POLEN FERTILITY OF *VICIA FABA* L., AFTER TREATMENTS WITH X-RAYS

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ABSTRACT

An attempt has been made to examine the fertility of polen from the Vica faba L., after short-term exposure to low dosages of X – rays (1X - 16,2 cGy; 2X - 32,4 cGy; 3H – 48,6 cGy; 4X – 64,8 cGy). The material has been analyzed during three consecutive generations M_1, M_2 and M_3. Dose-effect dependence was determined at the level of fertility and the morphology of the polen grains (the results are shown in tables in the text below). The irregularities in grain's normal form are result of the irregularities during the microsporogenesis in all the treatments. The filled grains with generally unchanged shape that differ from the typical ones in volume have been separated from the defective grains with lower vitality. Beside the empty (sterile) grains, a number of triangular grains with considerably enlarged volume have been detected, as well as grains with spherical shape in miniature dimension and in very low percentage grains with polygonal shape, squashed and with wrinkled surface. The decreased fertility of the polen grain and the possible abnormalities in the flower's constitution result with decrease in the total number of plants in the experimental groups especially in M_3 generation.

Keywords: *V. faba* L., X-rays, microsporogenesis, fertility of polen, frequency of defective grains in M_1, M_2 and M_3 generation

Introduction

The emergence and development of plants in the next generations, largely depends on the degree of pollen fertility. Known variability of this parameter in the modified conditions in the environment. (1, 3, 4, 7, 8, 9, 15). Having regard to the application of radiation in ionizating induced mutations in plants, was appointed to investigate the influence of X-rays on the fertility of pollen of *Vicia faba* L., depending on the dose and duration of irradiation, as one of the indicators the transmission of genetic changes in level which causes radiation.

Materials and Methods

In purpose to investigate the influence of ionizating radiation, when the seed is irradiated in germination phase, 90 h. *V. faba* var. *major* cultures are treated with different doses of X-rays. Irradiation is done with X-ray apparatus Shimadzu with voltage 90 KV, 20 mas, with a focal distance of the 22nd cm and measuring the dose of 0,05 Gy / minutes. 1X-group is exposed for a period of 3 minutes and 20 sec/16 2 cGy; 2X- group is exposed for a period of 6 minutes and 40 sec/32 4 cGy, 3X-group is exposed for a period of 10 minutes / 48 6 cGy, 4X- group is exposed for a period of 13 minutes and 20 sec/64, 8 cGy.

The control group of *V. faba* L., constitutes untreated seed planted in soil following the occurrence of early embryonic roots. Coloring of anthers is made with 2% acid-orcein and iodine-glycerine method by Vuchenovic and Petrovic (10). Polen fertility is determined based on analysis of pollen grains of 3000 by group (1000 pollen grains of a product) and is expressed in terms of 100^{th}.

Results and Discussion

A pollen mother cell (PMC) creates, after meiotic division, four haploid cells. These are microspores, which develop into the male gametophyte (pollen grain). The structure and form of the newly formed microspore depends on the regularity of

meiosis. The analysis of microsporogenesis established numerous irregularities of meiosis in treated plants compared with control. Short-term irradiation with X-rays leads to various abnormalities not only in the M1 but in M2 and M3 generation. **Fig. 1** is represented only part of the abnormal cells in various stages of this kariocinetical cycle which caused changes in microspores and later in pollen grains.

