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Effect of Air Pollution on Some Cytogenetic Characteristics, Structure, Viability and Proteins of *Zinnia elegans* Pollen Grains

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Abstract: There is now abundant evidence that the major effect of most air pollutants on plants is invisible rather than visible. This research try to detects some microscopic effect of air pollutants on pollen formation and structure. This research showed that Tehran polluted air could affect cell division process and chromosome segregation in polluted plants. Clumbing and stickiness of chromosomes were observed in some of the metaphase cells. In polluted plants observed the highest percentage of anaphase and telophase cells possessing laggard chromosomes and as well as low pollen fertility. Observation by scanning electron microscope showed that air born particles accumulated in surface of polluted pollen grains. Pollen shape and pollen's tectum could be changed in this condition. Also many vesicles released out from polluted pollen grains. SDS-PAGE showed that protein bands of polluted and non-polluted pollen grains are not different significantly, but in polluted pollen grains decrease protein content, as a result of air pollution that cause releasing of pollen proteins.

Key words: Pollen, laggard chromosome, cytogenetic, protein, air pollution, *Zinnia elegans*

Introduction

Over the centuries, air pollution has increased from a local nuisance to a global problem. Air pollutants have detrimental effect on organisms and many investigators research effect of air pollution on plants, animals and peoples. Increase in chromosome damage among the traffic policemen was reported (Hadnagy *et al.,* 1989; Anwar, 1988 and 1996). Mutagenicity and carcinogenicity of car exhaust and coal combustion emission was reported (Holmberg and Ahlborg, 1983, Mauderly 1986). There is a few report about effect of air pollution on the plant chromosomes: The percentage of mutations in the meristematic cells of plants growing in polluted areas was 2-6 times higher than in normal plants (Dnieproptetrovsk *et al.,* 1993). Nuclear envelope disrupte and direction of cell division changes during development of ovule and embryonic sac in polluted plants (Majd and Chehregani, 1992).

Air pollution can also affect pollen grains. Airborne pollen grains can be attacked directly by air pollutants. Changes in pollen shape and tectum under polluted condition were reported (Behrendt *et al.,* 1992 and 1997; Knox and Suphioglu, 1996). Tehran's polluted air has changed structure, ultra-structure, chemical compound and allergenicity of some pollen grains (Ghanati and Majd 1995). Allergenicity of pollen grain increased in polluted cities (Behrendt *et al*., 1997 and Helander *et al*., 1997).

Laboratory experiments have shown that NO_2, SO_2 and CO treatments can cause changes in the soluble protein composition of pollen grains. Some research showed that air pollutants could induce fractionation of proteins or formation of new proteins in polluted pollen (Ruffin *et al.,* 1983, Chakraborty *et al*., 1996 and Sanjukta *et al.,* 1998). Decreasing of total protein in polluted pollen was reported by Behrendt *et al.*(1997).

Materials and Methods

We chose Tehran as a high-polluted city for our studies, as a real condition. In Tehran the amount of air pollutants are several times more than the standard amounts (Table 1). In this research we study *Zinnia elegans* Facq. from Astraceae (2n=24) that growing in center area of Tehran as a polluted area and out of Tehran as a non-polluted area. Inflorescences having young flower buds were collected from 50 randomly selected plants from each groups and fixed in glacial acetic acid: ethanol (1:3) for 24h. Flower buds were washed and preserved in 70% ethanol at 4°C until used (Banerjee 1973). Cytological preparation used squash technique and 2% aceto-orcein as the stain. Photomicrographs were taken at X1000 magnification. One thousands pollen mother cells (pmcs)

Table 1: Comparison average amount of air pollutants in Tehran and standard amounts. Data evaluated at during spring and summer in two years, 2000 and 2001. Data obtained from 6-8 air pollution measurement stations. Standard amount accepted from Nation Ambient Air Quality Standards, USA. This table show that amount of some pollutants in Tehran are several times more than standard amounts

Pollutants	Carbon monoxide (ppm)	Sulfur dioxide (ppb)	Nitrogen dioxide (ppb)	Hydro carbons (ppm)	Respairable particle ($\mu g\ m^{-3}$)
Average in 2000	5.89	56	81	3.6	135
Average in 2001	4.39	72	78	2.47	112
Standard amount	9.5	35	54	_	<10μ; 51 >10μ; 15

Table 2: Cells having laggard chromosomes in polluted and non-polluted plants. Abbreviations: A1, anaphase I cells with laggard chromosomes; A2, anaphase II cells with laggard chromosomes; T1, telophase-I cells with laggard chromosomes; T2, telophase-II cells with laggard chromosomes; PF, pollen fertility

Specimen	A1%	A2%	T1%	T2%	PF%
Non-polluted	2.10	0.00	1.60	0.00	96.2
polluted	6.90	5.30	2.43	0.00	74.4

Table 3: Total protein content of polluted and non- polluted pollen grains of *Zinnia elegans* L.

