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Evaluation the urban atmospheric conditions in different cities using comet and micronuclei assay in *Tradescantia pallida*

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HIGHLIGHTS

- We examined and correlated methods for evaluation of pollution atmospheric in towns in region of Mato Grosso do Sul.
- We association the frequency of genotoxic and mutagenic damage in *T. pallida* with vehicular traffic in the towns monitored.
- Climatic factors influenced in formation of damage genetics in *T. pallida*.

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ABSTRACT

In the present study, genotoxicity and mutagenicity were investigated in *Tradescantia pallida* exposed to vehicular traffic at different sites in a high-altitude tropical climate. During March, May, July, September, and November 2014, a comet assay and micronucleus bioassays were conducted on young inflorescences and leaves of *T. pallida* collected from twelve towns in the southern region of Mato Grosso do Sul with different amounts of vehicular traffic. Weather parameters (temperature, relative humidity and rainfall) were measured and vehicles were counted to determine traffic levels in each town. A higher frequency of genotoxic and mutagenic damage was observed in the municipality of Dourados. The highest frequency of genetic damage was observed in September and November according to both assays. Relative humidity and rainfall were inversely proportional to the frequency of genetic damage in *T. pallida* during the collection period. Based on these results, we conclude that the bioassays are efficient for assessing the effects of vehicular traffic in these towns with respect to weather conditions over time. These bioassays can be applied to identify risk areas, which are determined by climatic conditions and air pollutants released.

1. Introduction

Air pollution resulting from anthropic activities that release pollutants through stationary and mobile sources causes a significant deterioration in air quality (Teixeira et al., 2008). Therefore, it is necessary to monitor sites in order to detect the effects of these stressors on living organisms (Klumpp et al., 2001; Rodriguez et al., 2011).

Biomonitoring facilitates evaluation of the effects of such pollutants based on bioindicators, i.e., living organisms (Crispim et al., 2014). Among these bioindicators, plants are often used to determine the levels of contaminants; they have various advantages over conventional methods, such as the potential for larger sample sizes, lower operational costs, and higher sensitivity (Catinon et al., 2008).

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Stress factors for plants, such as water deficits, temperature increases, and air pollution, are usually correlated, and may be associated with cellular, biochemical, physiological, and molecular responses (Allen Junior and Prasad, 2004; Crispim et al., 2012; Leakey et al., 2006).

Among the bioassays used to monitor air pollutants, the comet assay and micronucleus test are considered particularly useful to assess changes in genetic material (Boettcher et al., 2010; Sengottaiyan et al., 2012; Leite et al., 2013).

The comet assay can be used to detect levels of DNA damage in various cell types using electrophoresis based on the migration of DNA fragments outside the nucleus. In this manner, cells are scored according to damage, which is related to the extent of fragment migration (Collins, 2004; Speit and Hartmann, 2005).

The micronucleus assay using *Tradescantia pallida* (Trad-MCN) is a straightforward methodology, in which the sample material is easily accessible, and has high sensitivity to genotoxic agents. The Trad-MCN test estimates the frequency of micronuclei, which result from the breakage and loss of chromosomes that are not attached to the spindle during meiotic cell division, thereby forming small and round structures (Bortoli et al., 2009; Meireles et al., 2009; Crispim et al., 2014; Spósito et al., 2015).

Development in Brazil is associated with cattle-raising activities and the expansion of agricultural production, especially in the Cerrado and Pantanal regions, which are characterized by high altitude and tropical climate (Gomes and Silva, 2012). The increase in agricultural production and population growth in these regions has resulted in economic, social, and environmental changes, and vehicular traffic has increased in the southern Central-West region of Brazil to enable the flow of products from southern Mato Grosso do Sul to other states, which has affected air quality (Leal et al., 2008).

Few studies have examined genetic changes caused by air pollution in the southern region of Mato Grosso do Sul; accordingly, in this study, mutagenic and genotoxic effects of pollutants released by motor vehicles on *T. pallida* var. *purpurea* were investigated in the southern region of Mato Grosso do Sul.

