Macedonian Journal of Ecology and Environment
Vol. 19, issue 1
pp. 35-38
Skopje (2017)
eISSN 1857 - 8330
Original scientific paper
Available online at www.mjee.org.mk

Irregular mitosis and meiosis of *Vicia faba* L. after contamination with ^{131}I

Нерегуларности во митоза и мејоза кај *Vicia faba* L. по контаминација со ^{131}I

Gordana DIMESKA*, Lenka CVETANOVSKA

Institute of Biology, Faculty of Natural Sciences and Mathematics, Ss. Cyril and Methodius University, Skopje, Macedonia

In order to study the impact of radioactive substances on the cell cycle of *Vicia faba* L., which can be cultivated as food crop, experimental contamination was carried out with ^{131}I radioactive rain drops applied at 5 groups of plants in flowering phase. A detailed analysis was done of both karyokinesis during the cell cycle and pollen grains. The results are presented as MI (mitotic index and frequency of aberrations in the M1 generation) and as an original micrographs. There are indications that in terms of long irradiation, the disintegration of ^{131}I induced changes during mitosis and meiosis which depend on the absorbed dose, directly and indirectly influence the further growth and development of the treated crop. There have been detected different chromosome defects: chromosome breaks and structural rearrangements that occured in most of the chromosomes, adherence of the full complement of chromosomes, terminal connections and various interchromosomal links that contributed to single or multiple anaphase and telophase bridges which in some cases persist through the end of the cell cycle. There was high frequency of aberrant changes in meiotic karyokinesis I and II that are common in all of the treated groups, with few exceptions when dose-effect dependence was not determined. These irregularities resulted in defects in the construction of the pollen grains.
Key words: ^{131}I, aberration frequency, genetic variability, meiosis, mitosis, pollen grains. *V. faba* L.,

Со цел да се проучи влијанието на радиоактивните супстанции врз клеточниот циклус кај *Vicia faba* L., како важна прехранбена култура, беше направено истражување на ефектот од контаминацијата на 5 групи растенија во фаза на цветање со капки од раствор кој што содржеше радиоактивен изотоп ^{131}I. Направена беше детална анализа на кариокинезата за време на клеточниот циклус и анализа на поленовите зрна. Резултатите се презентирани како МИ (митотски индекс и фреквенција на аберации во М1 генерација) и во вид на микрографи. Постојат индикации дека во случај на долготрајно озрачување, распаѓањето на ^{131}I иницира промена за време на митозата и мејозата кои што зависат од апсорбираната доза, директно влијаејќи на понатамошниот раст и развој на третираната култура. Пронајдени се различни хромозомски дефекти: хромозомски прекини и структурни промени кои што ги опфаќаат повеќето од хромозомите, слепување на цели комплементи од хромозоми, терминални поврзувања и цели интерхромозомални врски кои што водат до појава на единечни или повеќекратни анафазни и телофазни мостови кои во некои случаи опстојуваат до крајот на клеточниот циклус. Постои висока фреквенција на нерегуларни промени во мејотската кариокнеза 1 и 2 кои се заеднички за сите третирани групи, со неколку исклучоци каде што не е утврдена зависност помеѓу апсорбираната доза и ефектот. Ваквите аберации резултираат со дефекти во конструкцијата на поленовите зрна.
Клучни зборови: ^{131}I, генетска варијабилност, мејоза, митоза, поленови зрна, фреквенција на аберации, *Vicia faba* L.

Introduction

Ionization is the strongest and most reliable mutagenic agent when experiments with inducing genetic variability in certain species are performed. The results are directly dependent on the dose, time of exposure as well as on the plant material nature. The application of relatively low doses causes significant DNA damage. In such cases, disruptions frequency enlarges, thus genetic variability in specific organism is increasing. The result is significantly different compared to higher doses usage, which is often lethal (Montezuma De Carvalho 1968; Crocker & Cattanach 1981; Hebrang & Petrovcic 1987; Aleksieva & Nikolov 1990; Myers 1993; Dubinin 1994).

Considering the existing literature data the aim of this research was to examine the impact of ^{131}I, applied externally, in the flowering phase of *Vicia faba* L.

Material and Methods

Five plant groups (*Vicia faba* L.) have been grown in pots in the same experimental conditions. There were 30 plants per group. In flowering stage, in all groups ^{131}I was applied with a total activity of 356 Bq, mass of 0,078 x 10-12g. ^{131}I application was in form of IODIURE ^{131}I DE SODIUM. After being applied in a form of radioactive rain

Submitted: 24.02.2017
Accepted: 09.05.2017

*Author for correspondence: dimeskag@yahoo.com

drops on the above ground plant parts, an activity measurement was performed using Brown-10 Geiger-Miler tube, SS-100, manufactured by the Institute of Nuclear Sciences "Boris Kidric" Vinca, and Belgrade. The results of the measurements are shown in Tab. 1.

