OPTIMAL CONDITIONS FOR ANDROGENESIS INDUCTION IN PLANTS

CONDIȚII OPTIME NECESARE PENTRU INDUCEREA ANDROGENEZEI LA PLANTE

LUCA M. A.[1], LEONTE C.[1]
e-mail: luca_mihai_alexandru@yahoo.com

Abstract. *The totipotency of the male reproductive cells was revealed by Guha and Maheshvari in 1964 (Aionesei, 2005), allowing the development and improvement of haploid plants breeding methods and their use in fundamental and applied research. Among the methods for haploid production, the androgenetic ones have the advantage of chromosome doubling during the first mitotic division and producing fertile plants with complete doubled chromosomes, compared with the ginogenetic ones, where the egg cell is difficult to access, the embryo representing often the first stage in which a treatment for chromosome doubling may be applied (Kasha, 2005). The literature review made by us shows general aspects of androgenesis and the genetic, physiological, and physical factors that influence plant androgenesis. The following aspect was investigated: different androgenetic methods for haploid production, the processes that the microspore undergoes during sporophytic development and the optimal conditions for androgenesis induction.*
Key words: androgenesis, haploids, microspores

Rezumat. *Demonstrarea totipotenței celulelor reproductive mascule de către Guha şi Maheshvari în 1964 (Aionesei, 2005) a permis dezvoltarea şi îmbunătățirea metodelor de obținere a plantelor haploide şi utilizarea acestora în cercetarea fundamentală şi aplicată. Dintre metodele pentru producerea haploizilor, cele androgenetice prezintă avantajul posibilității dublării cromozomilor în prima diviziune mitotică, cu obținere de plante cu garnitura cromozomică complet dublată şi fertile, față de metodele ginogenetice, în cazul cărora celula ou este dificil de accesat, deseori embrionul reprezentând primul stadiu în care poate fi efectuat un tratament pentru dublarea cromozomilor (Kasha, 2005). Studiul literaturii de specialitate efectuat de noi, expune aspecte generale asupra androgenezei, şi factorilor genetici, fiziologici şi fizici care influențează androgeneza la plante. Au fost investigate următoarele aspecte: semnificația termenilor, diferitele metode androgenetice pentru producerea haploizilor, procesele suferite de microspor în timpul schimbării dezvoltării normale cu dezvoltarea sporofitică şi condițiile optime necesare pentru inducerea androgenezei.*
Cuvinte cheie: androgeneza, haploizi, microspor

INTRODUCTION

The first natural haploid in flower plants was reported in 1922 by Belling and Blakeslee (Palmer and Keller, 2005), but only after the demonstration of the totipotency

[1]University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

of the male reproductive cells by Guha and Maheshvari in 1964 (Aionesei, 2005), the methodology of haploid plants production takes a step forward to the utilization of this type of plants in big number in different plant breeding programs. The genetic importance of haploid plants results from the full genetic constitution in phenotype (Leonte, 2003), recessive alleles expression (Raicu, 1980), time reduction in obtaining isogenic lines (Munteanu and Falticeanu, 2008; Gonzalez and Jouve, 2005), valuable lines particularly for heterogametic species (Radojevic, 2000). However, because of the monoploid condition, such plants are subject of the lethal and sub lethal genes action (Jacobsen and Ramanna, 1994 cited by Chani et. al., 2000).

Different methods are developed for haploid plants production at high frequencies. The literature review made by us presents general aspects of androgenesis.

MATERIAL AND METHOD

The study was based on literature analysis. We have investigated the significance of the terms, different androgenic methods for haploid plants production, the processes suffered by the microspore during the switch from the normal development to sporophytic development and the optimal conditions for androgenesis induction.

RESULTS AND DISCUSSIONS

Vegetal cells may express almost any part of their genome, having the ability to return to the embryogenic condition and regenerate hole plants (Reynolds, 1997, cited by Aionesei et. al., 2005), capacity called totipotency. Embryogenesis can be initiated not only from a zygote but also from the haploid generation cells, such as microspores (Oleszczuk et. al., 2005), a phenomenon called androgenesis.

