IJST (2013) A1: 63-68
Iranian Journal of Science & Technology

http://www.shirazu.ac.ir/en

The response of anther and pollen development, pollen cellular material release and pollen proteins to air pollution in *Petunia hybrida* Juss. (Solanaceae)

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Abstract

The study aimed to investigate the microsporogenesis, tapetum and pollen development in *Petunia hybrida* Juss. under control and air pollution condition. The connective shows a well-developed placentoid, giving the sporogenous tissue a crescent-shape. The sporogenous tissue of each of the four anther locules is surrounded by dimorphic tapetum. The outer (towards the epidermis) and inner (towards the connective) tapetal layers differ in shape, staining intensity and degree of vacuolization. During microsporogenesis and pollen maturation, the tapetum undergoes several changes and ultimately degenerates after pollen mitosis. The tapetum is the secretory type. Microsporogenesis with simultaneous cytokinesis forms tetrahedral tetrads of microspores. Mature pollen grains are prolate, tricolporate/tricolporoidate with furrows disposed along the polar axis and three lowly developed pores with striate-reticulate sculpture. Under air pollution, microsporogenesis was normal till tetrad stage. After this stage, some tetrahedral tetrads were still present and some pollen grains were irregular shaped, shrunk and fragile. Some tapetal cells were smaller and the number of their nuclei was less compared to those of the control. Cellular material release was higher in polluted pollen. SDS-PAGE pattern in polluted pollen did not show significant difference compared to the control.

Keywords: Airborne particulate material (APM); connective; microsporogenesis; protein bands; Tapetum

1. Introduction

The rapid increase in industrial and agricultural technology has been accompanied by a marked increase in the number and types of biologically active agents released into the biosphere. These agents can produce either mutagenic or physiological effects or a combination of both, and their effects can occur in any cell and at any stage of life cycle. Most of these agents act directly, but in some cases, they have unpredictable indirect effects [1].

Pollen grains house the male gamets (or their progenitor cells) and arise from microspore mother cells through programmed developmental stages, which are supported by the tapetum, the cell layer of the anther wall. Pollen grains are released into the air for fertilization. During this stage they can be attacked directly by air pollutants. Indirect effects of air pollution on anther and pollen are possible due to pollutants entering to the soil and this alters the pH [2, 3]. Root injury can result from the subsequent mobilization of trace element [2, 3]. Alternatively pollutants may interfere with aerial growth due to interruption of the metabolic pathway of respiration and photosynthesis [2, 3]. Verma *et al*. (2006) reported that air pollutants decrease chlorophyll and carotenoid contents, photosynthesis rate and stomatal conductance significantly [4]. Kaiser *et al*. (1993) showed that SO2 enters mesophyll cells where it reacts with water to form sulfuric acid which inhibits photosynthesis in the chloroplasts [5]. The combined influences result in reduced net productivity. This leads to the production of fewer, smaller pollen grains and an increased number of deformed pollen compared with plants of the same species growing in less polluted areas [2, 6].

Little attention has been given to effects of air pollution on anther development and microsporogenesis. The present study was done through microscopic observations on the development of anther and pollen and the effect of air pollution on these processes.

Pollen has 2.5% to 61% protein content. Most pollen proteins are likely to be enzymes that function during pollen tube growth and subsequent fertilization, but some pollen proteins are consumed by floral visitors, especially pollinators [7]. Studies on pollen proteins collected from polluted and unpolluted areas have shown contradictory results.

Received: 26 November 2011 / Accepted: 17 November 2012

Helender *et al*. (1997) did not observe any significant difference between protein bands of pollen grains collected from polluted and control areas [8]. Jilek *et al*. (1993) observed an increase of the major birch pollen allergen, Bet v 1, in areas where nitrogen loads are high [9], while Parui et al. (1998) found a decrease in Bet v 1 concentration due to air pollution [10]. SDS–PAGE pattern of pollen proteins in *Thuja orientalis* L. did not show significant differences in polluted pollen compared with those in the control group [11]. Due to the role of wall proteins and pollen coatings in adhering to vectors and components of the pistil, screening against UV radiation, prevention of desiccation, and retention of signaling molecules that participate in the pollen-stigma interaction [12] and alternatively the role of these proteins in allergenecity [13], assay of pollen proteins under air pollution is important.

