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ARTICLE

Genotoxic potential of the air in environments with photocopiers

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ABSTRACT: (Genotoxic potential of the air in environments with photocopiers). The contaminants most commonly found in environments with photocopiers are the volatile organic compounds (VOCs), aromatic polycyclic hydrocarbons, aldehydes, ketones, ozone, and particles. The present study aimed at the assessment of the genotoxicity of the air in environments with photocopiers. Six clones BNL 4430 of *Tradescantia* hybrid (*T. subcaulis* Bush x *T. hirsutifolia* Bush) had been kept for 15 days in two environments: one of them with four photocopiers working 12 hours per day (treatment 1), and the other with three photocopiers working eight hours per day (treatment 2). Other six plants comprised the check group and were kept in a greenhouse. The frequency of genic and chromosomal mutations was determined by assessment of the stamen hair cells and tetrads, after 15 days of exposition. There were quantified 415 stamen hairs and 1,500 tetrads per treatment. The plants from treatment 1 have showed significant differences in relation to the check group ($p \leq 0.05$) to the frequency of chromosomal damage. No significant difference was observed in the stamen hairs for genic mutation in relation to the check group.
Key words: chromosomal mutation, genic mutation, indoor genotoxicity, photocopier, toner, *Tradescantia*.

RESUMO: (Potencial genotóxico do ar em ambientes com fotocopiadoras). Os contaminantes mais comumente encontrados em ambientes com fotocopiadoras são os compostos orgânicos voláteis (VOCs), hidrocarbonetos policíclicos aromáticos, aldeídos, cetonas, ozônio e partículas. O presente estudo teve como objetivo avaliar a genotoxicidade do ar em ambientes com fotocopiadoras. Seis clones híbridos BNL 4430 of *Tradescantia* (*T. subcaulis* Bush x *T. hirsutifolia* Bush) foram mantidos durante 15 dias em dois ambientes: um deles contendo quatro máquinas fotocopiadoras que funcionam durante 12 horas/dia (tratamento 1) e outro com três máquinas, que funcionam durante 8 horas/dia (tratamento 2). Outras seis plantas constituíram o grupo controle e foram mantidas em uma casa de vegetação. A frequência de mutações gênicas e cromossômicas foi determinada a partir de cinco avaliações das células do pêlo estaminal e tétrades, após 15 dias de exposição. Foram quantificados 415 pêlos estaminais e 1.500 tétrades por tratamento. As plantas do tratamento 1 apresentaram diferenças significativas em relação ao grupo controle ($p \leq 0,05$) para a frequência de danos cromossômicos. Nos pêlos estaminais não foram observadas diferenças significativas para as mutações gênicas em relação ao grupo controle.
Palavras-chave: fotocopiadoras, genotoxicidade *indoor*, mutação cromossômica, mutação gênica, toner, *Tradescantia*.

INTRODUCTION

The quality of the air in the indoor environment is a new area of research and has attracted attention for demonstrating the positive association between the indoor pollutant sources and the worsening of health (Gioda & Aquino Neto 2003, Saldiva & Miraglia 2004). The indoor concentration of many pollutants is superior to the outdoor concentration, because the polluting sources are confined in a space that hinders the dispersion and increases the risk to health of the individual exposed.

Among the usually monitored contaminants in the indoor environments, there are the carbon dioxide and monoxide, total volatile, organic, and semi-volatile organic compounds, particulate matter, nicotine, and microorganisms (Gioda & Aquino Neto 2003). Specifically the environment with photocopiers is identified as high priority in the research for controlling the indoor contamination. The machines, besides producing electromagnetic fields, are know as sources of volatile organic compounds (VOCs), aromatic polycyclic hydrocarbons, aldehydes, ketones, ozone, and particulate matter (Hetes *et al*. 1995, Northein *et al.* 1998, Brickus & Aquino Neto 1999, California Environmental Protection Agency 2005). The emission of chemical compounds may happen as a direct result of machine operation or from its components such ink and toner. The toner is composed by resins (polystyrene, polyester, polyethylene, epoxy, polypropylene), black carbon, magnetite, silica, nitropyrene, benzene, and aromatic polycyclic compounds (Lofroth *et al*. 1980, Rosenkranz *et al.* 1980).

