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The role of cell selection for pollen grain fertility after treatment of barley sprouts (*Hordeum distichum* L.) with UV-B irradiation

Pomen izbora celic za plodnost pelodnih zrn po obravnavanju kalic ječmena (*Hordeum distichum* L.) z UV-B sevanjem

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Abstract: UV-B irradiation of barley sprouts within the range of 0.5-4.3 kJ/m2 induced an increase in the number of chromosome aberrations in the root meristem and pathologies in the reproductive system. Enhancement of cytomixis, increase of polymorphism and cytopathology of pollen grains were observed in the male generative system. The inverse trend was observed when intensity of cytomixis was compared to the pollen grain sterility. Damages induced by low doses of UV-B radiation were eliminated neither by DNA reparation nor by cell selection and were preserved in many cell generations. High UV-B level led to the activation of cytomixis due to which the population of microsporocytes was released from the excess load. It is presumed that cytomixis present a form of cell selection which was induced by an excess of microsporocyte disturbances.

Keywords: chromosome aberrations, root meristem, microsporangium, cytomixis, pollen grain sterility, cell selection, UV-B radiation, *Hordeum distichum* L.

Izvleček: Obravnavanje kalic ječmena z UV-B sevanjem (od 0,5-4,3 kJ/m^2) je povzročilo nastanek številnih kromosomskih aberacij v rastnem meristemu korenin in motnje v reproduktivnem sistemu. Pri moških generativnih organih smo zasledili povečanje citomikse, polimorfizma in patoloških sprememb pelodnih zrn. Ugotovili smo negativno povezanost med jakostjo citomikse ter pogostostjo patoloških sprememb pri tetradah mikrospor in sterilnostjo pelodnih zrn. Poškodbe zaradi nizkih odmerkov UV-B sevanja s popravljanjem DNA in z izborom celic niso izginile in so se ohranile več generacij celic. Visok odmerek je povzročil citomikso, zaradi česar se je sprostila populacija mikrosporocit. Predvidevamo, da citomiksa predstavlja način izbora celic, ki nastane zaradi večjega obsega motenj mikrosporocit.

Ključne besede: kromosomske aberacije, koreniski meristem, mikrosporangij, citomiksa, sterilnost peloda, izbor celic, UV-B sevanje, *Hordeum distichum* L.

Introduction

Ultraviolet in the range of 280-320 nm affect all levels of plant organization and also signal, regulatory and energetic functions (Jordan 1996, Ziska et al. 1992, Ziska and Teramura 1992, Caldwell et al. 2003, 2007; Zhang et al. 2003, Koti et al. *2004 a, b,* 2007*,* Hectors et al. 2010, Ballaré et al. 2011, Krasylenko et al. 2011). One of the most significant effects of enhanced UV-B radiation is an injury of the reproductive function of plants. It has been shown that additional UV-B radiation can exert genotoxic effect on the meristem, inhibit growth and development, influence pollination, decrease the quantity of the produced pollen and seed production of plants (Flint and Caldwell 1984, Conner and Neumeier 2002, Koti et al. 2004 a, b, 2007). Besides the direct action on generative organs, the main target of which is DNA of cells, UV-B radiation produces indirect effects which are realized by mechanisms connected with photoreception, transduction of signals and hormonal regulation (Tevini et al. 1981, Flint and Caldwell 1984, Santos et al. *1998;* Caldwell et al., 2007, Demkura et al. 2010, Keller et al. 2011*)*. The effect of action depends in many cases on genotype, ecotype, the stage of ontogenesis and other reasons (Jordan 1996, Torabinejad et al. 1998, Caldwell et al. 2007, Li et al. 2010). The data on mechanisms of the effect of UV-B radiation on the generative organs of plants are not available. In this connection, the main goal of the paper was to elucidate the character of damages in the generative system induced by UV-B radiation, its dose dependence, and also to estimate the role of cell selection in normalization of the pollen grain fertility.