2. Materials and methods

2.1. Study area

This study was performed in the southern region of Mato Grosso do Sul, Brazil, a region known as Grande Dourados, including the municipalities of Nova Alvorada do Sul, Rio Brilhante, Itaporã, Douradina, Dourados, Deodápolis, Fátima do Sul, Vicentina, Glória de Dourados, Caarapó, Juti, and Jateí (Fig. 1). The region has the majority of the vehicle fleet in the state (211,982 vehicles). The main highway (BR-163) passes through some municipalities, connecting the region of Grande Dourados to Campo Grande, the state capital

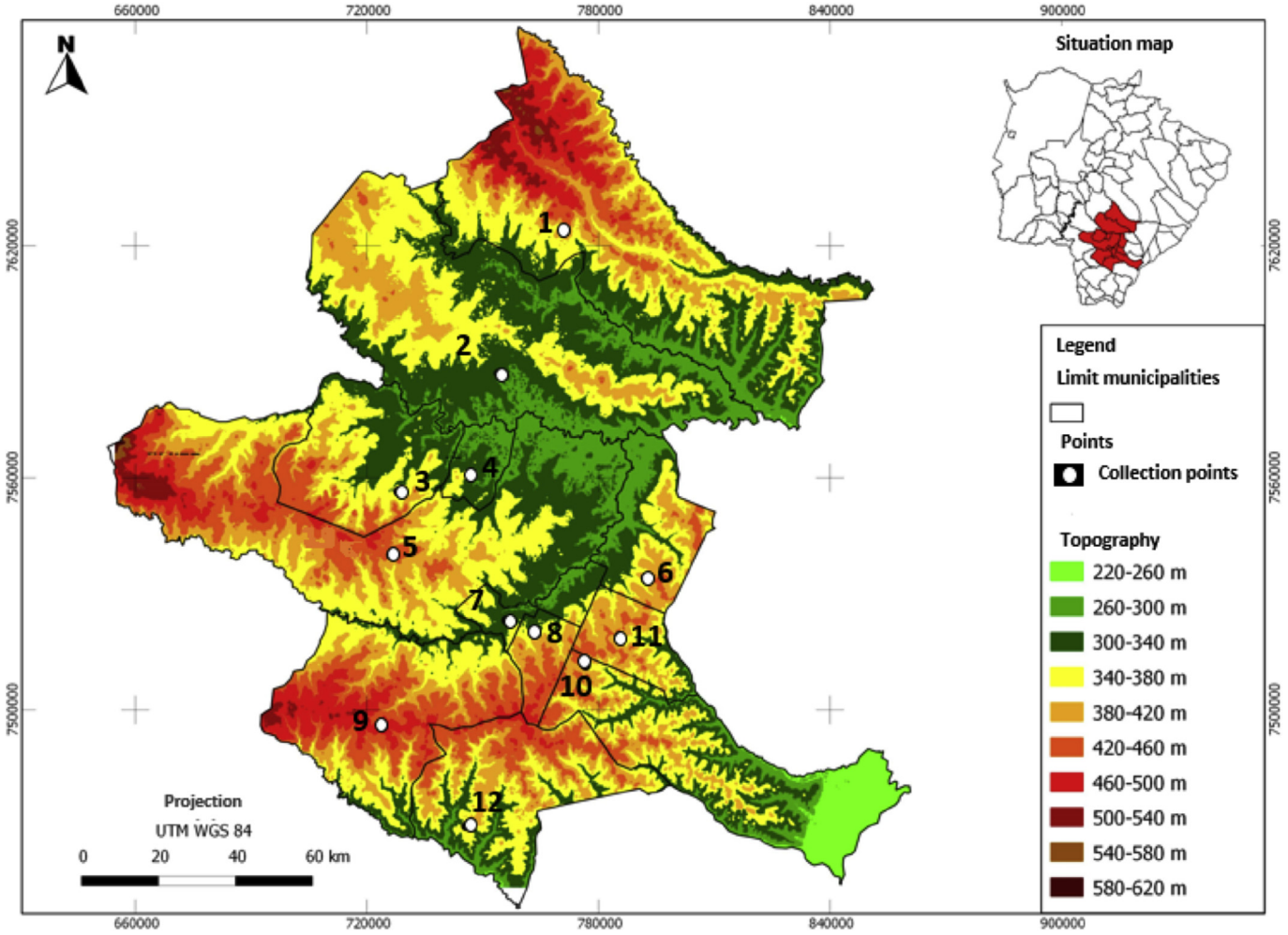

Fig. 1. Map of the southern region of Mato Grosso do Sul, illustrating altitudes and biomonitored sites: Nova Alvorada do Sul (1), Rio Brilhante (2), Itaporã (3), Douradina (4), Dourados (5), Deodápolis (6), Fátima do Sul (7), Vicentina (8), Caarapó (9), Jateí (10), Glória de Dourados (11) and Juti (12).

