Phytomonitoring of the Genotoxicity of Environmental Pollutants: An Application to Armenian Nuclear Power Plant

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Abstract - **Today the biosafety evaluation, a common problem of vital importance, is based on internationally proved test-systems, standards and techniques. The paradigm of biosafety includes multidisciplinary approach, a combination of physical, chemical and biological tests to monitor the environmental level of pollutants and needs to be improved by modern approaches. The genetic risk of environmental pollutions has long been studied by many researchers. In this study, used was the known sensitive plant test-system, clones of plant *Tradescantia* (spiderwort) able to detect gene mutations (frequency of mutational events and formation of micronuclei) in combination with chemical and, in some instances, with radiological measurements. In addition, male gametophyte generation of fruit trees was applied as bioindicators of genotoxicity. The obtained results did not show any significant increase along with wind direction. As for the male gametophyte assay, the fertility of the investigated fruit-trees near to NPP did not significantly differ from that of the control point. The influence of the NPP on the male generative system of the investigated taxa of fruit trees for the investigated year was not revealed. The system described needs to be expanded by species of interest (human) as there is a difficulty to transfer the revealed dose correlations to humans. The development of this idea includes various levels: population (epidemiological studies), individual, cellular, molecular (DNA), etc.**

Key words : Bioassay, Genotoxic effects, Monitoring, Environmental pollutants, NPP

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. A lot of biological systems have been applied to detect radioactive contamination of the environment, with some plants being the most sensitive assays to monitor genotoxic effects. Spiderworts (*Tradescantia* clones) have been broadly used as a biological indicator of air, water and soil mutagenity, as well as genotoxity of any chemical compounds and radionuclides released from NPPs (Aroutiounian *et al*. 1999; Shin *et al*. 1999; Kim *et al*. 2003; Kim *et al*. 2009; Panek *et al*. 2011). The stamen-hair systems of some *Tradescantia* clones, being heterozygous in their flower color (blue dominant, pink recessive), are shown to possess the highest sensitivity towards the induced mutations, even at low radiation levels. This system is consistent, accurate and reliable, inexpensive and requires a short training time (Ichikawa 1992). The *Tradescantia* Micronuclei (Trad-MCN) test, developed by Te-Hsiu Ma, reveals directly the magnitude of chromosome damage in the germ cells

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(Ma 1979, 1983, 1999; Kong and Ma 1999). Tests with *Tradescantia* require short terms, while for other plant indicators the same tests would have required several months, or even years. Mutagenic activity of soils from areas near the settlements along the main wind direction from the nuclear power plant was studied by means of the *Tradescantia* tests.

Male gametophyte generation of fruit trees can also be applied as bioindicators of genotoxicity. 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 species to estimate genotoxic potential in the examined locality or region (Micieta and Murin 1995). The selection on haploid level, and especially in masculine gametophyte generation, with the changes of haplo-diploid gene expression, can in short period induce changes in the structure of populations, becoming important factor of angiosperms evolution Such selection is more effective, than the selection in sporophyte generation, because in haploid phase the negative mutations are much more eliminated (Lyakh 1995).

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 NPP location area represents a hilled plain between 900 and 1000 m above the sea level. The climate is strictly continental, with strong variations of annual and daily temperature and 271 mm of annual atmospheric fallouts. 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 mentioned approach, allowing one to estimate the genotoxic action of environmental factors, is the test for the definition of plants pollen grains fertility. 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 (Bondar and Chastokolenko 1990). Pollen fertility for any kinds of plants including fruit trees, allows to estimate gametocide effects of environmental mutagens. Thus it is reasonable to use those kinds of plants that live in the investigated environment, including pollution components, sporophyte and gametophyte cycles of development. Pollen grains can be killed, fixed, and stored in ethanol for long periods of time; this facilitates the use of pollen traits, for studies of mutagenesis (Constantin 1982).

MATERIALS AND METHODS

Soil samples were collected at four sites around ANPP along the predominant wind direction; Metsamor, Aghavnatun, Armavir and Oshakan (Fig. 1). Soil from a green house of the Yerevan State University served as a control sample.

