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The *Tradescantia pallida* var. *purpurea* active bioassay for water monitoring: evaluating and comparing methodological conditions

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ABSTRACT

Tradescantia pallida var. *purpurea* cuttings with flower buds are utilized in bioassays to diagnose genotoxic effects of water. The literature describes different substances used to adapt and recover the cuttings before and after exposure to water samples and also describes the effects of different exposure times. This study evaluated and compared the micronuclei (MCN) frequencies in *T. pallida* when cuttings with flower buds were submitted to different methodological conditions. The bioassay was then applied bimonthly during seven months to assess the genotoxic potential of a site located on the Sinos River in Campo Bom, Rio Grande do Sul, Brazil. Micronuclei frequencies in buds of cuttings adapted and recovered in distilled water and in Hoagland solution were 3.0 and 2.9, respectively, for cuttings exposed to river water, and 1.19 and 1.23 in controls. No significant differences among MCN frequencies were observed when cuttings were exposed for 8, 24 or 32 hours to river water (from 3.07 to 4.73) and in controls (from 1.13 to 2.00) in all samplings during a year. Adaptation and recovery of cuttings in distilled water or Hoagland solution and exposure for different times did not influence the response of *T. pallida*, indicating that all the conditions tested are viable for biomonitoring of water genotoxicity. Water samples from the Sinos River presented genotoxicity during the period monitored, evidenced by the MCN frequencies recorded which were significantly higher than the frequencies of the controls.

Keywords: water bodies, genotoxic risk, micronuclei.

O bioensaio ativo com *Tradescantia pallida* var. *purpurea* para monitoramento de água: avaliação e comparação de condições metodológicas

RESUMO

Ramos de *Tradescantia pallida* var. *purpurea* contendo botões florais são utilizados em bioensaios para diagnosticar efeitos genotóxicos de água. Diferentes substâncias para adaptar

e recuperar os ramos antes e após a exposição a amostras de água e diferentes tempos de exposição são relatados na literatura. O objetivo do estudo foi avaliar e comparar as frequências de micronúcleos (MCN) em *T. pallida* quando ramos com botões florais foram submetidos a diferentes condições metodológicas. O bioensaio foi então aplicado bimensalmente durante sete meses para avaliar o potencial genotóxico de um sítio do Rio dos Sinos em Campo Bom, Rio Grande do Sul, Brasil. As frequências de MCN em botões de ramos adaptados e recuperados em água destilada e em solução de Hoagland foram de 3,0 e 2,9, respectivamente, quando ramos foram expostos a água do rio e de 1,19 e 1,23 nos controles. Não foram observadas diferenças significantes entre frequências de MCN em ramos expostos por 8, 24 ou 32 h a água do rio (de 3,07 a 4,73) e nos controles (de 1,13 a 2,00) em todas as amostragens ao longo do ano. Adaptação e recuperação de ramos em água destilada ou solução de Hoagland e exposição durante diferentes tempos não influenciaram a resposta de *T. pallida* de formas diferentes, indicando que todas as condições testadas são viáveis para biomonitoramento de genotoxicidade de água. Amostras de água do Rio dos Sinos apresentaram genotoxicidade ao longo do período monitorado, evidenciada pelas frequências de MCN registradas, que foram significativamente superiores às dos controles.

Palavras-chave: corpos d'água, risco genotóxico, micronúcleos.

1. INTRODUCTION

Pollutants released into watercourses change the physicochemical composition of the water, forming complex mixtures that can provoke toxic and genotoxic effects in living organisms (Ohe et al., 2004; Merlo et al., 2011).

The hydrographic basins with urban centers are more susceptible to various anthropic impacts which may compromise the quality of the physical, chemical and biological components of the ecosystems. The Sinos River Basin is located in the eastern part of the State of Rio Grande do Sul, Brazil, with a population of around 300 inhabitants/km^2, with about 95% of them living in urban areas (IBGE, 2014). The Sinos River, the main water body of this basin, has around 190 km, supplying water to a population of approximately 1.5 million people (FEPAM, 2014). The 32 municipalities of the basin contribute to the pollution of the river by discharges of direct release of industrial and domestic effluents, as well as by diffuse charges, which are produced by the runoff from various urban and rural effluents (Blume et al., 2010; Nunes et al., 2011; Oliveira et al., 2012).

