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Estimates of Genotoxic Effect by the Pollen Analysis of Vines and Fruit trees

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Abstract - The complex analysis of male reproductive system of vines and fruit-trees growing in zone of the Armenian Nuclear Power Plant (ANPP) is realized. The obtained data demonstrate that the homogeneous pollen with high fertility and optimal morphometric parameters in all variants is formed. The obtained results demonstrate that there is no one-sided effect of ANPP on the development and formation of investigated male generative system of vines and fruit trees. Thus, on the base of obtained results we can't confirm its deleterious effect on the environment.

Key words : Microgametophyte, pollen, fertility, genotype, homogeneity, *Rosaceae Juss.*, *Vitaceae Juss*

INTRODUCTION

The determination of spontaneous and induced mutation level around nuclear power plants allows one to evaluate the genotoxic action and genetic risk as a function of environmental pollution levels. The pollution of the environment with technogenic factors can change the evolutionary formed complexes of adaptive reactions. In situ indication of genotoxicity requires well-founded selection of indicator genotypes to estimate genotoxic potential in the examined locality or region (Ma 1979; Micieta and Murin 1995).

One of mentioned approaches, allowing to estimate the genotoxic action of environmental factors, is the test for the definition of plants pollen grains fertility. For such research are very convenient the sorts of *Vitis vinifera L.* of *Vitaceae Juss.* family and some fruit trees of *Rosaceae Juss.* family with low level of sterility. Some minimal sterility of pollen in any kinds of plants is detected even in normal functional anthers. It can be the result of abnormal distribution of chromosomes in meiosis (Staudt 1973). Flowering plants have been used as bioindicators of mutagenicity, phototoxicity and genotoxicity of environmental pollutants. The pathological changes shown in all stages of meiosis, considerably reduce generation of high-grade viable pollen. For the population sterility of pollen means reduction of a genetic variety and decrease of adaptive opportunities. Pollen grains fertility parameter for vines and fruit trees, allows to estimate gametocide effects of environmental mutagens. Thus, it is reasonable to use these taxons of plants that proceed in the investigated environment sporophyte and gametophyte cycles of development. Kordyum and Sidorenko (1997) and Shevchenko *et al.* (2002) investigated the genotoxic effect of radiation on different species of plants.

One of advantages in using microgametophyte as bioindicators of mutagenicity is the high sensitivity conditioned by the haploid state, when all lethal mutations affecting the development of pollen are immediately evident (Micieta and Murin 1995). The pollen abortion assay reflect deletions in virtually any part of the genome. However, the

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greatest advantage is that the species live in the polluted environment under evaluation, and this makes possible to evaluate the effectiveness of ecological factors including pollution components (Constantin 1984).

The reasons of formation of abortive pollen can be different. They can include the regulatory role of tapetum, RNA, i-RNA, intensity of all synthetic processes. Pederson *et al.* (1987) mentioned the difference in the gene expression on the different stages of formation and functioning of the pollen. In the species with three-cell pollen the genes can be expressed on both stages, and in two-cell pollen most of the genes are expressed during the pollen formation.

Penetration of different types of irradiation (UV, gamma, X-rays) varies in species, depending on the size and shape of the pollen grain, and the thickness of the pollen wall (Gilles and Prakash 1987). Low levels of normal pollen bring to low fertilization and abnormal seeds development.

Not only the fertility of pollen, but also the size of pollen is important indicator of environmental pollution. The changes in the size of pollen can be due to the distortions of growth and cell division in the formation of primary cells of archeospore and the tetrads of microspores. The variation in the cells size of plants grown in polluted area is larger than in control zone. For the viability of pollen is very important also its homogeneity because very large and very little pollen grains can decrease it.

MATERIALS AND METHODS

The Armenian Nuclear Power Plant (ANPP) industrial area is located in the western part of Ararat valley, near the settlement Metsamor, approximately 30 km NW of the capital, Yerevan. The ANPP location area represents a hilled plain between 900 and 1,000 m above the sea level. The climate is strictly continental, with strong variations of annual and daily temperature and 271 mm of annual atmospheric fallout. In the Ararat Valley the average temperature in July is +25···+27°C while in January +5···+7°C. Weak winds, up to 1 m sec^{-1}, are dominant.

