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ARTÍCULO ORIGINAL
ORIGINAL ARTICLE

Air quality assessment using the Pollen Abortion assay in *Tradescantia pallida* in a Mid-sized City in Southern Brazil

Evaluación de la calidad del aire utilizando el ensayo de aborto de polen en *Tradescantia pallida* en una ciudad de tamaño mediano en el sur de Brasil

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Abstract: Urbanization is an important source of air pollutants that can compromise human health. In developing countries, such as Brazil, most cities do not have air quality monitoring stations. Assessing air quality through plant species has gained recognized prominence, as they are sessile organisms and sensitive to environmental changes. Pollen abortion assay in *Tradescantia pallida* is a fast and low-cost bioassay that can be implemented in passive biomonitoring scenarios. The present study aimed to use the pollen abortion assay in *T. pallida* to assess air quality in the municipality of Rio Grande, RS, Brazil and the possible relationship with vehicular flow. A relation was found between the highest rate of pollen abortion and the sites where there was greater vehicular flow and ozone levels, while at the control point, the lowest rate of pollen abortion among the others was found, corroborating the hypothesis that air pollution together with high levels of ozone from vehicles, impair plant pollination.

Keywords: air pollution, biomonitoring, pollen, genotoxicity.

Resumen: La urbanización es una fuente importante de contaminantes atmosféricos que pueden comprometer la salud humana. En los países en desarrollo, como Brasil, la mayoría de las ciudades no tienen estaciones de monitoreo de la calidad del aire. La evaluación de la calidad del aire a través de especies vegetales ha ganado una importancia reconocida, ya que son organismos sésiles y sensibles a los cambios ambientales. El ensayo de aborto de polen en *Tradescantia pallida* es un bioensayo rápido y de bajo costo que puede ser implementado en escenarios de biomonitoreo pasivo. El presente estudio tuvo como objetivo utilizar el ensayo de aborto con polen en *T. pallida* para evaluar la calidad del aire en el municipio de Rio Grande, RS, Brasil y la posible relación con el flujo vehicular. Se encontró relación entre las tasas más altas de aborto de polen y los sitios donde hay mayor flujo vehicular y niveles de ozono, mientras que en el punto de control se obtuvo la tasa más baja de aborto de polen entre los demás, corroborando la hipótesis de que la contaminación del aire junto con altos niveles de ozono de los vehículos perjudica la polinización de las plantas.

Palabras clave: contaminación atmosférica, biomonitoreo, polen, genotoxicidad.

1. INTRODUCTION

Air pollution represents one of the biggest environmental risks to health(1). This pollution is characterized by a mixture of components such as particulate matter (PM), ozone (O_3), nitrogen oxides (NO_x), sulfur oxides (SO_x), carbon monoxide (CO), volatile organics, polycyclic aromatic hydrocarbons (PAHs) and metals at disproportionate concentrations in air, and is constantly modified by sunlight and temperature(2). In addition, air pollution is exacerbated near heavy traffic routes. Around the world, there are legal limits to the presence of these components in the air. In Brazil, these limits were recently updated by the Conselho Nacional do Meio Ambiente (CONAMA) in its 2018 resolution, seeking to ensure air quality for the population(3). The pollutants and limits set out in this resolution are based on those established by the World Health Organization and include: Particulate Material (PM_{10} and $PM_{2.5}$), NO_2, CO, O_3, SO_2 and Pb in the PM(4).

Although Brazil and other developing countries have legal provisions for controlling environmental pollutants, the number of studies monitoring air pollutants and their effects is limited(5). It is estimated that in Brazil, less than 2% of cities have air quality monitoring stations(6). The high cost of installing and maintaining monitoring stations combined with the low priority given to environmental issues by local governments has led scientists to seek low-cost alternatives to provide the population with safe and accurate answers regarding air pollution and its effects on the environment and health. In this regard, plants bioassays are suitable biomonitors and bioindicators of the genotoxicity of a polluted air due to their high sensitivity and ability to accumulate harmful substances[7]. Plant bioassays have been successfully used for in situ exposure studies of air quality monitoring[7,8,9,10,11,12]. Examples of these bioassays are the *Tradescantia pallida* micronucleus[11,12] and pollen abortion assay[7,8,9,10]. The latter is recognized for allowing the identification of the physiological response to short changes in the spatial gradient of air pollutants[9]. Furthermore, this methodology is simpler and less expensive than conventional air quality monitoring techniques, and can be used in areas without the support of sophisticated analytical laboratories[10].