(Denatran, 2014).

2.2. Collection period and sampling

2.2.1. Trad-MCN

The *T. pallida* MCN test was developed according to Ma et al. (1994) protocol, with modifications. Young inflorescences were collected only once during the months of March, May, July, September, and November (2014) of ornamental plantings. The inflorescences were fixed in an absolute ethyl alcohol and acetic acid (3:1) solution. After 24 h, the inflorescences were transferred to a 70% alcohol solution. Slides were prepared for each site and stained with acetocarmine (2%). The number of micronuclei in 300 tetrads per slide was counted using an optical microscope (Nikon YS2; Tokyo, Japan) at 400× magnification and the results are expressed as percentages (frequency of micronuclei in 100 tetrads).

2.2.2. Comet assay

An alkaline comet assay was performed according to the methods of Boettcher et al. (2010). Five random leaves of *T. pallida* were collected of ornamental plantings in May, July, September, and November of 2014, leaves were macerated with 1 mL of phosphate-buffered saline and 500 μL of EDTA (200 mM) (ultrapure water and EDTA-Titriplex 100 mM: $C_{10}H_{14}N_2Na_2O_6.2H_2O$, pH 10.0); 60 μL of macerated tissue was diluted in 1 mL of phosphate-buffered saline. Slices were prepared using 80 μL of cell suspension and 200 μL of low-melting-point agarose (0.5%, v/v) at 37 °C.

The slides were immersed in glacial lysis buffer (NaCl 2.5 M, EDTA-Titriplex 100 mM, Trizma base 0.01 M, DMSO 10%, Triton X-100 1%, pH 10.0) for 1 h at room temperature in the dark. The slides were placed in a horizontal electrophoresis chamber containing a freshly prepared electrophoresis buffer (NaOH 0.3 M, EDTA 0.001 M, pH 13.0), where they were maintained for 20 min to allow DNA to unwind. DNA migration in this buffer was allowed to proceed for 25 min at 25 V (300 mA, 0.8 V/cm). The slides were washed in Tris Base (0.4 M, pH 7.5) for 15 min at room temperature, dehydrated in ethanol (100%), and air-dried. Two slides were prepared for each replicate (n = 5) and stained with ethidium bromide (0.02 mol L^{-1}). Each image was scored according to the extent of DNA migration based on a visual analysis (0, undamaged; 1, mild damage; 2, moderate damage; 3, severe damage; 4, complete damage) using a fluorescence microscope (400× magnification, Labomed T121100; Los Angeles, CA, USA) by a single observer. A total of 100 cells on each slide were scored, and the total scores obtained for slide were between 0 and 400 arbitrary units (AU microgel_1) (Kocyigit et al., 2005). The total amount of DNA strand breakage was expressed in total arbitrary units (AU_T) defined as: $AU_T = N_0 \times 0 + N_1 \times 1 + N_2 \times 2 + N_3 \times 3 + N_4 \times 4$, where N_i is the number of nuclei scored in each category (Collins, 2002).

2.3. Environmental parameters and vehicular traffic

Relative humidity (%) was measured using a digital hygrothermometer (Minipa, MTH-1361, Houston, TX, USA) in May, July, September, and November. The monthly averages for temperature and rainfall were based on data provided by Climatempo, a weather forecast company, for March, May, July, September, and November (Table 1).

The topographical characteristics of the area based on SRTM (*Shuttle Radar Topographic Mission*) data were obtained from Embrapa Satellite Monitoring. These data consisted of digital elevation models generated by the National Aeronautics and Space Administration in 2001, and have a 90-m original grid resolution. SRTM data were processed using the GIS (Geographic Information System) application Quantum GIS, version 1.8 to generate a hypsometric map representing different elevation strata in the study area. The hypsometric map was used to identify the altitudinal ranges for the towns included in the present study.

Vehicular traffic was assessed by counting the number of vehicles per hour that passed the study sites at three different time of the day (from 7:30 a.m. to 8:30 a.m., 11 a.m. to 12 p.m., and 4 p.m.–5 p.m.). Following this, the average number of vehicles circulating at each site was calculated.

2.4. Statistical analysis

The analyses were performed using SAS University Edition (SAS, 2014). The Shapiro-Wilk test was used to verify the normality of the residuals, and the Bartlett test was used to examine the homogeneity of the variances.