The complex analysis of male reproductive system of vines and fruit-trees growing on distance of 3～5 km from the ANPP near the settlement Metsamor, in comparison with the control point on distance more than 30 km from ANPP is realized.

Comparative analysis of radionuclides content and specifically ^{137}Cs, in the zone around of ANPP and in control zone allows to define the possibility of estimation of mutagenic effects of radionuclides. Regular content measurements of instable isotopes were performed by the special NPP services during the whole period of ANPP operation, in different directions and at various distances from the plant. Most convenient for measurement and analysis is the ^{137}Cs, which is a source of easily detected gamma radiation and which is accumulated in the environment, having half-life period equal to 30 years and high activity for measurement at low concentrations.

The concentration of ^{137}Cs was estimated with the help of low background gamma-spectrometric analyzer with the GeLi semiconductor detector and supporting computer code. The error of measurement was equal to 5%. The soil samples were encoded for analysis.

The concentration of ^{137}Cs around of ANPP was 19,4 Bq/kg and in control point 12,0 Bq/kg (Aroutiounian *et al.* 2004).

The centre of ancient wine-making-Ararat valley-is still known as the main wine-making region with the majority of Armenian vineyards (60%). More than 200 sorts of grapes are grown in Armenia, the majority of them being native ones. Investigated 10 sorts of *Vitis vinifera* are Charentsi, Meghrabuyr, Burmunk, Shahumyani, Kishmish white, Ararati, Arakseni, Hadisi and from *Rosaceae Juss.* family investigated taxons are sorts of pear Malacha and Taparza (*Pyrus communis* L.), peach folk selection (*Persica vulgaris*), plum folk selection (*Prunus domestica* L.), cherry wild (*Cerasus vulgaris* Mill.), quince (*Cydonia vulgaris* Pers.). Biometric parameters of pollen are investigated by the method of the acetocarmine preparations analysis. In the fertile grains the cytoplasm is colored dark carmine-red. The sterile pollen graines are not stained or are stained non-uniformly. The data obtained on the basis of analysis of large quantities of pollen grains (about 10,000 in each variant). Sizes of 100 pollen grains for each variant were detected by ocular-micrometer.

RESULTS AND DISCUSSION

The results presented in Table 1 provide evidence for a

Table 1. Some parameters of pollen grains of some sorts of vines in different points of growth.

Sort of vine	Control point			Metsamor		
	Pollen fertility, %	Diameter, mkm	Volume, mkm^3	Pollen fertility, %	Diameter, mkm	Volume, mkm^3
Shahumyani	89.96±0.30	27.97±2.83	11451.33±3410,28	72.36±0.45*	28.75±2.94	12436.32±3806,36
Charentsi	95.16±0.21	26.02±2.29	9226.01±2557,63	95.36±0.22	25.05±2.92	8226.24±2852,0
Meghrabuyr	90.80±0.29	25.93±2.24	9145.01±2429,17	90.60±0.32	25.78±2.10	8962.41±2174,88
Burmunk	97.13±0.15	28.07±2.15	11488.10±2800,08	97.52±0.18	23.27±2.51	6596.28±2055,21
Kishmish white	87.70±0.33	26.91±1.45	106031.71±1683,56	84.90±0.36 *	27.83±2.18	11391.32±2546,33
Ararati	69.55±0.46	26.32±1.37	9541.93±1553,17	96.80±0.18 *	26.68±2.60	9871.92±2642,22
Arakseni	87.70±0.33	26.10±1.36	9326.03±1577,25	96.92±0.17*	26.38±1.40	9607.33±1585,20
Hadisi	97.03±0.17	26.68±2.24	9871.72±2484,64	94.17±0.24*	26.68±1.37	9871.96±1553,17

*Significantly different from control ($p<0.05$)

Table 2. Some parameters of pollen grains of some fruit trees in different points of growth.

