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Impact of air pollution on the mitotic activity in meristematic cells in shallot (*Allium cepa* L. var. *ascalonicum*)

Vpliv onesnaženega zraka na mitotsko aktivnost v meristemskih celicah šalotke (*Allium cepa* L. var. *ascalonicum*)

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Abstract. Test shallot plants *Allium cepa* L. var. *ascalonicum* were exposed to field conditions in research plots in the most polluted areas in Slovenia in the vegetation season in 1999. The intention of this research was to evaluate the influence of air pollution on mitotic activity in meristematic tissues of root tips of bioindication plants. At each sampling site the mitotic activity was determined under field conditions and in a pot experiment. The maturated bulbs were collected from the field and after winter dormancies cytogenetic analyses were run on them in a lab. Root tips were fixed in Clark´s fixative and afterwards stained with Schiff´s reagent.

Significant differences in the mitotic activity in different sampling plots in pot experiments were found. The results showed the presence of cytotoxic substances at chosen sampling sites which caused the decrease of mitotic cell division.

Key words: *Allium cepa* L. var. *ascalonicum*, cytotoxicity, mitotic index, environmental pollution.

Izvleček. Izbrane testne rastline šalotke (*Allium cepa* L. var. *ascalonicum*) so bile v času vegetacijske dobe izpostavljene naravnim razmeram na vzorčna mesta v bližini virov onesnaženja na ozemlju Slovenije. Namen raziskave je bil oceniti vpliv onesnaženega zraka na mitotsko aktivnost v meristemskih celicah koreninskih vršičkov bioindikatorskih rastlin. Vpliv onesnaženega okolja smo analizirali pri rastlinah izpostavljenih v lončnem in poljskem poskusu.

Posušene in očiščene čebulice so bile po zimski dormanci izpostavljene v laboratoriju, koreninski vršički so bili fiksirani v Clarkoven fiksativu in obarvani s Schiffovim reagentom.

Ugotovljeno je bilo, da se pojavljajo statistično značilne razlike med posameznimi lokacijami pri rastlinah izpostavljenih v lončnem poskusu. Rezultati kažejo, da so na izbranih lokacijah citotoksične substance, ki vplivajo na mitotsko aktivnost celic izpostavljenih rastlin.

Ključne besede: *Allium cepa* L. var. *ascalonicum*, citotoksičnost, mitotski indeks, onesnaženost okolja.

Introduction

Besides the analysis of chromosomal aberrations to evaluate genotoxicity a mitotic index is most frequently used as a parameter to evaluate cytotoxicity to show effect of chosen substances in cytogenetic bioindication. Mitotic activity decreases while the influence of environmental stress is stronger (Fiskesjö 1994, Paradiž 1996, 1998, Paradiž & al. 1996, Smaka-Kincl & al. 1996, Bavcon & al. 1999, Freiszmuth & al. 2000, Müller & al. 2000, Pavlica & al. 2000, Paradiž & Druškovič, 2001). In the *Alliaceae* family shallot is not commonly used for bioindication (Pavlica & al. 1997, 2000, Zoldoš & al. 1997).

Test shallot plants (*Allium cepa* L. var. *ascalonicum*) were exposed to field conditions at research plots of differently polluted areas of Slovenia in 1999: in the Šalek Valley the most polluted sites are either those which are very close to the thermal power plant (Veliki Vrh, ash dump of the Šoštanj Thermal Power Plant) or those which are highly polluted due to climatic conditions and topography but more distant from the thermal power plant (Zavodnje) or sites with relatively clean air in relation to the wind direction from the thermal power plant (Arnače, Škale); in the Zasavje region (Kovk) and in the Upper Meža Valley (Žerjav) two very polluted sites were taken for one vegetation period. At each sampling plot plants were growing under field conditions and pot experiment (see *Material and methods* for a short description of growing conditions). Mitotic activity was later determined in a laboratory.

In this study we attempted to determine the influence of air pollutants on plants (pot) and indirect, accumulated effects in soil exposed to environmental pollution on the cytogenetic material of the shallot. The aims of the study were to make a comparison among the sampling plots regarding the mitotic activity to define cytotoxic effect arising from environmental pollution and to confirm the applicability of chosen method for relatively fast, simple and cost-effective determination of cytotoxicity of polluted air. In comparison with the pots experiments (where plants were exposed primarily to air pollution) we expected decrease of mitotic activity in plants in field conditions, since these shallots were exposed to stress due to air and soil pollution.

Materials and methods

For every sampling site we used approximately equal-sized shallot bulbs (*Allium cepa* L. var *ascalonicum* Escalote de Jersey', 2n=16).

The choice of sampling sites and sampling procedure was carried out as described by Glasenčnik & al. (2002) and the method for cytogenetic analyses was performed as described by Fiskesjö (1994) and detailed by Glasenčnik & al. (2002).

