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Toxic Effects of Diesel Exhaust Particles on the Ovules and Embryonic Sac Development in *Phaseolus vulgaris* L.

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ABSTRACT

Diesel Exhaust Particles (DEP) are one of the most important air pollutants. Many researchers have reported the detrimental effect of DEP on peoples' health and that of different organisms. There are some reports about the effect of DEP on pollen grains and their allergic potential but this is the first investigation on its effect on ovule development in common bean. *Phaseolus vulgaris* L. plants were grown in similar-sized plots in different groups and treated with different concentrations (0.1, 0.3, 0.6 g/L) of water-soluble parts of DEP. Flowers and small pods were removed, fixed with FAA and subjected to developmental studies. Our results show that DEP can have some abnormal effect during of ovule development in common bean when treated with DEP (0.1, 0.3 and 0.6 g/l). The embryonic sac could not complete its growth and was smaller in DEP-treated plants than in control ones. In DEP-treated plants, vesiculation of the embryonic sac and plasmolysis of nucellar tissue were observed. Accumulation of dark particles, disruption of the nuclear envelope, and cell degradation in the embryonic sac are some of the results of treatment with DEP. SDS-PAGE showed that even though the protein pattern was the same in experimental and control plants, there was a slight quantitative increase of a band with molecular mass of 62 KD in 0.6 g/l DEP-treated plants.

Keywords: air pollution, embryology, DEP, megagametophyte, toxicology

INTRODUCTION

Over the centuries, concurrent with industrialization and population growth, air pollution has increased from a local nuisance to a global problem (Knox and Suphioglu 1996; Behrendt *et al*. 1997; Helender *et al*. 1997; Emberline 1998; Hwang *et al*. 2005). Diesel exhaust particles (DEP) are one of the most important air pollutants (Chehregani *et al*. 2006). DEP consist of a complex mixture of particulate matter, including elemental carbon and polycyclic aromatic hydrocarbons (PAHS; i.e., phenanthrene, florenes, naphthalenes, pyrenes, fluoranthrenes), as well as DEP aerosols, volatile organic compounds, various hydrocarbons, and gases including CO_2, CO, NO, NO_2, SO_2 (Air Resource Board 1998). After the combustion of diesel fuel, the exhaust components tend to aggregate into discrete, spherical, repairable particles approximately 0.1-0.5 μm in diameter (Bolands *et al*. 1999). These particles consist of an inert carbonaceous core with a large surface area, ideal for absorbing heavy metals and organic compounds (Peterson *et al*. 1996).

DEP appear to play a role in respiratory and allergic disease (Pandya *et al*. 2002) and may affect pollen grains indirectly via stress on the growth on the plant or directly through contamination of the anthers on the plant or during the flight of pollen grain through the air (Emberlin 1998). Some studies showed that air pollutants could induce the breakage of proteins or the formation of new proteins in pollen grains (Ruffin *et al*. 1983; Chakraborty *et al*. 1996; Chehregani *et al*. 2004). A decrease of total pollen protein in polluted areas was reported (Behrendt *et al*. 1997; Chehregani *et al*. 2004).

Although there are some studies that have shown the detrimental effects of environmental pollutants on pollen development and structure (Behrendt *et al*. 1992; Majd *et al*. 1992; Chehregani *et al*. 2004), we could not find any report about the effects of DEP on the developmental stages of plant ovules and embryonic sacs, which constitute an important step in the reproductive survival of plants.

MATERIALS AND METHODS

Plant material and treatments

Phaseolus vulgaris L. var. Talash (Fabaceae) plants were planted in different groups. Bean plants were grown from seeds in these plots. Beginning on the 13th July 2005, at 5 weeks of age (two weeks before flowering) and continuing for the next three weeks, during flowering and embryo sac development, each subset was treated by a group of DEP solutions. Four groups were treated by different concentrations of DEP (0.1, 0.3, 0.6 g/l). DEP were prepared in mixed form from 4 Mercedes buses (model 302), which is a popular bus in Iran. DEP was mixed with distilled water for 1 h and water-soluble parts were used after filtration with Whatman filter paper No. 4. Shoots of experimental plants (leaves and developing flowers) were sprayed with different concentrations of water-soluble DEP while controls were sprayed with distilled water. The shoot of each plant was sprayed with about 50 ml of the above mentioned solutions each day. Experiments were conducted at the research farm of Bu-Ali Sina University.

