Biologia Ambientale, **32**: I&D 9-13 (2018)
DOI 10.30463/id181.002

Towards a validation of *Tradescantia* micronucleus bioassay for genotoxicity monitoring in Alpine climates

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Pervenuto il 5.2.2018; accettato il 14.3.2018

Abstract

Tradescantia is a tropical plant, adapted to climates with high humidity, constant high temperatures and bright sunshine and is routinely used for genotoxicity monitoring. Its suitability has never been tested for screens in Alpine continental climates, characterised by cold, dark winters and periods of very low humidity. In this study the performance of *Tradescantia* micronucleus (MCN) test has been verified in an Alpine environment in Central Europe (South Tyrol). In situ monitoring was carried out from 2011 to 2014. Results indicate that the validity of the tropical plant *Tradescantia* for biomonitoring of genotoxic pollutants is strongly impaired by alpine climate, which results in strong fluctuations of micronucleus frequency and therefore to high standard variations. A solution for future measurements could be to quantify the effects of environmental factors like temperature and light exposure on micronucleus frequency, and to use this information to calibrate the system and standardize the results.

Key words: environmental monitoring / mutagenicity / air pollution / wood fire

Verso la validazione del test dei micronuclei con *Tradescantia* per il monitoraggio della genotossicità in ambiente alpino

La pianta appartenente al genere *Tradescantia*, di origine tropicale e quindi adattata a un clima con elevata umidità e temperatura, viene comunemente impiegata nel monitoraggio della genotossicità. In questo studio è stata verificata l'efficacia del test dei Micronuclei (MCN) con *Tradescantia* in condizioni climatiche estreme, come quelle continentali alpine in Alto Adige (Centro Europa). Il monitoraggio è stato svolto dal 2011 al 2014. I risultati indicano che la validità del test con la pianta tropicale *Tradescantia* per il biomonitoraggio degli inquinanti genotossici in un clima alpino è notevolmente alterata, comportando una forte variabilità della frequenza di micronuclei e di conseguenza un'elevata deviazione standard. Una soluzione per l'impiego futuro di questo test potrebbe essere la quantificazione degli effetti dei fattori ambientali, come la temperatura e l'irraggiamento solare, sulla frequenza di MCN, per calibrare e standardizzare i risultati.

Parole chiave: monitoraggio ambientale / mutagenicità / inquinamento atmosferico / riscaldamento a legna

INTRODUCTION

The World Health Organization (WHO) considers air pollution as one of the major threats to human health; several studies show that the number of people affected by cancer in the respiratory system is significantly higher in urban areas (Bernstein *et al*., 2004; Nyberg *et al*., 2000). These areas contains a number of potentially cancerogenic particles such as polycyclic aromatic hydrocarbons (PAHs), benzene and arsenic. These substances are the result of incomplete combustion of fossil fuels, produced by traffic and industrial activity. The distribution of particulate matter deriving from wood fire and diesel engines seems to be similar, and wider than other air pollutants (Nussbaumer *et al*., 2005). Chemical analyses indicate that wood smoke is among the top contributors to particle pollution, particularly in rural regions

(Moshammer *et al.*, 2009). The harmful emission of wood fire is composed of CO, organic particles produced during incomplete combustion, inorganic salts found in ashes, fine dust and NOx (Oser *et al.*, 2004). Usually, air monitoring includes only some of these harmful substances. Additionally, only the risk from single substances has been recorded in the past (Klumpp *et al.*, 2006). However, Klumpp *et al.* (2006) report that synergistic, antagonistic or additive effects of chemicals in complex pollutant mixtures cannot be recorded using traditional chemical analytical systems. Biological monitoring procedures are thus required to evaluate the genotoxic potential of ambient air in situ. The plant genus *Tradescantia* (Fig. 1) is considered an ideal indicator of atmospheric pollution and the genotoxic effect of aerosols (Rodrigues *et al.*, 1997), and is one of the most promising models of environmental monitoring (Mišik *et al.*, 2011). The *Tradescantia* micronucleus (Trad-MCN) bioassay has successfully been used to conduct long-term monitoring (Mišik *et al.*, 2007; Ma *et al.*, 1996; Monarca *et al.*, 2001). *Tradescantia* has the capacity of absorbing polluting particles through its buds. If exposed to genotoxic and/or mutagenic substances, the plant modifies the mother cells of its pollen. From the nucleus of the pollen-cell, small chromatin-fragments are separated. These fragments are called "micronuclei" (Fig. 2). The goal of our study is to test the suitability of *Tradescantia* MCN bioassays to monitor atmospheric pollution caused by traffic and also by wood burning for heating purposes. In particular, we sought to establish whether this tropical plant is suitable for biomonitoring in alpine climates, or whether the validity of data is affected by unsuitable climatic conditions.

