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Active monitoring of urban air with a simple short-term *Tradescantia pallida* var. *purpurea* bioassay under different temperature conditions

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ABSTRACT: (Active monitoring of urban air with a simple short-term *Tradescantia pallida* var. *purpurea* bioassay under different temperature conditions). The aim of this study was to investigate the influence of temperature regimes on the formation of micronuclei, and to assess the efficiency of a simple short-term exposure of *Tradescantia pallida* var. *purpurea* cuttings maintained in distilled water for *in situ* monitoring of air genotoxicity during different climatic seasons. The first experiment tested the effect of four temperature regimes and a reference temperature on the formation of micronuclei in growth chambers. For the second experiment, plant cuttings kept in distilled water were periodically exposed for 24 hours, from spring 2010 to winter 2011, in an urban site in the municipality of Novo Hamburgo, in the Sinos River Basin, State of Rio Grande do Sul (Brazil); in addition an indoor control was carried out. Micronuclei frequencies (MCN) were determined in young tetrads of pollen mother cells and expressed as MCN/100 tetrads. The MCN frequencies varied from 0.70 to 1.17, however there was no significant difference between the different temperature regimes (minimum and maximum temperatures of 6 and 38 °C) and the temperature of 26 °C. Under field conditions, MCN frequencies were significantly higher in samples from the urban site than in those from the control and did not vary significantly among seasons (4.0 in spring, 2.9 in summer, 3.1 in autumn and 4.3 in winter). The results indicated the validity of this simple method as a suitable experimental model for active air monitoring under the environmental conditions of the study.

Key words: bioindication, genotoxicity, air pollution, micronucleus.

RESUMO: (Monitoramento ativo simples de curta duração do ar urbano com o bioensaio de *Tradescantia pallida* var. *purpurea* sob diferentes condições de temperatura). O estudo objetivou investigar a influência de regimes de temperatura sobre a formação de micronúcleos e verificar a eficiência de um bioensaio de simples e rápida exposição de ramos de *Tradescantia pallida* var. *purpurea* mantidos em água destilada para o monitoramento *in situ* da genotoxicidade do ar ao longo das diferentes estações climáticas. No primeiro experimento, quatro regimes de temperatura e uma temperatura de referência foram testados quanto ao efeito sobre a formação de micronúcleos em câmaras de crescimento. No segundo experimento, ramos de plantas em água destilada foram periodicamente expostos por 24 horas, da primavera de 2010 ao inverno de 2011, a um ponto urbano no município de Novo Hamburgo, na Bacia do Rio dos Sinos, Rio Grande do Sul (Brasil), além de um controle em ambiente interno. As frequências de micronúcleos (MCN) foram determinadas em tétrades jovens de células-mãe de grãos de pólen e expressas como MCN/100 tétrades. Não foram observadas diferenças significativas entre frequências de MCN sob diferentes regimes de temperatura (temperaturas mínima e máxima de 6 e 38 °C) e a temperatura de 26 °C, variando de 0,70 a 1,17. Sob condições de campo, as frequências de MCN foram significativamente mais altas nas amostras do ponto urbano do que naquelas do controle e não variaram significativamente ao longo das estações (4,0 na primavera, 2,9 no verão, 3,1 no outono e 4,3 no inverno). Os resultados indicaram a validade desse método simples como um modelo experimental adequado para o monitoramento ativo do ar nas condições ambientais do presente estudo.

Palavras-chave: bioindicação, genotoxicidade, poluição do ar, micronúcleo.

INTRODUCTION

Biological indicators are useful tools that complement the conventional chemical monitoring of environmental quality, because they have an integrated response to synergistic, additive or antagonistic effects of chemicals in complex mixtures (Prajapati & Tripathi 2008). Various plant species are sensitive to the toxic effects of air pollutants and rapidly show cell or tissue disturbances. The presence of atmospheric genotoxic agents can be qualitatively and quantitatively detected

by genetic damage, such as chromosome breaking and DNA loss (Ma 1982).

The *Tradescantia* L. micronucleus (Trad-MCN) test is a widely used plant bioassay for *in situ* monitoring of air pollutant genotoxicity (Ma *et al.* 1994, Batalha *et al.* 1999, Guimarães *et al.* 2000, Klumpp *et al.* 2006, Carreras *et al.* 2009). This bioassay is based on the evaluation of micronuclei formed due to clastogenic and aneuploidic effects on chromosomes in early meiotic stages of the pollen mother cells of inflorescences (Ma 1979). If on the one hand, the *Tradescantia* clone 4430 is consi-

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dered highly efficient and sensitive to genotoxic substances (Isidori *et al.* 2003, Klumpp *et al.* 2006, Villarini *et al.* 2009), on the other hand, it is not well-adapted to tropical weather conditions, so that plant growth and flowering may be inhibited (Klumpp *et al.* 2004). Additionally, climatic conditions such as relative humidity and temperature are thought to influence micronuclei induction in the clone (Klumpp *et al.* 2004).