There are several evidences showing that magnetic fields and components of the toner, individually or in complex mixtures, are genotoxic (Rosenkranz *et al.* 1980, Iravathy Goud *et al.* 2001, Iravathy Goud *et al.* 2004, Valberg *et al.* 2006, Brody *et al.* 2007). The composition, complexity, and availability of these compounds depend on the size, number, and maintenance of the machines, the amount of copies obtained, dimensions of the environment where they are places, ventilation, air renewing, and the presence of filters for purifying the air (Hetes *et al.* 1995).

The monitoring of the exposure to these substances may be accomplished through the assessment of the biological and chemical parameters. In general, the chemical analyses demand sophisticated and expensive equipments, besides not offering directly the information

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regarding the threat to the biological systems. The assays with the animal and vegetal species, or bioindicators, are interesting alternatives due to their low cost, rapid, and reliable results. Although, the tests using animals are preferred for studying the air contaminants (Saldiva & Böhm 1998), the plants of the genus *Tradescantia* have shown to be a suitable model for the genotoxicity studies (Guimarães *et al.* 2004, Misik *et al.* 2006). According to the U.S. Environmental Protect Agency (1980), the assays with plants are more sensitive than other systems regarding the detection of genotoxic compounds present in the environment. The present study aimed at the assessment of the genotoxicity of the air in two environments with photocopiers using the stamen hairs and the micronucleus bioassay in clones BNL4430 of *Tradescantia* hybrid (*T. subcaulis* Bush x *T. hirsutifolia* Bush).

MATERIAL AND METHODS

In situ exposition

The assessment of genotoxicity was carried in two environments. The treatment 1 was performed in area of 6.76 m × 7.89 m, projecting windows disposed in two entire walls and four photocopiers working 12 hours per day. The treatment 2 was in area of 6.74 m × 7.89 m, it has ventilation flaps occupying two walls entirely and presents three photocopiers working 8 hours per day. In both environments, there is no system of exhaustion or filters.

Groups of six pots containing clones BNL 4430 of *Tradescantia* hybrid (*T. subcaulis* Bush x *T. hirsutifolia* Bush), donated by the Comissão Nacional de Energia Nuclear de Poços de Caldas, Minas Gerais State, Brazil, were exposed for 15 days in both places. Simultaneously, the plants of the control group were placed in greenhouse under controlled conditions. The substrate used in the pots is an organic compound (Campsulfértil®) obtained after several fermentations, followed by sterilization. The greenhouse and the two environments are located at Universidade do Contestado–UnC, in Concórdia, Santa Catarina State, Brazil.

Micronucleus (TRAD-MN) and stamen hair (TRAD-SHM) bioassay

After the exposition, young buds were collected and fixed in a solution of ethylic alcohol: acetic acid (3:1, v:v) and spared at -4 ºC until the micronuclei analyses of the tetrads (TRAD-MN). Other floral branches were placed under hydroponic system for analysis of the stamen hair cells (TRAD-SHM).

The procedures for determining the mutagenicity were done according to Rodrigues (1999a,b), Ichikawa (1992) and Ma (1990). The frequency of mutations was determined based on the five assessments performed in 415 stamen hairs and 1,500 tetrads per treatment. The assessments of the stamen hairs allowed the detection of genic mutations, and the assessments of the micronuclei in tetrads were indicative of chromosomal mutations.

The magnitude of the micronuclei and genic mutations variations were estimated by variance analysis followed by the Tukey's test. The analyses were performed using the Software SisVar (Ferreira 2003).

RESULTS AND DISCUSSION

The BNL 4430 clones used in the present study have demonstrated to be a suitable model for analysing the mutagenicity in indoor environments, corroborating with previous studies of Ma & Harris (1987a,b) and Alves *et al.* (2003).

The plants from treatment 1 exhibited rates of chromosomal damage (presence of micronuclei) with significant differences in relation to control group ($p \leq 0.05$) (Tab. 1, Fig. 1A). Regarding the stamen hairs, independently of the environment analysed, there was no significant difference for the genic mutation in relation to the control group (Tab. 2). The Table 2 presents the same stamen hairs features described by Ichikawa (1992), which demonstrated the absence of consequences upon the plant's flora structure.