2. Materials and Methods

Samples were collected from plants of *Petunia hybrida* Juss. var. Bravo cool water mix grown in control (National Botanical Garden, Paykanshahr, Tehran, 30 km far from Tehran) and polluted areas with heavy traffic (the city centre of Tehran). Both sites are located in a geographically limited area with fairly homogeneous conditions. Plants were grown under the same condition (fertilizer and irrigation). Reports by the air quality centre at the environment protection agency showed the type and monthly mean of air pollutants concentrations in flowering time in control and polluted areas at the sampling sites (Table 1). During collection month, few days were exceptional because short time peaks (3 h) of air pollutants were reported as 0.1 ppm (SO_2), 0.2 ppm (NO_2), 13.7 ppm (CO), 8.1 ppm (HC) and 191 μgm^{-3} (airborne particulate material, APM). The flower buds were collected from their beginning until flower opening at intervals each two days. Samples were fixed in FAA (formalin: acetic acid: alcohol ethyl 96, 2:1:17), dehydrated in a graded alcohol series and embedded in paraffin. Serial sections of 8-12 µm were prepared, stained with hematoxylin and Eosin and examined by light microscopy (LM) [14]. Some of pollen grains were acetolyzed to examine pollen structure [15]. Structure, agglomeration of APM and cellular material release were examined by LM and SEM [16]. Pollen specimens were prepared for scanning electron microscopy observation by dusting them directly onto sticky-tape coated aluminum stubs and gold coating. Photographs were taken with a JEOL JSM 633 OF SEM. Pollen grains were generally dusted onto a drop of water on a microscope slide for light microscopy. Specimens were viewed with an Olympus model Ah2 light microscope connected to camera.

Table 1. Mean of air pollutants concentration in control and polluted areas (reports of the air quality research centre at the environment protection agency, Tehran), APM=Airborne particulate material; ppm= Part per million

Type of air pollutant / Month	SO_2 (ppm)	NO_2 (ppm)	CO (ppm)	HC (ppm)	APM (μgm^{-3})
June (polluted area)	0.063	0.06	9.1	2.80	162
June (control area)	0.002	0.010	0.6	0.10	54

For protein analysis, the protein extracts were prepared by incubating pollen grains in 0.1M phosphate buffered saline, PBS, pH 7.4 in 15% ratio with stirring at 4–8^{o}C for 4 h. Suspensions were centrifuged at 10000 g for 20 minutes and supernatants were removed. Loading buffer (glycerol, sodium dodecylsulfate, mercaptoethanol, bromophenol blue) was added to each sample, vortexed briefly, heated in a boiling water bath for 5 min in the presence of reducing agent 2-mercaptoethanol. The extracts (25 µl) and a molecular weight ladder (8 µl; Feinbiochemica Gmbh and Co.) were run using discontinuous (4% stacking, 12% resolving) sodium dodecylsulfate-polyacrylamide gels (modified method of [17]). Proteins were stacked at 50 V and then resolved at 70 V overnight. Electrophoresis was performed in a vertical slab gel apparatus (Akhtarian, Ps-2000, Model 75, Iran) three times. The proteins were then stained with 0.2% Coomassie brilliant blue R250 and destained with a mixture of methanol, acetic acid and water (1:1:8). Statistical analyses were performed using ANOVA in SPSS and Duncan's multiple range test ($p \leq 0.05$).