Some studies have demonstrated that *Tradescantia pallida* (Rose) D.R. Hunt var. *purpurea* Boom can be used as efficiently as clone 4430 in genotoxic biomonitoring experiments (Guimarães *et al.* 2000, Suyama *et al.* 2002, Mielli *et al.* 2009). This species has many advantages over the clone, because it is well adapted to sub-tropical and tropical environmental conditions. *T. pallida* var. *purpurea* produces flowers throughout the year and is highly resistant to plagues; it is widely distributed and used for ornamental purposes in Brazil. Although climatic conditions also seem to interfere in the production of micronuclei of *T. pallida* var. *purpurea*, especially when plants are placed for a long time in exposure sites (Savóia *et al.* 2009), there is scant information from experiments under controlled conditions (Lima *et al.* 2009).

For active *in situ* monitoring of atmospheric air, *Tradescantia pallida* var. *purpurea* specimens are usually grown in pots with soil, which are exposed to sampling sites (Guimarães *et al.* 2000, Prajapati & Tripathi 2008, Carreras *et al.* 2009, Meireles *et al.* 2009). The main disadvantages of this method are the effort required to transport pots between the sampling sites and the laboratory, and the breaking and loss of branches with inflorescences during transport. Therefore, flowering branches of *T. pallida* var. *purpurea* placed in vessels that contain nutrient solution were used to screen the mutagenic potential of defined chemicals under controlled conditions (Batalha *et al.* 1999, Suyama *et al.* 2002, Alves *et al.* 2008, Lima *et al.* 2009).

The aim of this study was to investigate the influence of different temperature regimes on the formation of micronuclei under controlled conditions, and to assess the efficiency of a simple short-term exposure bioassay of *Tradescantia pallida* var. *purpurea* cuttings maintained in distilled water for *in situ* monitoring of air genotoxicity during different climatic seasons.

MATERIAL AND METHODS

Plants of *Tradescantia pallida* var. *purpurea* were individually cultivated in plastic pots (20 cm in diameter) containing 4 kg of commercial soil from the same batch and were watered three times a week. Once a week, 100 mL of 1/3-strength Hoagland solution was applied to each pot, and 100 mL of an N:P:K (nitrogen:phosphorus:potassium) fertilizer solution (10:10:10 v/v/v) was applied once a month. The pots were maintained in the open air without shade, at the University campus.

The first experiment tested the effect of four different temperature regimes and the reference temperature routinely used for laboratory control samples on the formation of micronuclei of *Tradescantia pallida* var. *purpurea* plant cuttings (Fig. 1A-E). The temperature values were selected as they represent the real field temperatures of the four climatic seasons in southern Brazil, as registered by the nearest Meteorological Station to the sampling sites for a one year-period (2009-2010). Each treatment included 15 plant cuttings obtained from potted plants and kept in a 2-L plastic vial filled with distilled water for 24 hours in a growth chamber; under artificial lighting with an intensity of 100 $\mu\text{mol.m}^{-2}/\text{s}$. One young inflorescence from each plant cutting was fixed in ethanol-acetic acid (3:1 v/v). After 24 h, they were transferred to 70% ethanol and stored in a refrigerator (Ma *et al.* 1994). The Trad-MCN bioassay was carried out as described by Ma (1982), with modifications. Each inflorescence was dissected under a stereomicroscope. The anthers were stained with 1% acetocarmine and macerated using a glass rod on a microscopic slide. After discarding anther fragments, the slide was covered with a cover slip, rapidly warmed, and examined under an Olympus CX31 microscope at 400 X magnification. The number of micronuclei in meiotic pollen mother cells in the early tetrad stage were counted. Ten slides were prepared per method and sampling site, and 300 tetrads per slide were scored. Micronuclei frequencies were expressed as the number of micronuclei in 100 tetrads.