Table 1. Results of measurements of application activity of ^{131}I

Plant groups	Activity (Bq)	Application activity of ^{131}I(g)
Group 1	35.6	0.008×10^{-12}
Group 2	55.1	0.012×10^{-12}
Group 3	69,6	0.017×10^{-12}
Group 4	56.9	0.012×10^{-12}
Group 5	65.1	0.014×10^{-12}

The control group consisted of untreated plants was grown in identical conditions likewise the experimental groups. They were treated with regular tap water. The experiment was carried out in the Botanical Garden of the Institute of Biology, Faculty of Natural Sciences and Mathematics. In the somatic chromosomes analysis, meristem tissue of the root was used. The material was analyzed according to Tjio & Levan (1950) cytological technique, as well as the standard "squash" method. The cytostatic 8-hydrooxyquinoline (0,002 M) was used for material treatment and then fixed according to Clark (alcohol-acetic acid- 3:1). A chromosome coloring was made with leucobasic fuchsin (Darlington & La Cour 1962), hematoxylin after Gomorry (Konstantinov et al. 1985). Pollen grains staining analysis was made with 2% acetate-orcein and with Iodine-glycerin method (Petrovic & Vucenovic 1992).

Results and discussion

The effects of long term exposure of the treated samples of *Vicia faba* during ^{131}I dissolution are visible on a cytological level in both karyokinesis and in mature pollen level. Detailed review of all changes during mitosis was made with complete analysis of the treated meristem root tissue compared to the results to the controlled group. Meristem activity was calculated and shown in mitotic index (MI) after detailed analysis of each phase. The research results are shown in Table 2 and 3.

It was determined that the changes of MI index depended on the dose amount, thus the first plant group treated with the lowest doses are closest to the control ones while in the other groups decreases of MI index were being noted, down to 1,72 in the group 5. The results of each separate group indicate changes in correlation with the ^{131}I doses used. Metaphase chromosomes do not differ morphologically compared with control ones, but the other groups (2, 3, 4 and 5) are characterized with altered morphology, visually elongated and thinner. Aberrant cells are relatively rare and mainly they were present as a full conglomeration of chromosomal complement or less common terminal connection between individual chromosomes.

The defects in further course of this karyokinesis were represented with lower percentage. They appeared as a consequence of interchromosomal links which have contributed to anaphase and telophase bridges that are mostly single but sometimes persisted till the end of the division (Fig.1).

Chromosomal and chromatic interruptions are noted in groups 2, 4 and 5. In Fig.2 there are shown more interruptions and rearrangements that affected most chromosomes of the complement. As a result of these interruptions acrocentric chromosomal fragments appeared which further on have formed laggards, significant for group 4 while rarely present in the other groups.

An incorrect chromosomal grouping in anaphase was detected, combined with a single or multiple bridges which resulted with uneven distribution of the genetic material in the nucleuses, producing the so called *somatic reduction* (Fig. 3).

All of the previous aberrations resulted in appearance of polynuclear cells with nuclear volume differences, as well as cells with one or more micro nucleuses. In meiotic karyokinesis analysis some division irregularities were found, as well as division absence in the last treated

Table 2. Mitotical index in the groups treated with ^{131}I in the flowering phase, M_1 generation

%		Control	Group 1	Group 2	Group 3	Group 4	Group 5
Mitotic index -MI		8.36	8.32	5.62	5.56	6.45	1.72
Interphase		91.64	91.63	94.38	94.37	93.42	98.21
Prophase		4.54	5.64	3.77	2.87	3.70	1.29
Prometaphase		1.00	0.79	0.26	0.07	0.48	0.18
Metaphase	Normal	2.00	0.40	0.17	/	/	0.17
	Aberrant	/	0.10	0.27	0.52	0.12	0.09
	Summary	2.00	0.50	0.43	0.52	0.12	0.26
Anaphase	Normal	0.24	0.35	0.46	0.17	0.03	0.17
	Aberrant	/	0.02	0.17	0.14	/	/
	Summary	0.24	0.37	0.63	0.31	0.03	0.17
Telophase	Normal	0.56	0.87	0.85	1.08	0.09	0.96
	Aberrant	/	0.15	0.36	0.70	/	0.17
	Summary	0.56	1.02	1.21	1.78	0.09	1.14
Other defects		0.05	/	0.07	0.12	0.06	

Table 3. Aberration frequency during mitosis in *Vicia faba* L., after the treatments with ^{131}I, in the flowering phase, M_1 generation (control and 5 plant groups)

