BIOLOGIA PLANTARUM 47 (1): 85-90, 2003/4

Structural modifications of the female gametophyte induced by temperature in *Nicotiana tabacum*

N. ENALEEVA* and L. LOBANOVA

Botanic Garden of Saratov State University, Astrakhanskaya str. 83, Saratov 410026, Russia

Abstract

Effect of environmental conditions on formation of *Nicotiana tabacum* L. megagametophyte was studied. It was established, that unfavorable temperatures can specifically modify the structure of embryo sacs (ES). At low temperature (9/5 °C), ES with a reduced number of cells or with egg-like synergide(s) can be formed; at high variable (40/25 °C) or constant (37 °C) temperatures, ES with excessive numbers of cells or with synergide-like egg cells arise. Total frequencies of the changed ES patterns varied from 8 up to 35 % per plant and depended on the plant genotype and conditions of exposure.

Additional key words: abnormal megagametogenesis, embryo sac, tobacco.

Introduction

Development of embryo sac (ES) and its morphological features are genetically determined, and this is expressed in its, as a rule, uniform presentation in plants of the same species. It is known, that the changes in structure of a mature ES can be caused by mutations affecting the separate stages of megasporogenesis or megagametogenesis. In particular, there is an information on mutations, manifesting in an arrest of ES development at early stage (Drews *et al.* 1998, Sheridan and Huang 1997), in reduction of mitosis number (Enaleeva 1997), in additional nuclei divisions in coenocytic phase (Lin 1978, 1981) or in autonomous division of egg cell (Chaudhury *et al.* 1997) or nuclei of the central cell (Ohad *et al.* 1996). However, the changes of ES features can be caused not only by genetic reasons, but also by influence of environmental conditions. One of the factors essentially modifying process of ES development, is temperature. There are findings about influence of temperature on ES development in *Tamarix odessana* and *T. parviflora* (Hjelmqvist and Grazi 1964), *Capsicum* (Dharamadhaj and Prakash 1978), *Scorzonera tau-saghyz* (Poddubnaya-Arnoldy *et al.* 1934), *Zea mays* (Chebotaru 1965). It is also established, that the degree of apomixis depends on temperature conditions in *Ranunculus* (Nogler 1984), *Dichantium* (Knox 1967, Knox and Heslop-Harrison 1963, Gupta *et al.* 1969-1970), *Eupatorium riparium* and *Themeda triandra* (Nogler 1984).

The aim of this paper was to study of influence of temperature on ES formation in *Nicotiana tabacum*.

Materials and methods

Field grown tobacco (*Nicotiana tabacum* L. line BG-6 and cv. Havana-Connecticut 2379) was used. On July 30, August 29 and October 8, ovaries of previously emasculated flowers were collected 2 - 3 d after the flowers opened and fixed in ethanol-acetic acid, 3:1 (v/v). The day/night temperature during ES development were 27/20, 24/16, and 9/4 °C, respectively. During the experiment, flowers pollinated spontaneously were removed.

Effect of high temperature was studied on plants of the line BG-6. The plants were grown in a greenhouse

Received 15 November 2001, *accepted* 14 August 2002.
Abbreviation: ES - embryo sac.
Acknowledgement: The work has been supported by Grant UR.07.01.016, Universities of Russia.
*To whom correspondence should be addressed; e-mail: botany@sgu.ru

(in a free soil). The flowering occurred in August at day/night temperature of 40/25 °C. In the other case, plants were grown in pots and on flowering they were transferred into growth chamber with constant temperature 37 °C and a 16-h photoperiod. On inflorescences, only emasculated buds of a certain size corresponding to the stage of one-nucleate ES (Lobanova and Enaleeva 1998) were left. Ovaries were collected and fixed, as in the above experiment.

The ES were isolated from the ovules and prepared for microscopic analysis by the method of enzymatic maceration (Enaleeva *et al.* 1972).

Results

In plants flowered at normal temperature (in ovaries fixed on July 30 and August 29), the frequency of abnormal ES varied from 0 to 1.3 %. However, in ovaries of the same plants fixed on October 8, the frequency of abnormal ES reached 8.3 - 11.3 % in the line BG-6, and 10.5 - 35.4 % in the cv. Havana-Connecticut 2379 (Tables 1, 2). The spectra of abnormal ES in all investigated plants of both genotypes were very similar. The structural disturbances were presented by two patterns: cellular ES with reduced number of cells (Fig. 1*G,H,I*) and 7 - 8 nucleate ES with

Table 1. Patterns of abnormal ES in *N. tabacum* cv. Havana-Connecticut 2379 under different environmental conditions.