3. Results

Young anthers are tetrasporangiate (Fig. 1A). In each one of the four corners, the cells of the outer primary parietal layer and inner primary sporogenous layer arise from periclinal divisions of the archeosporial cells (Fig. 1B). The outer layer cells divide periclinally to form the anther wall layers and give rise to an endothecial layer, two middle layers and a tapetal layer (Figs. 1C, D). The connective shows a well-developed placentoid (inward protrusion of septal parenchyma), giving the sporogenous tissue the typical crescent-shaped cross section (Figs. 1A, B). The tapetum is seen as the outer (towards the epiderm) and inner (towards the connective) whereby two tapetal layers exhibit a pronounced structural dimorphism in cell shape, staining intensity and degree of vacuolization (Figs. 1C, D). The internal part lines the placentoid and has an early origin, being recognizable as a

differentiation of the placentoid cells facing the sporogenous tissue (Figs. 1C, D). The sporogenous tissue of each one of the four anther locules is crescent shaped and surrounded by the tapetum (Figs. 1C-E). Sporogenous cells reveal longitudinal and transverse divisions, an increase size, dense cytoplasm and eventually give rise to microspore mother cells (Mmc) (Figs. 1E-G). Microspore mother cells (Mmc) surrounded by special callose wall undergo meiosis I and Π, with simultaneous cytokinesis (Figs. 1F-J; 2A, B). The disposition of the microspore in the tetrads is tetrahedral (Fig. 2B). After dissolution of the callose wall, microspores separate from the tetrads and form microspores (Figs. 2C, D). In the meiocytes with the initiation of meiosis, from prophase the onward, tapetal cells increase in size and become large and plurinucleate (bi-tetranuclate) (Figs. 1E, F, G, I; 2A, B). The secretory tapetum starts to vacuolate and degenerate after microsporogenesis during pollen development (Figs. 2C, D). The cells of the endothecium are thickened and bared during pollen development (Figs. 2E, F). Middle layer is degenerated during pollen maturity stage. Mature pollen grains are regular (87%) prolate (polar axis (P) 34µm, equatorial axis (E) 19.5µm, P/E ratio 1.74 µm), tricolporate/tricolporoidate with furrows disposed along the long (polar) axis and three lowly developed pores (Figs. 2G; 3A-F) and with striate-reticulate sculpture (Figs. 3C, D). Both acetolysed and non acetolysed pollen grains are regulate and triaperturate. However, non acetolysed pollen grains due to pollen hydration are somewhat bigger and show cellular material release (Figs. 3E, F).

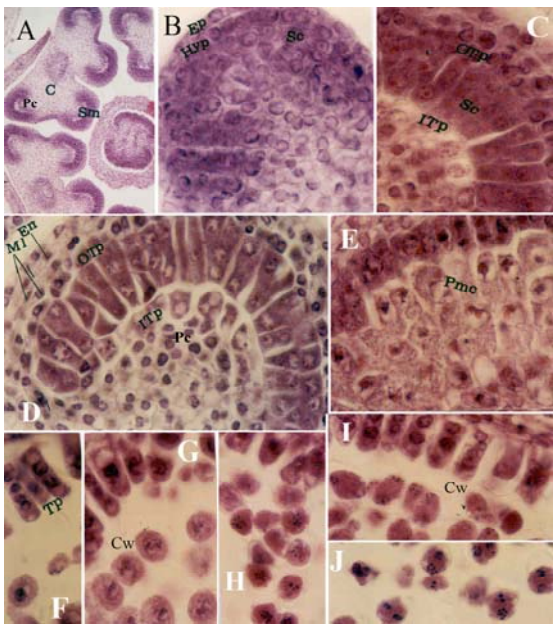

Fig. 1. (A-J) Light micrographs of cross-section of anther under control conditions, A. flower bud, connective (C), placentoid (Pc) and, sporogenous mass (Sm) are seen, ×100. B. Microsporangium with epidermis (Ep), an outer hypodermic layer (Hyp) (primary parietal cells) and an inner primary sporogenous cell or layer (SC), ×1000. C. The outer (OTp), inner tapetum (ITp) and sporogenous cells (Sc), ×1000. D. Formation of the anther walls (endothecium (En), middle layers (Ml) and the outer (OTp) and inner tapetum (ITp), placentoid (Pc) and premeiosis sprogenous mass, ×1000. E- G. Early meiosis I stage, tapetum (Tp) and callose wall (Cw), pollen mother cell (Pmc), Tapetum (Tp), ×1000. H, I. Mid and late meiosis I respectively and dyad cell formation, ×1000. J. Early meiosis II stage (Metaphase II), ×1000