As object of genetic monitoring *Tradescantia* clone 02 was applied, which is highly sensitive indicator widely used in experiments on mutagenesis (Ichikawa 1981, 1992; Arutyunyan *et al*. 1999). It is one of the most stable clones in terms of spontaneous somatic mutation frequency in the stamen hairs (Ichikawa 1992; Kim *et al*. 2003). Frequency of pink mutation events (PME), genetic-uncertain (colorless) mutation events (CME), in the test of *Tradescantia* stamen hairs (Trad-SH) and the formation of micronuclei in tetrads (Trad-MN) (revealing the level of non-disjunction or chromosomes damage) were defined.

Reproductive traits of male gametophyte of some fruit trees from *Rosaceae Juss*. family were analysed: cultivars Malacha and Dzmernuk of the pear (*Pyrus communis* L.), peach folk selection (*Persica vulgaris*), plum folk selection (*Prunus domestica* L.), quince (*Cydonia vulgaris* Pers.), cherry (*Cerasus vulgaris* Mill.) wild and Shpanka cultivar.

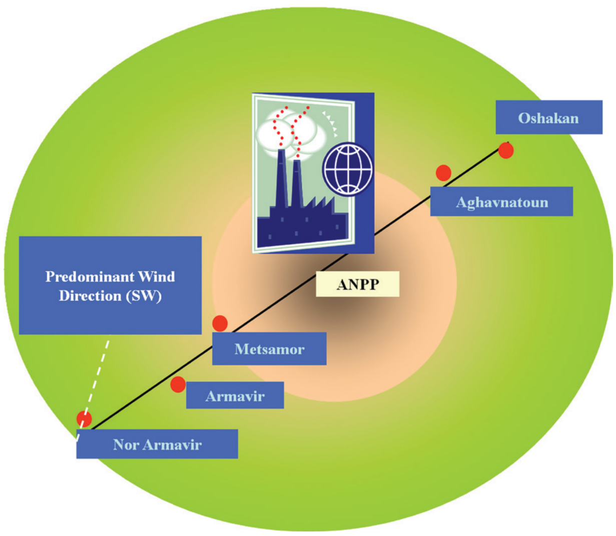

Fig. 1. Soil sampling points for genotoxicity analyses around the ANPP.

Investigated plants are cultivated on distances of 3~5 km from the ANPP - near to settlement Metsamor at their comparison with the control point on the distance more than 30 km from ANPP. Aceto-carmine staining was used for estimation of pollen sterility and morphometry. To determine pollen fertility, darkly stained pollen grains were recorded as fertile and viable, and unstained or very lightly stained ones were considered as sterile or non-viable. Estimation of pollen sterility was realized on the basis of analysis of 10000 pollen grains for each sample. Sizes of 100 pollen grains for each variant were detected.

Comparative analysis of radionuclids content and specifically ^{137}Cs, in the zone around of Armenian NPP and in control zone allows one to define the possibility of estimation of mutagenic effects of radionuclids. Regular content measurement of instable isotopes was 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. ^{137}Cs has mostly technogenic origin: nuclear tests and emergencies-Chernobyl disaster, NPP releases, etc. 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.

RESULTS

The obtained results demonstrate, that depending from soil samples the frequency of PME was 1.7 to 3.2 times higher, than the control level. The high level of PME was marked in the soils of Aghavnatun, 1.92±0.33 while the control sample demonstrated 0.41±016. The lowest level of PME was detected in the soils of Oshakan (0.92±0.23).

The frequency of CME was 1.7 to 3.2 times higher than the control level. The high level of CME was marked in the soils of Oshakan, 12.56±0.85 (3.93±0.48 in the control). The lowest level of CME was detected in the soils of Armavir (6.64±0.62).