Fig. 1. The plant genus *Tradescantia*.

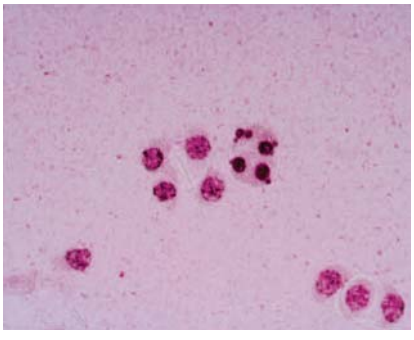

Fig. 2. Micronuclei in pollen mother cells of *Tradescantia*.

MATERIAL AND METHODS

Sampling sites

Samples from six sites in the Province of Bolzano (South Tyrol, Italy) have been analysed. The sites were independently distributed in urban and rural areas, and along the Brennero-highway A22, one of the main North-South axes in Europe (Fig. 3). The samples were taken five times from June 2011 to June 2014, with two samples taken in 2012.

Tradescantia – micronucleus assay

The plant used for the mutagenesis test belongs to the genus *Tradescantia*, and the clone used was #4430. It is a crossing of *T. hirsutiflora* Bush and *T. subcaulis* Bush. During the tests, we followed the procedure of Ma *et al.* (1994). For each sampling site, we used 15 young *Tradescantia* inflorescences. The stem was positioned in a glass beaker containing Hoagland's solution diluted 1:3, and exposed for 24 hours at the different sampling sites. Due to the low temperatures in November and December 2012, which potentially

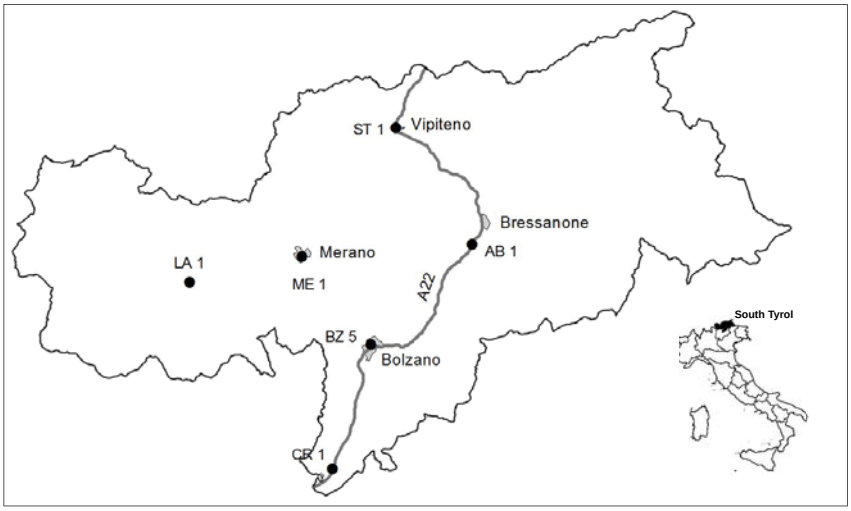

Fig. 3. Location of the *Tradescantia* sampling sites in South Tyrol, Italy. Dots represent the six sampling sites and the line the A-22 Brennero-highway, respectively.

led to plant damages, the plants were exposed only 6h a day. A control sample was handled in the lab at room temperature of 20+/-2°C. After this period of exposure the anthers containing the mother cells were separated and stained with acetocarmine stain. Afterwards, the early tetrads containing micronuclei and those without micronuclei were counted. For each sample at least five buds were checked and for each bud at least 300 tetrads were counted. The result was expressed as percentage of tetrads containing MCN.

Chemical analysis and statistical analysis

In addition to the analysis of MCN induction, the concentration of different chemical and physical parameters was measured, including mean air temperature, sun exposure, NOx and the particulate matters PM_{10} and $PM_{2.5}$. The statistical analyses were performed with SPSS Statistics 20. The micronucleus frequency was analysed with an analysis of variance with a significance level of $P<0.05$. Correlations between micronucleus frequency and the various chemical and physical parameters were also analysed using the Pearson correlation coefficient.