In the pot experiment plants were exposed in 15-litre self-watering pots (five per site, three bulbs per pot) and for experiment under field conditions plants were put directly in field soil (six bulbs per site) near the pots in the end of May 1999. After maturation all plant material was removed from sampling plots and bulbs were stored under dry conditions (4°C) for two months.

The soil from pots and field soil were analysed. The analysis of soils was carried out after homogenisation (ISO 11464) and digestionation (ISO 11466) using the methods for spectroscopy (ICP-MS, ET-AAS, CV-AAS).

Cytogenetic analyses were run after winter dormancies. Root tips were treated with using Feulgen "squash" technic. A mitotic activity was determined in five bulbs per sampling site, one slide per bulb and 2000 cells per slide. A mitotic index (MI) is a percentage of dividing cells scored.

All statistical treatments were performed using the Statistica for Windows 5.5 software package (STATSOFT 1999). Differences among sampling sites were tested using Kruskal-Wallis ANOVA. Cross comparisons between means were performed using the Mann-Whitney U test.

Results

Significant differences were found in MI among the sampling sites for shallots grown in pots (Kruskal-Wallis ANOVA: $H_{(6, 47)} = 19{,}49$, $p = 0{,}003$; *Fig. 1b*), while for plants grown in field soils no significant differences could be found ($H_{(5, 41)} = 7{,}68$, $p = 0{,}17$; *Fig. 1a*). Detailed statistics of pair-wise comparisons using Mann-Whitney U test are presented in *Table 1*.

The analysed plants material in pot experiment showed that the MI was between 3 and 14 %. The lowest value of MI was determined in Arnače near Velenje (3,7±2,5 %), a very low value was also at Veliki Vrh and at Kovk (6,1±2,6 % and 6,1±1,6 %), the highest was at Žerjav (10,93±3,5 %).

The results of shallot grown under field conditions showed that the value of MI was between 6 and 10 %, the lowest was determined at Veliki Vrh (6,4±3,7 %), the highest at the sampling site of Žerjav (10,93±3,5 %).

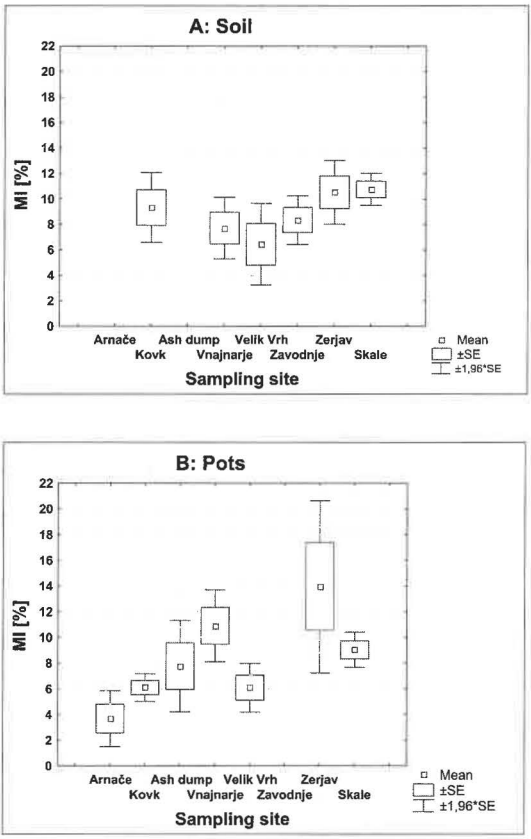

Figure 1a, b: Mitotic activity (%) in root tip cells of shallot, exposed to field conditions in soils (A) and in pots (B) at eight different localities in Slovenia in the vegetation season in 1999.

Table 1: Significance of differences in mitotic activity between sampling sites (Mann-Whitney U-test).

		Soil							
	1999 **MI**	ARNAČE	KOVK	ASH DUMP	ŠKALE	VNAJNARJE	VELIKI VRH	ZAVODNJE	ŽERJAV
Pots	ARNAČE		/	/	/	/	/	/	/
	KOVK	*		/	NS	NS	NS	NS	NS
	ASH DUMP	NS	NS		/	/	/	/	/
	ŠKALE	**	**	NS		NS	*	NS	NS
	VNAJNARJE	*	*	NS	NS		NS	/	NS
	VELIKI VRH	NS	NS	NS	*	*		NS	NS
	ZAVODNJE	/	/	/	/	/	/		NS
	ŽERJAV	*	/	NS	NS	NS	NS	*	