Initially, the original significance of the term "androgenesis" described the fertilization of the egg cell and then the inactivation of the female nucleus, but today it refers to the microspore embryogenesis (Segui-Simarro, 2010), although the haploid sporophyte appears without the gametophyte formation and the microsporogenesis that precedes the male gametophyte is not completed (Vagera and Havranek, 1983).

In some conditions, *in vitro*, the normal gametophytic development of the microspores can be changed to the sporophytic one (Iqbal and Wijesekara, 2007), structural and biochemical changes being identified at the microspore level. However, the "critical point" which designates the transition to the sporophytic organization is unknown (Babbar, 2004), the haploidy representing a problem in plant cell culture domain, under theoretical and practical aspect (Zagorska and Dimitrov, 1995).

Depending on the events during the division of *in vitro* cultured pollen, androgenesis is classified into two types: direct androgenesis – by modifying the normal development program of the haploid nucleus and it's mitotic division with the formation of a embryo-like structure and then a haploid plant – and indirect

androgenesis, by repeated divisions of the haploid nucleus with callus formation, tissue differentiation and haploid plants formation (Leonte, 2003).

The methods for *in vitro* androgenesis induction are anther culture and isolated microspore culture. Babbar et. al. (2004) identified five possible paths for androgenesis: the repeated division of the vegetative cell, the repeated division of the generative cell, the repeated division of both cells, and the symmetric division of the uninucleate microspore and the fusion products of the generative respectively vegetative cells.

For an optimal development of the *in vitro* androgenesis it is necessary that the androgenetic program to be designed considering the factors that influence androgenesis in plants.

Genetic factors. The androgenetic potential is high in few cultured plants, so that embryo production through this path, for double haploid lines use in plant breeding programs, is limited.

This limitation effect, manifested by the incapacity of morphogenesis in anther culture (Garcia et. al., 2009), determines the approach of those genotypes that manifests androgenetic potential (Yermishina et. al., 2004) and breeding for this character, as it is suggested by Beckert (1998) and Rudolf et. al. (1999) (cited by Segui-Simarro, 2010) because this androgenetic competence can be inherited by the descendants.

The androgenetic potential is genetically controlled by interactions of genes with non-additive effects, as it is the case of some triticale and secalotriticum species (Yermishina et. al., 2004), the three independent components involved in the anther culture response: callus induction, regeneration and plant development being under a polygenic control and inherited independently (Ekiz and Konzak, 1994, cited byt Chaudhary et. al., 2003) However, Kasha et. al. (2001a, cited by Oleszczuk et. al., 2006) suggests that a correct treatment may be the main solution for passing over the genotypic dependence.

Physiological factors. Even though the effects of donor plants physiological state are not studied as much as the effects due to the genotype, choosing the anther donor plants must be performed with discernment. Reed (2005) indicates good results if the anthers were collected from healthy plats with vigorous growth.

Ghiorghita and Nicuta (2005) states that are to be avoided those plants which suffered a form of stress and that the humidity, light and temperature the donor plants were grown are factors that influence the embryogenic potential of anthers about to be cultivated.

Donor plants age represents an important factor in designing an androgenesis induction program, the best androgenetic response being achieved with the anthers of the first set of flowers appeared on a plant (Leonte, 2011), even though in the case of *Nicotiana tabacum* var. *Havanna* there are no observable differences between the first and the last floral buds, regarding the androgenetic response (Johansson and Erikson, 1977, cited by Ghiorghita et. al, 2005).

The developmental stage of microspores is critical for androgenesis induction. There is a moment when the microspore can change the normal

gametophytic evolution to a sporophytic development. This moment differs within a species and even from one variety to another, but the general rule is that the sensitive period of microspores is between the uninucleate stage and bicellular pollen around the first mitosis, a moment in which a multitude of external stimuli can be applied on microspores to mask the gametophitic program and induce the sporophytic development (Babbar et. al., 2004).

Although Touraev et. al. (1997, cited by Babbar et. al., 2004) reveals that the microspores ability to switch the development program is present in a relatively large time window in *Brassica napus, Nicotiana tabacum* and *Triticum aestivum*, in the case of some recalcitrant species, time windows are small (Seggui-Simarro, 2010).