Specimen	Normal pollen	Pollen collected from polluted area	Pollen that 20 days exposed with pollution
Protein content mg/g	3.4	2.6	1.8

were analyzed for chromosome segregation during the anaphase and telophase stages. Pollen fertility was checked by staining a minimum of 1000 pollen grains from each group using acetocarmine: 50%glycerine (1:1) for 1h. Well stained and perfect pollen grains were taken as fertile, while unstained /empty pollens were considered as infertile (Sheidai & Inamdar 1992).

We collected pollen grains from central regions of Tehran as polluted area and out of Tehran as non-polluted area.Some of the pollen grains were applied directly and others were exposed to Tehran's polluted air for 20 days. For this purpose, pollen grains kept in a box and Tehran's polluted air was circulated in the box after passing through a filter with 5-10 μm diameter pores. Polluted and non-polluted pollen grains were studied by photo and scanning electron microscopes. After coating with gold, samples were directly analyzed using a scanning electron microscope model SEM-EDS, EX 300 Link, England.

Pollen protein extraction, including polluted and non-polluted pollen grains, was made separately at 4°C in Tris-HCl buffer, pH 7.6. Finally, 12% SDS- polyacrylamid gel electrophoresis was performed for the total soluble protein according to the method of Laemmli(1970). The extraction of soluble proteins was made in sample buffer (0.125M Tris-HCl, pH 6.8, 4% SDS, 20% glycerol, 10% β-mercaptoethanol, 0.1% bromophenol blue dye) with heating for 3-4 minutes at 100°C before loading. The amount of protein was 5μ g per channel and the total current was 14 mA. The gel run was made in Tris-glycine buffer (pH 8.3) with 0.1% SDS. The gel was calibrated with a marker protein obtained from Sigma Co. An estimate of the protein concentration of the pollen extract was determined by using the method of Lowry *et al.* (1951).

Results

Cytological studies showed that in polluted plants, in during meiosis chromosome segregation have some abnormality, some of metaphase cells showed clumbing and sticking of the chromosomes and some chromosomes didn't enter in metaphase arrangement (Fig. 1), while some of the anaphase I and II as well as telophase showed presence of 1-6 laggard chromosomes (Table 1 and Fig. 2). Statiscally analysis (spss) showed that percentage of cells with laggard chromosomes were increased in polluted plants significantly (Fig. 7a). Our observation showed that fertility of pollen decrease in polluted plants (Table 2 and Fig. 7b).

Ultra-structural studies of pollen grains of *Zinnia elegans L.* showed that they are spherical apolar, 17-20 μm in diameter having a thick exine with three visible colpes.

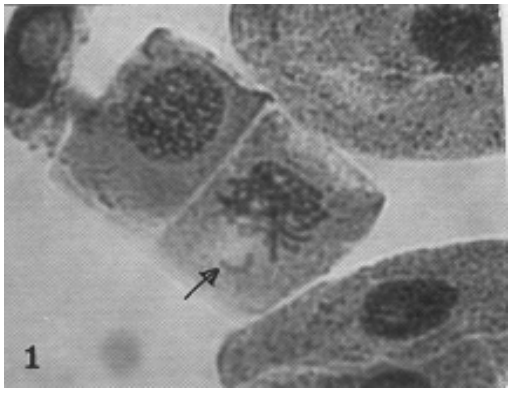

Fig. 1: In polluted plants some metaphase cells showed some chromosomes (→) that didn't enter in metaphasic arrangement (X1500).

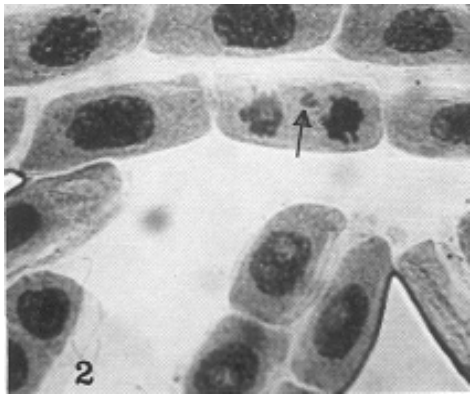

Fig. 2: In polluted plants some anaphase and telophase cell showed laggard chromosomes(→).