Analysed groups	Metaphase							Anaphase			Telophase				
	Summary	Normal	Aberrant					Summary	Normal	Aberant	Summary	Normal	Aberrant		
	%	%	Summary	Partial stickness	Total stickness	Breaks	Translocations	%	%	Bridges	%	%	Summary	Lagrads	Bridges
Control	100	100	/	/	/	/	/	100	100	/	100	100	/	/	/
1	100	80	20	/	20	/	/	100	93.33	6.67	100	85.36	14.63	/	14.63
2	100	66.67	33.33	/	/	33.33	/	100	100	/	100	84.61	15.38	/	15.38
3	100	/	100	6.67	93.33		/	100	55.55	44.44	100	60.78	39.21	/	39.21
4	100	38.89	61.11	5.55	44.44	5.55	5.55	100	73.08	26.92	100	70	30.0	2.0	28.00
5	100	/	100	/	75	25	/	100	100	/	100	100	/	/	/

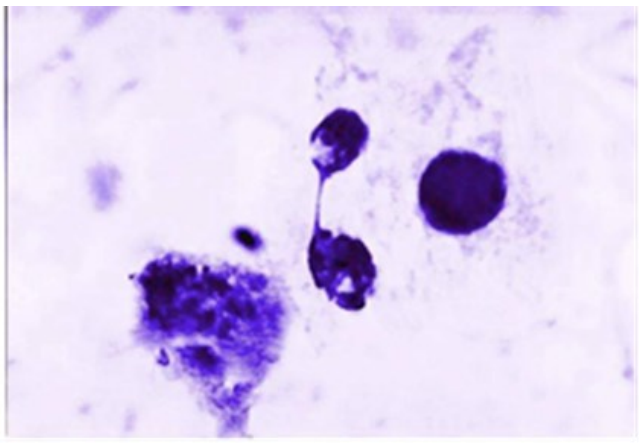

Figure 1. Interphase bridge which persisted through the end of the cell cycle in mitosis

