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IMPACT OF CITY POLLUTION ON MICROSPOROGENESIS OF *Chelidonium majus* L. PLANTS

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Abstract

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The study was carried out at the area of the capital of the Slovak Republic – Bratislava. To study influence of city pollution on microsporogenesis of wild plant species *Chelidonium majus* L. was used. During two years (1999–2000) numbers of abortive microspores were evaluated. The criterion for the selection of seven monitored sites was the different level of pollution. The results of the study indicated different level of impact of pollution in monitored sites.

Key words: pollen grains, *Chelidonium majus* L., genotoxicity, pollution, in situ monitoring

Introduction

In many sprawling conurbation quality of environment is deteriorated impaired by rapidly growing industry and emission from motor vehicles. Plants provide the best means for in situ determination of the effects of environmental chemical under complex actual living condition (Cebulska-Wasilewska, Plewa, 2003). Various plant species and methods have been proposed and used for biomonitoring of air pollutants (e.g. Mičieta, Murín, 1996; Kordyum, Sidorenko 1997; Cańas et al., 1997; Paradiž, Lovka, 1999; Kammerbauer, Dick, 2000; Isidori et al., 2003). Reproduction processes, meiosis and mainly the process of microsporogenesis, are very sensitive to stress and therefore, they are frequently used to detect genotoxicity of environment. The aim of the presented study was to evaluate genotoxic impact of city pollution on microsporogenesis of wild plants in in situ condition. The study was carried out during two years at the Bratislava city. The frequency of abortive and anomal pollen grains of *Chelidonium majus* L. was evaluated according to Mičieta, Murín (1997).

Material and methods

Seven sites in Bratislava were monitored (Fig. 1):

Site 1: station of city traffic "Chatam Sófer", "Žižkova ul.", very busy car and bus traffic predominantly contaminated by vehicle exhaust emissions

Site 2: at the entrance to area "Železná studnička" part of Bratislava forest park, about 150 m from the train station "Červený most" – site essentially free of direct motor vehicle exhaust emission

Site 3: behind the station of city traffic "PKO", "Žižkova ul." street, very busy car and bus traffic – predominantly contaminated by vehicle exhaust emissions

Site 4: 200 m from the industrial plant "Technické sklo", near the Roman ruins "Villa rustica" – predominantly contaminated by near pollution from technical glass plant

Site 5: city incinerator – "Spaľovňa komunálneho odpadu", Podunajské Biskupice – predominantly contaminated by close petrochemical plant and combustion products

Site 6: at the entrance to "Líščie údolie" street, Karlova Ves – control site essentially free of direct motor vehicle exhaust emission, no industry plant in vicinity, border of close protected lanscape area "Malé Karpaty Mts"

Site 7: at the end of street "Údolná ul.", near forsaken garden – site essentially free of direct motor vehicle exhaust emission, part of the old town in the center of the city.

The frequency of abortive pollen grains was evaluated according to methods by Mičieta, Murín (1997). As a model species *Chelidonium majus* L. was used. *Ch. majus* have blooming period for about five months and fulfill all the criteria for using wild plant species for this kind of monitoring (see Mičieta, Murín, 1996, 1997). During the period of 1999–2000, each two weeks from the second half of May to end of the September samples were collected. They were fixed in mixture of ethanol and acetic acid (3:1), which was after 24 h substituted, by 75% ethanol. Pollen grains were stained with 0.05% aniline blue in lactophenol. They were evaluated for size, form, and staining ability with deviation considered as evidence for lack of viability – abortion. As anomal pollen grains were evaluated grains with cytoplasmatic content at least more than 1.5 times larger than normal (see Fig. 2). For one sample minimum 9000 pollen grains were counted. As control population site 6 was chosen. Most of the obtained data have not parametric distribution therefore non-parametric Wilcoxon test was used.