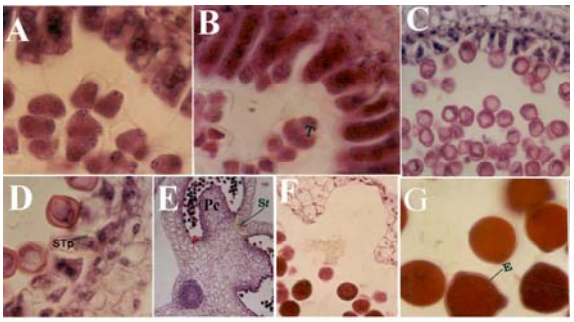

Fig. 2. (A-G) Light and electro micrographs of cross-section of anther under control conditions, A. Late meiosis II stage, ×1000. B. Tetrad stage, note the grown of tapetal cells, ×1000. C, D. Pollen development stage, note the touch of developing pollen with secretory tapetum (STp), respectively ×400 and ×1000. E. Cross section of anther before dehiscence, note the dissolution of tapetum, Stomium (St), placentoid (Pc) ×100. F. Anther dehiscence stage, note the absence of endothecium in stomium, ×1000. G. Structure of mature pollen and its exine (E), ×1000

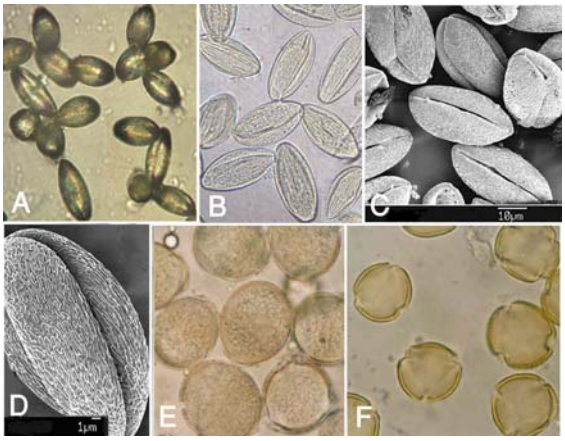

Fig. 3. (A-F) Light and electro micrographs of pollen grains under control conditions, A, B. prolate pollen grains, long colpic are obvious whereas pori are small and undeveloped, respectively ×400 and ×1000. C, D. Electron micrographs of pollen structure of the plants grown under control condition, the pollen grains and exine are regular, scale bars show the magnification. E, F. Cellular material release in acetolysed and nonacetolysed pollen respectively, ×1000

Structural abnormality was not observed in anther exposed to air polluted until tetrad stage (Figs. 4A, B). In this stage, after dissolution of callose, some microspores are still closed together in the tetrad. Size of tapetal cells is smaller than the control ones (Figs. 4A, B). Some pollen grains showed abnormalities such as pollen shape irregularity, detaching and fragility (breakage) of exine and the mean of pollen regularity reduced to 65% compared with control (87%) (Figs. 4C-F). Pollutant materials deposition is seen in the pollen collected from polluted area (Figs. 4E- H). A decrease of pollen size was observed in polluted area (P 28µm, E 17.5.5µm, P/E ratio 1.6 µm).

