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Effects of Acid Rain on the Developmental Stages of Ovules and Seed Proteins in Bean Plants (*Phaseolus vulgaris* L.)

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Abstract: The aim of this research was to detect some microscopic effects of acid rain on ovule formation, development, structure and protein content in plants. Bean plants were grown in plots in different groups and treated by different acid solutions of HNO_3, H_2SO_4 and both HNO_3, H_2SO_4. The pH of each group regulated at 2, 3, 4, 4.5. Treatments were taken for 4 weeks, one in each day. Developmental stages of ovules were compared in acid treated and control plants. Results showed that developmental process of ovules show some abnormalities. Embryo sac was not completed its growth and was smaller in the treated plants than control ones. In treated plants, vesiculation of embryo sac and plasmolysis of nucellar tissue were seen. Accumulation of dark particles and disruption of nuclear envelope in embryonic cells are the results of acid treatments. Treatments by HNO_3 with pH 2, was more effective than other acid solutions. SDS-PAGE showed that protein pattern was the same in experimental and control plants but quantity of protein bands was different.

Key words: Acid rain, embryo sac, ovule, ovule development, seed protein, electrophoresis

INTRODUCTION

Anthropogenic acid deposition- rain, fog and snow-is one of the main pollution types that being a major problem for the countries in Europe, Asia and North America (Gimeno *et al*., 2001; Bouwman *et al*., 2002). In natural conditions, atmospheric precipitation is slightly acidic due to the dissolution of atmospheric Carbon Dioxide (CO_2). At present, deposition with pH lower than 5.6 is defined as Acid Rain (AR) (Wyrwicha and Sklodowska, 2006). Acidic rain is caused mainly by dissolution of sulphur dioxide, nitric acid and hydrochloric acid. These pollutants originate from human activity such as the combustion of burnable waste and fossil fuels within thermal power plants and automobiles (Percy *et al*., 1987).

Acid deposition may cause decline in health and growth of trees as well as other plants including crops (Kaya *et al*., 2005; Wyrwicha and Sklodowska, 2006). Direct contact of acid rain with leaves causes pH dependent anatomical changes (Paparozzi and Tukey, 1983; Stoynora and Velikova, 1998) ultrastructural abnormality (Gabara *et al*., 2003) and biochemical changes (Velikova *et al*., 2000; Gabara *et al*., 2003). Appearance of this kind of changes depends on pH of acid rain and acidifying cations and anions present in it (Soares *et al*., 1995). Histological examination of plant tissues has been a useful tool for diagnosing the sensitivity of plants to pollutants and acid rain (Alves, 1988).

We could not find any report about effect of acid rain on the ovule formation and development. The aim of this research was to elucidate some microscopic effects of acid rain on the ovule and embryo sac development, which is important in reproduction and survival of plants.

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MATERIALS AND METHODS

Plant Materials and Treatments

We used bean plants (*Phaseolus vulgaris* L.) belonging to Fabaceae as an experimental model. Experiments were conducted at research farm of Bu-Ali Sina University in 2004. In June 2004, four plots with each containing earth soil were divided into 16 subsets. Bean plants were grown from seeds in these plots. Beginning on 13 July 2004, at the age of 5 weeks and continuing for the next three weeks, each subset was subjected once to one pH level of simulated acid rain. Plants sprayed with distilled water pH (6.8) were regard as the control. The following groups were experimental spraying treatments:

- The plants sprayed with distilled water pH 6.8 as control.
- The Plants treated by HNO_3 solution pH 4.5, 4, 3 and 2 separately.
- The plants treated by H_2SO_4 solution pH 4.5, 4, 3 and 2 separately.
- The plants treated by mixed solution of HNO_3 and H_2SO_4, pH 4.5, 4, 3 and 2 separately.

All experimental and control groups were treated by about 30 mL of above mentioned solutions at each times. Treatments were done one time at each day.

