Environ Sci Pollut Res (2012) 19:718–723
DOI 10.1007/s11356-011-0612-3

RESEARCH ARTICLE

Biomonitoring the genotoxic effects of pollutants on *Tradescantia pallida* (Rose) D.R. Hunt in Dourados, Brazil

Bruno do Amaral Crispim · Jussara Oliveira Vaini · Alexeia Barufatti Grisolia · Tatiane Zaratini Teixeira · Rosilda Mara Mussury · Leonardo Oliveira Seno

Received: 4 April 2011 / Accepted: 29 August 2011 / Published online: 13 September 2011

Abstract
Purpose This study aimed to associate the intensity of vehicular traffic in the city of Dourados (Mato Grosso do Sul State, Brazil) with mutagenic effects and alterations in leaf physiology as measured by the quantity of micronuclei and the leaf surface parameters of *Tradescantia pallida*.
Methods Five collections of inflorescences were undertaken for 24 weeks to determine the quantities of micronuclei using the *Tradescantia* Micronuclei (Trad-MCN) bioassay. Leaf surface parameters, including stomatal index (SI), stomatal density, and the size of the stomatal ostiole opening size (SO), were evaluated in addition to Trad-MCN. Collections were made at four sampling points with different vehicular traffic intensities. Statistical analyses were performed with SAS software using the Tukey's and Kruskal–Wallis test. Additionally, associations of the characteristics were verified using Pearson's simple correlation analysis.
Results Significant effects were observed with the Trad-MCN bioassay ($p<0.01$) that were related to the collection period and location, as well as significant differences ($p<0.05$) for the effects of the collection points using the Kruskal–Wallis test. In general, the locations with greatest vehicular traffic had plants with the greatest stomatal density values. The characteristics SI and SO did not demonstrate significant differences ($p>0.05$) in relation to the collection sites. The simple correlation analysis demonstrated a negative association (−0.65) between SI and Trad-MCN ($p<0.05$).
Conclusion Plants growing in localities with more intense vehicular traffic had greater quantities of micronuclei as well as higher frequencies and average numbers of stomata than localities with less traffic, indicating the presence of atmospheric contaminants that damaged their DNA.

Keywords Pollution · Stomatal density · Leaf anatomy foliar · Micronuclei · Mutagenesis · Stomatal index · Vehicular flux

Responsbile editor: Elena Maestri

B. A. Crispim (✉) · J. O. Vaini · A. B. Grisolia · T. Z. Teixeira · R. M. Mussury
Faculdade de Ciências Biológicas e Ambientais, Universidade Federal da Grande Dourados, Dourados, MS, Brazil
e-mail: brunocrispim.bio@gmail.com

L. O. Seno
Faculdade de Ciências Agrárias, Universidade Federal da Grande Dourados, Dourados, MS, Brazil

1 Introduction

The economic growth of the last few decades has brought improvements to many developing countries in terms of their access to consumer goods and increased levels of well-being. However, this same growth has also increased environmental degradation and polluted water resources, the air, and the soil through the uncontrolled growth of large urban centers (Luiz et al. 2005).

Concentrations of atmospheric pollutants have rapidly risen in Brazil due to increasing ownership and use of motor vehicles. According to statistics supplied by the National Department of Transit (Denatran 2010), Brazil now has approximately 62 million motor vehicles. Mato Grosso do Sul State (MS) alone has 945,000 vehicles, and large numbers of vehicles are likewise present in the city of Dourados—which currently has a fleet of 92,000 vehicles.

There have been no investigations of biologic damage caused by atmospheric pollutants in this locality.

According to Martins et al. (2002), increasing emissions of polluting gases from motor vehicles are causing significant damage to the natural environment and to human health. Among the environmental problems caused by these emissions are the acidification of rivers and forest soils and the loss of their fauna and flora, the corrosion of buildings and historical monuments, and global environmental changes. In terms of human health, some of the principal problems involve respiratory ailments in children, older people, and those with previous existing conditions, higher rates of asthma and bronchitis, alterations of the immunological systems of otherwise normal people, and circulatory problems (Eyre et al. 1997).

The atmospheric pollutants most common in urban centers are carbon monoxide, carbon dioxide (CO_2), nitrogen oxide, sulfur dioxide, hydrocarbons, and particulate materials. Some of these substances have mutagenic and carcinogenic characteristics (Skov et al. 2001; Colvile et al. 2001; Umbuzeiro et al. 2008).