Various organisms are recognized scientifically as indicators of pollution, although their respective responses are dependent on the types of pollutants and experimental conditions to which they are exposed (Ma et al., 1994; Mielli et al., 2009). When water bodies are assessed by active monitoring, bioindicators are exposed to water samples for short periods which vary from a few hours to days (Ma et al., 1994; Jiang et al., 1999; Thewes et al., 2011).

Tradescantia pallida (Rose) D.R. Hunt. var. *purpurea* Boom is a species that is well-adapted to both subtropical and tropical climates and has been utilized in bioassays to diagnose genotoxic effects of water (Umbuzeiro et al., 2007; Thewes et al., 2011; Endres Jr. et al., 2014; Kieling-Rubio et al., 2014). Assessments have shown that the response of this species to mutagenic agents is as efficient as the response of the *Tradescantia* 4430 clone (Suyama et al., 2002; Mielli et al., 2009), which is recognized as a bioindicator of genotoxicity due to increased frequencies of micronuclei (MCN) in the cytoplasm of cells in the tetrads phase (Ma et al., 1994).

Biomonitoring of watercourses using *Tradescantia* micronuclei (Trad-MCN) bioassay is conducted by partially immersing cuttings with young inflorescences into water samples and maintaining them in a non-polluted environment throughout the test (Ma et al., 1994; Endres Jr. et al., 2014). It is important to monitor the abiotic conditions where the plants from which

the cuttings will be sampled are cultivated, in order to avoid using plants for the bioassays that have a background MCN rate above that expected as a result of spontaneous mutations (Ma, 1983; Pereira et al., 2013). Young inflorescences are used for *Tradescantia* genotoxicity tests because they have flower buds that are in the process of cell division and are therefore more sensitive to mutagenic agents, since chromosomal damage occurs in prophase I of meiosis. One advantage of active monitoring using the Trad-MCN bioassay is the short time period needed to complete the test with water samples, generally comprising adaptation, exposure and recovery periods. The period of adaptation prior to exposure of *Tradescantia* cuttings is important in order to avoid chromosomal damage caused by possible genotoxic pollutants at the cultivation site or by stress caused by cutting, while recovery is the time necessary for the meiotic cells to achieve the stage of tetrads when the micronuclei are scored (Ma, 1983; Ma et al., 1994).

Various exposure times have been described in the literature for *Tradescantia* (Ruiz et al., 1992; Umbuzeiro et al., 2007; Kieling-Rubio et al., 2014). Further, different substances have been used for the periods of adaptation and recovery of cuttings, including Hoagland solution (Ma et al., 1994; Mielli et al., 2009), tap water (Ruiz et al., 1992; Jiang et al., 1999), mineral water (Monarca et al., 2003; Crebelli et al., 2005), and distilled water (Thewes et al., 2011; Kieling-Rubio et al., 2014). Therefore, with the goal of assessing methodological conditions of the active bioassay for water monitoring using *Tradescantia pallida* var. *purpurea*, the objectives of this study were to: (a) compare the micronuclei frequencies observed in the cuttings adapted and recovered in distilled water and in Hoagland solution; (b) investigate the influence of the time of exposure of the cuttings on MCN formation, and (c) apply the bioassay to assess the genotoxic potential of water at a sampling point of the Sinos River in Campo Bom, Rio Grande do Sul, Brazil.

2. MATERIAL AND METHODS

2.1. Water sampling

Water samples were collected at a sampling point of the Sinos River (29°41'30.28"S and 51°02'42.43"W), in Campo Bom. This municipality is located in the lower section of the Sinos River Basin, Rio Grande do Sul, Brazil, with intense urbanization and industrialization (IBGE, 2014). Physico-chemical parameters showed a high degree of water pollution in the lower section of the river (Blume et al., 2010). The assays were conducted from January 2012 to September 2013, totaling nine samplings. Water samples from the surface were transported to the laboratory in accordance with a protocol published by the Brazilian Association of Technical Norms (ABNT, 1987) and the Standard Methods for the Examination of Water and Wastewater (APHA, 2005).

2.2. Biological material

Specimens of *Tradescantia pallida* var. *purpurea* to be used for the Trad-MCN bioassays were grown in plastic pots (37 cm x 20 cm x 20 cm) containing 4 kg of commercial soil in a non-polluted area of the Universidade Feevale. Plants were watered three times per week and 100 mL of N:P:K fertilizer solution (nitrogen:phosphorus:potassium, 10:10:10, w:w:w) were applied monthly (Thewes et al., 2011). All of the plants were derived from vegetative propagation.