Sampling and Microscopic Studies

Flowers and young pods were removed from treated and control plants separately. The specimens were fixed in FAA_{70} (formaldehyde, glacial acetic acid and 70% ethanol, 5:5:90), stored in 70% ethanol. Specimens embedded in paraffin and sectioned at 5-12 μm with a Leitz 1512 microtome. Staining was carried out with PAS (Periodic acid Schiff) according to the protocol suggested by Yeung (1989) and contrasted with Meyer's Hematoxilin. Several sections were studied under a light microscope Zeiss Axiostar Plus for each developmental stages of ovule. Developmental stages of ovules were compared in treated and control plants. For each developmental stage, at least 20 ovules were studied and differentiations between treated and control plants were analyzed.

Protein Studies

Mature seeds were harvested from experimental and control plants at the same time (at beginning of legume drying). Water soluble seed storage protein extractions of normal and treated plants 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) with heating for 3-4 min at 100°C before loading. The amounts of proteins were 10 μg per lane; protein marker standards (Sigma, St. Louis, Mo) were run in parallel.

RESULTS

Results of Microscopical Studies

The survey of microscopic specimens that were prepared from acid treated plants showed that the developmental stages in these plants are not the same to control ones. In this paper, we avoid from description of ovule developmental stages in control plants (Fig. 1a) and focused on description of abnormalities in acid treated groups. According to present results, in plants which were treated by different acidic solutions, some abnormalities were seen during ovule development. Some of these effects are in common in all acid treated groups and others are the specific in the some treated groups.