Material and methods

We used barley (*Hordeum distichum* L., 2n=14) of Scarlet variety of French selection. Three-day sprouts were irradiated by a 20 W Philips TL ultraviolet lamp with filter cutting off the short-wave region of the ultraviolet spectrum. Radiation doses were 0.5, 2.2 and 4.3 kJ/m^2 with the intensity 0.5 W/m^2s^1. One group of sprouts (about 100 plants) was fixed 24 and 48 hour after irradiation. The other group of sprouts (about 100 plants) was cultivated in soil to study the development of reproductive organs. The material was fixed with Navashin mixture; temporal slides were prepared according to the standard cytological protocol (Pausheva 1984). The fixation of spike was made from the differentiation stage of microsporocytes to maturation of pollen grains. The slides were stained with acetoorcein following enzymic maceration (for root meristem) and either acetocarmin or Schiff's reagent (Feulgen protocol) for microsporangium. The quantity of anomalies in cell systems was counted and measurements were made.

The calculation of the number of chromosomal aberrations was carried out by the ana- telophase method, the mitotic index (MI) was defined by percentage of mitotic dividing cells. We estimated 10 roots per stage, 50-70 anthers with microsporocytes and 20 anthers for the analysis of pollen grains (PG) per stage. The data were statistically processed by the Microsoft Excel software.

Results

The effects of UV-B-radiation on the root meristem.

In the first mitosis the quantity of chromosome aberrations increased in proportion to the radiation dose, in the second mitosis the dose dependence varied: at high dose the level of chromosome aberrations decreased, while the number of degenerated cells increased (Table 1, Fig. 1). The cell degeneration occurred by the apoptosis type. It was particularly remarkable that in individual cases under maximal exposition of UV we observed the phenomena of cytomixis connected with migration of the injured chromatin along the plasmodesmal channels. Therefore, the increasing of UV-B-radiation dose resulted in increased number of aberrations in the first mitosis, while in the second, the induction dynamics of chromosome aberrations exhibited both direct and inverse dose-dependence (Fig. 1).

The effects of UV-B radiation on the reproductive system of plants.

Microsporogenesis: Microsporogenesis was with the formation of tetrads of the isobilateral

N/N Experimental variant. Radiation dose		Root meristem				Microsporogenesis and the stage of the development of the pollen grain				
		24 h after irradiation		48 h after irradiation		Microsporogenesis, degree of cytomixis, %	Tetrads with anomalies, %	Sterility of microspores, B %	Sterility of two-celled PG, %	Sterility of the three-celled PG, %
		MI, %	Aberrant anaphases, %	MI, %	Aberrant anaphases, %					
1	Control	5.8	3.05±0.65	4.5	2.20±0.29	4.3	4.5±0.3	1.9±0.1	3.8±0.9	2.9±0.7
2	0.5 kJ/m^2	6.2	2.61±0.73	5.2	5.75±0.96	7.3	6.0±0.6	2.1±0.3	9.0±1.7	11.3±1.4
3	2.2 kJ/m^2	6.6	5.92±0.96	5.1	6.24±0.80	6.3	8.1±1.0	2.7±0.4	9.7±1.5	9.1±1.8
4	4.3 kJ/m^2	5.8	8.82±1.10	5.0	4.52±0.82	19.7	8.5±0.7	3.7±0.4	6.8±1.5	3.8±0.8

Table1: The indices of cytogenetic disturbances in the root meristem of *H. distichum* sprouts and the generative sphere of the plants in the process of ontogenesis.
Tabela 1: Indeksi citogenetskih motenj v meristemu korenin kalic vrste *H. distichum* in generativnih organih v procesu ontogeneze.

structure is presented in Figures 3a and 4a. Under the influence of UV-B-radiation, cytomixis was the main type of pathology in microsporogenesis. We consider that one should distinguish between weak (local), intensive and destructive (pathological) cytomixis. Local cytomixis is a physiological norm for barley. UV-B radiation, similar to other stress factors, intensifies the destructive character of cytomixis. In barley under maximal exposition of UV intensive cytomixis affected up to about 20% of microsporocytes (Fig. 3b). In this case stickenessand fluidity of chromatin increased in microsporocytes. We observed a kind of transitional chromatin (fragments of nuclei, chromosomes, micronuclei, bands of chromatin from cell to cell). Not all microsporangia were affected by cytomixis. Most of microsporocytes completed meiosis with the formation of normal, only rarely nonbalanced, tetrads of microspores (Figs. 4a, b). In barley the bulk of »transitional« chromatin usually remained either in the composition of cynticia or in the intracellular space. Destructive cytomixis usually occurred in less developed flowers and in immature spikes of the second growth and is, presumably, a way to eliminate the nonviable cell system.

