

## CYTOLOGICAL EVIDENCE OF SPOROPHYTE DEVELOPMENT OF MICROSPORES IN CULTURE OF TRITICALE ANTHERS WITHOUT COLD TREATMENT

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### S u m m a r y

**In the culture of anthers, obtained from donor hexa- and octoploid triticale plants and the Students variety, by the cytological techniques the authors studied the development of microspores without action on anthers by cold shock. It was revealed, that shock temperatures are not required for an induction of androgenesis.**

**Key words:** triticale, anther culture, sporophytic development, cold pretreatment.

Anther culture is one of techniques used for mass production of haploid plants of cereals. This method is based on formation of copies of zygotic embryos (embryoids) from microspores - androgenesis (haploid embryogenesis, pollen embryogenesis, androclina), which occurs in plant anthers isolated in certain development stage and cultivated in nutrient medium with hormonal additives. Homozygous lines obtained on the basis of diploidized haploids are widely used in creation of varieties and source material for breeding work on spring and winter wheat, barley, rice and triticale (1).

It has been demonstrated in numerous experiments the possibility of sporophytic development of microspores from donor plants under the induced action on spikes and anthers. To increase its frequency, the action of low positive temperature is in a wide-spread use. In fact, cold exposure has become a routine procedure in a world practice of creation haploids. There are different opinions about aftereffects of such cold stress on further development of sporophytic plants during their cultivation. It has been established in physiological studies, that cold pre-treatment reduces respiration intensity in anthers and thereby prolongs their vitality (2).

It is believed that cold stress causes reorientation in the spindle of I division resulting in formation of microspores with two equal nuclei (3). Low temperatures delay the destruction of anther cell wall, which causes an adverse impact on development of isolated microspores in culture (cited from 4), and, at the same time, cold prolongs viability of microspores, as well as the period suitable for introduction of the anthers to in vitro culture ("embryogenic window"). This fact is important in cases when it takes time to deliver donor's material to laboratory. The effect of cold is assumed to switch off the genes or to inhibit functions of gene products responsible for gametophytic program (5). It is also believed that cold stress applied under a certain regime works as the trigger of sporophytic development of microspores in critical multi-vacuolized stage (6).

At the same time, it has been revealed an ambiguous influence of this factor on cultured anthers of cereals. Thus, cold exposure wasn't necessary for induction of divisions in microspores of wheat anthers taken from donor plants grown in field. An ambiguous effect of cold stress was observed in different seasons (7). The cold pre-treatment of harvested barley spikes (duration 3 - 14 days) didn't provide any positive results in anther culture (8). In experiments with two widely cultivated wheat varieties Acheloos and Vergina and hybrids from their crosses, it has been shown no need in cold stress for formation of haploids in anther culture: the main factors were donor genotypes and temperature of cultivation the anthers (9).

The study of cold exposure effects on soft wheat of domestic selection (Saratov) having different degree of response revealed sporophytic development of microspores in anthers of freshly harvested ears; in some genotypes it occurred even more frequent than in anthers subjected to cold stress (1, 10).

The purpose of this work was cytological study of sporophytic development of microspores in freshly harvested anthers not exposed to cold stress.