Microscopic studies

After flowering, flowers and young pods were removed from experimental and control plants, fixed in FAA_{70} (formalin: acetic acid: ethanol, 2:1:17), stored in 70% ethanol, dehydrated in a graded alcohol series, embedded in paraffin and sectioned at 7-10 μm with a microtome (Leitz 1512, Germany). Staining was carried out with Hematoxilin-Eosin (Mayer 1976). Prepared sections were studied under a light microscope (Zeiss Axiostar, Germany) for

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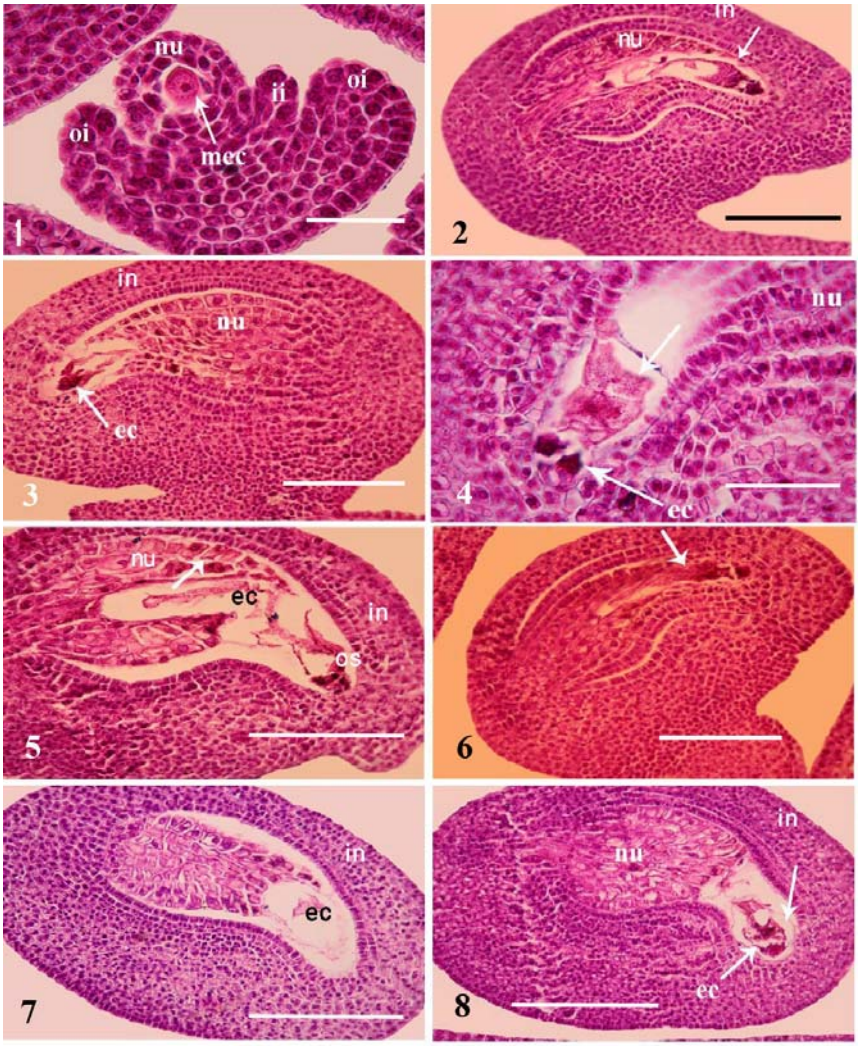
Short Communication