The dose dependence of cytomixis incorporation into microsporogenesis was of nonlinear character (Fig. 1). In this case, low correlation was observed between the intensity of cytomixis and frequency of pathologies in tetrads of microspores. Though cytomixis might be the reason for formation of the unbalanced tetrads, both types of disturbances were, most likely, a result of the same reason associated with genetic instability (prolonged mutagenesis) induced by the effect of UV-B- radiation.

The development of pollen grain: Under normal conditions, the development of male gametophyte in barley, similar to most of cereals, begins to release microspores from the microsporocyte envelope and includes the stages completing the formation of sporoderm, growth and polarization of microspore. Then follows the first asymmetric mitosis, polarization of two-celled pollen grain, the second mitotic division which are accompanied by the synthesis of cytoplasm, and then by the deposition of reserve substances in the vegetative cell cytoplasm (Batygina 1974, Poddubnaya-Arnoldi 1976, Heslop-Harrison 1979, Mascarenhas 1989).

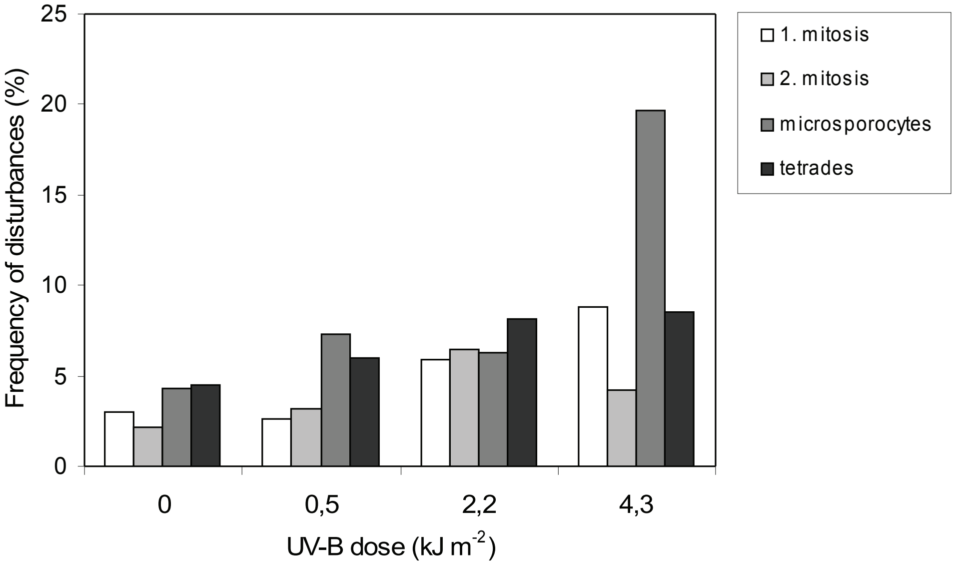


Figure 1: UV-B dose dependences of the number of cytogenetic disturbances in *H. distichum* sprouts in the first mitosis in the root meristem and microsporogenesis.

Slika 1: Število citogenetskih motenj v odvisnosti od UV-B sevanja pri kalicah *H. distichum* pri prvi mitozi v koreninskem meristemu in mikrosporogenezi.