All characteristics that met the assumptions of normality and homogeneity were used for an analysis of variance and posterior averages were compared using the Tukey's test at the 5% probability level. Pearson's chi-squared test was also applied to calculate correlations between variables. The level of significance was 5%.

3. Results

The analysis of variance results considering the effects of city, period, and their interaction on micronucleus frequency (MCN) and DNA damage (arbitrary units) are summarized in Table 2.

The numbers of MCN and DNA Damage (Arbitrary Unit) were

Table 1
Sampling sites, altitude (m), average number of vehicles per hour (ANV), temperature °C (temp), relative humidity % (RH), and pluviosity mm^3 (Pluv) at each month during the collection period.

Collections sites	Altitude	ANV	March			May			July			September			November		
			Temp	RH.	Pluv	Temp	RH.	Pluv	Temp	RH.	Pluv	Temp	RH.	Pluv	Temp	RH.	Pluv
Caarapó	470.2	561.87	24.6	–	4.2	19.8	53.95	3.9	18.85	53	3	25.05	45.45	2.6	25.05	41.9	25.05
Deodápolis	407.5	219.33	25.2	–	4.8	20.3	43.9	3.9	19.2	56.15	4	25.05	52.95	4.5	25.7	32.95	25.7
Douradina	341.1	40.33	24.85	–	5	19.9	49.35	3.8	19.05	59.05	3.2	25.05	56.05	2.8	25.25	55.55	25.25
Dourados	450.2	559.16	24.9	–	5.1	19.75	56.1	3.2	18.85	44.35	3.3	25	43.2	0.4	25.2	37.95	25.2
Fátima do Sul	337.4	274.49	25.2		5	20.3	73.2	3.8	19.2	52.95	3.2	25.05	43	2.8	25.65	35.9	25.65
Glória de Dourados	411.5	356	25.2		4.8	20.3	45.6	3.9	19.2	45.15	4	25.05	46.25	4.5	25.65	36	25.65
Itaporã	350.3	110.83	24.85	–	5	19.85	69.35	3.6	18.85	70.55	3.3	25	54.6	0.4	25.2	35.95	25.2
Jateí	395.6	52.66	25.2	–	4.8	20.3	48.9	3.9	19.2	47.5	4	25.05	48.7	4.5	25.65	38.5	25.65
Juti	375.1	35.99	25.45	–	4.6	20.15	61.5	4	18.95	54.55	6	25	51.75	4.7	25.7	41.15	25.7
Nova Alvorada do Sul	419.0	64	25.55	–	4.7	20.5	55.25	4.7	19.6	59	3.5	25.15	73.9	3.6	25.85	41.75	25.85
Rio Brilhante	318.0	674	25.45	–	7.3	20.1	52.7	6.6	19.2	56	4.5	25	67.1	7.5	25.8	44.4	25.8
Vicentina	359.9	251	25.2	–	4.8	20.3	64.95	3.9	19.2	47.45	4	25.05	43.95	4.5	25.65	38.6	25.65

(–) No collection.

Table 2
Analysis of variance data for the average micronucleus frequency (MCN) and DNA damage (AU), considering the sampling city and collection period in *Tradescantia pallida*.

Cause of the Variation	MCN		DNA Damage (Arbitrary Unit)	
	DF	Quadratic means	DF	Quadratic means
City	12	24.22***	12	1977.18***
Periods	4	174.28***	2	7831.24***
City*Periods	38	4.77***	36	314.54***
CV (%)	23.38		15.68	
R^2	0.93		0.85	

Significance: ***$p < 0.0001$; CV, Coefficient of variation; DF, degrees of freedom.

significantly influenced by the variable city and periods, as well as by the interactions of these variables.

The frequency of MCN in *T. pallida* tetrads in March did not differ significantly between cities. The highest MCN frequencies were observed in September and November. Among the cities, Dourados had the highest level of genetic damage (Table 3).

The comet assay results are summarized in Table 4. In May, there were no significant differences among cities, with the exception of Dourados, which showed a high frequency of DNA damage (arbitrary units, 72.75). Based on the average values across all months, Dourados had a higher frequency of DNA damage than other monitored towns using both tests.

With regard to the sampling periods, according to the Trad-MCN and comet assays, all monitored towns showed a higher frequency of DNA damage in September and November than in other months. There were significant positive or negative correlations among most variables (Table 5).