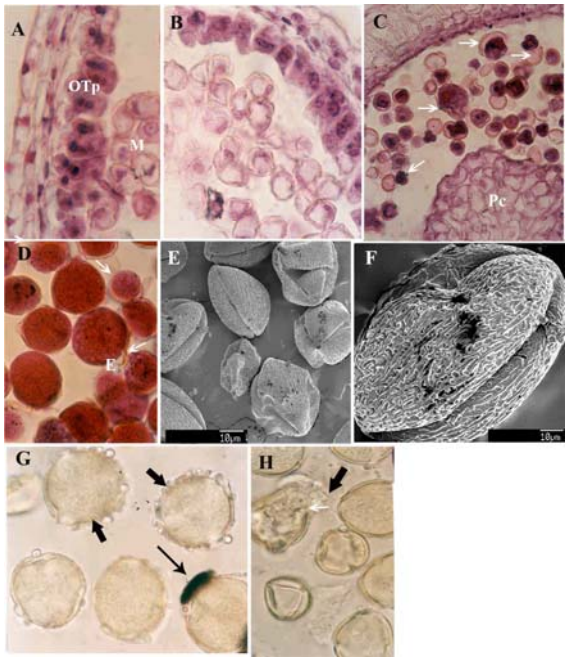

Fig. 4. (A-H) Light and electro micrographs of cross-section of anther under polluted conditions, ×1000, A, B. Late tetrads stage, note the reduction of tapetum (OTp) size, the contact microspores (M) together and their staining intensity. C. Abnormality of pollen, note to irregular size and fragility (breakage) of pollen (white arrows), placentoid (Pc). D. Pollen with Fragile and abnormal exine (E) (white arrows). E, F. Electron micrographs showing irregularity and breakage of the pollen grainges in plants grown under air pollution conditions. Scale bars show magnification. G, H. Aglomerization of air pollutants (black long arrow) and cellular material release (black short arrows) in acetolysed (G) and nonacetolysed (H) pollen respectively, in acetolysed pollen, pollen fragility is seen as well, ×1000

Cellular material release was higher in polluted pollen than in the control. Therefore, membrane vesicles from the cell surface are widely observed in polluted samples compared to control (Figs. 4G, H). SDS-PAGE pattern of soluble protein bands of pollen extracts did not show any obvious difference between the polluted and control extracts (Fig. 5).

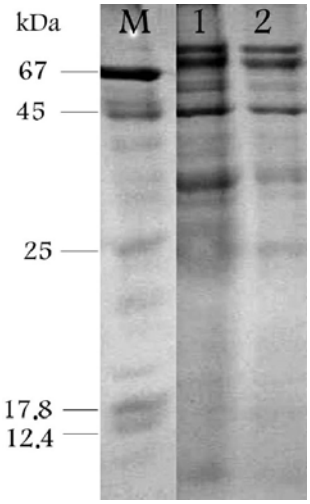

Fig. 5. SDS-PAGE pattern of soluble proteins of pollen extracts, 1, 2 respectively the pollen extracts of control and polluted area. M, marker

4. Discussion

The tapetum of *Petunia hybria* Juss. is the secretory (parietal) type. Studies on some genera in solanaceae have supported this kind of tapetum [18, 19]. In contrast to these results, Majd and Mofrad (2001) observed a plasmodial tapetum in *Datura* [20]. The major function of the tapetum is to provide essential metabolites to the development of the microspores [18, 21- 23]. The outer and inner tapetum cells differ in shape, stainability and degree of vacuolization. Tapetal cells from the parietal layer are isodiametric while those from connective cells are irregularly polygonal in shape. This is similar to the findings in *Lycopersicon esculentum* Mill. [18]. The significant differences in organelle content and configuration between outer and inner tapetum were observed. A character of the thecae that occurs only in a very few families is a pollen sace placentoid, a protrusion of parenchymatic tissue into each pollen sac from the connective side. It occurs in most families of Lamiales and in Solanaceae, Acanthaceae, Bignoniaceae, Scrophulariaceae and some convolvulaceae [24]. In Scrophulariaceae, this placentoid is reduced in size in some Antirrhineae and *Veronica*, while in the Lindernieae, it is lacking completely [25]. A developmental study of anther tapetum in *Tecoma stans* has shown that the inner secondary parietal layer gives rise to tapetum. The remainder of the tapetum on the inside (connective) is contributed by the parenchymatous connective cells lying just outside the sporogenous cells. The tapetum thus follows the dicotyledonous type of ontogeny. It also shows a distinct dual origin and is structurally dimorphic. In prophase I of meiosis, the tapetal

cells are bi- tetranucleate. They enlarge until tetrad stage and, after tetranucleate stage, during pollen development start to vacuolate and eventually degenerate.