The micronuclei are formed during the telophase, when the nuclear envelope is reconstituted around the chromosomes of the daugther-cells. These micronuclei may have been a result of acentric chromosomal fragments or entire chromosomes that were not included in the main nucleus due to structural damages (Fenech 2000), as consequence of action of physical, chemical or biological agents (Fenech 2000, Stopper & Müller 1997, Kirsch-Volders *et al.* 1997).

The compounds under dispersion in these environments were able to induce germinative mutations, but have not exerted significative effect on the somatic tissue, as the

Table 1. Mutation rate ± SD (mutant events numbers/300 tetrads) detected by the bioassay of micronucleus in pollen mother cells (TRAD-MN) of clones BNL 4430 of *Tradescantia* hybrids (*T. subcaulis* Bush x *T. hirsutifolia* Bush) exposed to the photocopiers in the two environments and in the greenhouse

Assessments	Environment 1	Environment 2	Greenhouse (control)
1	82±2.83	34±1.30	0
2	69±3.12	68±2.91	4±0.30
3	124±4.70	31±1.12	0
4	17±1.12	21±1.01	0
5	45±1.93	65±3.02	0
Mean	67.4a	43.8ab	0.8b

*Means in the row followed by the same letter do not differ statistically by the Tukey's test ($p \leq 0.05$)

Table 2. System of stamen hairs and mutant events ± SD (in 415 stamen hair) of clones BNL 4430 of *Tradescantia* hybrids (*T. subcaulis* Bush x *T. hirsutifolia* Bush) exposed to the photocopiers in the two environments and in the greenhouse.

Characteristics	Environment 1	Environment 2	Greenhouse (control)
Mean of hairs per stamen	41.2±1.12	41.9±1.63	41.2±0.93
Mean of cells per hairs	25.55±0.90	25.86±0.98	25.55±0.70
Estimation of hairs per flower	247.2±4.20	251.4±3.94	247.2±3.63
Number of mutant events	0a	2a±0.27	0a

*Numbers in the row followed by the same letter do not differ statistically by the Tukey's test ($p \leq 0.05$)

stamen hair. Previous studies regarding the genome of the *Tradescantia* species accomplished by de Sax and Edmonds (1933), Steinitz (1944) and Underbrink *et al.* (1973) have already demonstrated that the meiotic chromosomes are more susceptible to the breakage than the others under rest. According to Underbrink *et al.* (1973), both tests are extensively used to detect mutations in *Tradescantia*. However, the efficiency of micronucleus test displays approximately 36 times greater. Studying the effects of deficiency in magnesium (Mg), sulfur (S) and calcium (Ca) in *Tradescantia paludosa*, Steffensen (1953, 1954 and 1955) also described a greater sensitivity of microspores compared with root tips, according to preliminary evidence of increased susceptibility of meiotic to mitotic cells.

The highest sensitivity of the meiotic chromosomes is guaranteed by the lowest specificity of the damage necessary to produce micronuclei when compared to the mutation in the stamen hairs. In this case, it is possible to assume that numerous loci in each one of the 12 chromosomes of BNL 4430 clones is subjected to breakages, which lead to the formation of micronuclei. Oppositely, in the stamen hairs, the mutation may occur in a specific locus in a chromosome for the production of pink cells (Ma *et al.* 1978).

Other explanation to a lower rate of somatic mutation is that the cells present a repair system for mutation that makes possible its reversion after 15 days of exposition. The DNA repair system was already observed by Iravathy Goud *et al.* (2001), when assessing the chromosomal damages in leukocytes of the photocopier's operators. In the study, there were observed the significative increase in the rate of mutation when the individuals were assessed after 15 and 30 minutes from the beginning of the exposition, which decreased gradually after 45 minutes. According to the authors, this increase happened due to the breakages in one of the DNA strands that were converted while the cell passed through repair process, pointing therefore the interaction between the complex chemical mixtures and the DNA.