Investigated taxon	Control point			Metsamor		
	Pollen fertility, %	Diameter, mkm	Volume, mkm^3	Pollen fertility, %	Diameter, mkm	Volume, mkm^3
Pear, sort Malacha	94.84±0.22	39.11±4.69	31307.03±10392,78	95,44± 0.21	39.14±4.99	31379.11±14351,92
Pear, sort Dzmernuk	86.90±0.34	36.31±4.01	25052.85±8172,06	79.71±0.40*	33.17±2.65	19563.1±4925,62
Peach, folk selection	95.61±0.29	54.52±4.52	84809.80±23791,81	88.69±0.32*	56.72±3.19	95456.80±16705,8
Plum, folk selection	87.60±0.33	41.09±5.28	44153.74±11679,96	96.93±0.17*	37.86±3.64	28471.10±8347,62
Cherry wild	74.67±0.44	45.47±5.97	49198.66±18528,07	68.38±0.47*	45.45±3.88	49133.77±12365,09
Quince	98.31±0.13	46.07±5.64	53389.19±18534,31	86.24±0.34 *	47.40±3.10	53703.31±11073,85

*Significantly different from control ($p<0.05$)

high level of pollen fertility in investigated sorts of vines. The highest level of pollen fertility discovered for sort Burmunk (in control 97,13±0,15%, in experiment 97,52± 0,18%). The data for sorts Charentsi, Meghrabuyr, Burmunk, Arakseni, Hadisi have shown the fertility level more than 87% in both points.

Sizes of pollen grains for investigated sorts of vines vary from 23,27±2,51 mkm for Burmunk sort to 28,75±2,94 mkm for Shahumyani sort. It is revealed high quality of the pollen population for all investigated sorts.

The results presented in Table 2 show a high level of pollen fertility in investigated fruit trees. The highest level of pollen fertility is defined for peach, plum and quince (more than 86% in both points). For pears the fertility level vary from 79,71±0,40% to 95,44±0,21%. The lowest level of pollen fertility was shown for cherry: from 68,38± 0,47% near the ANPP and 74,67±0,44% in control point.

Sizes of pollen grains from fruit trees had shown large inter-species variation, that was caused by genotype specificity of investigated fruit cultures. The largest pollen grains were detected for peach 54,52±4,52 mkm in control and 56,72±3,19 mkm in surroundings of ANPP and the smallest for different sorts of pears in area of ANPP 33,17 ±2,65 mkm and 39,14±4,99 mkm.

As a result, for two sorts of vines Ararati ($p<0,001$) and Arakseni ($p<0,001$) and one taxon of plum (folk selection) ($p<0,001$) is revealed the decrease of pollen sterility level in in the area of ANPP in comparison with control point. For two sorts of vines Shahumyani ($p<0,001$) and Kishmish white ($p<0,001$) and four taxons of fruit trees Pear, sort Dzmernuk ($p<0,001$), Peach, folk selection ($p<0,001$), Cherry wild ($p<0,001$) and Quince ($p<0,001$) had revealed the increase of pollen sterility in the area of ANPP. Three sorts of vines: Charentsi, Meghrabuyr, Burmunk and one sort of pear Malacha-all growing in the area of ANPP had shown no significant differences in the high pollen fertility compared to control point.

The obtained results demonstrate that there is no one-sided effect of ANPP on the development and formation of investigated male generative system of vines and fruit trees. Thus, on the base of obtained results we can't confirm its deleterious effect on the environment.

CONCLUSION

The obtained results demonstrate high fertility of pollen of investigated vines and fruit trees growing around the area of ANPP. The negative directional influence of the ANPP on the male generative system of the investigated sorts of vines and species of fruit trees has not revealed. For some investigated sorts is shown increase of sterility, for others decrease, for some of them no significant difference between plants from the area of ANPP and control point.

The further monitoring of pollen fertility is necessary at the different plants species, growing around the area of ANPP, for the definition of their suitability for more informative bioindication of action of environment factors.

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