Tradescantia pallida (Rose) D.R. Hunt (Commelinaceae) is a 15–25-cm tall, herbaceous, succulent plant originally from Mexico (Lorenzi and Souza 2001) that can be annual or perennial, rarely epiphytic, erect to decumbent, and frequently emitting roots at the nodes; the leaves are alternate, entire, with leaf sheaths surrounding the stems (Ribeiro et al. 1999) and are purple, pubescent, and highly decorative (Lorenzi and Souza 2001); the inflorescences are cymose, racemose, or capitate, with small to large bracts, frequently spathaceous.

The anatomical characteristics of the leaves of *T. pallida* can be easily discerned. It flowers during the entire year, is easily handled and cultivated, requires little care, is inexpensive to acquire, and its biomonitoring responses are reliable and easily evaluated. This species has been extensively used as a bioindicator because many somatic mutations induced by mutagens or carcinogenic agents such as benzene, mercury, or aromatic polycyclic hydrocarbons present in the air, soil, or water will disrupt its chromosomes and are associated with the formation of micronuclei (Carvalho 2005).

The *Tradescantia* Micronuclei (Trad-MCN) bioassay is important in epidemiological studies seeking to relate the effects of air pollution with human health. Increasing levels of atmospheric pollutants have been observed to increase the frequency of micronuclei in plant cells, reinforcing the perception that these pollutants will have detrimental effects on humans (Guimarães et al. 2004; Prajapati and Tripathi 2008). According to Rodrigues et al. (2005), micronuclei are formed from chromosomal fragments or intact chromosomes dispersed in the cytoplasm near the nuclei that were not linked to the spindle fibers during meiosis of the pollen grains.

Gaseous pollutants penetrate into plant leaves principally through stomatal pores during gas exchange and can cause alterations in their physiology, metabolism, ultrastructure, and cell structure. Interestingly, increasing levels of pollutants often result in greater numbers of large stomata on the leaf surfaces of plants (Brobov 1955; Larcher 2000).

Considering the inexistence of reliable information about air quality in the city of Dourados (MS) and the importance of environmental monitoring as a preventive measure for public health problems, the present study examined the relationship between the intensity of vehicular traffic in the municipality of Dourados (MS) and the quantity of micronuclei, stomatal index, stomatal density, and the size of the stomatal pores in *T. pallida*.

2 Methods

2.1 Collection localities

The city of Dourados is located in the southern region of MS in central-western Brazil (22°13′18″ S; 54°48′23″ W). It is the second largest city in the state in terms of population and has a total area of 4,086 km². The regional climate is tropical–humid (Dourados 2010).

The four sampling locations used in the present study were areas of ornamental plantings (including *T. pallida*) situated at different locations in the municipality. These localities were selected according to the intensities of their vehicular traffic: (1) Rua Hayel Bom Faker (22°14′52″ S; 54º48′19″ W) with intense traffic (approximately 1,900 vehicles per hour); (2) Rua Ponta Porã (22°13′8″ S; 54º48′38″ W); (3) Rua Monte Alegre (22°13′16″ S; 54º48′41″ W), both sites with intermediate traffic (approximately 1,100 vehicles and 1,040 vehicles per hour, respectively); and (4) Cidade Universitária (22°11′47″ S; 54º56′1″ W) with very low traffic levels (approximately 20 vehicles per hour). The latter site was considered the experimental control.

2.2 Data and biological material collections

Young inflorescences of *T. pallida* were collected during the months of March through August of 2010. After collection, the floral buds were fixed in Carnoy's solution (3:1 ethanol/acetic acid) for 24 h and subsequently conserved in 70% ethanol. To analyze for the presence of micronuclei, 0.5 cm long floral buds were selected as these buds have cells (tetrads) in an ideal phase for observing micronuclei.

During the final sampling period (August 2010), leaf impressions were taken from different individual plants in addition to collecting their inflorescences. To take the epidermal impressions, five random leaves were removed from plants at each sampling site and Super Bonder® glue was applied to the adaxial face; the dried glue with the leaf impressions were then peeled away. These leaf impressions were then examined under a microscope to determine the parameters of stomatal index (SI), stomatal density (SD) (per square millimeter), and stomatal ostiole opening size (SO). In order to quantify these three parameters, photomicrographs were taken of ten fields on five slides (totaling 50 fields) using a binocular microscope with a coupled digital camera; parameters measurements were made using the Moticam 2300 3.0MP Live Resolution program. The stomatal index of each point was calculated using Salisbury's formula, as presented by Wilkinson (1979).

2.3 Evaluation of the biological material

The biological characteristics of quantities of micronuclei (MCN), SI, SD, and SO were evaluated in the Laboratório de Biologia Geral da Faculdade de Ciências Biológicas e Ambientais at the Universidade Federal da Grande Dourados.