According to Carvalho (2005), the numerous similarities between the genetic constitution of superior plants and mammals may suggest similar effects of a mutagen upon the plant and mammal's DNAs. However, there are organizational and physiological differences between them, mainly in the morphogenesis and metabolism. These differences may be a result of different reactions if considered a chromosomal lesion. In the human being, e.g., only a minimal fraction upon the DNA may or may not lead to mutations, while in *Tradescantia* most of the lesions results in mutation. A determined increase of the frequency on the initial damage will increase in the same proportion the mutation incidence rate in both human being and *Tradescantia* (Ichikawa 1992, Ichikawa 1997). Thus, a relative increase in the somatic mutation frequency in *Tradescantia* may indicate a proportional increase of the risk of mutation for human beings (Carvalho 2005).

Several authors describe the mutagenic and carcinogenic effects of some chemical substances found in the toner

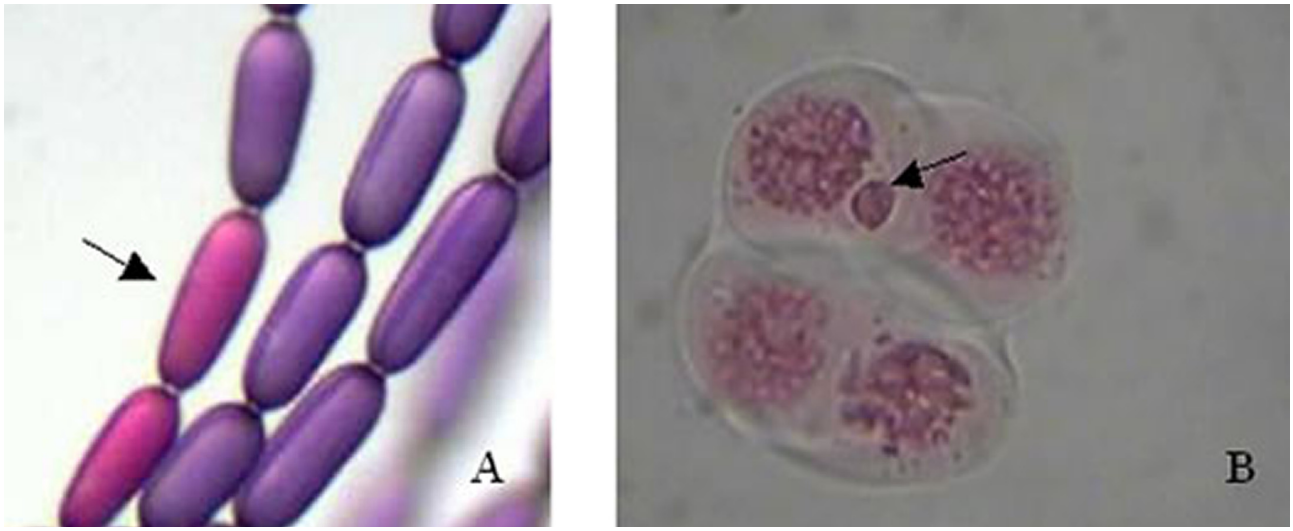

Figure 1. Pink mutant cell (arrow) in stamen hair (A) and tetrad with micronucleus (B, arrow) in clones BNL 4430 of *Tradescantia* hybrids (*T. subcaulis* Bush x *T. hirsutifolia* Bush) exposed to the photocopiers in the two environments.

such as styrene that is able to induce chromosomal mutation and change among the sister chromatids (Vaghef & Hellmann 1998), benzene that causes breakages in one of the DNA strands (Andreoli *et al.* 1997), and the aromatic polycyclic ones that cause oxidative damages and induce breakage in the DNA (Popp *et al*. 1997).

The results found here have shown that the bioassay of BNL 4430 clones may be used in the quality control of the indoor air in environments with photocopiers. The plants exposed to the environment with a higher photocopiers number and during a longer functioning time have presented higher genetic damage rates. Among them, the predominance of chromosomal mutations indicates that there were a high sensibility to the PMCs during the detection of mutagens present in the air. Moreover, the results reinforce the need of investigation and identification regarding the search of ways to reduce and control of the substances emission, which may induce mutations.

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