Mature pollen grains are prolate, tricolporate/tricolporoidate with furrows disposed along polar axis and three lowly developed pores, with a striate-reticulate sculpture. Pollen of *P. hybrida* has previously been described by Natarjan [26] as prolate, 37.5×27.5 (μm), yellow in colour, tricolpate with equidistant furrows disposed along the long or polar axis and three hidden germ pores, and with a thick reticulate exine (cited by [27]). Stafford and Knapp reported that pollen grains in *Petunia* type are small-moderate (polar axis generally ≤ 39μm); 3– 4 colporate or occasionally syncolporate; colpi relatively long and narrow; ornamentation striate-reticulate [27]. Ito et al to test MALES TERILITY1 (MS1) gene function in heterologous garden petunia (*Petunia hybrida*), studied pollen phenotype of control and transgenic plants [28]. Although they did not explain aperture structure, scanning electron microscopy images of pollen grains did not show any obvious pore in aperture. Striate-reticulate exine and lowly developed and unclear pores were also seen in scanning micrographs of pollen grains of *Petunia hybrida* in studies of Herrero and Dickinson on pollen tube development. Consistent with our results, they reported that pollen grains were characterized with long colpis and inconspicuous and lowly developed pores (apparently without pore), and with a striate-reticulate exine [29].

Air pollution leads to tapetum abnormality and an increased number of irregular, fragile and deformed pollen grains. Results of this study were in agreement with those of the previous studies that showed air pollution caused pollen fragility and abnormality [2, 3, 30-32]. Tapetal anomalies are considered to be the causal mechanisms that produce cytoplasmic and genetic male sterility [33, 34].The tapetum has a nutritive role and its premature disintegration deprives the developing microspores for each essential metabolite. In this study it has been shown that pollen fragility and cellular material release are induced in pollen collected from the polluted areas. Because of the important roles of pollen cellular material in the interaction of stigma-pollen as well as pollen germination and pollen tube growth, inhibition of these caused male sterility.

Results of SDS-PAGE of proteins showed no difference between protein bands and the pollen extracts collected from the nonpolluted and polluted areas. Behrendt and Tomczok reported that there is a significant difference between total protein and Phl p 5 (major allergen), being lower in supernatants from rural timothy grass pollen grains compared to areas near high-traffic roads [35]. Also, studies on *Lagerstroemia indica* L. showed that total protein content and protein bands staining intensity decrease due to air pollution [36]. Chehregani et al. (2004) reported that the protein content of *Zinnia* pollen grains decreases in response to air pollution, causing the release of pollen proteins [37]. Similar to our results, Helender et al. (1997) did not observed any significant difference between the protein bands of the polluted and control areas [8]. In addition, the study of content and electerophoretic profile of pollen proteins in *Spartium junceum* L. [38] and pollen proteins bands in *Thuja orientalis* L. [11] showed no significant differences in polluted pollen grains compared with controls. Studies show contradictory results; therefore, it is possible that type of plant species, thinning of exine under air pollution or pseudo increase of pollen weight due to pollutants deposition on pollen surface caused these differences, which require further study. In conclusion, anthers were sensitive to air pollution during and after the meiosis. Thus, reduced growth and premature degeneration of the tapetum (tapetum malfunction) are responsible for the pollen abnormalities.

References

[1] Pfahler, P. L. (1981). *In vitro* germination characteristics of maize pollen to detect biological activity of environmental pollutants. *Environ. Health. Perspect*., *37*, 125-132.

[2] Emberlin, J. (1998). The effects of air pollution on allergenic pollen. *Eur. Respir. Rev*., *8*, 164-167.

[3] Emberlin J (2000). The problem of pollen. *Allergy*, *8*, 25-28.