2.4 The Trad-MCN test

After fixation and conservation of the inflorescences, the slides of selected buds containing early tetrads were made following a methodology adapted from Ma (1981). At least five slides were prepared from specimens from each sampling site. Micronuclei counts and analyses were made of 300 tetrads on each slide using an optical microscope (Bioval) with a total magnification of ×400. All of the slides were examined by two previously trained evaluators.

2.5 Statistical analyses

The MCN characteristics, SI, SD, and SO were submitted to the Shapiro–Wilk test to verify the normality of the residuals and to Bartlett test to examine the homogeneity of the variances. The statistical analyses were performed with the aid of SAS 9.2 software (SAS 2000).

The characteristics MCN and SO that obeyed the presumptions of normality and homogeneity were submitted to variance analysis and posterior comparisons of their averages using the Tukey test at a 5% level of probability. SE and SD values that did not fulfill the presumptions of normality were evaluated by the Kruskal–Wallis nonparametric test ($p<0.05$).

Pearson's test was also applied to calculate the simple correlations between the variables. The level of significance adopted was 5%.

3 Results

The numbers of MCN were significantly greater ($p<0.01$) at monitoring point 1, followed by points 2, 3, and 4 during the entire biomonitoring period, demonstrating that the numbers of micronuclei observed were related to the numbers of vehicles circulating in that area. It is important to note that during the months of July and August, collections were not made at collection point 3 because the plants were unexpectedly trimmed by city workers. Nonetheless, the quantities of micronuclei were significantly higher at this point as compared to the numbers observed at point 4, indicating that this environment contained contaminating compounds emitted by motor vehicles.

The quadratic means of the numbers of MCN and SO encountered at the four collection points are summarized in Table 1. The numbers of MCN were significantly influenced by the variable period and collection point, as well as by the interactions of these variables. The collection point did not significantly influence the SO.

Table 2 displays the results of the interactions between collection period and collection point ($p<0.01$). In general, it was possible to confirm that the point with the lowest vehicular flux (point 4) had the lowest number of micronuclei in relation to those points with intermediate (points 2 and 3) and elevated fluxes (point 1). Additionally, a significant increase in the numbers of micronuclei could be seen with increasing vehicular flux. It was also observed that the greatest MCN counts occurred in March at all of the collection points, which was probably due to the unusually high temperatures (average 28°C) during this collection period.

Of the characteristics evaluated by the Kruskal–Wallis nonparametric test, only SD showed a significant relationship ($p<0.05$) with the collection point. This result indicates that vehicular flux is related to increased stomatal densities on the leaves of *T. pallida* (Table 2). In a previous analysis of this

Table 1 Causes of the variation, degrees of freedom (*df*), and quadratic means of the MCN and SO encountered in *Tradescantia pallida*

Causes of the variation	*df*	Quadratic means	
		MCN	SO
Collection	4	310.65***	
Point	3	1620.84***	2.00, NS
Collection×point	10	20.36**	
CV (%)	16.73		33.24
R^2	0.91		0.08

NS not significant ($p>0.5$), *CV* coefficient of variation, *MCN* micronuclei, *SO* stomatal ostiole opening size

**$p<0.1$, ***$p<0.001$

Table 2 Averages of the numbers of micronuclei, stomatal frequencies, and average stomatal densities observed in *Tradescantia pallida* at the four sampling points during the collection period

Average numbers of micronuclei at the collection points						Frequency of stomatal density (%)		Average stomatal density (mm^2)
	1 (March)	2 (May)	3 (June)	4 (July)	5 (August)	≤22 mm^2	>22 mm^2	
Point 1 (~1,900 vehicles/h)	34.20 aA	27.74 aB	22.60 aB	21.20 aB	23.36 aB	76	24	27.56 a
Point 2 (~1,100 vehicles/h)	32.32 abA	17.80 abB	19.40 abB	20.80 aB	20.20 aB	86	14	23.75 ab
Point 3 (~1,040 vehicles/h)	24.04 bA	13.80 bB	15.80 bB	–[a]	–[a]	92	8	22.87 b
Point 4 (~20 vehicles/h)	9.40 cA	4.00 cA	4.60 cA	7.80 bA	6.20 bA	94	6	21.99 b

Averages of micronuclei followed by the same upper case letter on a given line, or lower case letter in a column, do not significantly differ by the Tukey's test at a 5% probability level. Averages of stomatal frequencies and average stomatal densities followed by the same letters do not significantly differ according to the Kruskal–Wallis test at a 5% probability level

[a] No collection

characteristic, it was verified that large numbers of leaves had stomatal densities ≤22 mm^2. When the frequencies of stomatal density at the different collection points were compared, it was seen that plants that had smaller percentages of leaves with stomata densities ≤22 mm^2 were largely found at the sampling point with the greatest flux of motor vehicles, and that the frequency of leaves with stomata densities within this interval increased with reductions of vehicular traffic.