Analogical relations were detected also for the application of Trad-MN test. The frequency of tetrads with micronuclei exceeds the control level 1.5~3.3 times. The highest mutagenic activity was also detected in the soils of Oshakan, 32.0±0.85 (9.8±0.54 in the control). The lowest level of tetrads with micronuclei was detected in the soils of Armavir (14.72±0.65).

The frequency of micronuclei formation in tetrads exceeded the control level 1.6~4.6 time. The highest mutagenic activity was also detected in the soils of Oshakan, 61.0±0.89 (13.4±0.62 in the control). The lowest level of CME was detected in the soils of Armavir (21.8±0.75) (Fig. 2).

It is demonstrated significant correlation of CME, Tetrads with MN and MN in tetrads parameters with the estimation of ^{137}Cs content in the soil (Table 1).

For the investigated period the concentration of ^{137}Cs around Armenian NPP was in the range between 19.4 Bq kg^{-1} and 65.2 Bq kg^{-1} and 12.0 Bq kg^{-1} in control point (Fig. 2).

The data of analysis of pollen fertility and morphometric traits of fruit trees demonstrated no significant difference in

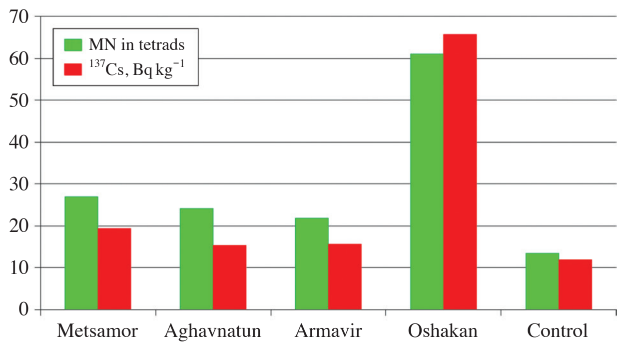

Fig. 2. The ratio between % of MN in tetrads of *Tradescantia* and ^{137}Cs content (Bq kg^{-1}) in the soil samples from different points (Aroutiounian *et al*. 2004).

Table 1. Correlation of the Trad-SH and Trad-MN test results with the estimation of ^{137}Cs content in the soil

Variant	PME, 1000	CME, 1000	Tetrads with MN, %	MN in tetrads, %
Pearson correlation coefficient (r) with levels of ^{137}Cs in the soil	0.200	0.887*	0.981**	0.984**
p (2-lateral)	0.747	0.045	0.003	0.002

*Significantly different from control ($p<0.05$), **Significantly different from control ($p<0.01$)

the high pollen fertility and non-changed size of pollen in all investigated taxa compared to those of the control point (Table 2, Table 3).

DISCUSSION

On the basis of obtained results is established high fertility at the investigated fruit-trees near to settlement Metsamor that significantly does not differ from the control point. The influence of the ANPP on the male generative system of the investigated taxons of fruit trees for the investigated year is not revealed.

When accidents occur at the nuclear facilities like Fukushima Daiichi NPP, the release of radioactivity to the environment includes radionuclides such as ^{131}I ($t_{1/2}$ = 8.0 d), ^{134}Cs ($t_{1/2}$ = 2.1 yr) and ^{137}Cs ($t_{1/2}$ = 30.1 yr) which pose potential risks to human and ecosystem health (Kanda 2013). A trace amount of ^{137}Cs exists in the environment as it originated from the fallout of nuclear weapon test in the mid-1960s. In that regard, ^{134}Cs data, if available, can give rise to reality of radioactive contamination arisen from the nuclear facilities. Radioactive materials leaked from the nuclear facilities can be transported into the environment along three pathways: via atmospheric circulation; via ocean currents; and via biological pathways. Phytomonitoring is one way of analyzing the biological pathways as it uses genetic and/or morphological changes in reproductive cells which can be induced by the environmental pollutions. The result of this study did not deal with ^{134}Cs data, which could be shortcoming of the present study, the study to find relationship between biological indications as assessed by the Trad-MN assay or pollen grain sterility and environmental pollutions, particularly in terms of ^{137}Cs concentration, can be of practical importance in monitoring around nuclear facilities NPP.