RESULTS

The values of gene mutation found at the sampling site of Chiusa (AB1) in June 2011, April 2012 and April 2013, were significantly higher compared to those of the negative control. The values of MCN for Vipiteno (ST1) recorded in April, December 2012 and April 2013 were significantly higher than the negative control. Significantly higher MCN values were also measured at the sampling site in Laces in June 2014 (Table I). Due to the relatively high variability of MCN data we didn't find any other statistically significant differences between the sampling sites and the negative control. Pearson's correlation analysis revealed a significant and strong negative correlation between the temperature and the micronucleus frequency (correlation coefficient = -0.613, P = 0.01) and between the sun exposure and the micronucleus frequency (correlation coefficient = -0.478, P = 0.05). The mean values of NOx, PM_{10} and $PM_{2,5}$, respectively, the temperature, and the sun exposure of the sampling sites Bolzano (BZ5), Cortina all'Adige (CR1), Merano (ME1), Laces (LA1), Chiusa (AB1) and Vipiteno (ST1) are reported in table II and table III.

DISCUSSION AND CONCLUSION

We found a significantly higher concentration of micronuclei in the analyzed *Tradescantia* buds in areas close to the A22 highway and thus with a high traffic volume compared to areas with lower traffic. The narrow profile of the Isarco valley, through which the highway A22 runs, seems to increase

Table I. Mean Trad-MCN/100 tetrads found at the six sampling sites on the respective sampling date.

	Trad-MCN/100 tetrads (Mean ± Standard deviation)				
Sites	**June 2011**	**April 2012**	**Nov./ Dec. 2012**	**April 2013**	**June 2014**
BZ 5 Bolzano	4.1 ± 0.85	5.2 ± 2.0	n.a.	4.9 ±3.1	n.a.
AB 1 Chiusa	7.1 ± 3.2**	7.7 ± 1.6*	7.4 ± 2.6	5.1 ±0.6**	5.7 ± 1.8
ST 1 Vipiteno	4.7 ± 1.7	8.1 ± 1.8*	16.0 ± 4.2**	7.8 ±3.3*	4.1 ± 2.0
CR 1 Cortina	4.4 ± 1.5	2.9 ± 0.3	n.a.	2.3 ±0.7	n.a.
ME 1 Merano	3.2 ± 1.2	3.3 ± 1.5	n.a.	4.6 ±2.2	n.a.
LA 1 Laces	3.4 ± 1.4	4.2 ± 1.2	7.7 ± 3.4	5.4 ±1.5	8.7 ± 3.2**
Control	2.6 ± 1.0	4.3 ± 0.7	5.4 ± 3.9	2.1 ±0.4	2.2 ± 1.3

n.a.= not assessed (no sampling performed).
* Asterisks indicate statistical significance compared to the negative control (Dunnett's t-test, P ≤ 0.05).
** Asterisks indicate statistical significance compared to the negative control (Dunnett's t-test, P ≤ 0.01).

Table II. Mean values of NOx, particulate matter 10 μm (PM_{10}), particulate matter 2.5 μm ($PM_{2.5}$), temperature (Temp), and solar irradiation (RAD) at the three sampling sites of Bolzano (BZ5), Cortina all'Adige (CR1) and Merano (ME1).

Station:	**Bolzano**					**Cortina a/A**					**Merano**				
Parameter	**NO_x** μg/m³	**Temp** °C	**RAD** W/m²	**PM_{10}** μg/m³	**$PM_{2.5}$** μg/m³	**NO_x** μg/m³	**Temp** °C	**RAD** W/m²	**PM_{10}** μg/m³	**$PM_{2.5}$** μg/m³	**NO_x** μg/m³	**Temp** °C	**RAD** W/m²	**PM_{10}** μg/m³	**$PM_{2.5}$** μg/m³
21/06/2011	51.6	24.5	49464	23.4	18.0	16.8	23.9	40008	21.9	14.9	32.5	23.0	37516	19.4	15.8
18/04/2012	68.8	17.9	33264	9.8	8.9	25.0	10.7	22422	4.4	4.8	92.2	16.2	39890	12.9	8.2
22/04/2013	68.8	14.6	29854	11,9	8.9	49.5	13.0	15920	11.7	11.8	53.0	14.5	20929	16.0	13.7

n.a.= not assessed (no sampling performed).

Table III. Mean values of NO_X, Particulate matter 10 µm (PM_{10}), Particulate matter 2.5 µm ($PM_{2.5}$), temperature (Temp), and solar irradiation (RAD) at the three sampling sites of Laces (LA1), Chiusa (AB1) and Vipiteno (ST1).