The second experiment evaluated the air genotoxicity at the outer edge of the Parque Municipal Henrique Luís Roessler, a fragment of the Atlantic Forest in the urban area of Novo Hamburgo municipality (29°40'S, 51°06'W) in the Sinos River Basin. *Tradescantia pallida* var. *purpurea* cuttings were partially immersed in distilled water during a one year-period (from 2010 to 2011) during each season. During the same period, a negative control was used with plant cuttings exposed to air in a growth and acclimatization room in the laboratory, with constant temperature of 26 °C and natural light. For each sample, fifteen cuttings kept in a 2-L plastic vial filled with distilled water were exposed in the morning and collected after 24 h exposure. The subsequent steps of the experiment were carried out in the same manner as the first experiment (described above). Ten slides were prepared per treatment, and 300 tetrads per slide were scored.

The climate of the urban site of the study is Cfa type, according to the Koeppen classification; the warmest month's mean temperature is higher than 22 °C and rainfall is distributed throughout the year (Moreno 1961). Local data on rainfall, relative humidity and temperature were supplied by Station no. 83961 (the closest weather station to the sampling site), in the municipality of Campo Bom (29°41'S; 51°03'W).

Data analysis was carried out using the SPSS 19.0 statistical package (SPSS, Chicago, IL, USA). Data of

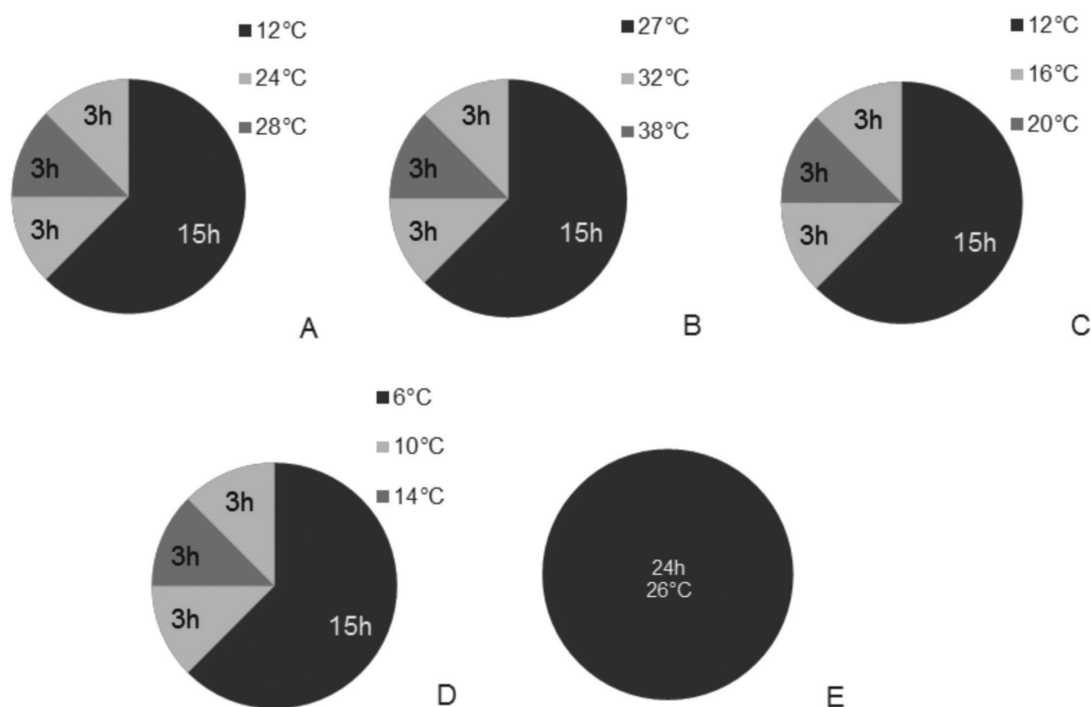


Figure 1. Temperature regimes (A-E) used in Experiment 1. The sequence of the temperatures in each regime is presented in Table 1.

micronuclei were expressed as mean $\pm$ standard deviation (SD). The Shapiro-Wilk test was used to confirm normal data distribution and homogeneity of variances was tested by the Levene test. In the first experiment, data were compared using the parametric analysis of variance (ANOVA). In the second experiment, means of micronuclei frequencies between sampling sites in each season were compared using the Student t test, at the level of 5% significance. Mean frequencies of micronuclei in each sampling site throughout the seasons were submitted to the parametric analysis of variance (ANOVA), and differences were tested by the Tukey test, at 5% significance.

RESULTS AND DISCUSSION

Comparing the micronuclei frequencies observed in the different temperature regimes and the standard exposure temperature of 26 °C under controlled conditions, no significant differences were observed. Values ranged from 0.70 to 1.17 (Table 1). The results showed that mutation rates in meiotic cells of *Tradescantia pallida* var. *purpurea* cuttings were similar at temperatures from 6 to 38 °C.