***: p<0.001; **: p<0.01, *: p<0.05; NS: not significant.

Discussion

The inhibition of mitotic activity was often used for tracing cytotoxic substances. Cytotoxicity was defined as a decrease in the mitotic index and as an increase in the frequencies of chromosomal aberrations (Fiskesjö 1994). The analysis of chromosomal aberrations for evaluation of genotoxicity was already done (for results see Glasenčnik & al. 2002). In our study a decrease in the mitotic index of shallot root meristems was found depending on the chosen location in pot experiment with probability p<0,01. We used the same soil for all pots and therefore plants grown in pots were exposed only to air pollutants. Analysis of chromosomal aberrations showed no significant differences, but the frequency of chromosomal aberrations was at least two-fold higher in comparison to published data for a control environment (3%, e.g. Vidakovič & al. 1993). Therefore air pollution had a strong influence on mitotic activity and also on the frequency of chromosomal aberrations. These results are in accordance with the results of fumigation experiments of a research group in Graz: ozone treatments consistently increased the rate of chromosomal aberrations, the MI remained unchanged, with fumigation with SO_2 and H_2S the MI was lower and the chromosomal aberrations were also consistently increasing (Müller & al. 2000).

Arnače sampling plot showed, against expectation, a very small mitotic activity, though the finding was in accordance with the recent results from other research fields (Svetina 1999, Kugonič & Stropnik 2001), which revealed elevated levels of Co, Cr and Ni in meadow soil and higher levels of heavy metals in vegetables and forage were found in comparison with other sampling sites in the bottom of the Šalek Valley. The highest frequency of chromosomal aberrations (for details see Glasenčnik & al. 2002) in our pot experiment confirmed the presence of stress inducing factor at this sampling site.

Since the cleaning device was built on the Šoštanj Power Plant in 1995, the emissions have been drastically reduced, which has reflected in an improvement of ecosystem conditions (Ribarič-Lasnik & al. 1999b). This is not a case at some sites, which are still polluted due to their position or due to accumulation of pollutants in the past. At the sampling site of Veliki Vrh just facing power plant chimneys and the prevailing wind direction a high concentration of air pollutants is directly brought from chimneys to this highly exposed site. This was confirmed by several studies (e.g. high levels of Cd in soil and vegetables Kugonič & Stropnik 2001, high levels of Cd, As and Hg in fungi Al Sayegh-Petkovšek & al. 2002, the most acid precipitation (pH<5,6) Sevšek & al. 2000). The low value of MI determined and the highest frequences of chromosomal aberrations (see Glasenčnik & al. 2002) at that sampling site were in agrement with the above mentioned studies.

Considering the extreme pollution with heavy metals in the Upper Meža Valley (see RIBARIČ-LASNIK & al. 1999a and references therein) the high value of MI in both experimented plots in Žerjav were surprising because we expected a low value of MI at this site due to extreme value of heavy metals in the surroundings (e.g. 4,99±6,92 $mgkg^{-1}$Cd, 1496±1071,02 $mgkg^{-1}$ Pb, 498,22±546,0 $mgkg^{-1}$ Zn KUGONIČ & STROPNIK 2001). More investigations are needed at this research plot to verify the results.

Contrary to our expectation no significant differences in the mitotic activity could be found among localities in the experiment under field conditions. We found significant differences in the frequency of chromosomal aberrations in different sampling sites concerning shallot grown in the field (see GLASENČNIK & al. 2002). The results are in accordance with the statement of FISKESJÖ (1997) saying that when chromosomal aberrations occur, there are almost always some growth restrictions. These results indicated that the influenece of chronic exposure (soil) is far more genotoxic while the acute exposure (pots) is more cytotoxic. Results obtained with our investigation were confirmed also with the results of an assessment of cytogenetic hazard for plants caused by highway traffic: the mitotic activity decrease and the frequency of chromosomal aberrations increase with the duration of traffic influence in the onion (*Allium cepa* L.) exposed in short-lasting *in situ* exposure near highways in Slovenia (PARADIŽ & DRUŠKOVIČ 2001). In spruce and other perennial plants growing spontaneously on experimental sites near the highways as bioindicators of long-lasting exposure, cytogenetic damages increased in correlation with the duration of traffic, but only at sites with more than 20 years' traffic influence (*ibid*).

Nevertheless, we also found out that the values of MI in the field conditions and in the pot experiment were lower than the average values from the literature for the control (10-15 %; e. g. Oud & RICKARDS 1999; 5-10%; e. g. PARADIŽ 1996) and these results showed a decrease in mitotic activity and a cytotoxic effect of chosen sites.

Considering the present results we can conclude that the polluted air does have a cytotoxic effect on mitotic activity with the decrease of MI and that more polluted sites have a higher effect on mitotic activity. We can also say that the polluted air and soil together probably had a stronger effect at the majority of investigated plots. Therefore we conclude that the mitotic activity is the appropriate testing system for determination of MI in shallot root tip meristematic cells for quick, easy and useful estimation of cytotoxic effect of environmental pollution.

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