2.3. Trad-MCN bioassays

Bioassay I - comparison between MCN frequencies observed in cuttings adapted and recovered in distilled water and in Hoagland solution: In January 2012, 20 cuttings of *Tradescantia pallida* var. *purpurea* with flower buds, each cutting from a plant, were partially immersed in vessels containing 2 L of distilled water (treatment 1) or Hoagland solution

(Hoagland and Arnon, 1950) (treatment 2) and left for 24 hours to adapt. For exposure, cuttings were partially immersed in 2 L of water from the Sinos River for 8 hours. After this, cuttings were transferred to distilled water (treatment 1) or Hoagland solution (treatment 2) for 24 hours. Negative controls were set up simultaneously, following the same methodology, but replacing the river water with distilled water (treatment 1) or Hoagland solution (treatment 2).

Bioassay II - influence of the exposure time of the cuttings on micronuclei formation: This experiment was conducted from March 2012 to January 2013, with a seasonal periodicity, totaling four samplings. Cuttings were adapted for 24 hours partially immersed in vessels containing 2 L of distilled water. After this, 20 cuttings were exposed for 8, 24 or 32 hours to water samples from the Sinos River. Only cuttings that had been exposed to river water for 8 hours were then transferred to distilled water for a 24 hour recovery period. Simultaneously, negative controls were set up, using the same exposure times, but replacing the water sample from the river with distilled water.

Bioassay III - applying the bioassay to assess the genotoxic potential of the water from the Sinos River: This experiment was conducted from March to September 2013, with bimonthly periodicity, totaling four samplings. For each sampling, the same number of cuttings were used as in Bioassay I. Cuttings were exposed to water from the sampling point of the river for 8 hours, under the same conditions as Bioassay I, with adaptation and recovery periods as described for Bioassay II. Simultaneously, negative controls were set up, replacing the water sample from the river with distilled water.

All bioassays were conducted in a climate-controlled room with temperature at 26 ± 1°C.

2.4. Inflorescence fixation, slide preparation and micronuclei score

After each bioassay, inflorescences with flower buds were fixed in 3:1 (v/v) ethanol/acetic acid for 24 hours and stored in 70% ethanol under refrigeration. One bud from each inflorescence was dissected, and the anthers were squashed in 1% acetocarmine stain on a slide. Only preparations with early tetrads were included in the analysis. Ten slides were prepared for each sample, and the number of MCN in a random set of 300 tetrads per slide was scored under 400x magnification (Olympus CX4 microscope). Micronuclei frequencies were calculated by dividing the total number of micronuclei by the total number of tetrads scored, and the results were expressed as MCN/100 tetrads (Thewes et al., 2011).

2.5. Statistical analyses

Statistical analyses were conducted using SPSS 20 for Windows. Micronuclei frequencies were verified for normality using the Shapiro-Wilk test and compared using the Student *t* test at 5% probability (Bioassays I and III) and the analysis of variance (ANOVA) followed by the Tukey test at 5% probability (Bioassays II and III).

3. RESULTS AND DISCUSSION

The MCN frequencies recorded for the flower buds exposed to water samples of the Sinos River, adapted and recovered in distilled water, did not differ significantly from those of samples adapted and recovered in Hoagland solution (Table 1). Also, there was no significant difference between MCN frequencies recorded for negative controls with distilled water or with Hoagland solution, which were respectively 1.23 e 1.19, significantly lower than the frequencies in the respective bioassays with water from the river (Table 1). These results indicated that the increase in genetic damage in meiotic cells of *Tradescantia pallida* var. *purpurea* cuttings can be explained by the genotoxic effect of the water from the river and that the response of this bioindicator should not have been influenced by both methods applied for adaptation and recovery of cuttings. The MCN frequencies recorded for the

negative control remained below the background rate considered as resulting from spontaneous mutations in *T. pallida* var. *purpurea* plants cultivated in non-polluted environments (Pereira et al., 2013). In previous studies, negative controls using distilled water presented similar MCN frequencies to those recorded in the present work (Thewes et al., 2011; Kieling-Rubio et al., 2014).

Table 1. Frequency of micronuclei (MCN) in *Tradescantia pallida* var. *purpurea* cuttings adapted and recovered in distilled water or Hoagland solution after exposure to water from the Sinos River and the negative control.