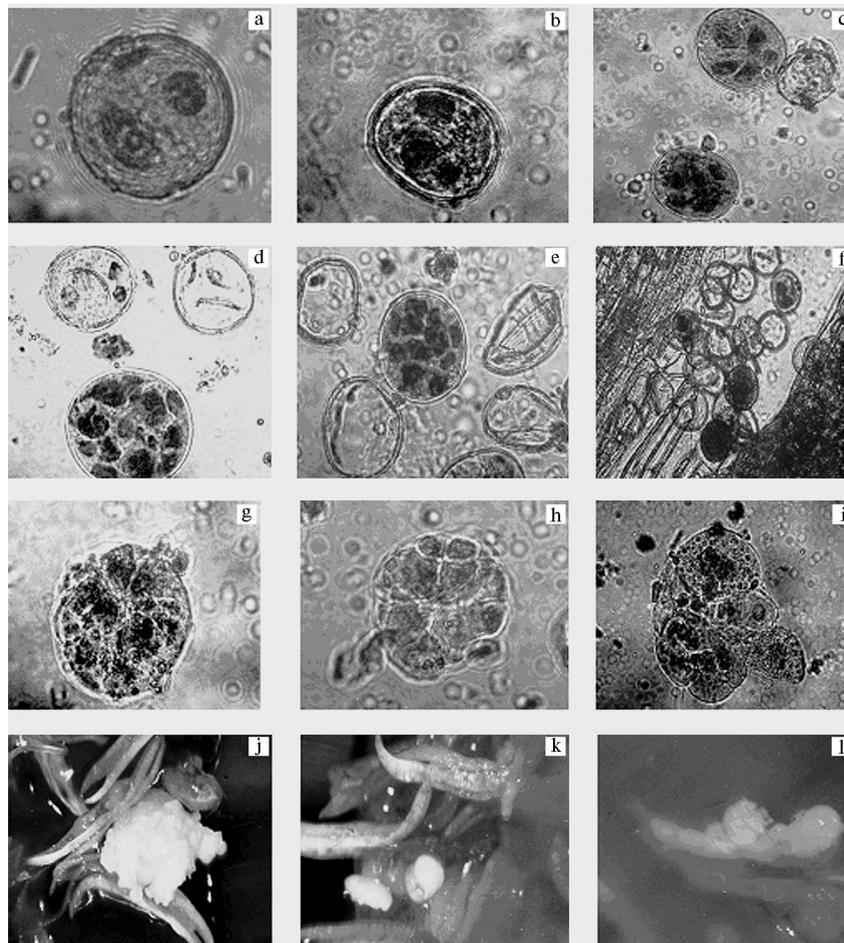
*Technique.* The object of study - donor plants of primary hexa- and octaploid triticale grown in field conditions (Saratov province, 2006-2008). Ancestors of the studied triticale - domestic varieties of winter rye derived from breeding work in Saratov province and the variety Student approved for use in the same region. Freshly harvested ears with anthers containing vacuolized microspores were sterilized according to standard procedures and cultivated in nutrient medium N 6 with the addition of growth regulators 2,4-D (1,5 mg/l) and kinetin (0,5 mg/l); sucrose content - 6%. After 4; 9; 14 and 21 days of cultivation, the anthers were fixed in Carnoy solution (3:1) (50 pcs. for each period of maturation) and stained with aceto-carmin; pressed preparations were made by conventional cytological methods (11). Cytological examination and a consequent photomicrography were performed on the microscope IM35 ("Opton", Germany).

The obtained data were statistically processed by variance analysis using the program Agros.

*Results.* Cytological studies revealed various events during cultivation of freshly harvested anthers: the majority of microspores died even by the 7<sup>th</sup> day of cultivation, some - continued to develop in gametophytic program with further degeneration of division products; a very small population of cells passed to sporophytic development. The latter demonstrated an equal division of microspore (location of the pore and two resulting nuclei corresponded to an equilateral triangle) (Fig. 1), or the division leading to formation of 1 vegetative and 1 generative cells (with subsequent mitotic activity of the vegetative cell) (see Fig., 2).

A number of divisions resulted in formation of multicellular structures located inside a microspore capsule (see Fig., 3-6). The intensive growth of these newly-formed structures caused tension and break of microspore capsule and then - anther cover (see Fig., 7-9). Have studied the frequency of formation of various androgenetic structures (embryoids and calli), it was shown that androgenesis in triticale anthers culture could be induced without exposure to shock temperatures. Moreover, two genotypes (the amphidiploid AD-1 and the variety-standard Student) manifested sporophytic development of microspores only in anthers of freshly-harvested ears not subjected to storage at low positive temperatures (frequencies, respectively, 1,7 and 7,8%) (Table).

Thus, observations of cytological events in triticale anther culture not exposed to cold shock revealed different stages of sporophytic development of microspores previously described for anthers affected by cold stress (3, 12).



**Cytological events of sporophytic development of microspores in triticale anther culture not subjected to cold stress** (composition of cultivation medium - see "Technique"): a, b— first divisions of microspores; c, d, e - formation of multicellular structures; f— general view of an anther; g, h, i— breaking of microspore capsule; j, k— formation of androgenetic structures (calli and embryoids); l— general view of an anther with androgenetic structures; a-i — staining with aceto-carmin; optical magnification  $\frac{1}{2}10^{\frac{1}{2}}60$ .

It is believed, that stress is a key signal responsible for switching of microspores into alternative, i.e., sporophytic, development (13). In anther culture of barley (14), rice (15), wheat (16, 17), such stress can be affected by deficit in anthers of nitrogen or carbohydrates, in wheat anther culture (18) and isolated microspores (13) – by temperature (over 30 °C).

**The frequency of sporophytic development of microspores in anther culture of triticale the different genotypes subjected to cold stress or without cold impact.**

Genotype	Number of anthers, pcs.	Frequency of embryogenic anthers, %	Total frequency of newly formed structures, %
AD-1:			
experiment	235	0,85	1,70
control	456	0	0
AD-2:			
experiment	1871	1,12	1,34
control	4690	0,38	0,41
AD-3:			
experiment	240	2,92	2,92
control	507	0,99	0,99
The variety Student (standard):			
experiment	51	3,92	7,80
control	326	0	0
HCP <sub>05</sub>		0,08	0,09

Note: composition of cultivation medium – see "Technique". Experiment – without cold stress, control – exposed to cold impact.