Date and temperature	Plant	Number of ES investigated	Abnormal ES [%]	Abnormal ES [%] with nuclei number < 7 cellular	7 or 8 egg-like synergide	another differences
30 July	1	300	0.7	0.0	0.7	0.0
27/20 °C	2	300	0.0	0.0	0.0	0.0
	3	300	0.3	0.0	0.3	0.0
	4	300	2.0	0.0	0.0	2.0
29 August	1	300	1.3	1.3	0.0	0.0
24/16 °C	2	300	0.3	0.3	0.0	0.0
	3	300	1.0	0.7	0.3	0.0
	4	300	0.7	0.0	0.3	0.3
8 October	1	300	20.0	13.0	6.7	0.3
9/4 °C	2	86	10.5	5.8	4.7	0.0
	3	130	35.4	20.8	13.8	0.8
	4	300	22.3	14.7	7.6	0.0

Table 2. Patterns of abnormal ES in *N. tabacum* line BG-6 under different environmental conditions.

Date and temperature	Plant	Number of ES investigated	Abnormal ES [%]	Abnormal ES [%] with nuclei number < 7 cellular	7 or 8 egg-like synergide	another differences
30 July	1	300	0.0	0.0	0.0	0.0
27/20 °C	2	300	0.3	0.0	0.0	0.7
	3	300	1.4	0.7	0.0	0.7
	4	300	0.7	0.7	0.0	0.0
	5	300	0.3	0.3	0.0	0.0
29 August	1	300	0.7	0.7	0.0	0.0
24/16 °C	2	300	0.9	0.3	0.0	0.6
	3	300	0.3	0.0	0.0	0.3
	4	300	2.0	1.0	0.7	0.3
	4	300	1.0	0.3	0.7	0.0
8 October	1	300	11.3	3.0	8.0	0.3
9/4 °C	2	300	11.1	3.0	7.3	0.7
	3	300	11.4	4.7	6.7	0.0
	4	300	8.2	4.3	3.3	0.6
	5	300	8.3	5.3	3.0	0.0