Anther wall. The anther wall function in androgenesis is still discussed, some of the authors reporting that it has a stimulating effect, others that it is inhibitory. Ghiorghita and Nicuta (2005) remember Nitsch and Norrel (1973) that support the stimulatory effect of the anther wall because the aqueous extract of anthers induces the growth of isolated pollen. According to Suderland et.al. (1994, cited by Wang et. al., 1999) for androgenesis induction the tapetum and the anther wall properties are critical, much like the properties of anthers undergoing maturation, close to anthesis.

Culture medium. The culture medium composition is another factor that influences callus formation and *in vitro* organogenesis induction (Zagorska and Dimitrov, 1995) or embryogenesis. A series of species that were considered as recalcitrant came to be intense utilized in adrogenesis programs due to the optimization of culture conditions. It is possible that the proliferation of the callus is not a genotype dependent alternative in embryogenesis induction, but a result of suboptimal culture conditions (Segui-Simarro, 2010).

According to Gonzalez and Jouve (2005) the culture medium and the genotype affect: the rate of survival of microspores during *in vitro* androgenesis, the percentage of symmetrical divisions, the number of division per 100 viable microspores and the numbers of microspores with four or more nuclei.

The multitude of culture mediums and different utilized concentrations of phytohormones make it impossible to create a single culture media for all plant species in order to promote androgenesis.

The aggregation state of the culture medium influence the androgenic response, the liquid ones providing better conditions than the solid ones, though there is the disadvantage that those liquid media do not ensure anther floatability (Ghiorghita et. al., 2005). To overcome this impediment two phase medias were created.

Physical factors. Pretreatments became an essential condition for improving androgenetic response in many species (tab. 1), stress or suboptimal conditions creating the possibility of a such a response.

For many plants, low temperatures represent the factor that disturbs the inner stability of the anther, with changes in microspore development and embryo formation (Oleszczuk et. al., 2006), but there can't be indicated an universal level of stress with positive effects on androgenesis and plant regeneration.

Table 1

Pretreatments used for androgenesis induction

Pretreatment	Species	Cited bibliography
4°C for 4 days and gamma irradiation 1Gy	Medicago sativa L.	Zagorska and Dimitrov, 2005
35°C for 12 hours	Solanum phuraja Juz. X S. Chacoense Bitt.	Chani et. al., 2000
Manytol	Hordeum vulgare L.	Li et. al., 2001

Temperature. In general, anther cultures are incubated at 24-25 °C (Reed, 2005), but there are cases when higher temperatures or lower ones with initial heat shock give better responses.

The climatic conditions in which the donor plants were grown influence the production of floral bud production. In the case of higher temperatures during donor plant growth, in potato, the temperature brings benefits on to the embryo production in a higher manner than photoperiod (Chani et. al., 2004). Low androgenesis frequencies are recorded due to the long day conditions in which the anther donor plants are cultivated (Vagera and Havranek, 1983) in the case of the species *Nicotiana tabacum* L. and *Datura inoxia* Mill.

CONCLUSIONS

1. Even if the androgenic potential is high for few cultured plants, adequate treatments represent a solution to overcome the genotypic dependence;

2. Chousing anther donor plants must be effectuated considering their health and development, avoiding plants that are exposed to different forms of stress;

3. In general, the sensitive period for androgenesis induction in microspores is between the uninucleat stage and the bicellular pollen around first mitosis;

4. The correct election of culture media for anthers or isolated microspores, supplementation with optimal concentration of phytohormones and pretreatments are essential for the androgenesis programs.

REFERENCES

1. **Aionesei Tatiana, Touraev A., Bors E. H., 2005** – *Pathways to microspore embryogenesis.* Biotechnology in Agriculture and Forestry, vol. 56, p. 11-34.
2. **Babbar S. B., Agarwall P. K.,Sahay S., Bhojwani S. S., 2004 a** – *Isolated microspore culture of Brassica: An experimental tool for developmental studies and crop improvement.* Indian Journal of Biotechnology, vol. 3, p. 185-202.
3. **Babbar S. B., Kumari N., Mishra J. K., 2004 b** – *In vitro androgenesis: events preceding its cytological manifestation*. Plant Biotechnology and Molecular Markers, Anamaya Publishers;
4. **Chani E., Veilleux R. E., Boluarte-Medina Tatiana, 2000 –** *Improved androgenesis of interspecific potato and efficiency of SSR markers to indentify homozygous regenerants.* Plant Cell, Tissue and Organ Culture, nr. 60, p. 101-112.