Figs. 1-8 Ovule development in *Phaseolus vulgaris* L. (**1**) Longitudinal section through an ovary and distinct young ovule. Outer and inner integuments have initiated and nucellus cells and megagametocyte are seen at this stage. (**2**) Longitudinal section through a young ovule with a mature embryonic sac (×400). (**3**) Nucellus tissue shows irregularity and degradation in DEP-treated plants at any concentration. Arrow shows changing shape of nuclei in embryo sac (×400). (**4**) Decomposition of embryonic sac in DEP-treated plant at 0.6 g/l (×400). (**5**) Irregularity and halted formation of the nuclear envelope in embryonic sac and irregularity in nucellus tissue (↑) (×400). (**6**) Deficiency of growth of embryo sac (↑) and accumulation of dark particles in embryonic sac seen in the group treated with 0.3 and 0.6 g/l (×400). (**7**) Degradation of nuclei in embryonic sac (↑) in the DEP-treated plants at 0.6 g/l (×400). (**8**) Deficiency in growth and irregularity of embryo sac shape and size are signs of decomposition of the embryonic sac in DEP-treated plants with 0.3 and 0.6 g/l (×1000). Abbreviations: ec, embryonic sac; ii, inner integument; mec, mega gametocyte; nu, nucellus tissue; oi, outer integument. Bar = 80 μm in **1** and 200 μm in **2-8**.

each developmental stage of ovules and embryonic sacs. The developmental stages of experimental and control samples were compared. At each developmental stage, at least 20 flowers were studied and differentiation between experimental and control plants were microscopically determined.

SDS-PAGE

Seed protein extractions of normal and DEP-treated pollen grains were carried out at 4°C in Tris-HCl buffer (pH 7.6). SDS-polyacrylamide gel electrophoresis over a 12% gel was performed on the soluble proteins according to the method of Laemmli (1970). The extraction of soluble proteins was made in sample buffer (0.125 M Tris-HCl, pH 6.8, 4% SDS, 20% glycerol, 10% β-mercaptoethanol, 0.1% bromophenol blue dye) by heating for 3-4 minutes at 100°C before loading. The amount of protein was about 10 μg per lane; protein marker standards (Sigma, St. Louis, Mo) were run in parallel. Staining of gels was carried out using Comassi Blue Brilliant (Sigma, USA).

RESULTS

Microscopic studies

Phaseolus vulgaris L. ovules are anatropous, bitegumic with the outer integument completely surrounding the inner integument (**Figs. 1, 2**). Embryo sac formation conforms to the polygonum type (monosporic, seven cells, eight nucleate embryo sac consisting of three antipodal cells; a large binucleate central cells; and egg cell adjacent to two synergids). Although antipodal cells are formed, they are short-lived and appear to degenerate prior to embryonic sac maturation.

A survey of microscopic specimens that were prepared from DEP-treated plants showed that the pollen developmental stages in these plants were not the same as control ones. According to our results, in plants which were treated by different DEP solutions, some abnormalities were seen during ovule development. The embryonic sac was smaller (data not shown) in plants that were treated by DEP solutions, with a maximum decrease of 32% at 0.6 g/l; furthermore, the stability of nucellar tissue resulted in a decrease in penetration of the embryo sac in the surrounding nucellus tissues (**Fig. 3**), i.e., digestion of nucellus tissues can not take place in DEP-treated plants (note: the growth of the embryo sac needs the degradation of surrounding cells). In DEP-treated plants the shape of the embryo sac changed dramatically. In normal plants, the shape of the embryonic sac was ovate (**Fig. 2**) but in DEP-treated plants it was zigzagged and had irregular shapes (**Figs. 3, 5, 6**). A change in the shape of the nucleus (arrows in figures) in the embryo sac is another consequence of treatment with DEP (**Figs. 3, 4, 8**). An increase in vacuole volume was evident in the nucellar cells of treated plants (**Figs. 5, 6**), so that cytoplasm and nucleus are swept to one side and separated from the cell wall. The abundance of vesicles and particles is a sign of the decomposition of the embryo sac (**Figs. 3, 4**), visible in DEP-treated plants. This phenomenon was more evident in polar nuclei than egg apparatus cells (**Fig. 6**). Accumulation of dark, unknown particles in the embryo sac is another effect of DEP treatments (**Figs. 7, 8**). The accumulation of these particles takes place in the egg apparatus more than in other cells.