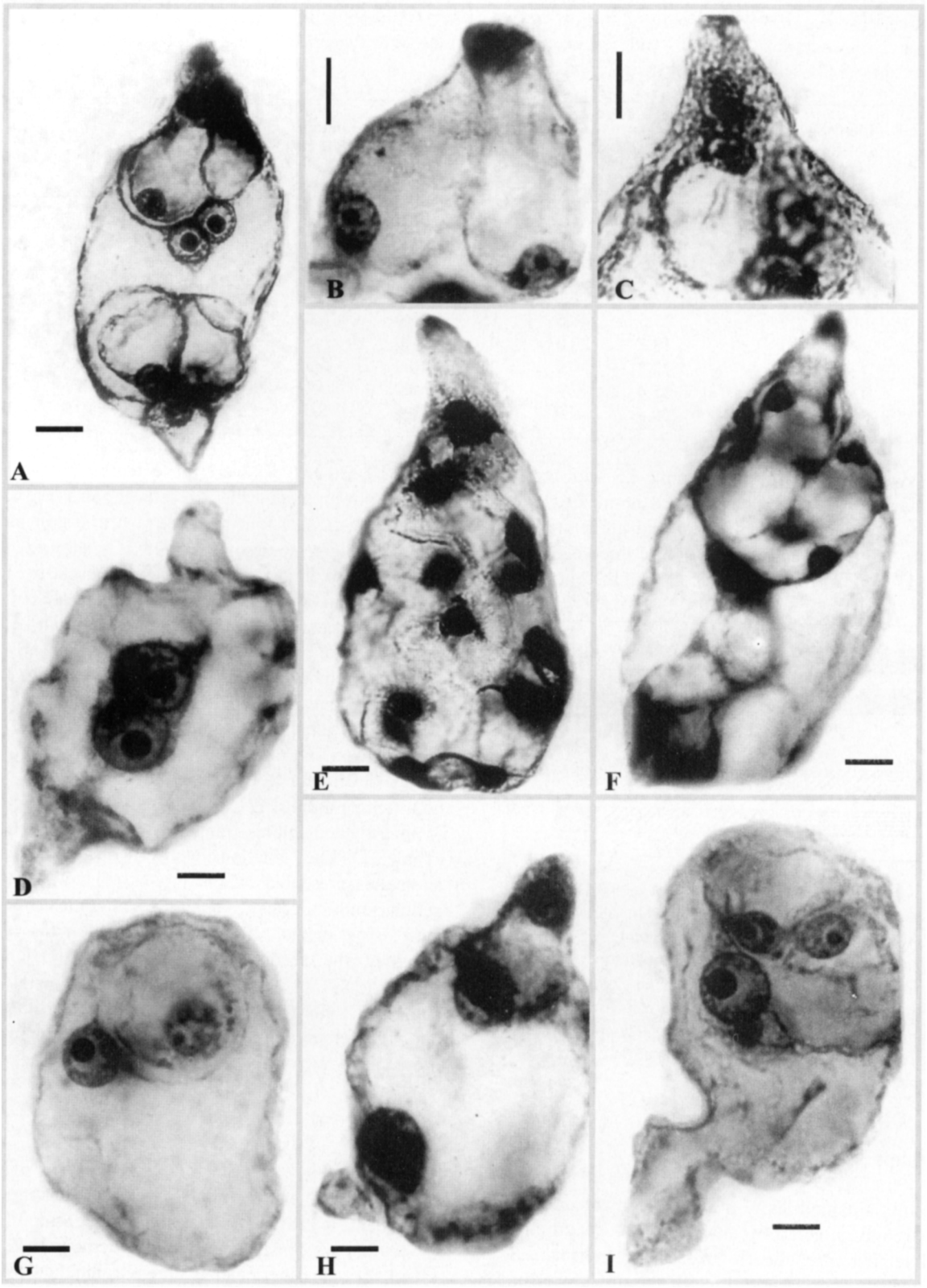

Fig. 1. Abnormal ES patterns produced at unfavourable temperatures: *A* - ES with normal structure; *B,C* - micropylar parts of ES, *B* - egg-like synergide, *C* - synergide-like egg cell; *D,E* - coenocytic ES, *D* - two-nucleate ES, *E* - ES with nuclei number more than 8; *F* - ES with excessive number of cells in egg apparatus; *G,H,I* - cellular ES with reduced number of cells, *G* - two-celled ES containing one polar cell and "central" cell with one nucleus, *H* - three-celled ES with two cells at opposite ends, *I* - four-celled ES with three cells at one pole. *Bars* = 10 μm.

Table 3. Patterns of abnormal ES in *N. tabacum* line BG-6 induced by high temperature. Abnormal ES [%] with nuclei number < 7 (1 - cellular, 2 - coenocytic), 7 or 8 (3 - with egg-like synergide(s), 4 - with synergide-like egg cell, 5 - another differences, 6 - coenocytic), and > 8 (7 - cellular, 8 - coenocytic).

Temperature	Plant	Number of ES investigated	Abnormal ES [%]	Abnormal ES [%] with nuclei number							
				< 7		7 or 8				> 8	
				1	2	3	4	5	6	7	8
Variable	1	159	8.8	0.0	0.0	0.0	1.3	0.0	0.0	7.5	0.0
40/25 °C	2	200	8.0	0.5	0.0	0.0	2.5	0.0	0.0	5.0	0.0
	3	200	10.0	1.0	0.0	0.5	1.5	0.0	0.0	7.0	0.0
	4	175	12.6	1.1	0.0	0.0	1.1	0.0	0.0	10.3	0.0
Constant	1	200	21.5	0.5	1.0	0.0	6.0	1.0	1.5	9.0	2.5
37 °C	2	200	13.5	0.0	0.0	0.0	6.5	0.0	0.0	7.0	0.0
	3	200	23.5	0.0	0.0	0.0	3.5	2.0	3.0	10.5	4.5
	4	130	35.4	2.3	0.8	0.0	13.1	3.1	3.1	11.5	1.5
	5	200	20.5	0.0	0.5	0.0	6.0	1.0	1.5	10.0	1.5

egg-like synergide(s) (Fig. 1*B*). Among ES with reduced cell number, two- and three-celled ES dominated. Two-celled ES contained one polar cell and a "central" cell with one or two nuclei (Fig. 1*G*), three-celled ES contained two polar one-nucleate cells and a "central" cell