Ozone is one of the air pollutants characterized by being highly phytotoxic in high concentrations[13]. This photochemical pollutant is recognized for its chronic effects on cultural crops, causing reduction of growth or yield, thus impacting global food security[14]. It is possible that the use of a plant genotoxicity assay (pollen abortion assay) in conjunction with measurements of ozone concentrations may serve as an alternative for assessing regional air quality. The study, carried out by Fleck et al.[15] in a city with a high demographic density, demonstrated the possible relation between high ozone concentrations and genotoxicity in plants through the pollen abortion test. In addition, this pollutant can be measured through passive monitoring, which, in addition to being a simple and less expensive apparatus, also allows to obtain a pollution gradient for the studied region.

Our study was performed in the city of Rio Grande, located in the state of Rio Grande do Sul, Brazil. This city has a population density considered to be medium sized[16]. It is characterized by its industrial activity and one of the largest ports in Brazil, and has already served as a scenario for studies that investigated the influence of the industrial complex on the environmental quality of the municipality[17,18,19,20]. Rio Grande is the city with the highest ozone levels in its region[21]. Therefore, the aim of the present study was to evaluate the air quality in a medium sized city with high influence of industrial pollution by means of passive monitoring of ozone as well as to assess the genotoxic effects of these pollutants by using the pollen abortion assay in *T. pallida*.

2. MATERIALS AND METHODS

Study area and sample collection

The city of Rio Grande is located in the state of Rio Grande do Sul, in the extreme south of Brazil. There are approximately 211,005 inhabitants in this area[22]. The climate is humid subtropical with heavy and regular precipitation throughout the year. In winter, the average minimum temperature is 2 ºC and the total average is 13.4 ºC. In summer, the average minimum temperature is 18 ºC and the general average is 22.6 ºC. The relative humidity of the air varies between 77% and 90%. The predominant winds are from the northeast, followed by southeast and southwest.

The sampling was carried out during the autumn months of April and May. *T. pallida* samples were obtained at four different sites, which are explained in Table 1.

Table 1. Synoptic table containing the characteristics of the sampling sites

	Location	Characteristics	Coordinates
Site 1 (control)	Campus of the Universidade Federal do Rio Grande – FURG	Small population density and away from the city center and the industrial hub	32°04'33.9"S 52°10'05.0"W, altitude 6 m
Site 2	Dr. Nascimento street	Downtown area with high population density	32°02'10.8"S 52°05'51.1"W, altitude 6 m
Site 3	Luiz Otero avenue	Recreational place in the city and away from industrial and downtown areas	32°10'39.0"S 52°09'06.4"W, altitude 4 m
Site 4	Buarque de Macedo street	Located away from the industrial hub but close to the city center	32°02'43.3"S 52°06'54.3"W, altitude 7 m

The inflorescences of *T. pallida* collected came from outdoor gardens and ornamental beds in the city, characterizing a passive biomonitoring study. The collected material was stored in a container with fixative (acetic acid and ethanol) and transported immediately to the laboratory.

The selection of *T. pallida* to act as an air quality monitor in our study is due to two main reasons: (1) in the study region, this species was introduced through cultivation (several years prior to this study), having its presence already disseminated in multiple sites of the city. Thus, it is considered an ornamental species in the region; (2) the genus Tradescantia has already been used to monitor air quality through cytogenetic tests such as micronucleus (Trad-MNC) or chromosomal aberrations, thus promoting the hypothesis that the pollen abortion test would allow monitoring of air quality.

Traffic flow in the studied sites

According to municipal officials, Rio Grande has about 129,485 vehicles(23). In order to better characterize the local exposures suffered by the studied species, the flow of vehicles in the studied places was obtained during peak hours (07:30 am to 8:30 am, 11:30 am to 12:30 pm, 6:30 pm to 7:30 pm), from 10 measurements. This data was kindly provided by the institute responsible for urban mobility in the city of Rio Grande (Urban Mobility Secretariat). Only motor vehicles were counted.

Passive Ozone Measurement

O_3 measurement was conducted by passive sampling during the same period of time as the pollen abortion assay. Cellulose filters were impregnated with indigotine disulphonate (IDS) solution (400 µL) following the methodology of Scheeren and Adema (1996). Then they were inserted into open diffusion tubes and placed at monitoring sites for 24 h. Blanks were obtained from filters exposed in the same conditions but were sealed from atmosphere contact. After exposure, the filters were removed from the samplers, placed into glass tubes with distilled water (5 mL) and sonicated in an ultrasonic bath for 5 min. Supernatants were then analyzed by spectrophotometry at 620 nm.