[4] Verma, R. B., Mahmooduzzafar, Siddiqi, T. O. & Iqbal, M. (2006). Foliar response of *Ipomea pes-tigridis* L. to coal smoke pollution. *Turk. J. Bot*., *30*, 423-417.

[5] Kaiser, W. M., Hofler, M. & Heber, U. (1993). Can plants exposed to SO2 excrete sulfuric acid through the roots? *Physiol. Plant*., *87*, 61-67.

[6] Omura, M., Matsta, N., Moriguchi, T., Kozaki, I. & Akihama, T. (1989). Variation in the physiological and genetic characteristics and pollen grain number in Japanese pear depending on the growing conditions. *Bull. Fruit Trees Res. Stn. Ser*., *16*, 11-24.

[7] Roulston, T. H, Cane, J. H. & Buchmann, S. L. (2000). What governs protein content of pollen: pollinator preferences, pollen-pistil interactions, or phylogeny? *Ecol. Monogr*., *70*, 617-643.

[8] Helender, M. L., Savolainen, J. & Ahlholm, J. (1997). Effect of pollution and other environmental factors on birch pollen allergens. *Allergy*., *52*, 1207-1214.

[9] Jilek, A., Swoboda, I. M., Breiteneder, H., Fogy, I., Ferreira, F., Schmid, E., Heberle-Bors, E., Scheiner, O., Rumpold, H., Kraft, D., Koller, H. T. & Breitenbach, M. (1993). Biological functions, isoforms and environmental control in the Bet v 1 gene family.

In: *Molecular biology and immunology of allergens*, Kraft, D. & Sehon, A. (Eds), pp. 245-271. Boca Raton, FL: CRC Press., *270*, 2607-2613.

[10] Parui, S., Mondal, A. K. & Mandal, S. (1998). Protein content and protein skin test sensitivity of the pollen of *Argemone mexicana* on exposure to SO_2. *Grana*, *37*, 121-124.

[11] Rezanejad, F. (2009). Air pollution effects on structure, proteins and flavonoids in pollen grains of *Thuja orientalis* L. (Cupressaceae). *Grana*, 48, 205-213.

[12] Dickinson, H. G. (2000). Pollen coating-chimaeric genetic and new function. *Sex. Plant. Reprod.*, *12*, 302-309.

[13] Knox, B. & Suphioglu, C. (1996). Environmental and molecular biology of pollen allergens. *Trends. Plant. Sci*., *1*, 156-164.

[14] Ruzin, S. E. (1999). *Plant Microtechnique and Microscopy*. New York: Oxford University Press.

[15] Erdtman, G. (1960). The acetolysis method. A revised description. *Svensk. Bot. Tidskr.*, *54*, 561-564.

[16] Rezanejad, F. (2009). Air pollution effects on structure, proteins and flavonoids in pollen grains of *Thuja orientalis* L. (Cupressaceae). *Grana*, *48*, 205-212.

[17] Laemmli, U. K. (1970). Cleavage of structural protein during the assembly of the head of bacteriophage T4. *Nature, 227*, 680-685.

[18] Polowick, P. L. & Sawhney, V. K. (1993). Differentiation of the tapetum during microsporogenesis in tomato (*Lycopersicon esculentum* Mill) with special reference to the tapetal cell wall. *Ann. Bot*., 72, 595-605.

[19] Sazima, M., Vogel, S., Cocucci, A. & Hausner, G. (1993). The perfume flowers of *Cyphomandra* (Solanaceae): pollination by euglossine bees, bellows mechanism, osmophores, and volatiles. *P1. Syst. Evol*., 187, 51-88.

[20] Majd, A. & Mofrad, R. (2001). Comparative study of structural, anatomical and developmental characteristics of two species of *Datura* and assay their antimicrobial effects. *The 1st. Iranian. Cong. Appl. Biol*., Tehran, Iran.

[21] Hardy, C. R., Stevenson, D. W. M. & Kiss, H. G. (2000). Development of the gametophytes, flower, and floral vascular in *Dichorisandra thyrsiflora* (Commelinaceae). *Am. J. Bot*., *87*, 1228-1239.