Based on Pearson correlation analyses, there was a negative association between MCN, RH, and pluviosity, and a positive association between MCN, AU, temperature, and traffic. The observed associations indicate that the frequency of MCN was inversely proportional to RH and pluviosity; that is, higher numbers of vehicles and temperatures were associated with an increased frequency of MCN and AU in *T. pallida*. Increases in pluviosity and relative humidity were accompanied by a decrease in the frequency of MCN.

Caarapó, Deodápolis, Dourados, Glória de Dourados and Nova Alvorada do Sul are at high altitudes, ranging from 407.5 to 470.2 m (Fig. 1). Although the Pearson's correlation coefficient was low, indicating that there was no correlation between altitude and genetic data (MCN and AU), we observed a significant negative correlation between these parameters and rainfall.

4. Discussion

These results demonstrated that *T. pallida* was susceptible to the effects of air pollutants from vehicles. Similar results have been observed for ginkgo (*Ginkgo biloba*), pothos (*Epipremnum aureum*), periwinkle (*Vinca rosea*), and *T. pallida* plants exposed to air pollutants. DNA damage was detected in these plants using the micronucleus test and comet assay. We confirmed the utility of these techniques for determining mutagenicity and genotoxicity in different plant structures (leaves and inflorescences) (Costa and Droste, 2012; Sriussadaporn et al., 2003).

Compared to the other monitored sites, Dourados has the largest vehicle fleet (119,060) (Denatran, 2014), and consequently showed a high incidence of genetic damage according to the results of both tests. According to Costa and Droste (2012) and Spósito et al. (2015), a higher incidence of genetic damage in bioindicator plants is associated with urban areas with large vehicle fleets and high concentrations of airborne pollutants.

Based on an evaluation of mutagenicity in the tetrads of anthers and genotoxicity in the leaves, *T. pallida* from Rio Brilhante and Caarapó showed a high frequency of DNA damage, which can be attributed to pollutants associated with the vehicle fleets of the aforementioned towns (13,000 and 10,505 vehicles, respectively) (Denatran, 2014). Other factors might also contribute to the increase in damage. In Rio Brilhante, the high frequency of genetic damage might also be associated with traffic-control devices, such as speed bumps, electronic speed control devices, and roundabouts located near plant collection sites. Traffic-control devices placed at the streets increase the concentration of air pollutants released by vehicles due to acceleration and consequently increase fuel consumption (Moura and Fernandez, 2012).

In the case of Caarapó, the plant collection site was located along a main access road to the municipality (BR-163), where light and heavy vehicle traffic is continuous and intense, and this resulted in an increased frequency of genetic damage compared to other towns with a low traffic flow. The emission of gas pollutants depends on the vehicle and fuel type. Petroleum derivatives commonly used by heavy vehicles for cargo and passenger transport are responsible for the majority of the greenhouse gas emissions (MMA, 2009).

Based on the correlation analysis, temperature and collection period were positively related to the occurrence of genetic damage. In particular, there was a higher frequency of genetic damage according to both tests (Trad-MCN and a comet assay) in September and November than in the other monitored months. These results might be related to the lower relative humidity in these periods than in the other months. Similar observations were reported by

Table 3
Average micronucleus frequency observed in tetrads of *Tradescantia pallida* at the collection sites, periods and average collection periods, indicating their statistical differences as determined by the Tukey's test at a 5% significance level.

Collections sites	March	May	July	September	November	Average
Nova Alvorada do Sul	1.11 bA	1.55 bA	1.55 bB	–	5.22 aB	2.35 C
Rio Brilhante	0.77 cA	2.39 bcA	5.11 abA	5.78 aB	5.66 aB	3.94 B
Dourados	1.33 eA	4.39 dA	6.11 cdA	13.33 aA	9.11 bA	6.85 A
Douradina	0.44 cA	1.16 bcB	2.39 abcB	–	4.44 aB	2.10 D
Fátima do Sul	0.55 dA	4.22 bcA	3.89 cA	6.66 abcB	7.66 aA	4.59 B
Glória de Dourados	0.33 cA	1.44 bcB	3.38 abA	5.22 aB	5.83 aB	3.24 C
Vicentina	1.33 bA	2.66 abA	2.78 abB	3.50 abC	4.28 aB	2.90 C
Jateí	1.22 aA	–	2.50 aB	–	–	1.73 D
Deodápolis	0.33 cA	2.61 bcA	2.89 abcB	5.44 abB	5.50 aB	3.35 C
Juti	0.94 eA	2.44 cdeA	2.11 deB	3.00 bcdeC	8.11 aA	3.31 C
Caarapó	0.33 dA	1.77 cdA	3.38 bcA	7.33 aB	7.11 aA	3.98 B
Itaporã	0.78 dA	2.66 cdA	3.11 bcdB	–	7.00 aA	3.38 C

Averages followed by the same lowercase letters within a row represent comparisons of the variables among months and average with the same uppercase letters within a column represent comparisons among cities. Averages followed by the same letter did not differ significantly; (–) No collection.