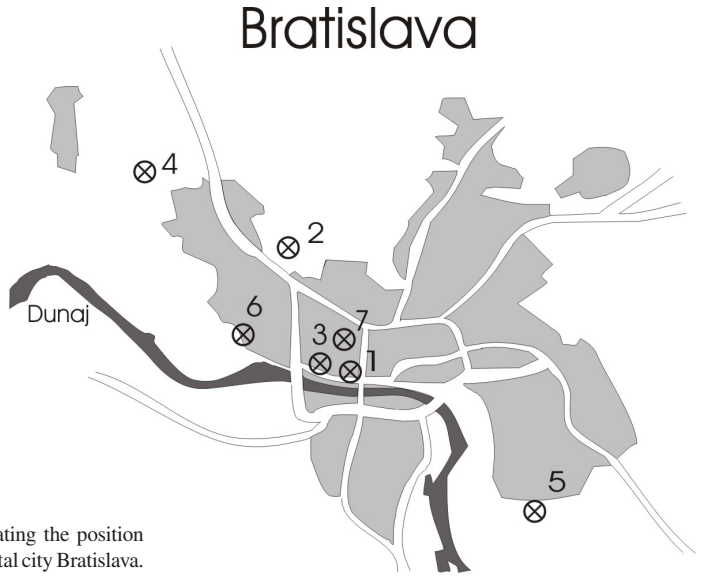

Fig. 1. A schematic map illustrating the position of monitoring sites in area of capital city Bratislava.

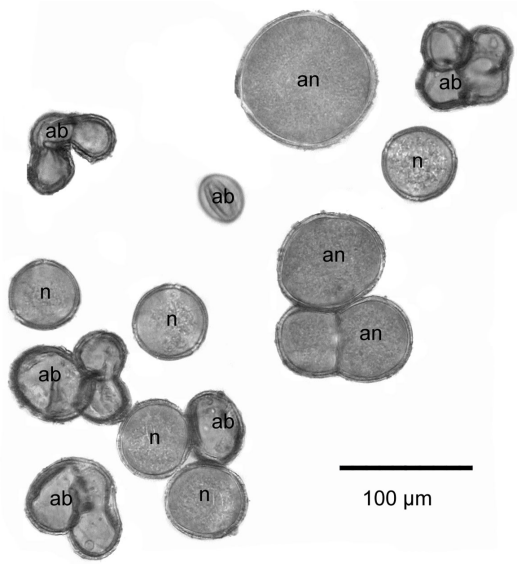

Fig. 2. Example of different kinds of evaluated pollen grains. ab – abortive pollen grains, an – annomal pollen grains, n – normal pollen grains.

Results

The results of the study are presented in Table 1–4.

Together with frequency of abortive pollen grains, the frequency of anomal pollen grains was monitored (Table 3–4). The Pearson correlation coefficient between anomal and abortive pollen grain on samples from the site 7 was 0.75 ($p = 0.051$). It was impossible to compare frequencies of abortive and anomal pollen grains from other sites because of insufficient data. As well the results from site 2 (Železná studnička), where the recorded frequency of abortive pollen grain is high, are unexpected. On the other hand frequencies of the abortive pollen grains were relatively low in the site 5 (City incinerator) at this site there were high differences between samples recorded. The locality with the highest mean year frequency of abortive pollen grains in 1999 was site 1 (6.1%) even through, in the next year same level frequency was recorded at this site (5.8%). Other localities like site 7 (9.88%) and site 2 (8.4%) had higher frequencies in that year. The reason for it could be partly in different climatic condition during the year, high drought in the summer 2000. This emphasizes importance of using suitable controls collected each time during the monitoring.

T a b l e 1. Mean of abortive pollen grain frequency to 100 pollen grains on the monitoring site in 1999