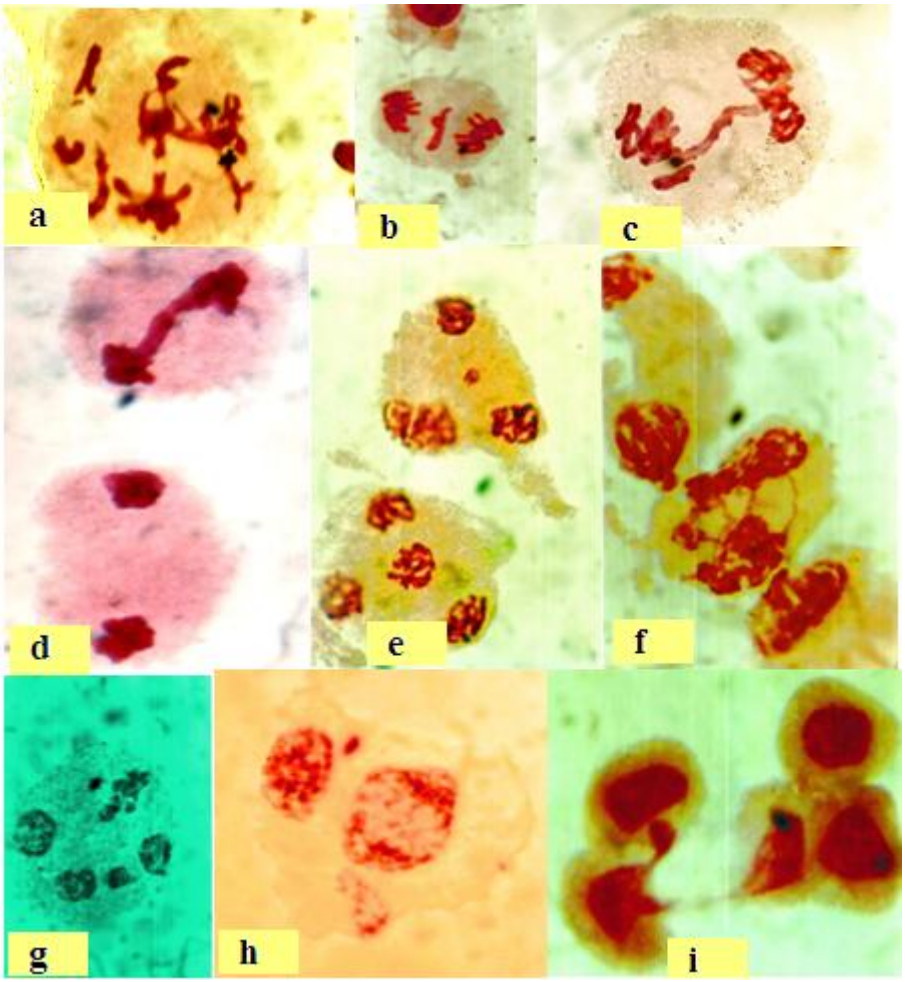

Fig. 1. a-irregular conjugation of chromosomes, bivalents and polyvalents in poliploid cell, diakinesis; **b**-lagard inactivated bivalent in late anaphasis; **c**-bridge of anaphasis; **d**- persisting bridge in telophasis I; **e**- defectiv cell, telophasis II with micronukleus; **f**-multiple bridges; **g**- defectiv telophasis II; **h**- defectiv cell with different genetical material in the nucleuse; **i**- defectiv microspores with micronucleuse and citomixis

Normal pollen grains are threecolporated, elliptical and about 1.7, times longer than the width (11). They contain a number of organic substances that in the presence of iodine stain darker, while sterile pollen remain colorless (**Fig. 2, Fig. 4**). This phenomena is confirmed in literature data (2, 5). As a result of irregularities which persist (remain) until the end of meiotic division and defects in microspores, appear defective mature pollen grains, which is evident from the analysis presented in **Table 1** and **Fig. 3**.

It was found a significant difference in pollen fertility between the control plants (95%) and treated groups, with 2X, the decline of 86%. The reduction in fertility by increasing the dose is worse in M2 and M3 generation and considering the fact that some anthers are completely empty, that shows dependence is exactly here. The stamina that are analyzed and measured fertility is sufficiently high but many flowers were sterile, so the weight of the fruit in M1 and M2 and especially M3 generation is much lower than the control.