Sample	**MCN frequencies** (mean ± standard deviation)		*t*	p
	Hoagland solution	Distilled water		
Sinos River	3.00 ± 0.47**	2.95 ± 0.30**	0.225	0.826
Negative control	1.19 ± 0.38	1.23 ± 0.53	-0.192	0.851
t	7.924	7.400		
p	<0.001	<0.001		

** indicate highly significant difference between frequencies recorded for river water and negative controls, according to Student *t* test (p=0.05).

For the samples collected from March 2012 to January 2013 for Bioassay II, no significant differences were observed among MCN frequencies in flower buds exposed for periods of 8, 24 and 32 hour to water from the Sinos River, in any of the months monitored. There were also no significant differences among MCN frequencies observed in flower buds after 8, 24 and 32 hours exposure in each negative control sampling (Table 2).

Table 2. Frequency of micronuclei (MCN) in *Tradescantia pallida* var. *purpurea* cuttings exposed for different times to water from the Sinos River and the negative control.

Month	**Sample**	**MCN frequencies** (mean ± standard deviation)			F	p
		8 h	24 h	32 h		
March 2012	Sinos River	3.67 ± 0.85	3.16 ± 0.58	4.25 ± 1.77	0.884	0.443
	Negative control	1.27 ± 0.28	1.47 ± 1.12	1.33 ± 0.41	0.104	0.902
June 2012	Sinos River	4.73 ± 0.49	4.73 ± 1.94	4.60 ± 1.09	0.017	0.983
	Negative control	1.80 ± 0.51	2.00 ± 0.94	1.33 ± 0.33	1.398	0.285
October 2012	Sinos River	4.16 ± 2.12	3.53 ± 0.96	3.40 ± 0.49	1.321	0.317
	Negative control	1.27 ± 0.37	1.27 ± 0.43	1.13 ± 0.38	0.190	0.829
January 2013	Sinos River	4.27 ± 1.34	4.13 ± 0.69	4.67 ± 0.94	0.365	0.702
	Negative control	1.47 ± 0.56	1.53 ± 0.51	1.47 ± 0.38	0.031	0.969

The exposure time of the cuttings in the Trad-MCN bioassay can vary depending on the quality of the water samples (Ruiz et al., 1992). Exposure times tested in the present work

were based on methodologies described in the literature. In the majority of published studies, exposure times of 4430 *Tradescantia* clone or *Tradescantia pallida* var. *purpurea* in water samples of water bodies were either of 8 hours (Kieling-Rubio et al., 2014), 12 hours (Duan et al., 1999), 24 hours (Umbuzeiro et al., 2007; Endres Jr. et al., 2014) or 30 hours (Ruiz et al., 1992). In the present study, cuttings exposed for 8 hours to samples of river water were allowed to recover for 24 hours to allow time to finalizing cell division, since at the last stage (the tetrads stage) it is possible to observe the micronuclei that resulted from genetic damage that took place during the prophase I of the corresponding meiosis (Ma, 1983; Ma et al., 1994). In contrast, if the exposure time of inflorescences to potential genotoxic agents is 24 hours or longer, there is no need for a recovery period, since meiotic division takes 24 to 30 hours to complete (Ma, 1983; Ma et al., 1994). In view of this, cuttings exposed to water samples for 24 and 32 hours were not submitted to a recovery period.

The MCN frequencies recorded in Bioassay II varied from 3.07 to 4.73 in samples of water from the Sinos River and from 1.13 to 2.00 in negative controls (Table 2), and were within the range of MCN frequencies obtained in other studies. Endres Jr. et al. (2014) observed frequencies varying from 1.81 to 6.48 MCN in inflorescences exposed for 24 hours to water from a stream in the municipality of Novo Hamburgo (Rio Grande do Sul, Brazil) and from 1.19 to 1.62 MCN in negative controls. Similar frequencies (from 1.87 to 6.22 MCN) were observed by Umbuzeiro et al. (2007) in plants exposed for 24 hours to water from the Cristais River (São Paulo, Brazil). Ruiz et al. (1992) observed frequencies ranging from 2.5 to 9.0 MCN in inflorescences exposed for 30 hours to water from a canal of the Queretaro River (Mexico) and a mean of 1.5 MCN in negative controls. Frequencies of 3.19 and 8.73 MCN were observed in plants exposed for 12 hours to samples of water from the Panlong River (China), while negative controls presented a mean of 2.49 MCN (Duan et al., 1999).