During the *in situ* monitoring experiment throughout the four seasons of the year, typical climatic conditions were observed for the region of the inferior third of the Sinos River Basin, in which Novo Hamburgo is located (Table 2). Mean minimum and maximum temperatures varied from 2.9 to 16.6 °C and from 14.3 to 31.4 °C, respectively, from winter to summer (Table 2). The relative humidity only varied by 13% during the study

period (Fig. 2), following the general pattern observed in the region (Buriol *et al.* 2007). Accumulated rainfall two days before and on the day of *in situ* exposure of *T. pallida* var. *purpurea* cuttings was zero in summer, autumn and winter, and 1.8 mm in spring (Table 2).

Under the environmental conditions of the four seasons studied, the MCN frequencies were significantly higher in samples from the urban site than in those from the control and did not vary significantly among seasons (Table 2). The highest percentage of increase in the number of MCN relative to the control occurred in winter (194%), followed by spring (177%), autumn (152%) and summer (148%). The results indicated that the characteristics of the studied urban site, with mo-

Table 1. Number of micronuclei and micronuclei frequency (MCN/100 tetrads) in pollen mother cells of *Tradescantia pallida* var. *purpurea* exposed for 24 hours to four varying temperature regimes and one constant temperature.

Temperature regimes in °C	No. of MCN ¹	MCN frequency (mean $\pm$ SD ²)
A - 24/28/24/12	35	1.17 $\pm$ 1.25
B - 32/38/32/27	22	0.73 $\pm$ 0.66
C - 16/20/16/12	21	0.70 $\pm$ 0.39
D - 10/14/10/6	30	1.00 $\pm$ 0.59
E - 26	29	0.97 $\pm$ 0.64
		F = 0.6543
		P = 0.6269

1. From a total of 3,000 tetrads.

2. Standard deviation.

Table 2. Number of micronuclei and micronuclei frequency (MCN/100 tetrads) in *Tradescantia pallida* var. *purpurea* pollen mother cells seasonally exposed for a 24-h period to atmospheric air in an urban site of Novo Hamburgo relative to the control, and temperature and rainfall conditions at the exposure times.

Season/Year	Temperature ¹ (°C)		Rainfall ² (mm)	Urban site MCN frequency (mean $\pm$ SD ³)	Control MCN frequency (mean $\pm$ SD)	t	P
	Max.	Min.					
Spring 2010	27.2	11.5	1.8	4.00 $\pm$ 1.83	1.13 $\pm$ 0.57	4.730	0.001
Summer 2011	31.4	16.6	0.0	2.87 $\pm$ 1.41	0.97 $\pm$ 0.64	3.887	0.002
Autumn 2011	20.5	8.5	0.0	3.13 $\pm$ 1.10	1.03 $\pm$ 0.67	5.140	<0.001
Winter 2011	14.3	2.9	0.0	4.27 $\pm$ 1.89	1.10 $\pm$ 0.94	4.738	<0.001
				F = 1.786 P = 0.167	F = 0.100 P = 0.959		

1. Maximum and minimum temperature of the day of *Tradescantia* cuttings exposure.

2. Accumulated rainfall two days before and at the day of exposure.

3. Standard deviation.

tor vehicle traffic, may produce a significant level of genotoxic pollution. Villarini *et al.* (2009) carried out an active biomonitoring study with a 24-hour exposure of *Tradescantia* clone 4430 to three sampling sites in Perugia (Italy), and found MCN frequencies up to 4.7. Several working groups have studied the genotoxic potential of the air through passive monitoring, exposing *T. pallida* var. *purpurea* at selected outdoor sites for several months. Although these studies used potted plants, the results can be compared, since both methods of exposure (plant cuttings and potted plants) are equally sensitive to air genotoxicity (data not published). In São Paulo city, southeastern Brazil, Guimarães *et al.* (2000) observed high MCN frequencies (up to 7.1) at two sites of heavy motor vehicle traffic. In Santo André, a town in the São Paulo metropolitan area with intense industrial activity and heavy motor vehicle traffic, Savóia *et al.* (2009) found MCN frequencies of up to 4.6. Meireles *et al.* (2009) observed MCN frequencies up to 2.1 close to avenues of heavy traffic in the city of Feira de Santana, northeastern Brazil. In Córdoba, one of the most polluted cities in Argentina, the highest mean MCN frequency observed was 3.5 (Carreras *et al.* 2009). In Varanasi, in India, tetrads of *T. pallida* var. *purpurea* had MCN frequencies of 3.9 to 4.3 in areas

near the city center and 2.4 at a location away from the city (Prajapati & Tripathi 2008).