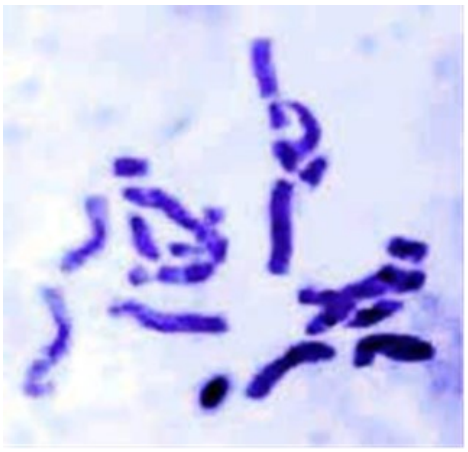

Figure 2. Breaks and structural rearrangements the chromosomes

Figure 3. Multipolar spindle fibers

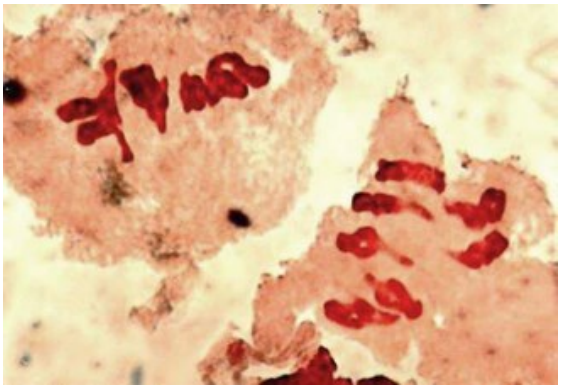

Figure 4. Defects in metaphase I of meiosis

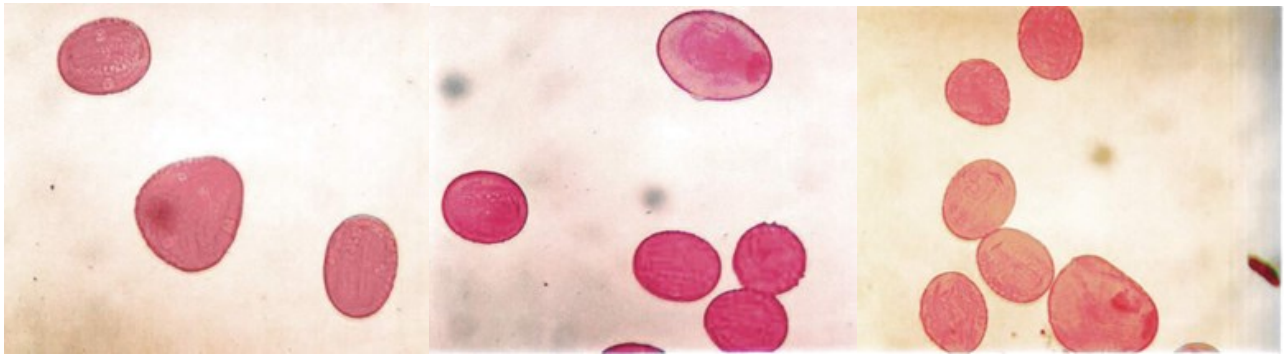

Figure 5. Different types of pollen grains

group. In the early stages, as a result of the irregular conjugation, prevalent and unusual bivalent for this species appeared. Changes that were detected during meiosis 1 and 2 are common for all treated groups and could generally be correlated with the dose amount. There were exceptions in the frequency of these aberrations, probably because of the separate analysis of flowers.
In Fig. 4 a cell with 7 bivalents in the material from the first treatment group is presented. In this group defective cells were rare and conglomeration and bridges that persist till the end of meiosis 2 were noted. In all analyzed groups tetrad aberration with micro nucleuses or with reduced core number were common. These defects were most common in the 3rd group which was treated with the highest dose. Core deficiency of micro spores appeared as well as gigantic micro spores where two cores were present. Some of the changes have not yet been confirmed with literature data but refer to treatments with other isotopes and to short term exposure whereby effects are monitored mainly on mitotic karyokinesis level (Montezuma De Carvalho, 1968; Errico et al., 1983).

Normal *Vicia faba* grain is three colored, elliptic and about 1.7 times longer than wide (Sjödin 1971; Reille 1992). The above mentioned changes in meiosis contribute to irregular formation of pollen with altered morphology. At I-treated group, pollen grains have smaller dimensions but some atypical patterns with slight crimping were noticeable. Highest variability regarding pollen morphology was determined in group 3. There were some spherical shapes with previously mentioned changes, but with gigantic pollen grains with triangle and oval shape (Fig. 5). Spherical grain shape with visually smaller volume have been determined by Sjödin (1971) during pollen analyzes on *V. faba*, when treated with neutrons (140 rad) during treatment of seed material with X-rays (4000 R), a mutant was gained (po-1) which was characterized with triangle pollen grain shape.

Conclusions

The effects of long term exposure at low doses during ^{131}I decay applied in flowering phase in 5 plant groups of *V. faba* was determined. Faba beans are highly radiosensitive when grown in the presence of this radioactive isotope. Contamination with ^{131}I is causing significant cytological changes which are expressed though irregularities during somatic division in the sex cells. Also, genotoxic and mutagenic impact was concluded by decreased mitotic activity in the root regardless of the dose amount. This means that long-term exposure can be harmful as well as acute short-term high doses.

References

Aleksieva A. & Nikolov C. (1990). Influence of gamma-radiation on some radiobiological and genetic reactions in plants from four different varieties of soybean. *Genetics and Selection*, **23**(5) :416-423.

Crocker M. & Cattanach B. M. (1981). X-ray induction of traslocations in mice carryng metacentrics (Robertsonian fusions); detection of whole arm chromosome exchanges. *Mutation Research*, **91**: 353-357.

Darlington C. D. & La Cour L. F. (1962). *The handling of chromosomes*. Allen and Unwin, London, 264 pp.

Dubinin, N. P. (1994). Some Problems in Modern Genetics. In: Glazko, TT, Sozinov, LA. and Glazko, VI (eds). J. Reports of Russian Ac. of Agr, Sci 2 (1994): 29-31.

Errico A., Saccaedo F., Conicella C., Monti L. M. (1983). Variability induced by a dicentric chromosome in *Vicia faba* L. *Genetica Agraria,* **37**: 161-162.

Hebrang, A. & Petrović, F. (1987). Radijacija i zaštita u medicinskoj dijagnostici. Medicinska knjiga, Beograd-Zagreb.

Konstantinov, G. H., Belcheva, R.G., Goranov, A. K., Ralchev, K. H., Genova, G. K. (1985). *Laboratory manual in genetics*, Sophia.

Montezuma de Carvalho, I. (1956). X-ray experiments on mitosis. I. Analysis of chromosome changes in colchitetraploid X 2 cells. *Bot. da Sociedade Broteriana*, **30**: 221-240.

Myers, M. J. (1993). *Genetic and somatic effects of ionising radiation and how to assess their risks. Radiation protection of patients*. Cambridge University Press, USA. 19-34.

Petrović, S., Vučenović, M. (1992). Laboratory manual in cytogenetics. Faculty of Agriculture, University of Novi Sad. (In Serbian) [Petrović, S., Vučenović, M. (1992). *Praktikum iz citogenetike*. Poljoprivredni fakultet, Univerzitet u Novom Sadu].

Reille M. (1992). *Pollen et spores D'Europe et D'Afrique du nord.* Cedex, Marseille, 243 pp.

Sjödin J. (1971). Induced morphological variation in *Vicia faba* L. *Hereditas,* **67**: 155-180.

Tjio, J. H., Levan, A. (1950). The use of oxyquinoline in chromosomes analysis. *Anal. Extac. Exp. Aula Dei*, **2** (1): 21-64.