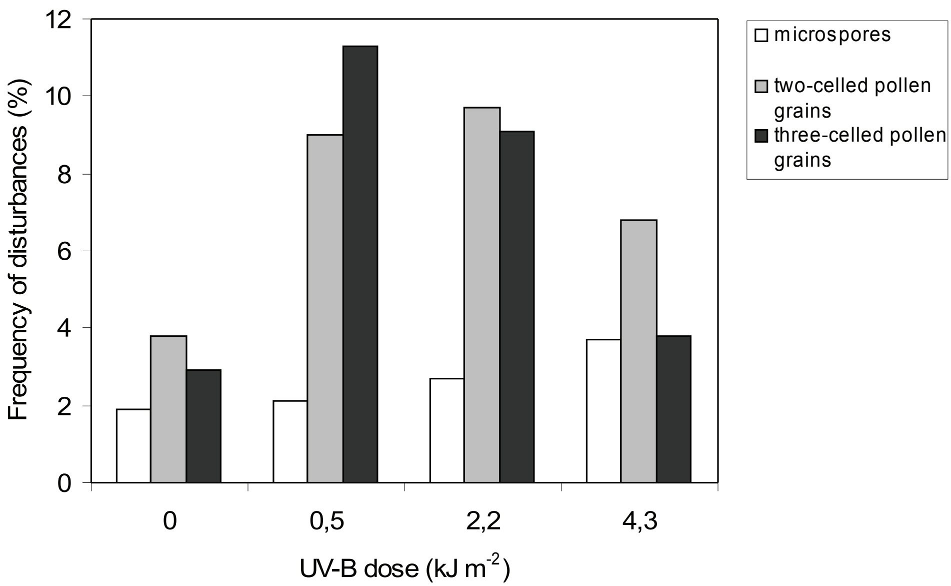


Figure 2: UV-B dose dependences of the number of cytogenetic disturbances of *H. distichum* in the phases of development of pollen grain.

Slika 2: Število citogenetskih motenj pri vrsti *H. distichum* v fazah razvoja pelodnega zrna.

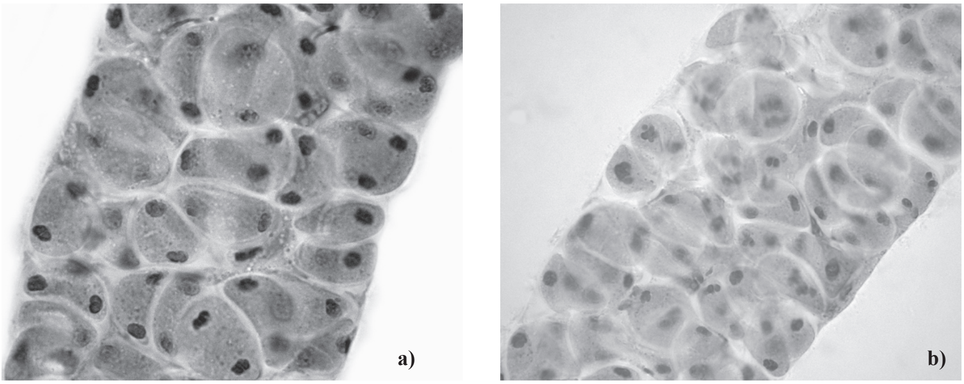


Figure 3: Microsporogenesis: a – telophase of 2^{nd} divisions of meiosis, control; b – telophase of 2^{nd} divisions of meiosis, UV-B dose: 4.3 kJ\m^2; intensive cytomixis.
Slika 3: Mikrosporogeneza: a – telofaza druge delitve pri mejozi, kontrola; b – telofaza druge delitve pri mejozi, UV-B odmerek: 4,3 kJ\m^2, intenzivna citomiksa.

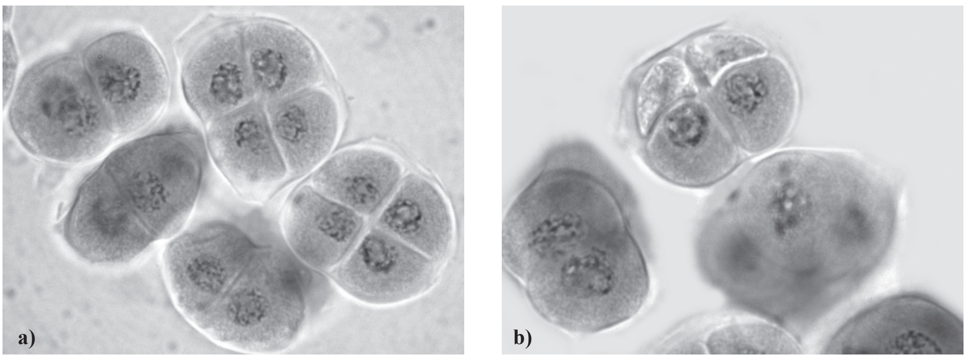
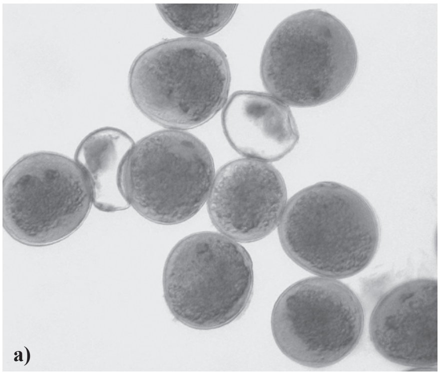


Figure 4: Formation of the tetrads: a – UV-B dose: 0.5 kJ\m^2; b – UV-B dose: 4.3 kJ\m^2.
Slika 4: Nastanek tetrad: a – UV-B odmerek: 0,5 kJ\m^2; b – UV-B odmerek: 4,3 kJ\m^2.