The obtained results on cultivation of anthers from triticale and wheat doesn't support the opinion about low positive temperatures as the trigger of sporophytic development of microspores (6). Harvesting the ears from donor plant is a stress itself, which factor combined with in vitro cultivation of anthers can lead to repression of gametophytic genes and sporophytic development awoken without any other influences.

Have analyzed the frequency of formation of androgenetic structures (calli and embryoids), it was found that shock temperatures are not required for induction of androgenesis in triticale anther culture. Consequently, the current procedure of anthers cultivation can be simplified by eliminating one of its stages - pre-treatment of anthers with low positive temperatures.

REFERENCES

1. D'yachuk T.I., Technological and Selection Aspects of Haploidy (on Example of Wheat and Barley), *Doct. Sci. Dissertation*, Saratov, 2003.
2. Ouyang J.W., He D.G., Feng G.H. and Jia S.E., The Response of Anther Culture to Culture Temperature Varies with Growth Conditions of Anther-Donor Plants, *Plant Sci.*, 1987, vol. 49, pp. 145-148.
3. Sukhanov V.M., Androclina and Its Peculiarities in Wheat, *Cand. Sci. Dissertation*, Saratov, 1983.
4. Reznikova S.A., *Tsitologiya i fiziologiya razvivayuschegosya pyl'nika* (Cytology and Physiology of Developing Anther), Moscow, 1984.
5. Chen Y., Anther and Pollen Culture of Rice, in: *Haploids in Higher Plants In Vitro*, Springer Verlag Publishers, 1986, pp. 3-25.
6. Kruglova N.N., Batygina T.B., Gorbunova V.Yu. and Sel'dimirova O.A., *Embriologicheskie osnovy androklinii pshentisy: atlas* (Embriological Basics of Androclina in Wheat: Atlas), Moscow, 2005.
7. Henry Y. and de Buyser J., Wheat Anther Culture, in *Biotechnology in Agriculture and Forestry (Wheat)*, Bajaj Y.P.S., Ed., Springer-Verlag, 1990, pp. 283-326.

8. Aleksandrova L.G. and Lukyanyk S.F., Anther Culture of Barley: Efficiency and Reproducibility, in *Mat. nauch. konf. po s.-kh. biotekhnologii* (Proc. Sci. Conf. on Agricultural Biotechnology), Tselinograd, 1991, pp. 63-65.
9. Xynias I.N., Zamani I.A. and Goul-Vavdinoudi E., Effect of Cold Pretreatment and Incubation Temperature on Bread Wheat (*Triticum aestivum* L.) Anther Culture, *Cer. Res. Com.*, 2001, vol. 29, pp. 331-338.
10. Tkachenko O.V., In Vitro Tissue Culture of Short-Straw Soft and Durum Wheat, *Extended Abstract of Cand. Sci. Dissertation*, Saratov, 2001.
11. Pausheva Z.P., *Praktikum po tsitologii rastenii* (Practical Works on Plants Cytology), Moscow, 1970.
12. Anapiyaev B.B., Experimental Morphogenesis and Biotechnology of Obtaining Haploids in Culture of Wheat Microspores, *Extended Abstract of Doct. Sci. Dissertation*, Moscow, 2001.
13. Touraev A., Indrianto A., Wratchko I. and Heberle-Bors E., Efficient Microspore Embryogenesis in Wheat (*Triticum aestivum* L.) Induced by Starvation at High Temperature, *Sex Plant Reprod.*, 1996, no. 9, pp. 209-215.
14. Hoekstra S., Zijderveld M.H., Heidekamp F. and Mark F., Microspore Culture of *Hordeum vulgare* L.: the Influence of Dencity and Osmolality, *Plant Cell Reprod.*, 1993, no. 12, pp. 661-665.
15. Ogawa T., Fukuoka H. and Ohkawa Y., Induction of Cell Division of Isolated Pollen Grains by Sugar Starvation in Rice, *Breed. Sci. Jpn.*, 1994, vol. 44, pp. 75-77.
16. Hu T.C., Ziauddin A., Simion E. and Kasha K.J., Isolated Microspore Culture of Wheat (*Triticum aestivum* L.) in a Defined Media. 1. Effects of Pretreatments, Isolation Methods and Hormones, *In vitro Cell Dev. Biol. (Plant)*, 1995, vol. 31, pp. 79-83.
17. Mejza S.J., Morgant V., Di Bona D. and Wong I.R., Plant Regeneration from Isolated Microspores of *Triticum aestivum* L., *Plant Cell Rep.*, 1993, vol. 12, pp. 149-153.
18. Huang B., Wheat Anther Culture: Effect of Temperature, in *Biotechnology in Agriculture and Forestry (Wheat)*, 1990, vol. 13, pp. 403-415.
19. Li H.C., Qureshhi J. and Kartha K.K., The Influence of Different Temperature Treatments on Anther Culture Response of Spring Wheat (*Triticum aestivum* L.), *Plant Sci.*, 1988, vol. 57, pp. 55-61.