This indicates that significant changes occurred and the level of the structure of the flower, particularly in the construction of the gynaeceum. Fertility treated in 1X-generation M3 group decreased to 65% and is due to the presence of type mutant irregular shape pollen grains whose frequency is relatively high (85%). The insignificant % (low%), these grains are represented in the pollen 3X and 1X group. Reduction of fertility and percentage of surviving plants by increasing the dose noted in experiments in *V. faba* introductory treatments with gamma rays 8 and 12 krad / 80-120 Gy with higher dose, induce high sterility in plants as a greater frequency of mutations, primarily physiological and morphological achieved with lower dose (6).

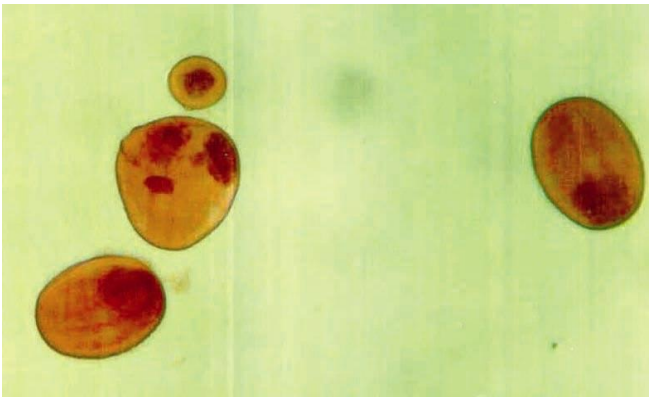

Fig.2. Defective pollen grains of in *Vicia faba* L. colored using acidorcein method

TABLE 1

Frequency of defective grains in M_1, M_2 and M_3 generation

		Normal pollen grains (%)	Defectiv pollen grains (%)										
			Summary	Frilly full	Raised large full	Spherical full	Spherical small,full	Spherical small, empty	Empty with normal form	Triangle full	Small filled	Large filled	Other forms
M1 generation	C	95.10	4.90	2.50	/	2.50	/	/	0.30	/	/	/	/
	1X	88.80	11.2	2.77	0.03	0.17	1.23	0.70	/	1.50	0.73	3.50	0.56
	2X	86.50	13.5	2.97	/	1.27	1.30	2.13	0.47	0.83	1.37	1.87	1.25
	3X	88.30	11.7	4.40	0.03	0.63	0.73	0.20	0.27	2.50	0.97	1.10	0.86
	4X	88.70	11.3	3.30	/	1.10	1.03	1.47	0.10	1.83	0.57	1.60	0.26
M2 generation	C	94.70	5.30	1.83	/	1.67	/	/	0.30	0.97	0.43	/	0.10
	1X	92.50	7.50	1.97	/	0.33	1.73	1.17	0.30	0.53	0.43	0.93	0.10
	2X	91.90	8.10	2.33	/	0.63	0.63	0.27	0.07	1.53	0.33	1.60	0.70
	3X	94.50	5.50	2.57	/	0.37	0.50	0.43	0.07	0.37	0.27	0.77	0.13
	4X	89.90	10.1	2.30	0.03	1.43	0.47	1.23	0.83	0.07	0.07	3.43	0.23
M3 generation	C	91.93	8.07	2.37	/	1.57	0.13	/	0.03	2.20	0.93	0.43	0.36
	1X	65.90	34.1	3.30	/	0.83	0.43	0.63	0.10	0.10	0.17	1.07	27.43
	2X	/	/	/	/	/	/	/	/	/	/	/	/
	3X	93.50	6.50	1.80	/	0.33	1.10	0.63	/	0.30	0.50	1.40	0.47
	4X	/	/	/	/	/	/	/	/	/	/	/	/