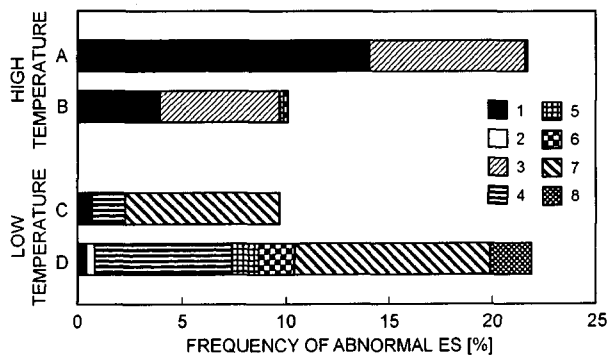

Fig. 2. Frequencies of different patterns of abnormal ES in plants, which flowered under different environmental conditions (summarized data). *A,B* - in October under low temperature 4/9 °C, *A* - the cultivar Havana-Connecticut 2379, *B* - the line BG-6; *C,D* - in the line BG-6, *C* - under variable temperature 40/25 °C, *D* - under constant temperature 37 °C starting from one-nucleate ES. For explanation of symbols 1 to 8 see Table 3.

(Fig. 1*H*). Other types were presented by 3 - 5-celled ES (unipolar or bipolar) with 1 - 3 cells at poles (Fig 1*I*).

Investigation of four plants grown in a greenhouse at high variable temperatures (40/25 °C) showed that the frequency of abnormal ES varied from 8 to 12.6 % (Table 3). Qualitative composition of anomaly patterns in different plants was found to be very similar. Most frequent were cellular ES with more than 8 nuclei, excessive number of cells in egg apparatus (Fig. 1*F*) and /or in antipodal complex, and with additional polar nuclei. The second most abundant was ES with synergide-like egg cell (Fig. 1*C*).

ES developed from a one-nucleate stage at constant high temperature (37 °C), the level of anomalies in 5 investigated plants ranged from 13.5 to 35.4 % (Table 3). The relationships of different types of anomalies presented on histograms (Fig. 2), testify that cellular multinuclear ES appeared as the most frequent in both experiments. ES with synergide-like egg cell occupied the second place. Characteristic for plants grown at 37 °C was the occurrence of coenocytic ES (Fig. 1*D,E*), not recorded in the experiment with variable day/night temperature. Among coenocytes, structures with more than 8 nuclei were observed (Fig. 1*E*).

Discussion

The results obtained show that development of tobacco ES is strongly affected by environmental conditions. At normal temperature, the frequency of abnormal ES in plants investigated did not exceed 2 %. In October under unfavourable conditions, the number of structure abnormalities increased significantly – up to 35.4 %. Comparison of two genotypes (cv. Havana-Connecticut 2379 and the line BG-6) for percentage of abnormal ES revealed different value(s), suggesting that a response of megagametogenesis to unfavorable conditions is genotype dependent. It is assumed that structural ES modifications in October are caused mainly by low temperatures, but an effect of some other changes in environmental conditions such as photoperiod or air humidity cannot be excluded. Further special experiments should be carried out to elucidate their role in inducing of megagametophyte disturbances.

If flowering occurred at high temperature, the

frequency of ES anomalies also substantively increased (to 8 - 35.4 %). It was found that the level of ES anomalies was higher in plants growing in pots under constant high temperature than that in plants growing in free soil under variable temperatures. This is probably because of a more severe heat stress under the constant temperature of 37 °C.

The low and high temperatures have a sharply defined specificity of morphogenetic effect. The low temperatures cause formation of ES with reduced number of nuclei or formation of egg-like synergide(s), but the high temperatures mainly induce an increase of the number of nuclei in ES or formation of synergide-like egg cell. The observation of similar patterns of ES anomalies in all plants of the same experimental variant testifies the existence of certain cytological laws causing these structural changes.

As follows from publications devoted to study of influence of the temperature factor on female generative sphere of plants of different systematic groups, some morphogenetic effects of low and high temperatures, revealed by us in tobacco, are most likely universal. In particular, it seems that reduction of nuclei number is characteristic for low temperatures, and that, on the contrary, its increase is induced by high temperatures. In *Tamarix. odessana* and *T. parviflora* normally exhibiting ES of different types (*Adoxa, Fritillaria, Drusa, Chrysanthemum cinerariifolium* and *Plumbagella*) in the same plants, mainly ES with a reduced number of divisions develop at low temperatures (Hjelmqvist and Grazi 1964). In *Zea mays* at high temperature, an increase of number of nuclei in antipodal cells was observed (Chebotaru 1965). In a *Linum usitatissimum* line, characterized by formation of two egg cells (as a result of division of the initial egg cell), frequency of these events increased from 31 to 52 % with increasing temperature from 16 to 28 °C (Huyghe 1987).