The O_3-8h concentration was estimated from the equation:

$$C = K\left(\frac{\Delta E}{t}\right)$$

C is the concentration (µg/m3), K is the calibration factor (902), ΔE is the difference between the sample and the blank absorbance, and t is the time of exposure in hours.

Pollen abortion assay using T. pallida

The pollen abortion assay followed the protocol established by Mičieta and Murín(23) and was performed with *T. pallida* flower buds from specimens located within a maximum radius of 20 meters from the study sites. Flower buds were collected concomitant with the monitoring period of O_3. Samples were fixed in an ethanol and acetic acid solution (3:1 v/v) and transferred to 70% ethanol solution after 24h. Pollen grains were extracted from anthers and pressed onto slides, which were stained with 0.5% aceto-carmine for microscopic evaluation. Slides were photographed with a digital camera directly attached to the microscope (Zeiss, Germany). Three hundred cells were evaluated per slide, and for each site ten slides were made, resulting in a total of 3000 cells per area.

Pollen grains were evaluated in terms of size, form and staining ability, with deviations considered to be evidence of abortion. The criteria used to establish pollen abortions were: presence of altered forms of pollen and staining deficiency(24). Young pollen grains were excluded from the analysis.

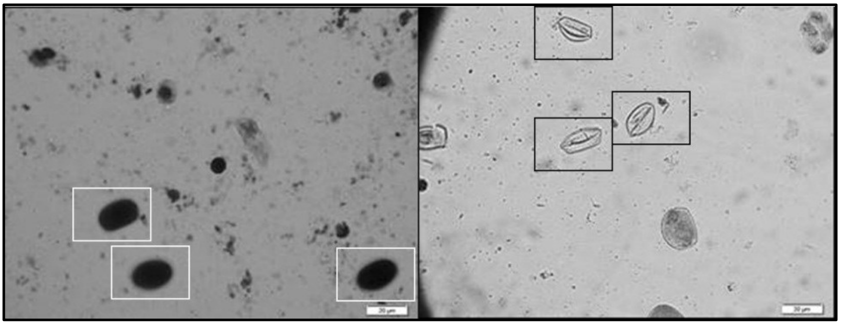

Figure 1. Normal (left, white rectangles) and aborted (right, black rectangles) pollen grain*
*The white ruler comprises 20μm.

Statistical analysis

Analysis of the data was performed using the PRISM 5 software. The means of the O_3 concentrations and vehicular flow were compared by one-way ANOVA followed by Tukey test, and pollen abortion frequency was analyzed by the non-parametric Kurskal-Wallis test and the means compared by the Dunns test, considering a critical value $p < 0,05$.

3. RESULTS AND DISCUSSION

The purpose of this study was to evaluate air quality throughout a medium sized city by using low-cost methodologies with simple procedures. This study made measurements of ozone and plants genotoxicity in locations with different intensities in the vehicular flow. Figure 2 shows the averages of pollen abortions in the evaluated sites. Sites 2 and 4 had a significantly higher number of abortions compared to site 1 (control). Regarding the number of motorized vehicles, site 2 had a higher number in relation to the other studied sites, also, sites 2,3 and 4 had a greater significant flow in relation to the control. Ozone levels had a similar behavior, with sites 2, 3 and 4 registering higher levels of ozone compared to control (Table 2).

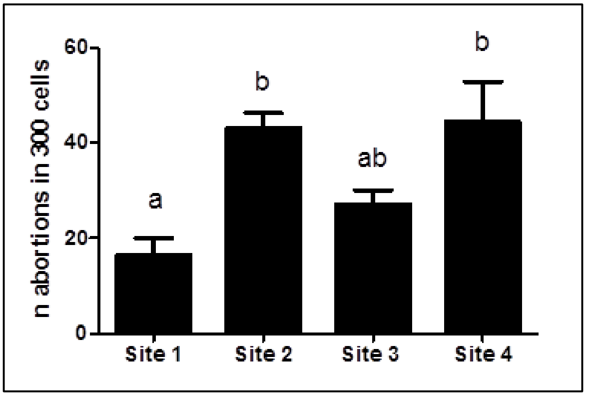

Figure 2. Average pollen abortion in 300 cells of *T. pallida* in the evaluated sites.