The mature pollen grain of barley has a pair of arrow-shaped sperms and a vegetative cell nucleus, the cytoplasm of which is filled with amyloplasts. Ultraviolet-B radiation led to an enhancement of polymorphism and to disturbance of polarity in pollen grains, unsynchronized development, the increase of the frequency in the formation of oligoplasm pollen grains (Fig. 5a). The latter present an evidence for nonspecific character of gametic disturbances caused by different stress factors. The appearance of oligoplasm pollen grains might be associated by either mutations of specific genes of pollen grain, whose expression intensifies after the first mitosis or by mutation which determines the male cytoplasmic sterility (Mascarenhas 1990, Nirmala and Kaul 1994). Such pollen grains are late or interrupt in their development, and their sperms do not complete the cycle of their differentiation. According to the morphological traits degeneration of microspore nucleus, generative cell, sperms and nucleus of the vegetative cell in the pollen grain occur by apoptosis (Fig. 5b). In reality, the recent publication reports that UV-B irradiation can indeed initiate apoptotic processes in plant cells (Lytvyn et al. 2010).

Thus, the range of cytological disturbances in pollen sacs had a nonspecific character.

Induction of disturbances in the course of pollen grain development correlated negatively with the UV-B radiation dose and with the degree



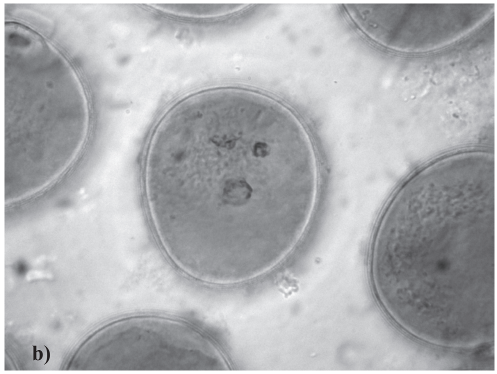


Figure 5: Mature three-celled pollen grains: a – normal and »oligoplasm« pollen grains, UV-B dose: 2.2 kJ\m^2; b – apoptosis degradation of pollen grain nuclei, UV-B dose: 4.3 kJ\m^2.
Slika 5: Zrela tri-celična pelodna zrna: a – normalna in »oligoplazemska« zrna, UV-B odmerek: 2,2 kJ\m^2; b – apoptozni razpad jedra pri pelodnemu zrnu, UV-B odmerek: 4,3 kJ\m^2.

of cytomixis (Table 1, Fig. 2). An enhancement of the UV-B dose in first step increased the number of anomalous pollen grains, but further on it decreased. At the higher dose the level of pollen sterility was close to control.

Discussion

This might evidenced the threshold character of the effect and the induction of the recovery mechanisms. It is known that in response to increased level of cytogenetic disturbances the DNA reparation systems become more active and leading to apoptosis induction or to proliferative death of non-repaired cells via the cell selection (Calendo 2001). Due to cell selection, namely, to the haplontic cell selection (taking place at the ontogenesis haplophase) the cells with recessive lethal mutations that are not subjected to the action of diplontic cell selection are eliminated (Gaul 1959). The action of haplontic selection is evident at the stage of the pollen grain maturation.

Paradoxically, but disturbances induced by low doses of UV-B radiation were not eliminated neither by reparation nor by cell selection and are preserved in many cell generations. They remained beyond the reach even of the haplontic competition resulting in relatively high percentage of the pollen grain sterility. Cytomixis began to act at higher UV-B-radiation dose, due to which the population of microsporocytes released from the excess of genetic load. Consequently, in the response of plants to radiation the threshold effect was observed which may be caused by damages of a number of DNA and other molecules initiating the reparation processes and cell selection (Calendo 2001). The intensification of cell selection occured in ontogenesis at the end of diplophase (microsporogenesis) and also at the end of haplophase (formation of gametes). Thus the long-term effects of UV radiation on reproductive system are likely causing the prolonged mutagenesis which was induced by irradiation. Decrease of disturbance number in reproductive tissues at the higher dose UV is probably connected with the threshold effect and activation of restore processes.