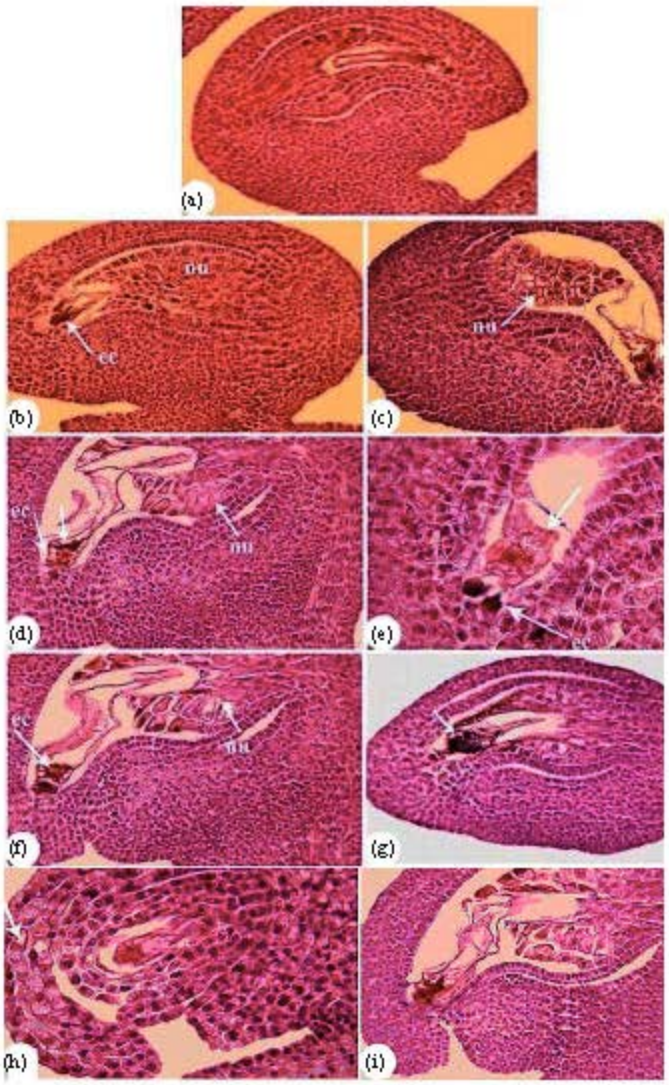

Fig. 1: (a) Longitudinal section through an ovule and embryo sac of control plant *Phaseolus vulgaris* L (x400). Ovule is anatropous and there is not any abnormality. (b-i) Ovule development in plants that treated by different acid solutions: (b) Longitudinal section through an ovule and embryo sac. Embryo sac was remained small and could not nourish from nucellar cells (x400), (c) The shape of embryo sac was changed and the signs of decomposition were seen in embryo sac of treated plants (x400), (d) any vesicles and particles were not seen in cytoplasm of embryo sac in treated plants, but few small vesicles are visible in control plants. Nucleus envelope was not formed in some case of treated plants by acid solution (x400), (e) polar nuclei in plants that were treated by acid solutions were vesiculated and have less stain ability than control ones (x1000), (f) plasmolysis and vacuolization of nucellar cells is one of HNO_3 acid treatment results (x400), (g) accumulation of black particles (starch grains) in embryo sac were seen in plants that treated by acid solution (x400), (h) Vacuolization of ovule integument cells (↑) and more growing of them are another effect of acid treatments (x400) and (i) Degradation of nuclei in embryo sac cells and vacuolization of nucellar cells (↑) are other obvious effect of acid treatment (x400). Abbreviations: mu, nucellus tissue; ec, embryo sac

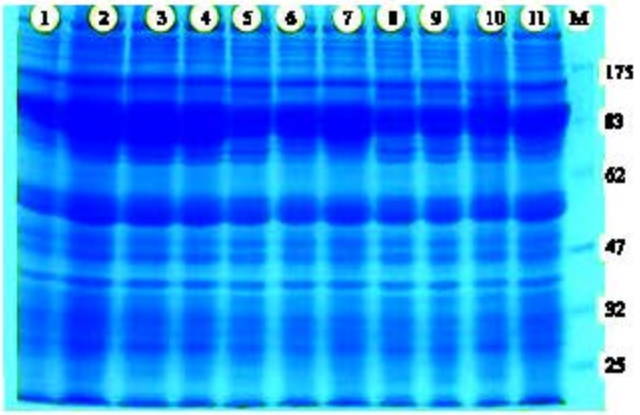

Fig. 2: Seed protein bands in acid treated bean plants and control ones. Lane 1: The bands of treated plants by H_2SO_4, pH2. Lane 2: The bands of treated plants by HNO_3, pH2. Lane 3: The bands of treated plants by both HNO_3, H_2SO_4, pH2. Lane 4: The bands of treated plants by H_2SO_4, pH3. Lane 5: The bands of treated plants by HNO_3, pH3. Lane 6: The bands of treated plants by both HNO_3, H_2SO_4, pH3. Lane 7: The bands of treated plants by H_2SO_4, pH4. Lane 8: The bands of treated plants by HNO_3, pH4. Lane 9: The bands of treated plants by both HNO_3, H_2SO_4, pH4. Lane 10: The bands of treated plants by H_2SO_4, pH4.5 and Lane 11: The bands of control plants. M: protein markers (kDa)

Embryo sac was smaller (about 34%) in plants that were treated by acid solutions and nucellus stability causes the decrease in penetration of embryo sac (Fig. 1b). In plants that were treated by H_2SO_4 and mixed solution of acids, penetration of embryo sac into nucelar tissue was very poor. Our results indicate that in plants that were treated by different acid solutions, the shape of embryo sac was changed dramatically. Changing of nucleus shapes in embryo sac is the results of acid treatments (Fig. 1c). The abundance of vesicles and particles is the sign of decomposition of embryo sac (Fig. 1d) that were visible in acid treated plants. This phenomenon was more evident in polar nuclei than egg apparatus cells (Fig. 1e). Increasing of vacuole volume was evident in the nucellar cells of treated plants (Fig. 1f), so that cytoplasm and nucleus sweep in one side and separate from cell wall. Accumulation of dark particles in the embryo sac is the effect of acid treatments (Fig. 1g). It seems that they are starch particles. The accumulation of these particles takes place in egg apparatus more than others. In plants treated by H_2SO_4 solution, ovule integuments have greater and faster growth and therefore micropyle canal was closed early (Fig. 1h). In treated plants by HNO_3 solution, the cells of ovule integuments were vacuolated (Fig. 1i). This effect was neither seen in plants that were treated by other acid solutions nor in control ones.

These abnormalities cause to decrease in the seed number of mature legumes. Our results showed that in normal plants legume contains 5-6 healthy seeds but in acid treated plants number of seeds were decreased. Decreasing is considerable (average 3) in the groups that treated by acid solutions pH 2. Differences between normal plants and treated groups (pH 2) are significance ($p \leq 0.05$).