Date	Monitoring site (mean ± S.D.)						
	1	2	3	4	5	6	7
3.5.1999	3.7 ± 0.9*	1.8 ± 0.7*	3 ± 0.2*	4.5 ± 0.8*	2.6 ± 0.1*	1.5 ± 0.1	3.5 ± 0.6*
18.5.1999	3.4 ± 0.9*	6.4 ± 1.7*	1.4 ± 0.1	6.0 ± 3.6*	2.7 ± 0.2*	1.3 ± 0.1	3.8 ± 0.8*
2.6.1999	4.5 ± 0.9*	4.2 ± 1.6*	1.6 ± 0.1	4.4 ± 0.1*	5.6 ± 1.1*	2.6 ± 0.2	
14.6.1999	12.2 ± 2.5*	2.9 ± 0.3	2.1 ± 0.4		3.1 ± 0.1	2.7 ± 0.2	
7.7.1999	4.3 ± 1.2*	9.2 ± 5.0*	4.1 ± 2.0*	4.1 ± 0.4*	2.9 ± 0.1*	1.6 ± 0.5	3.6 ± 2.3*
14.7.1999	5.3 ± 1.6*	2.2 ± 0.4	4.8 ± 0.8*	2.9 ± 0.8	2.3 ± 0.3	2.4 ± 0.1	5.8 ± 2.5*
29.7.1999	4.0 ± 0.4		3.1 ± 0.5	3.8 ± 0.6	2 ± 0.3	3.5 ± 0.4	3.8 ± 1.4
11.8.1999	3.7 ± 0.3	7.8 ± 0.7	2.1 ± 0.2	3.6 ± 0.6	2.5 ± 0.3		
28.8.1999	9.0 ± 3.9*	4.2 ± 1.2*	6.0 ± 0.9*	4.7 ± 0.5*	2.9 ± 0.8*	1.5 ± 0.2	1.8 ± 0.2*
7.9.1999			2.5 ± 0.4*		2.5 ± 0.4*	1.9 ± 0.2	
22.9.1999	11.3 ± 2.6				4.3 ± 1.0		

Control – the data from the site 6 were taken as control
mean ± S.D., mean of abortive pollen grain frequency to 100 grains ± standard deviation
* – significant difference from the control ($p \leq 0.05$)

T a b l e 2. Mean of abortive pollen grain frequency to 100 pollen grains on the monitoring site in 2000

Date	Monitoring site (mean ± S.D.)						
	1	2	3	4	5	6	7
12.5.2000		7.2 ± 1.3			1.9 ± 0.3		
23.5.2000	5.4 ± 0.6*	3.2 ± 0.4		8.7 ± 5.9*	1.5 ± 0.2	4.2 ± 0.1	4.0 ± 0.5
2.6.2000							4.4 ± 0.3
14.6.2000				5.5 ± 1.1	2.8 ± 0.2		
4.7.2000			4.7 ± 0.4*			3.8 ± 0.1	25.0 ± 3.5*
14.7.2000		10.8 ± 4*	2.3 ± 0.3	11.0 ± 4.1*	6.0 ± 6.2*	4.1 ± 0.3	9.8 ± 2.7*
1.8.2000	5.3 ± 0.2*	12.6 ± 4.6*	5.7 ± 2.5*	12.8 ± 3.3*	2.1 ± 0.3	4.0 ± 0.2	8.0 ± 3.8*
14.8.2000	7.4 ± 5.5*	12.6 ± 5.4*	3.0 ± 0.1	5.1 ± 1.2*		3.3 ± 0.3	4.9 ± 0.5
28.8.2000				8.2 ± 1.0*	7.1 ± 6.5*	3.0 ± 0.1	22.6 ± 2.5*
12.9.2000			3.4 ± 0.4*		2.4 ± 0.3	2.7 ± 0.2	5.9 ± 0.6*
1.10.2000	5.2 ± 0.5	4.0 ± 0.4	4.5 ± 0.4	5.0 ± 0.8			4.4 ± 0.2

Control – the data from the site 6 were taken as control
mean ± S.D., mean of abortive pollen grain frequency to 100 grains ± standard deviation
*– significant difference from the control ($p \leq 0.05$)

Table 3. Mean of anomal pollen grain frequency to 100 pollen grains on monitoring site in 1999

Date	Monitoring site (mean ± S.D.)						
	1	2	3	4	5	6	7
18.5.1999	–	–	–	–	–	–	–
2.6.1999	–	–	–	–	–	–	
14.6.1999	–	–	–		–	–	
7.7.1999	–	–	–	–	–	–	–
14.7.1999	–	–	–	–	–	–	2.21 ± 3.04
29.7.1999	–		0.03 ± 0.03	–	–	–	0.36 ± 0.54
11.8.1999	–	–	–	–	–		
28.8.1999	–	–	–	–	–	–	–
7.9.1999			–		–	–	
22.9.1999	–				–		

– no anomal pollen grains in sample were detected
mean ± S.D., mean of abortive pollen grain frequency to 100 grains ± standard deviation

Table 4. Mean of anomal pollen grain frequency to 100 pollen grains on monitoring site in 2000