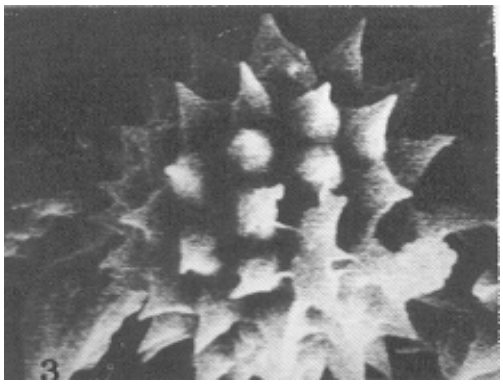

Fig. 3: Scaning micrograph of Zinnia pollen grains that collected from non-polluted regions. Pollen grain are spherical having spinate tectum.(X1700).

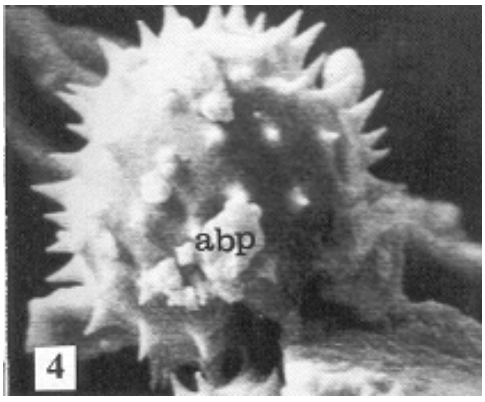

Fig. 4: Scaning micrograph of Zinnia pollen grains that collected from polluted regions. Pollen grains are folded, tectum disrupted and air born particles(abp) accumulated on the surface of pollen (X1700).

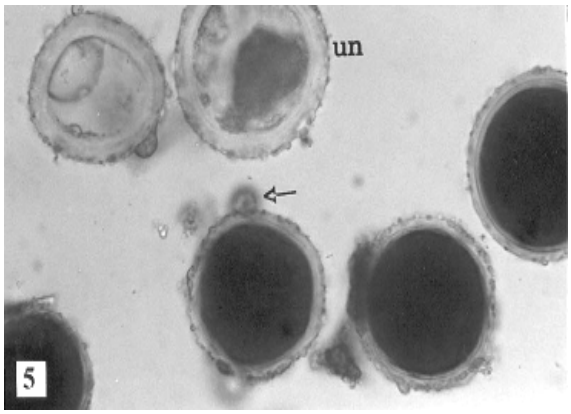

Fig. 5: Photo micrograph of Zinnia pollen grain that collected from polluted area. Air pollutants(abp) accomulated on the surface of pollen and pollen material released out of pollen grain (→).

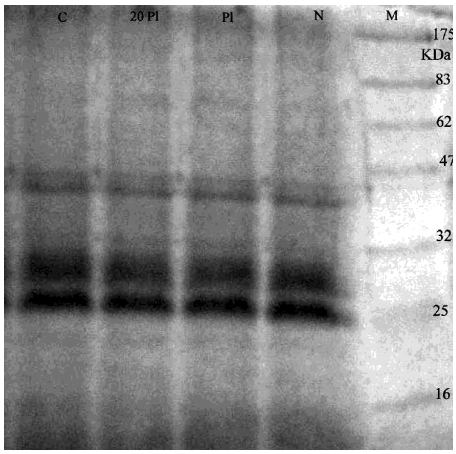

Fig. 6: Protein profile of pollen grains. Polluted and non-polluted pollen grains have the same bands. M, markers; N, normal pollen; P, polluted pollen; 10p, pollens that 10 days exposed with pollution; 20pl, pollen grains that 20 days exposed with pollution. C, Control group that treated by clean air.

SEM analysis showed tectum on pollen grain is long needles (Fig. 3). On the surface of non-polluted pollen grains no atmospheric fine dust nor agglomeration was visible. After contamination with polluted air color of the pollen grains changed dramatically and darkened considerably. When pollen grains exposed to polluted air, they became folded, vermicular particles accumulated in surface of pollen grains (Fig. 4). Pollen grains collected from polluted area covered with high amounts of pollutants. In addition to, tectum of polluted pollen grains