The character of ES changes, induced by temperature, simulates some known female gametophyte mutations. For example, ES with reduced number of cells arising at low temperature, are similar to a phenotypic manifestation of the mutation, characteristic for the tobacco line BG-141.4 (Enaleeva 1997), but the effect of increased temperature coincides with an effect of *ig*-mutation, causing the additional mitotic divisions in the coenocytic phase of ES (Lin 1978, 1981).

According to the present view, modifications that are similar in appearance to mutations, are qualified as phenocopies. It is known, that such modifications can result from disturbances of gene expression on various levels such as transcription, translation or post-translational protein modifications. It means, that changes of the ES development, caused by environmental factors and mutations, could be realized on the basis of the same cytological mechanisms, and a further research of both types of ES variability would promote their comprehension.

References

Chaudhury, A.M., Ming, L., Miller, C., Craig, S., Dennis, E.S., Peacock, W.J.: Fertilization-independent seed development in *Arabidopsis thaliana* (ovule apomixis). - Proc. nat. Acad. Sci. USA **94**: 4223-4228, 1997.

Chebotaru, A.A.: [To a question on experimental embryology of maize]. - In: Kovarsky, A.E. (ed.): Genetika, Selektsiya i Semenovodstvo Kukuruzy. [Genetics, Selection and Seed Improving of Maize.] Pp. 169-181. Kartya Moldovenyaske, Kishinev 1965. [In Russ.]

Dharamadhaj, P., Prakash, N.: Development of the anther and ovule in *Capsicum*.- Aust. J. Bot. **26**: 433-439, 1978.

Drews, G.N., Lee, D., Christensen, C.A.: Genetic analysis of female gametophyte development and function. - Plant Cell **1**: 5-17, 1998.

Enaleeva, N.: A tobacco mutant with a reduced cell number in embryo sacs. 1. Expression of the mutation in plants of different generations at the mature gametophyte stage. - Sex. Plant Reprod. **10**: 300-304, 1997.

Enaleeva, N.Kh., Tyrnov, V.S., Khokhlov, S.S.: [Dissection of the embryo sacs of some angiosperms by means of tissue maceration]. - Tsytol. Genet. **6**: 439-441, 1972. [In Russ.]

Gupta, R.K., Roy, R.P., Singh, A.P.: Aposporous apomixis: seasonal variation in tetraploid *Dichantium annulatum* (Forssk.) Stapf. - Port. Acta biol. **A11**: 253-260, 1969-1970.

Hjelmqvist, H., Grazi, F.: Studies on variation in embryo sac development. - Bot. Notiser **117**: 141-166, 1964.

Huyghe, C.: [The haploid-diploid polyembryony in flax (*Linum usitatissimum* L.)]. - Etudes cytol. physiol. agron. **7**: 567-573, 1987. [In French.]

Knox, R.B. Apomixis: seasonal and population differences in a grass. - Science **157**: 325-326, 1967.

Knox, R.B., Heslop-Harrison, J.: Experimental control of aposporous apomixis in a grass of the *Andropogoneae*. – Bot. Notis. **116**: 127-141, 1963.

Lin, B.Y.: Structural modifications of the female gametophyte associated with the indeterminate gametophyte (*ig*) mutant in maize. - Can. J. Genet. Cytol. **20**: 249-257, 1978.

Lin, B.Y.: Megagametogenetic alterations associated with the indeterminate gametophyte (*ig*) mutation in maize. - Rev. bras. Biol. **41**: 557-563, 1981.

Lobanova, L., Enaleeva, N.: The development of embryo sacs in *in vitro* ovaries of *Nicotiana tabacum* L. - Plant Sci. **132**: 191-202, 1998.

Nogler, G.A.: Gametophytic apomixis. - In: Jory, B.M. (ed.): Embryology of Angiosperms. Pp. 475-518. Springer-Verlag, Berlin 1984.

Ohad, N., Margossian, L., Hsu, Y.C., Williams, C., Repetti, P., Fischer, R.L.: A mutation that allows endosperm development without fertilization. - Proc. nat. Acad. Sci. USA **93**: 5319-5324, 1996.

Poddubnaya-Arnoldy, V.A., Steshina, N.A., Sosnovets, A.G.: [Material to biology of flowering and reproduction of *Scorzonera tau-saghyz* Lipsh. et Osse] .- Bot. Zh. **19**: 338-364, 1934. [In Russ.]

Sheridan, W.F., Huang, B.Q.: Nuclear behavior is defective in the maize (*Zea mays* L.) lethal ovule 2 female gametophyte. - Plant J. **11**:1029-1041, 1997.