5. Chaudhary H. K., Dhaliwal I., Singh S., Sethi G. S., 2003 – *Genetics of androgenesis in winter and spring wheat genotypes.* Euphytica, nr. 132, p. 311-319;
6. Garcia Amelia M. T., Galati B. G., Anton A. M., 2002 – *Microsporogenesis, macrogametogenesis and pollen morphology of Passiflora spp. (Passifloraceae).* Botanical Journal of the Linnaean Society, vol. 139, p. 383-394;
7. Ghiorghiță G., Nicuță Petrescu Daniela, 2005 – *Biotehnologiile azi.* Editura Junimea, Iaşi.
8. Gonzalez J. M., Jouve N., 2005 – *Microspore development during in vitro androgenesis in triticale.* Biologia Plantatum, nr. 49, p. 23-38;
9. **Iqbal M. C. M., Wijesekara Kolitha B., 2007** – *A brief temperature pulse enhances the competency of microspores for androgenesis in Datura metel.* Plant Cell. Tiss. Organ Cult., nr. 89, p.141-149;
10. **Kasha K. J., 2005** – *Chromosome doubling and recovery of doubled haploid plants.* Biotechnology in Agriculture and Forestry, Haploids in crop improvement 2, Springer, p. 123-152;
11. **Leonte C., 2003** – *Ameliorarea plantelor.* Editura Ion Ionescu de la Brad, Iaşi.
12.**Leonte C., 2011** – *Tratat de ameliorarea plantelor.* Editura Academiei Române, Bucureşti.
13. Munteanu N., Fălticeanu Marcela, 2008 – *Genetica şi ameliorarea plantelor ornamentale.* Editura Ion Ionescu de la Brad, Iasi.
14. Oleszczuk S., Sowa S., Zimny J., 2006 – *Androgenic response to preculture stress in microspore cultures of barley.* Protoplasma, nr. 228, p. 95-100.
15. **Palmer C. E. D., Keller W. A., 2005** – *Overview of Haploidy*. Biotechnology in agriculture and Forestry, Haploids in crop improvement 2, Springer;
16. **Radojevic Ljiljana, Marinkovic N., Jevremoviv S., 2000** – *Influence of the sex of flowers on anrogenesis in Aesculus hippocastanum L. anther culture*. In Vitro Cell. Dev. Biol. Plant, nr. 36, p. 464-469.
17. **Raicu P., Anghel I., Stoian Veronica, Duma Doina, Taisescu Elena, Badea Elena, Gregorian Liliana, 1983** – *Genetica, metode de laborator.* Editura Academiei Române, Bucuresti.
18. Reed Sandra M., 2005 – *Haploid cultures.* Plant Development and Biotechnology, CLC Press LLC.
19. **Segui-Simarro J. M., 2010** – *Androgenesis revisited.* Bot. Rev., nr. 76, p. 377-404.
20. **Vagera J., Havranek P., 1983** – *Regulation of androgenesis in Nicotiana tabacum L.cv. White Burley and Datura inoxia Mill. Effect of bivalent and trivalent iron and chelating substances.* Biologia Plantarum (Praha), nr. 25 (1), p. 5-14.
21. **Wang M., Hoekstra S., van Bergen Sandra, Lamers Gerda E. M., Oppedikj B. J., van der Heijden M. W., Priester W., Schilperoort R. A., 1999** - *Apoptosis in developing anthers and the role of ABA in this process during androgenesis in Hordeum vulgare L.* Plant Molecular Biology, nr. 39, p. 489-501.
22. **Yermishina N. M., Kremenevskaja E. M., Gukasian O. N., 2004** – *Assessement of the combining ability of tritiacale and secalotriticum with respect to in vitro androgenesis characteristics.* Russian Journal of Genetics, vol. 40, nr. 3, p. 282-287.
23. **Zagorska N., Dimitrov B., 1995** – *Induced androgenesis in alfalfa (Medicago sativa L.).* Plant Cell Reports, nr. 14, p. 249-252.