Results of Protein Studies

Water soluble seed protein pattern of acid treated plants, pH 2, 3, 4 and control ones were investigated. Seed storage proteins (water soluble ones) were extracted and carried out on SDS-PAGE after preparation. Results showed that the protein band pattern and numbers of bands were the same in all groups (both treated and control ones) and there was not any distinctive differences between them (Fig. 2). In all studied groups, 19 protein bands were visible. Acid treatments cause to reduce

the quantity (density) of some protein bands only. Density decreasing is more obvious in the band with 83 kDa weight in the groups that treated by HNO_3 pH 3, mix of HNO_3 and H_2SO_4, pH 3 and H_2SO_4 pH 3.

DISCUSSION

Air pollution has become a serious global problem. Acid deposition-rain, fog and snow, is one of the main results of pollution, being a major problem for the most countries (Gimeno *et al*., 2001; Bouwman *et al*., 2002). Acid rain cause to damage different plant organs, but there is not any report about effect of acid rain on the development of reproductive organs.

Microscopic studies showed that developmental process of ovules in bean plants were taken to ordinary process described by Buvat (1989) in dicotyledonous plants (Fig. 1a). In the base of our studies, ovules in bean plant are anatropous, according to Johansson and Walles (1994). But some abnormalities were seen during ovule developmental processes in bean plants treated by different acid solutions. Some of these effects were general and are visible in all acid treated groups and some are specific in a treated group. In general, embryo sac was smaller in experimental groups than control ones. The reason of this effect is more stability of nucellus tissue that does not permit the growth of embryo sac. Decomposition of nuclei in embryo sac were evidence in acid treated groups. It seems that acidic treatments like other stresses and pollutants cause to degradation in structure and function of endoplasmic reticulum and therefore, in this case, formation of nuclear envelope was prevented (Majd and Chehregani, 1992).

Many vesicles and particles were formed in embryo sac. The shape of embryo sac came out from natural status (crescendo shape) and changed to zigzag form (Fig. 1c and d). It seems that these are the signs of degradation of embryo sac. These findings are new as effect of acid rain but they are accordance with findings of some prior reports concerning environmental pollutants (Majd and Chehregani, 1992; Chehregani *et al*., 2005). Decreasing of seeds in the mature legumes of experimental groups, pH 2 especially, was confirmed decomposition of ovules and embryo sacs. In all treated groups, nucellar cells showed evidence of plasmolysis (Fig. 1f) that is new report about effect of acid rain but accordance with finding of Majd and Chehregani (1992) about effect of SO_2 on the soybean ovules.

Treatment with HNO_3 solution, instead of other treatments, cause to more growth of embryo sac and greater penetration into nucellar tissue. Probably reason of this effect, is supplement of nitrogenous nutrient for plant by this treatment. In this experimental group, sever vacuolization of ovule integument cells were seen.

In plants that were treated by H_2SO_4 solution, ovule integuments have greater growth, so that micropylar channel was closed sooner than normal ones (Fig. 1h). In addition, cells of ovule integuments are more condensed. It seems that these effects are related to existence of high sulphur concentration that in this case, is according to findings of Majd and Chehregani (1992) concern to treatment with SO_2.

SDS-PAGE studies indicate that protein bands are the same in all treated and control groups. It seems that acidification of rain could not affect protein patterns, but quantity of some bands was changed in acid treated plants. This is meaning that acid stress could be affected gene regulation and tend to decrease production of some proteins (83 kDa, for example). Based on our results although acid treatments cause to some abnormality during ovule development, but it seems that remaining seeds have the same protein patterns with normal ones.