[22] Hermann, P. M. & Palster, B. F. (2000). Stamen development in the Ericaceae. *Am. J. Bot*., *87*, 934-957.

[23] Suzuki, K. & Tajeda, H. (2001). Ultrastructural study on degeneration of tapetum in anther of snap bean (*Phaseolus vulgaris L*.) under heat stress. *Sex. Plant. Reprod.*, *13*, 293-299.

[24] Eames, A. J (1961). *Morphology of the Angiosperms*. New York: McGraw-Hill.

[25] Kadereit, J. W. (2004). *Flowering Plants, Dicotyledons: Lamiales* (*except Acanthaceae including Avicenniaceae*) Series: *The Families and Genera of Vascular*: *Vol. 7*. Germany: Springer.

[26] Natarjan, A. T. (1957). Studies in the morphology of pollen grains: Tubiflorae. *Phyton*, *8*, 21-42.

[27] Stafford, P. & Knapp, S. (2006). Pollen morphology and systematics of the zygomorphic-flowered nightshades (Solanaceae; Salpiglossidae sensu D'Arcy, 1978 and Cestroideae *sensu* D'Arcy, 1991, *pro parte*): A review. *Sys.t biodivers.*, *4*, 173-201.

[28] Ito, T., Nagata, N., Yoshiba, Y., Takagi, M. O., Ma, H. & Shinozaki, K. (2007). Arabidopsis MALE STERILITY1 encodes a PHD-type transcription factor and regulates pollen and tapetum development. *The Plant Cell*, *19*, 3549-3562.

[29] Herrero, M. & Dickinson, H. G. (1981). Pollen tube development in *Petunia hybrida* following compatible and incompatible intraspecific matings. *J. Cell Sci*., *47*, 365-383.

[30] Majd, A. & Mohamady, S. (1992). Effect of certain toxins and air pollutants on pollen development of *Glycine max. J of Sci., Tehran Islamic Azad Univ*., 649-651.

[31] Bherendt, H., Becker, W. M., Friedrichs, K. H., Darsow, U. & Tomingas, R. (1992). Interaction between aeroallergens and airborn particulate matter. *Int. Arch. Allergy Immunol*., *99*, 425-428.

[32] Bherendt, H., Becker, W. M., Friedrichs, K. H. & Ring, J. (1997). Air pollution and allergy. *Int. Arch. Allergy Immunol*., *113*, 69-74.

[33] Horner, H. T. & Roger, M. A. (1974). A comparative light and electron microscopic study of microsporogenesis in male fertile and cytoplasmic male sterile sunflower (*Helianthus annuus*). *Can. J. Bot*., *52*, 435-441.

[34] Nakashima, H., Horner, H. T. & Palmer, G. (1984). Histological features of anthers from normal and ms3 mutant soybean. *Crop Sci*. 24: 735-739.

[35] Behrendt, H. & Tomczok, J. (1999). Timothy grass (*Phleum pratense* L.) pollen as allergen carriers and initiators of an allergic response. *Int. Arch. Allergy Immunol*., *118*, 414-418.

[36] Rezanejad, F., Majd, A., Shariatzadeh, S. M. A., Moein, M., Aminzadeh, M. & Mirzaeian, M. (2003). Effect of air pollution on pollen soluble proteins, structure and cellular material release in *Lagerstroemia indica. Acta Bot. Cracov. Ser. Bot*., *45*, 129-132.

[37] Chehregani, A., Majde, A., Moin. M., Gholami, M., Shariatzadeh, S. M. A. & Nassiri, H. (2004). Increasing allergy potency of Zinnia pollen grains in polluted areas. *Ecotoxicol. Environ. Saf*., *58*, 267-72.

[38] Rezanejad, F. (2007). The effect of air pollution on microsporogenesis, pollen development and soluble pollen proteins in *Spartium junceum* L. (Fabaceae). *Turk. J. Bot.*, *31*, 183-191.