum* L.) microspores treated with inducer chemicals. Plant Cell Rep. 20: 685-690.



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