The Pearson simple correlation analyses among the characteristics identified a negative association of −0.65 ($p<0.01$) for SI and MCN, and a positive association of 0.53 ($p<0.05$) for SI and SD. The former association indicates that the quantity of MCN is inversely proportional to the quantity of SI, that is, increasing numbers of vehicles were associated with increasing quantities of MCN and diminishing stomatal indexes in *T. pallida*. In the latter association, increases in SI were accompanied by increases in SD, as was to be expected.

4 Discussion

The results of the present study demonstrate that *T. pallida* is susceptible to the effects of pollutants and can therefore be used to biomonitor air quality, corroborating observations made by Klumpp et al. (2006) and Meireles et al. (2009). Environmental biomonitoring has been adopted in many countries and has frequently resulted in the introduction of effective measures to minimize problems generated by pollution.

Vehicular flux (and the consequent liberation of pollutants emitted by burning fossil fuels) has been shown to provoke clastogenic events in the genetic material of the cells evaluated in the present study. Our analyses demonstrated that larger quantities of micronuclei were observed at the sampling points with the highest intensities of motor vehicle flux, and vice versa. According to Meireles et al. (2009), the greatest numbers of micronuclei observed in *T. pallida* occur in urban areas because these have higher concentrations of atmospheric contaminants.

Schaub et al. (2005) noted that alterations in the opening and closing of stomatal pores commonly occur in polluted environments. The analysis of leaf stomatal density of *T. pallida* demonstrated that plants growing in localities with higher vehicular fluxes have higher frequencies and larger average numbers of stomata. This observation indicates that the pollution levels in these localities are unfavorable to the normal physiological development of these plants, and that increasing numbers of stomata are required to attend their physiological necessities of gas exchange under these conditions. Similar observations were reported by Ferdinand et al. (2000), Alves et al. (2003), Maranho et al. (2006), and Alves et al. (2008).

Under normal conditions, the stomatal index is related to the percentage of epidermal cells that become stomata (Castro et al. 2009), and this percentage can vary in response to a number of environmental and/or genetic factors. The plants exposed to the largest numbers of circulating vehicles demonstrated increases in the numbers of epidermal cells—thus resulting in diminishing stomatal indexes. The environmental stress caused by the atmospheric pollutants apparently resulted in increasing the numbers of epidermal cells and stomatal density.

Micronuclei testing using plants of the genus *Tradescantia* (Trad-MCN) and Clone 4430 are considered one of the most efficient and sensitive methods of biomonitoring with plant models to investigate the action of genotoxic agents (Batalha et al. 1999; Meireles et al. 2009). As *T. pallida* has been found to be as useful as its clone in the Trad-MCN assay, researchers have been able to examine the specimens of *T. pallida* growing as ornamental plants in parks and public gardens in many parts of Brazil (Batalha et al. 1999; Guimarães et al. 2004) under real-life situations (Mielle et al. 2009).

Plants that grow in localities with elevated concentrations of CO_2 normally demonstrate low stomatal indexes. This phenomenon was demonstrated by Costa (2004) in a study of leaves of herbarium samples (collected between 1919 and 1959) in which it was observed that preserved leaves had twice the stomatal indexes of the leaves of living plants exposed to high concentrations of CO_2. The negative association observed between MCN and SI can be explained by the high flux of motor vehicles and a consequent increase in CO_2 levels in the atmosphere, reflected directly by the high average numbers of MCN. The higher incidence of MCN observed in March may be related to climatic factors such as rainfall, temperature, or humidity that can differ slightly from one location to another (Meireles et al. 2009), with differing effects on pollutant dispersal.

The present work is the first examination of biomonitoring of genotoxic effects of air pollutants in the city of Dourados (Mato Grosso do Sul State, Brazil), but our results corroborate the frequencies of micronuclei in *T. pallida* observed by Meireles et al. (2009) and Prajapati and Tripathi (2008) in Feira de Santana (Bahia State, Brazil) and Varanasi (India), respectively, two cities with similarly high levels of motor vehicle traffic.

5 Conclusions

The results of the research allow us to conclude that those localities with greater motor vehicle traffic in Dourados, MS have significantly higher indexes of atmospheric contamination by genotoxic agents that can damage cell DNA—thus supporting the validity of this technique for biomonitoring air quality. It can also be concluded that the techniques used for analyzing the leaf surfaces were efficient and indicated that *T. pallida* demonstrates physiological differences in terms of its stomatal density, index, and opening in highly polluted environments.

Acknowledgment The authors are grateful to the Universidade Federal da Grande Dourados (UFGD) for their logistic support.

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