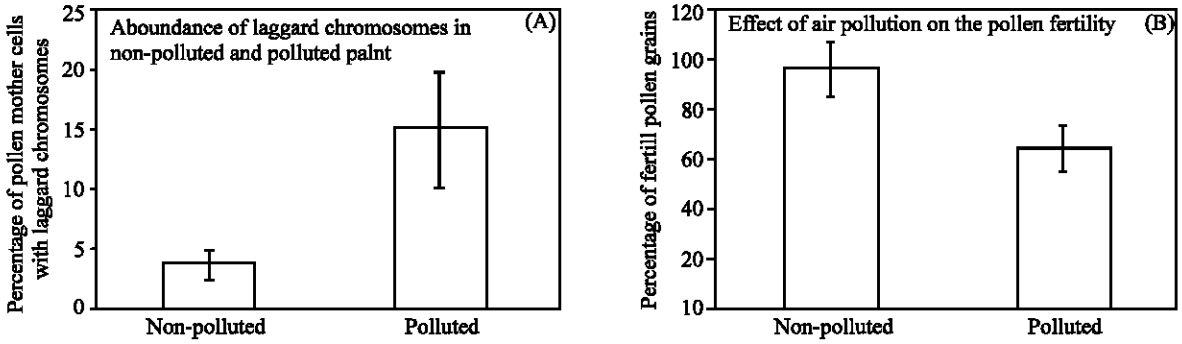

Fig. 7: Increasing of laggard chromosomes and decreasing of fertile pollen have been seem in polluted plants. Polluted and non-polluted plants are different significantly.

disrupted (Fig. 5). Study of polluted pollen grains by using of photomicroscope showed that pollen material released out of pollen grain and aggglomerated on the surface of pollen grain (Fig. 5).

The SDS-PAGE protein profile of different pollen samples including polluted and non-polluted are showed in Fig. 6. The bands are presets in the molecular weight range 12 K Da and 67 K Da. SDS-PAGE showed that five bands observed in non-polluted pollen grains. Protein bands of polluted and non-polluted pollen grains are not significantly, but in polluted pollen protein content was decreased (Table 3). Significant changes were found in total concentration of pollen protein per gram of pollen grains, between polluted and non-polluted pollen grains.

Discussion

The present study showed that air pollution could affect cell division and chromosome segregation. In polluted plants, some of metaphase cells showed abnormal arrangment of the chromosomes while some of the anaphaseI and II as well as telophaseI showed presence of 1-7 laggard chromosomes (Figs. 1, 2 and Table 2). Increasing abnormality in chromosome segregation in polluted regions can resembled as a type of aneoploidy or mutation, wich is in accordance with the finding of Hadnagy *et al.* (1989) and Anwar (1988 and 1996) in human and that of Holmberg and Ahlborg (1983) and Mauderly (1986) on plant meristematic cells. Viability of pollen grains in plants that grown in polluted regions decrease considerably. Our results indicate that these changes in pollen viability can due to abnormality in cell division and pollen development.

According to our observation a large amount of air pollutants adsorbed on pollen surface (Figs. 4,5). Agglomeration of air borne particles were seem on the surface of pollen collected from highly polluted regions (Fig. 4). This is in agreement with those reporting of Behrendt *et al.* (1992), Ghanati *et al.* (1995) and Knox and Suphioglu (1996). The conclusion from these experiments is that organic substances adsorbed to air born particles mediate the agglomeration of particles on the pollen surface following by changes in pollen permeability in the coated pollen grains. Under this condition aqueous compounds may then induce the release of proteins from pollen grains (Fig. 6) and give raise to formation of submicronic particles (Behrendt *et al.*, 1992). A consequence of the release of allergen molecules as aerosols is that the molecules are free to interact with other types of air born particles, such as diseal exaust particles, that are associated with air pollution. Findings of several researchers support this idea (Behrendt *et al.*, 1992, 1997). In this condition, sensitized individuals have more contact with pollen proteins (allergens) than in the non-polluted condition. This phenomenon is one of the reasons of increase allergy and asthma frequency in polluted cities.

The results of 12% SDS-PAGE analysis for soluble protein (Fig. 5) demonstrate the same bands in polluted and non-polluted pollen. It seems that air pollution had not significant effects on pollen proteins, wich is opposite with findings of the Ruffin *et al.* (1983), Chakraborty *et al.* (1996) and Sanjukta *et al.* (1998). According to our results, total protein of pollen grains significantly decreased in polluted pollen grains (Table 3). This is in accordance with the finding of Behrendt *et al.* (1997), but not with the finding of Ruffin *et al.* (1983). In order to study the interaction between pollen and particulate pollutants cause to release pollen material and agglomeration of organic particles on to pollen surface.

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