Station:	Laces					Chiusa					Vipiteno			
Parameter	NO_X µg/m³	Temp °C	RAD W/m²	PM_{10} µg/m³	$PM_{2.5}$ µg/m³	NO_X µg/m³	Temp °C	RAD W/m²	PM_{10} µg/m³	$PM_{2.5}$ µg/m³	NO_X µg/m³	Temp °C	RAD W/m²	PM_{10} µg/m³
21/06/2011	18.4	20.4	49893	12.1	12.7	116.0	22.5	53404	21.9	17.8	41.0	19.6	34702	15.6
18/04/2012	25.6	14.7	43482	9.2	8.8	141.7	8.7	27684	10.4	7.8	39.4	5.0	15039	7.9
06/11/2012	17.8	7.3	8778	5.0	2.5	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
05/12/2012	n.a.	n.a.	n.a.	n.a.	n.a.	552.8	0.3	5899	34.4	20.6	105.0	-0.2	5421	17.1
22/04/2013	22.6	12.2	22159	16.9	15.6	141.0	12.0	21482	17.5	14.2	29.0	9.7	19696	11.9
03/06/2014	17.8	16.4	35576	6.6	8.1	126.7	15.8	32395	8.9	9.1	38.0	13.1	32704	8.6

n.a.= not assessed (no sampling performed).

air pollution and MCN frequency, especially in combination with low temperatures, which is highlighted by the strong negative correlation found between the temperature and the MCN frequency. Similar results are reported by Isidori *et al.* (2003), who revealed a higher number of micronuclei in *Tradescantia* placed along highways with elevated traffic volume, particularly during the wintertime with low temperatures. *Tradescantia* is a plant originating from the tropical forest and is therefore not well adapted to prolonged periods with low temperatures which occur in alpine areas during winter. Thus, it was not always possible to conduct the mutagenesis test, especially in the coldest winter months. In addition, the control plants in the laboratory showed a slight increase of MCN values during winter, which hampered the detection of a statistical significant difference between the control plants and plants of the different sampling sites. This applies particularly to the sampling sites of Chiusa and Laces, which showed relatively high MCN values in December 2012, although the highest NO_X and PM_{10} values were recorded during this month and the exposition time of the plants was reduced from 24h to 6h a day. A reduction of the exposure time was necessary because the temperatures dropped below zero during night time which potentially led to plant damages. Laces is situated in a valley with a high use of wood heating during the cold season. We could reveal an increase of the mutagenicity in the coldest winter months compared to the control plants. Due to a high variability of data in winter recordings, this increase was, however, not statistically significant. A significant increase of the mutagenicity was only recorded in June 2014 for this sampling site, although there was no correlation with any physical-chemical collected data. Experiments conducted in the field like the present study generally show a higher variability of the data than laboratory experiments under strictly controlled conditions. Consequently, statistically significant differences between different sampling sites are much more difficult to reveal (Klumpp *et al.*, 2006). Variable climate conditions such as changes in the relative humidity, temperature, precipitation, and wind speed can affect the frequency of MCN in response to the presence of gene harming particles in the environment and can highly influence the performance of the experiments (Isidori *et al.*, 2003; Blasior *et al.*, 2005; Costa *et al.*, 2012; Klumpp *et al.*, 2004; Savoia *et al.*, 2009). The results of this study indicate that differences in air pollution can be detected using the higher plant genus *Tradescantia* as a biological indicator, but in areas with alpine climate low temperatures and light exposure seem to be limiting factors, restricting the suitability of *Tradescantia* to annual seasons with moderate to high temperatures and sufficient daylight. Furthermore MCN frequency variations along time draw attention to the importance of monitoring at various time points throughout the year (Junior *et al.*, 2015). To avoid these problems, in future studies it would be necessary to correct for such additional variables by quantifying the effect of factors like exposure to UV and visible light, temperature and humidity on the frequency of MCN. Therefore, a standardization especially of the exposure technique is necessary to reduce the variability of the data due to varying environmental conditions, and to allow the application of the *Tradescantia* MCN test for routine monitoring, also in areas with alpine climate or otherwise challenging conditions.

Acknowledgements

We thank the employees of the Laimburg Agency, who cultivated *Tradescantia* for us. Additionally we thank the people of the Chemical-Physical and Biological Laboratory of the Environmental Agency of Bolzano who helped realizing this research and M. Spitaler who provided language help and writing assistance.

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