By Kordyum and Sidorenko (1997) the low percentage (0.5~2.0%) of anomalies of meiosis in microsporogenesis, sporades and pollen grains as well as mitosis in root meristematic cells has been revealed in the majority of 94 species of angiosperms (from 28 families) growing in different ecological conditions in the zone with higher radionuclide pollution after the Chernobyl NPP accident. Only in certain species (8~10%) the correlation between an increase of the number of anomalies and an increase of the radiation level was shown. In that research were mostly not presented the species specific for *Transcaucasia*, and especially fruit trees of local species (Fig. 3).

Table 2. Properties of pollen fertility of fruit trees in different points of growth

Investigated sample	Pollen fertility, % ± SE	
	Control point	Metsamor
Pear, Malacha cultivar	94.84 ± 0.22	95.44 ± 0.21
Pear, Dzmernuk cultivar	86.90 ± 0.34	79.71 ± 0.40
Peach	95.61 ± 0.29	88.69 ± 0.32
Plum	87.60 ± 0.33	96.93 ± 0.17
Cherry wild	74.67 ± 0.44	68.38 ± 0.47
Cherry, Shpanka cultivar	82.68 ± 0.38	84.40 ± 0.36
Quince	98.31 ± 0.13	86.24 ± 0.34

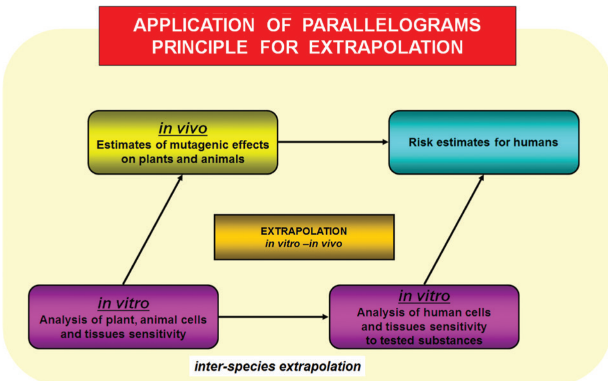

Fig. 3. Application of parallelograms for extrapolation.

Table 3. Properties of pollen size of fruit trees in different points of growth

Investigated sample	Control point		Metsamor	
	Diameter, μm ± SD	Volume, μm^3 ± SD	Diameter, μm ± SD	Volume, μm^3 ± SD
Pear, Malacha cultivar	39.11 ± 4.69	31307.03 ± 10392.78	39.14 ± 4.99	31379.11 ± 14351.92
Pear, Dzmernuk cultivar	36.31 ± 4.01	25052.85 ± 8172.06	33.17 ± 2.65	23102.10 ± 4925.62
Peach	54.52 ± 4.52	84809.80 ± 23791.81	56.72 ± 3.19	95456.80 ± 16705.80
Plum	41.09 ± 5.28	44153.74 ± 11679.96	37.86 ± 3.64	28471.10 ± 8347.62
Cherry wild	45.47 ± 5.97	49198.66 ± 18528.07	45.45 ± 3.88	49133.77 ± 12365.09
Cherry, Shpanka cultivar	42.98 ± 4.86	41550.63 ± 9831.23	49.79 ± 3.91	43944.30 ± 86134.21
Quince	46.07 ± 5.64	53389.19 ± 18534.31	47.40 ± 3.10	53703.31 ± 11073.85

CONCLUSION

The obtained results did not show any significant increase along with wind direction. As for the male gametophyte assay, the fertility of the investigated fruit-trees near to NPP did not significantly differ from that of the control point. The system described needs to be expanded by species of interest (human) as there is a difficulty to transfer the revealed dose correlations to humans.

The further monitoring of pollen fertility is necessary at the different plants species, growing around the nuclear facilities, for the definition of their suitability for bioindication of action of environment factors. The formation of gametophyte with the low level of sterility can be important argument for the formation of system of bioindication.

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