Micronuclei frequencies of the control were similar to values found for inflorescences of *Tradescantia pallida* var. *purpurea* by Klumpp *et al.* (2006) and lower than those registered by Batalha *et al.* (1999), Suyama *et al.* (2002) and Alves *et al.* (2008). These data indicate that cultivation and exposure procedures do not affect the plants and that the environmental conditions of the urban site were stressful enough to increase DNA damage in pollen mother cells.

In this study, MCN frequencies did not vary significantly even when inflorescences were exposed to the highest thermal amplitude tested (16 °C). However, Lima *et al.* (2009) found an inverse relation between daily thermal amplitudes (from zero to 20 °C) and the frequencies of micronuclei when exposing *T. pallida* var. *purpurea* cuttings for 3 hours to ozone treatments in fumigation chambers. Previously, Ma *et al.* (1994) showed that cuttings of *Tradescantia* clone 4430 should not be cultivated under temperatures lower than 15 °C and higher than 36 °C in a greenhouse. The authors did not make further considerations about abiotic conditions during exposure tests. The effect of temperature on this clone was investigated by Klumpp *et al.* (2004), who found an increase of more than 100% in the spontaneous mutation rate of tetrads at the constant temperature of 11 °C compared to 22 °C. A significant increase in micronuclei frequency was also found by the authors when exposing the cuttings to 11 and 17 °C for 3 hours, followed by 18 hours at 22 °C. The sensitivity of clone 4430 to low and high temperatures limits the reproducibility of the Trad-MCN and Klumpp *et al.* (2004) recommended that, for best test results, the ideal exposure temperature is between 15 and 30 °C. Due to its genetic characteristics, *Tradescantia* clone 4430 may be more sensitive to abiotic environmental alterations than *T. pallida* var. *purpurea*, since the latter is known to have high adaptability and is resistant to different environmental conditions.

The high relative humidity registered throughout the year in the Novo Hamburgo region did not seem to affect spontaneous MCN frequencies of the control

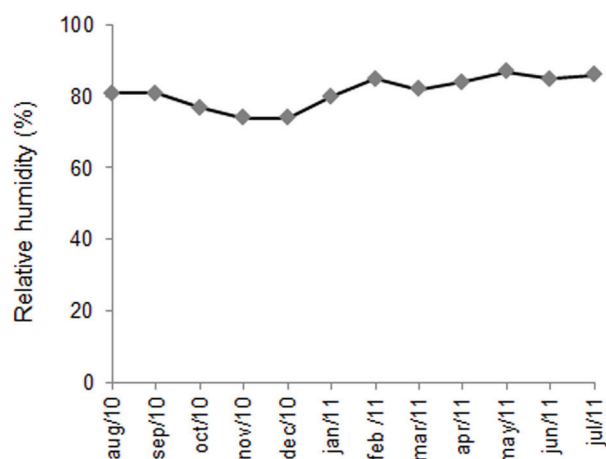


Figure 2. Monthly relative humidity in Novo Hamburgo, Rio Grande do Sul State, Brazil, during August 2010 to July 2011.

and MCN frequencies in the inflorescences exposed to the urban site. Klumpp *et al.* (2004) observed no differences in MCN frequencies of the control among 35 and 80% of relative humidity. Under high humidity conditions, stomata may remain totally or partially open and the uptake of gaseous pollutants can be maintained, even at high summer temperatures.

Rainfall can also interfere with the MCN frequency (Savóia *et al.* 2009). Nevertheless, since plants for active air monitoring were only exposed for a short time to the sampling points, no direct comparisons can be made with data from passive monitoring studies. Previous experiments have shown that significantly higher MCN frequencies were observed in the seasons in which *Tradescantia* exposure coincided with greater rainfall volumes (unpublished data). In order to avoid the influence of this parameter on the formation of MCN, the day of exposure can be selected by making sure there was no precipitation during the days immediately before the assay.

In the literature, most *Tradescantia* cuttings were maintained in nutrient solution for adaptation before exposure and for recovery after treatments. This procedure is stressful (Lima *et al.* 2009) and may increase the risk of contamination, because the nutrient solution is previously prepared in the laboratory and carried to the open environment, where it is exposed to variable weather conditions. In this study, the *Tradescantia pallida* var. *purpurea* cuttings were kept in distilled water, which proved to be efficient even when subjected to complex mixtures of various components of the air and to variable temperatures. The results indicate the validity of this simple method as a suitable experimental model for active air monitoring under the described environmental conditions. Future studies should be carried out to test more extreme temperatures and variable relative humidity, which would yield more information about the relevance of these abiotic variables on the induction of micronuclei in field conditions.

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