Cytomixis as a form of cell selection, occurring in microsporocytes before and at the beginning of meiosis, deserves special attention. We presumed that *via* cytomixis the population of microsporocytes regulated its excess, and simultaneously eliminated from the mutational load and solved the problems of nutritional character. Space continuity and uniqueness of microsporocytes as a cenocytic system of pollen sac were realized via cytomixis (Heslop-Harrison 1966a, 1966b, Welan 1974, Guo and Zheng 2004). Cytomixis might play a special role in provision of repairing processes in initials of male gametes.

In reality in the case of local cytomixis the chromatin loops penetrate the intercellular channels and united microsporocytes into groups in the early prophase of meiosis. Such contacts do not entail negative consequences for future meiosis. *Vice versa*, the microsporocytes that are not included in the network may fall out of the developmental program. They may be delayed in the prophase-metaphase of the first division of meiosis and undergo proliferative death. It is known the callose wall of microsporocytes is not an impermeable barrier and does not prevent the migration of chromatin and organelles topenetrate through wide intercellular channels along which not only chromatin but also cytoplasmic organelles, signal molecules and trophic factors may pass (Risueno et al. 1969, Zheng et al. 1987, Souza and Pagliarini 1997, Hecht 2000). It is believed that the intercellular contacts ensure not only the synchronization of meiosis but also the cellular population homogeneity in microsporocytes and equalize the qualitative state of pollen grains which is required for a rapid and successful pollination.

Cellular selection in the population of microsporocytes occurs through the so-called autonomous apoptosis which unlike morphogenetic apoptosis, is not programmed but is initiated by the microsporocyte population itself. This assumption is supported by the irregularity of cytomixis, which does not occur simultaneously in all microsporangia or microsporocytes in the same anther. Intensive cytomixis is induced by UV-B radiation, hybridization and other stress factors. Many researchers assume that the nature of intensive and destructive cytomixis is connected with the genetic disbalance (disturbance of homeostasis) of polyploids, haploids, aneuploids, mutants, hybrids and apomicts (Poddubnaya-Arnoldi 1976, Mantu and Sharma 1982, Bedi 1990, Orlova 1994). Stress factors, such as radiation, hybridization, chemical agents and herbicides usually enhance the destructive effect of cytomixis (Bobak and Herich 1978, Dwivedi et al. 1988, Bellucci et al. 2003). Usual environmental conditions and their seasonal fluctuations exert no marked effect on cytomixis, which is obviously under the genetic control (Mantu and Sharma 1982, Bellucci et al. 2003).

The intensive and destructive (to a larger extent) cytomixis made the meiosis pattern more complicated and may led to serious genetic consequences. However, this is mainly peculiar to genetically unbalanced, sterile forms and in spikes of the second growth or nonviable individuals. Basing in the experimental material and data from literature we assumed that cytomixis reflected the mechanisms of the cellular selection during which cellular population limits the number of functioning microsporocytes, thus regulating redundancy, and eliminating the mutation load.

Conclusion

UV-B radiation of barley sprouts within the range of 0.5-4.3 kJ/m^2 induced an increase in the number of chromosome aberrations in the root meristem and pathologies in the reproductive system. Enhancement of cytomixis, increased polymorphism and cytopathology of pollen grains was observed in the male generative system. Injuries caused by UV-radiation were of nonspecific character. The negative correlation was observed between the intensity of cytomixis and frequencies of pathologies in tetrads of microspores and also between the sterile level of pollen grains. The induction of disturbances during development of the pollen grain revealed negative dose dependence. Under the maximal exposition of UV-B radiation the index of pollen sterility approaches to that obtained at the control. Injuries induced by low doses of ultraviolet were not eliminated, either by DNA reparation either by cell selection and were preserved in many cell generations. An enhancement of the radiation dose led to the activation of cytomixis due to which the population of microsporocytes was released from the excess load. We presume that cytomixis present a form of cell selection which is induced by an excess of the injuries a threshold level of microsporocytes. It possibly limits mutagenesis, regulates the state, quantity of microsporocyte population solving simultaneously the problems of nutritional character and thus promotes preservation of the pollen grain fertility.

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