For the monitoring of the genotoxic potential of the river water from March to September 2013 (Bioassay III), MCN frequencies did not differ significantly among samples ($F=0.273$, $p=0.844$), but were significantly higher than their respective negative controls, which in turn did not differ from each other ($F=0.561$, $p=0.644$) for any of the sampling months (Figure 1).

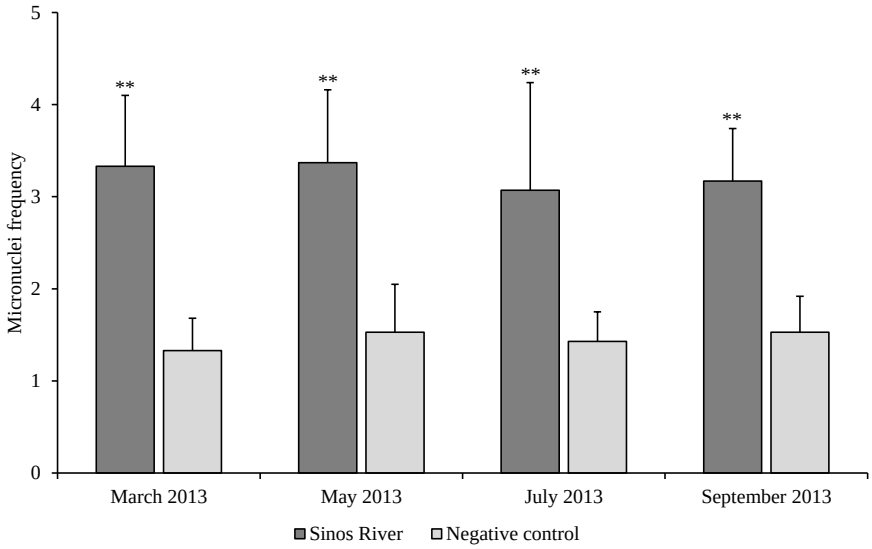

Figure 1. Frequency of micronuclei (mean ± SD) in *Tradescantia pallida* var. *purpurea* exposed for 8 hours to water from the Sinos River and the negative control, from March to September 2013. Asterisks indicate highly significant difference between frequencies recorded for river water and negative controls, according to the Student *t* test (p=0.05).

The low variation between the lowest and highest MCN frequencies observed in tetrads of flower buds exposed to samples of water from the Sinos River indicates that the site exhibited relatively constant genotoxic potential throughout the monitored period. Micronuclei frequencies significantly higher than those of the negative control may be due to the presence of substances with genotoxic potential in the water of the Sinos River. Physicochemical analyses performed to evaluate the water quality at different points of the Sinos River reported the occurrence of pollutants. Excessive levels of biochemical oxygen demand, chemical oxygen demand, total nitrogen and total phosphorus were detected at different sampling points of the river (Blume et al., 2010). A study conducted by the State Foundation of Environmental Protection of the State of Rio Grande do Sul indicated rates of heavy metals above those established by the National Environmental Council (CONAMA) Resolution 357/2005 for class 3 water bodies (Brasil, 2005), especially in the lower section of the basin (FEPAM, 2014). The presence of hexavalent chromium in water sampling points in the lower section of the river was associated with increased frequency of MCN in meristematic root cells of *Allium cepa* and *Eichhornia crassipes* (Oliveira et al., 2012).

The release of domestic and industrial effluents as well as the use of agricultural chemicals are the main sources of pollution of water bodies in the Sinos River Basin (Lemos et al., 2009; Figueiredo et al., 2010). Recently, the active micronuclei bioassay using *Tradescantia pallida* var. *purpurea* was applied in monitoring studies of water bodies in the lower section of the Sinos River Basin evidencing genotoxicity in water samples from the Estância Velha/Portão and Pampa streams (Kieling-Rubio et al., 2014) and from the Vila Kunz stream (Endres et al., 2014).

4. CONCLUSION

Adaptation and recovery of cuttings with flower buds in distilled water or Hoagland solution and exposure of cuttings for different time periods did not influence the response of *Tradescantia pallida* var. *purpurea*, indicating that all the methodological conditions tested are viable for *ex situ* biomonitoring of water genotoxicity. Under the experimental conditions of the present study, the bioassay allowed the detection of genetic damage on the bioindicator, probably as a response to synergic effects of the complex mixture of pollutants in the water of the Sinos River. It is important to maintain the bioassay under controlled conditions, in order to ensure that any cell damage observed is only related to the effects of genotoxic agents present in the water body being analyzed.

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