SDS-PAGE

Seed protein extractions of normal and treated plants were subjected to SDS-PAGE and protein bands were compared

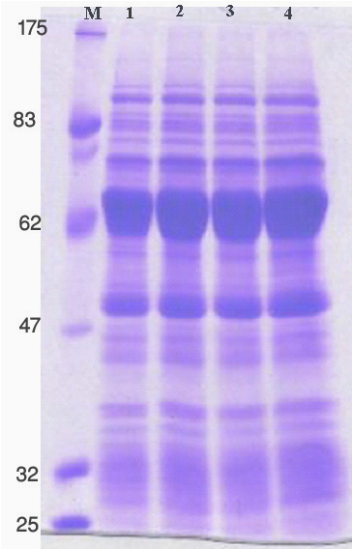


Fig. 9 Protein pattern of seed proteins in DEP-treated and control plants. All bands were the same in both treated and control plants, but the quantity of one band with molecular mass of 62 KD was higher in DEP-treated plants with 0.6 g/l. Lanes: M, protein marker; 1 = control, distilled water-treated plants; 2-4 = 0.1, 0.3 and 0.6 g/l DEP-treated plants.

in treated and normal plants. Protein patterns were the same in both groups, although plants treated with DEP showed more protein of molecular mass of about 62 KD than the control (**Fig. 9**).

DISCUSSION

Air pollution has become a serious global problem. DEP is one of the main pollutants, being a major problem for most countries (Behrendt *et al*. 1997; Helender *et al*. 1997; Emberline 1998; Hwang *et al*. 2005; Chehregani and Kouhkan 2007). Pollution with DEP causes damage to different plant organs, but there is not yet any report about the effect of DEP on the development of ovules and embryonic sacs.

Microscopic studies showed that the development of ovules in bean plants follows a standard process as described by Buvat (1989) in dicotyledonous plants (**Figs. 1, 2**). Ovules in the bean family are anatropous (Johnson and Walles 1994).

The development of ovules is very important for a plant's reproductive survival, so that any abnormality it suffers can cause a decrease in seed production and plant survival. Some abnormalities were seen during ovule developmental when treated by the water-soluble part of DEP at 0.1, 0.3 and 0.6 g/l. The embryonic sac was smaller in treatment plants than in controls (**Fig. 3**). Under normal conditions, the embryo sac digests surrounding nucellar tissue allowing it to enlarge into space provided by the enlargement of surrounding cells. A reason for this effect may be that the nucellar tissue is more stable and does not permit its digestion and subsequent growth of the embryo sac. In addition, the shape of nuclei in DEP-treated embryonic sacs changed (**Figs. 3, 4, 8**). It seems that DEP treatments, like other stresses and pollutants, cause a degradation in the structure and function of the endoplasmic reticulum and therefore, in this case, formation of the nuclear envelope may have been prevented (Majd and Chehregani 1992; Chehregani and Kavianpour 2007), although this is difficult to confirm since this is the first report about the effect of DEP on the embryo sac of ovules.

Many vesicles and particles were formed in the embryo sac. The shape of the embryo sac emerged from its natural state (ovate to crescendo shape) and changed to a zigzag form (**Figs. 5, 6**), possibly signs of degradation of the embryo sac. These findings are in agreement with those of some prior reports concerning other environmental stresses and pollutants (Deshpande *et al*. 1974; Bor 1978; Chehregani *et al*. 2006). In all treated groups, nucellar cells showed evidence of plasmolysis (**Fig. 5**), similar to findings of Majd and Chehregani (1992) about the effect of SO_2 on soybean (*Glycine max* L.) ovules.

SDS-PAGE studies indicate that protein bands are the same in all treated and control groups. It seems that DEP treatments could not affect protein patterns of seeds, although the quantity of some bands changed in DEP-treated plants. Our prior report (Chehregani and Kouhkan 2007) indicates that DEP caused protein patterns to change in pollen grains. We can conclude that protein synthesis is more sensitive to DEP in pollen grains than in ovules.

DEP as an important pollution factor has some detrimental effects on plants (Behrendt *et al*. 1997; Chehregani and Kouhkan 2007). Results of this study indicate that DEP can induce several abnormalities during ovule development, affecting thus the fertility and survival of bean plants.

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