Date	Monitoring site (mean ± S.D.)						
	1	2	3	4	5	6	7
12.5.2000		0.01 ± 0.04			–		
23.5.2000	0.03 ± 0.18	–		–	–	–	0.59 ± 0.83
2.6.2000							0.17 ± 0.27
14.6.2000				–	–		
4.7.2000			–			–	–
14.7.2000		0.03 ± 0.05	–	–	–	–	5.95 ± 2.48
1.8.2000	–	–	–	0.01 ± 0.02	–	–	1.08 ± 1.4
14.8.2000	–	–	0.01 ± 0.03				0.6 ± 1.64
28.8.2000				–	–	–	8.3 ± 0.15
12.9.2000			–		–	–	0.01 ± 0.04
1.10.2000	–	–	–	–			–

– no anomal pollen grains in sample were detected
mean ± S.D., mean of abortive pollen grain frequency to 100 grains ± standard deviation

Discussion

Murín (1987) as the first made some important conclusions about the use of microsporogenesis of wild plants in the detection of genotoxicity, in this work other authors e.g. Mičieta, Murín (1996, 1997) have continued.

From the obtained data we can see that sensitivity of microsporogenesis is very high, which is manifested in variability at the data obtained from the same site (Table 1, 2). It is possible that the influence of in situ conditions may be higher than we have expected, this emphasizes importance of sufficient number of samples taken during the monitored period. However, this variability could therewithal indicate that monitored populations are under influence of some probably genotoxic factors. Higher level of the genome mutation frequency manifests itself in higher frequency of the anomal pollen grains at some sites (Table 3, 4). The high percentage of anomal pollen grains in samples from the site 7, could be connected with illegal dump of garbage, which appeared there in 2000. We suppose that anomal pollen grains are probably rising as the result of mutation on genome level, by damage or disruption of spindle mechanisms and cytoskelet. They would be regarded as unreduced diploid, triplod or tetraploid pollen grains. The same kind of pollen described Mičieta, Murín (1998) on *Pinus nigra* A r n. This assumption partly approves high correlation coefficient (k = 0.75) between the frequency of anomal and abortive pollen grains. Abortivity of the pollen grains as the result of mutation on gene level could be in relation with the anomality of the pollen grains that rises from mutation on genom level during meiosis and tetradogenesis. However in present time we have not any evidence for this suggestion. We also could not exclude particular contribution of worst climatic condition, mainly drought in season 2000, on the other hand this have had probably synergic effects with pollution, which leads to higher frequency of abortive and anomal pollen grains. Mortality of pollen grains as errors of meiosis are also studied recently in *Typha latifolia* L. (Berdnikov et al., 2002) but only in dependence to mutation frequency. Jackson et al. (2000) described pollen wall mutation on *Haplopappus gracilis* (N u t t.) G r a y. They suppose, "that some organizing factor(s) during pollen walls formation were disrupted by the mutation interfering with proper assembly of the ektexine" (Jackson et al., 2000), but they recorded only mutation of pollen wall, there were no markedly enlarged pollen grains.

Conclusion

Bio monitoring in situ will not and cannot substitute for physicochemical air monitoring, but it will allow the adverse effects of pollution on the environment to be detected and evaluated. Evaluation of impact of all environmental factors in in situ monitoring is principal advantage of field experiments. On the example of *Chelidonium majus* impact of city pollution on microsporogenesis was presented. The advantage of this bioassay is that it is low in cost and easy to apply. It is expected, that results will provide some basic informa-

tion that will be used for designing this bio monitoring systems. The results show a different level of pollution on monitored sites and high sensitivity of microsporogenesis.

Translated by the authors

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Mišík M., Mičieta K.: **Vplyv mestského znečistenia na mikrosporogenézu** *Chelidonium majus* L.

Na príklade druhu *Chelidonium majus* L. sme sledovali vplyv znečistenia a environmentálnych faktorov na mikrosporogenézu. V priebehu pokusu v rokoch 1999–2000 sme hodnotili frekvenciu abortívnych peľových zŕn. Výsledky poukazujú na rozličný stupeň záťaže jednotlivých monitorovaných lokalít a na vysokú citlivosť mikrosporogenézy na vplyv vonkajších podmienok.