Acid rain as a result of air pollution has some detrimental effects on the plants (Kaya *et al*., 2005; Wyrwicha and Sklodowska, 2006). Anatomical changes ultrastructural abnormality (Gabara *et al*., 2003) and biochemical deficiency (Velikova *et al*., 2000; Gabara *et al*., 2003) were reported in the acid treated plants. Present results, in addition to, indicate that acid rain can induce several abnormalities

during ovule development. Abnormality during development of ovules is more evidence in the groups that were treated by pH 2 acid solutions, especially HNO_3. These abnormalities are dangerous for plant survival. Our results indicate that fertility of plants and seed number was decreased by half. This is the first report about effect of acid rain on the ovule development that should be considered together with prior reported effects. Hence ovule safety is an important factor in surviving of plants, we can conclude that acid rain is harmful factor for yielding and surviving of plants.

REFERENCES

Alves, P.L.C.A., 1988. Efeiots nutricionais da chuva ácida simulada e do Latossolo Vermelho-Amarelo de Cubatão-SP, em soja (*Glycine max* L.) Merril. M.Sc. Thesis, Universidade Federal de Viçosa, Minas Gerais, Brasil.

Bouwman, A.F., D.P. Van Vuuren, R.G. Derwent and M. Posch, 2002. A global analysis of acidification and eutrophication of terrestrial ecosystems. Water Air Soil Pollut., 141: 349-382.

Buvat, R., 1989. Ontogeny, Cell Differentiation and Structure of Vascular Plants. Springer-Verlag.

Chehregani, A., B.E. Malayeri, F. Mohsenzade and R. Golmohammadi, 2005. Effects of heavy metals on the developmental stages of ovule and embryo sac in *Euphorbia cheiradenia*. Pak. J. Biol. Sci., 8: 622-625.

Gabara, B., M. Sklodowska, A. Wyrwicha, S. Glinska and M. Gapinska, 2003. Changes in the ultrastructure of chloroplasts and mitochondria and antioxidant enzyme activity in *Lycopersicon esculentum* Mill. leaves sprayed with acid rain. Plant Sci., 164: 507-516.

Gimeno, L., E. Marin, T. Del Teso and S. Bourhim, 2001. How effective has been the reduction of SO_2 emissions on the effect of acid rain on ecosystems. Sci. Environ., 275: 63-70.

Johansson, M. and B. Walles, 1994. Functional anatomy of the ovule in broad bean (*Vicia faba* L.): Ultra structural seed development and nutrient pathways. Ann. Bot., 74: 233-244.

Kaya, M., M. Atak, K. Mahmood, C.Y. Çefçi and S. Özcan, 2005. Effect of pre-sowing seed treatment with zinc and foliar spray of humic acid on yield of common bean (*Phaseolus vulgaris* L.). Int. J. Agric. Biol., 6: 875-878.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. J. Nat., 227: 680-685.

Majd, A. and A.H. Chehregani, 1992. Studies on developmental processes in ovules of Soja *Glycine max* L. plants and effects of certain toxins and environmental pollutants. Acta Hortic., 319: 431-436.

Paparozzi, E.T. and H.B.J. Tukey, 1983. Developmental and anatomical changes in leaves of yellow Birch and red kidney bean exposed to simulated acid precipitation. J. Am. Soc. Hortic. Sci., 108: 890-898.

Percy, K.E. and E.A. Baker, 1987. Effects of simulated acid rain on production, morphology and composition of epicuticular wax and on cuticular membrane development. New Phytol., 107: 577-589.

Soares, A., Y.J. Ming and J. Pearson, 1995. Physiological indicators and susceptibility of plants to acidifying atmospheric pollution: A multivariate approach. Environ. Pollut., 87: 159-166.

Stoynora, D. and V. Velilova, 1998. Effects of simulated acid rain on chloroplast ultrastructure of primary levels of *Phaseolus vulgaris*. Biol. Plant., 40: 589-598.

Velikova, V., I. Yordavow and A. Edreva, 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: Protective role of exogenous polyamines. Plant Sci., 151: 59-66.

Wyrwicha, A. and M. Sklodowska, 2006. Influence of repeated acid rain treatment on antioxidative enzyme activities and on lipid peroxidation in cucumber leaves. Environ. Exp. Bot., 56: 198-204.

Yeung, E.C., 1989. Histological and Histochemical Staining Procedures. In: Cell Culture and Somatic Cell Genetics of Plants. Vasil, I.K. (Ed.), Academic Press, Orlando, Fla, pp: 686-697.