Table 2. Vehicular flow and ozone levels measured at the studied sites.

	Site 1	Site 2	Site 3	Site 4
Number of vehicles	$2223^{a} \pm 105$	$7301^{b} \pm 205$	$4595^{c} \pm 123$	$5741^{d} \pm 317$
Ozone (µg/m³)	$10.28^{a} \pm 1.88$	$55.42^{b} \pm 7.09$	$23.86^{c} \pm 0.07$	$49.79^{b} \pm 1.07$

^{a,b,c,d} different superscript letters indicate statistical difference using ANOVA followed by Tukey test ($p < 0.05$)

Our data show that sites with higher ozone concentrations have greater genotoxicity in the studied plant, and that satisfactory results were obtained with the pollen abortion test. In addition, we highlight the feasibility of using plants of the genus *Tradescantia*, which is normally used for mutagenic and chromosomal aberration tests (*Tradescantia* micronucleus assay), in simple genotoxic techniques such as the pollen abortion assay.

Usually, epidemiological studies that assess air quality in cities are based on results obtained from conventional networks of air quality monitoring systems. However, as pointed out by Carvalho-Oliveira et al. [10], this type of monitoring does not allow the spatial variation of pollutants throughout an entire city to be determined with the precision required to minimize possible errors in an exposure risk assessment. In view of this, there is the option of obtaining direct measurements in a study area, ensuring better spatial resolution, however this approach becomes too expensive and with complex logistical execution when the research needs to be performed in larger areas. In this context, the use of plants as air pollution biomonitors represents a good alternative for air quality management.

Our findings are in line with other studies that indicated that the pollen abortion assay is a sensitive tool for investigating air pollution effects[7,8,9,10,24,25,26]. This assay is highly sensitive since the target cells (microspores) are haploid and detect lethal mutations which affect the development of pollen[25]. Another advantage of this experimental model is that the indicator species can be plants cultivated in the region (as in our study) and also with native species as pointed out by Solenská, Mičieta and Mišík[27]. Furthermore, this technique is able to identify the physiological response to short changes in the gradient of air pollutants[9] and can be used in larger areas without the support of sophisticated analytical laboratories.

To the best of our knowledge, only the study by Fleck, Moresco and Rhoden[26] sought to assess the relationship between ozone and the pollen abortion test. However, in that study, the authors found a strong positive correlation between NO_2 concentration and the result of the test, but it did not show a correlation with ozone. In contrast to this, our results demonstrated that the measured ozone levels and the number of pollen abortions reveal a strong positive correlation. This result is somewhat expected, since ozone is highly phytotoxic in high concentrations and is recognized for its chronic effects on plants. In addition, ozone is a pollutant formed by vehicular emissions at ground level, so its values were also correlated with the vehicular flow of each location.

An important aspect of our study is that both ozone and genotoxicity markers were obtained from passive biomonitoring. This offers advantages over other methodologies where the data related to air quality are obtained at fixed points because, despite being the usual procedure, this measure represents an average of local pollution, but ignores the different pollution gradients throughout an entire city. This approach does not accurately reflect the exposure of the population in the different scenarios of a city. Thus, the techniques used in our study were simpler and less expensive than conventional air quality monitoring and allow for monitoring of extensive areas, resulting in improved spatial resolution of air quality monitoring.

Another aspect to be emphasized is the use of the species *T. pallida* for the pollen abortion assay. Usually, the genus *Tradescantia* is used to monitor air quality through cytogenetic tests such as micronucleus (Trad-MNC) or chromosomal aberrations(25). However, these tests require more time and skill from analysts. On the other hand, the pollen abortion test, besides having already scientific support from other studies(7,8,9,10,16,25), is easy to evaluate because it is based on the simple confirmation of the pollen grain color or not. Our results allow us to confirm the sensitivity of this species to assess air quality through pollen abortion testing.

4. CONCLUSION

The pollen abortion test using *T. pallida* proved to be an effective method and was associated with air pollution in a municipality with an intense urban flow. It is a simple and accessible bioassay with a close relationship with vehicular traffic and ozone levels. There may be the possibility of associating the test with more air quality data to obtain a more accurate bioassay result. Moreover, tests using plants as bioindicators can help monitoring air quality and also assess possible genotoxic risk for humans.

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