Sjödin (12), treating seeds with X-rays with doses of 4000 R gets mutant Po-1 which is characterized by triangle form of pollen grains. About 73% of pollen in this mutant is fertile, compared fertility of the parental form -Primus which is 97%. Treatment with neutrons-140 rad gets the Po-2 mutant which is characterized by spherical shape of pollen grains. Their volume is considerably lower than that of Primus varieties and the percentage of fertility decline (reduced) to 81%. The same author in (12) concluded partial and complete male sterility in treatments with ionizing radiation and chemical mutagenic agents (X- rays, 6000-9000R; γ- rays, 4000-7000R; neutrons, 70-260rad, and

chemical mutagenic agents with concentrations: EMS, 0.10-0.25%; MMS, 0.01-0.025%; EOC, 0.16-1.28%). Thereby partially sterile lines were significantly higher frequency of treatments with radiomimetics substances compared with ionizating rays.

Chart 1. Percentage ratio between fertile and sterile grains of *Vicia faba* treated with different dosages of X - rays

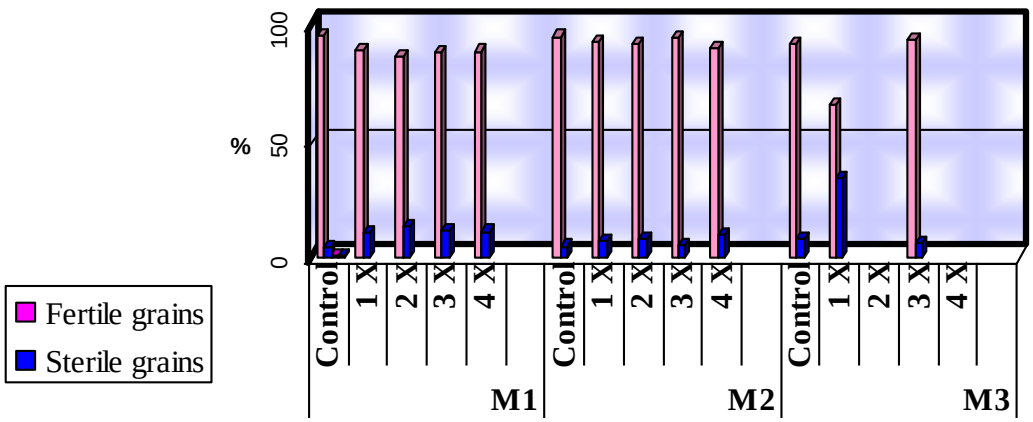

Fig. 3. TLC analyses for detection of GOS after transformation of lactose for 4 h (a) and 24 h (b). (**A**) yeast strains belonging to Group 1; (**B**) yeast strains belonging to Group 2; (**C**) yeast strains belonging to Group 3; (**D**) yeast strains belonging to Group 4

Fig. 4. Fertile and sterile pollen grains of *Vicia faba* L., colored using iodine – glycerin method

Conclusions

From the results obtained is evident negative effect of short-term irradiation with X-rays with visible radiation weakens the body and reduces the ability to develop this culture,

except in 3x-group in which the negative effect on the level of morphology of plant organs is less expressed. But in any case, this portion can not be assessed as positive. In terms of low fertility have seen slight decline in values with increasing dose with respect to control values in M1 and M2 generation and a significant decrease in the percentage of M3 generation. The discrepancies in the morphology of the basic type of pollen grains of *Vicia faba* L., is regarded as defects systematized in several groups. Most frequent were raised, spherical, triangular in shape, full and empty grains with higher and lower volume, while the characteristic poliagonal shape, side flait and irregularly shaped pollen grains are represented by a low percentage. About 80.5% of irregular form of pollen grains, in the M3 generation plant is considered mutant type. During the M1 and M2 generation fertility of the pollen is kept to a satisfactory level, but in principle all the pollen grains with changed morphology are sterile or have no ability for normal fertilization and together with other negative effects in the structure of reporoductive organs in the treatment groups leads to significant reduction of number of plants in M3 generation. Based on extensive literature review and data on faba been studies from our laboratory, a model for androgenetic capacity of pollen grain is proposed.

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