Table 4
Average DNA damage in the leaves of *Tradescantia pallida* based on a comet assay in sites in the southern region of Mato Grosso do Sul.

Collections sites	DNA Damage				
	May	July	September	November	Means
Nova Alvorada do Sul	22.75 cB	34.25 bcC	70.75 aA	57.00 abB	46.18 C
Rio Brilhante	31.00 cB	49.75 bcB	84.00 aA	83.00 aA	61.93 B
Dourados	72.75 aA	88.50 aA	70.00 aA	71.50 aA	75.68 A
Douradina	35.25 aB	39.00 aB	42.50 aB	45.50 aB	39.50 D
Fátima do Sul	33.50 bB	61.25 aB	56.75 abB	55.50 abB	51.75 C
Glória de Dourados	26.75 bB	58.50 aB	62.50 aA	63.25 aA	52.75 B
Vicentina	29.25 bB	63.00 aB	59.25 aB	57.75 aB	52.31 B
Jateí	19.25 bB	44.50 aB	52.25 aB	55.50 aB	41.08 D
Deodápolis	32.50 bB	56.25 abB	60.50 aA	60.00 aA	52.31 B
Juti	34.75 bB	56.75 abB	61.75 aA	70.75 aA	56 B
Caarapó	43.00 bB	73.50 aA	66.25 abA	65.75 abA	62.12 B
Itaporã	33.25 aB	57.25 aB	55.75 aB	56.50 aB	50.68 C

Averages followed by the same lowercase letters within a row represent comparisons among months and the same uppercase letters within a column represent comparisons among cities. Averages followed by the same letter did not differ significantly based on the Tukey's test at a 5% significance level.

Table 5
Correlations (and associated p-values) among collection date, micronucleus frequency (MCN), arbitrary units (AU, comet assay) in *T. pallida*, relative humidity (RH), temperature (Temp), rainfall (Plu), altitude, and vehicular traffic.

	MCN	AU	RH.	Temp.	Plu.	Altitude	Vehicular traffic
Collection	**0.70**	**0.52**	**−0.47**	**0.29**	**−0.47**	0.00	–
MCN		**0.68**	**−0.51**	**0.20**	**−0.45**	0.11	**0.40**
AU			**−0.44**	**0.31**	0.06	0.08	**0.66**
RH.				**−0.38**	0.05	−0.09	**−0.32**
Temp.					−0.05	−0.02	−0.07
Plu.						**−0.28**	0.08
Altitude							0.11

Significant correlations ($p < 0.05$) are indicated in bold.
(–) Traffic data were only collected once.

Spósito et al. (2015), who found that low temperatures and relative humidity increase the incidence of air pollutants and consequently increase genetic damage.

These findings are consistent with those of Pereira et al. (2013), who demonstrated that relative humidity and rainfall are positively related to the frequency of genetic damage in *T. pallida*. Another underlying factor was the difference in vehicular traffic between monitored towns. Towns with a larger vehicle fleet had significantly higher levels of air pollutants, i.e., genotoxic and mutagenic agents, which compromise environmental quality.

Although there was no significant correlation between altitude and genetic damage, a negative correlation between altitude and rainfall was observed; these factors negatively influenced the frequency of MCN in the study areas. According to Roldão et al. (2012), climate modifiers, such as altitude, are typically correlated with temperature and rainfall. These factors might directly influence the frequency of damage, thus corroborating the results of this study.

5. Conclusion

In conclusion, the development of reliable and low-cost techniques based on plants might complement and reinforce conventional techniques used to evaluate pollution. Despite the greater availability of biological material, i.e., inflorescences, the cost of the comet assay is higher than that of the Trad-MCN test, and it requires more expertise for sample preparation. Nevertheless, the results of the two bioassays were consistent, indicating that the techniques are both effective for detecting DNA damage in plants subjected to environmental stressors, such as air pollutants.

Bioassays using *T. pallida* might be used to support the adoption of environmental policies in other municipalities in Mato Grosso do Sul and can enable the identification of risk areas and draw attention to weather conditions on a regional scale.

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