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Effect of Acid Rain on the Development, Structure and Viability of Pollen Grains in Bean Plants (*Phaseolus vulgaris)*

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Abstract: Acid rain is one of the great importance stresses that are associated with atmospheric pollutions. This research tries to detect some microscopic effects of acid rain on pollen formation, development and structure. Bean plants were growed in plots in different groups and were treated by different acid solutions of HNO_3, H_2SO_4 and both HNO_3, H_2SO_4. The pH of each group regulated from 2-4.5. Experimental plants were sprayed with different acid solutions and control plants sprayed by distilled water. Treatments were taken for 4 weeks, one in each day. Young buds and flowers were removed and fixed by using FAA and stored in 70% ethanol. The specimens embedded in paraffin and sectioned at 8 μm with microtome. Staining was carried out with Hematoxylin and developmental stages were compared in treated plants and controls. Studies of microscopic preparation showed that acid rain could affect developmental process of pollen grains in plants that treated by acid solutions. In treated plants, numbers of pollen grains and also amount of fertile pollen grains were decreased in each anther significantly. Exine was not formed in some case of plants that treated by different acid solutions. Degradation of tapetum cells was take later in treated plants than control ones. This is cause to non-well feeding of developmental pollen grains that trend to some abnormalities during pollen development. Vesiculation of cytoplasm and accumulation of black particles are the results of acid treatments that were more evidence in treated plants. Treatment by HNO_3, pH 2, is more effective than others regarding induction of abnormalities during pollen development and decreasing of pollen fertility.

Key words: Acid rain, pollen grain, pollen development, pollen fertility

INTRODUCTION

As a result of human activities, acidification of rainwater, i.e., acid rain, has become a serious global problem (Wang *et al*., 2002). Acidity of precipitation in the world has increased in the last several years. This increase in acidity has been attributed to an increase in sulfuric and nitric acids (Likens *et al*., 1972). Brosset (1973) proposed that anthropogenic sulfur dioxide is either oxidized to SO_3 and then hydrated to H_2SO_4 or first hydrated to H_2SO_3 and then oxidized to sulfuric acid (Evans *et al*., 1977).

Among atmospheric stress factors, which reduce germination, growth and survival, acid rain is potentially one of great importance (Sheppard *et al*., 1993). Acidity of rain damage membrane lipids and proteins as well as nucleic acids which result in a reduction of plant growth and development. Acid rain is a serious environmental problem that has an impact on agriculture, forestry and human health (Shvetsova *et al*., 2002).

Acid rain induces changes in the cellular biochemistry and physiology of the whole plant. Biological effects of acid deposition on plants are numerous and complex and include visible symptoms of injury (chlorosis and/or necrosis) and invisible effects such as reduced photosynthesis, nutrient loss from leaves, altered water balance, variation of several enzyme activities (Ferenbaugh, 1976; Evans, 1982). Kratky *et al*. (1974) showed that germination of pollen grains and pollen tube were decreased significantly in tomato plants that traded by acid solution. Decreasing number of pollen grains was reported in acid rain condition by Waldron and Cracker (1989).

Ecological effects of acidified rain have been determined in some areas (Abrahamsen *et al*., 1976; Beamish *et al*., 1975; Whittaker *et al*., 1974) but more investigations are needed to evaluate the importance of increased inputs of acidic components in rainwater.

The aim of this research is to elucidate microscopic effects of acid rain on the developmental stages of pollen grains and their viability. We could not find any report about acid rain on the development of anther and pollen grain in 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. In 7 June 2004, four plots with each containing earth soil were divided in to 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 simulated acid rain. The plants were irrigated with distilled water before treatments. Plants sprayed with distilled water (pH 6.8) were regard as the control.

The following variants were experimented, applying single spraying treatments:

1. Plants sprayed with distilled water pH 6.8 were regarded as the control.
2. Plants were treated by HNO_3 solution pH 4.5, 4, 3 and 2 separately.
3. Plants were treated by H_2SO_4 solution pH 4.5, 4, 3 and 2 separately.
4. Plants were treated by mixed solutions of HNO_3 and H_2SO_4 pH 4.5, 4, 3, 2 separately.

Sampling and microscopic studies: Flowers and young pods from plants had removed from treatment and controlled plots separately. The flower were fixed in FAA_{70} (formaldehyde, glacial acetic acid and 70% ethanol, 5:5:90), stored in 70% ethanol. Specimens were embedded in paraffin and sectioned at 5-12 μm with a Leitz 1512 microtum. 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 anther and its developmental stages. Developmental stages of treated and controlled samples were compared. At each developmental stage, at least 20 anthers were studied and differentiations between treated and control plants were analyzed. Pollen fertility was checked by staining a minimum of 1000 pollen grains from each group using acetocarmine: 50% glycerin (1:1) for 1h.Well stained and perfect pollen grains were taken as fertile, while unstained/empty pollens were considered as infertile (Sheidai and Inamdar, 1992).

RESULTS

Results of microscopic studies showed that each anther of *Phaseolus vulgaris* consist of four pollen sac. The four pollen sac display relatively synchronous development, are initiated very early during flower development and occupy the majority of each flower. Pollen development was taking as other dicotyledonous plants. But in plants that were treated by different acidic solutions, some abnormalities were seen during pollen development. Tetrads were formed as spherical shape in normal plants but changing of tetrad shape to polygonal form is one of the treated effects by acid solutions (Fig. 1). In normal plants, tapetum layer cells were degrade very soon and plasmodium results that fed developmental pollen grains (Fig. 2), but degradation of tapetum cells were take later in treated plants (Fig. 3). Shape of pollen grains is spherical to triangle in normal plants, but abnormal shapes including vibrate and asteroid shapes were seen in plants that treated by acid solutions (Fig. 4). Exine was not formed in some case of treated plants by acid solutions (Fig. 5). Many vesicles and vacuoles were seen in cytoplasm of pollen grains in treated plants (Fig. 6), but few small vacuoles visible in control plants. Accumulation of black particles are evidence in pollen grains of plants that treated by acid solutions (Fig. 7). It seems that starch grains were accumulated in treated plants. Viability of pollen grains was checked by staining a minimum of 1000 pollen grains from each group by using acetocarmine: 50% glycerin (1:1) for 1h.Well stained and perfect pollen grains were taken as fertile, while unstained /empty pollens were considered as infertile. Results showed that in plants that treated by acid solutions viability and fertility of pollen grains were decreased dramatically (Fig. 8). In treated plants, also numbers of pollen grains and fertile pollen were decreased significantly in each anther (Table 1). Differentiations between normal plants and treated are significant ($p<0.01$), regarding both pollen fertility and abundance of pollen grains in each anther.

Table 1: Results showed that number of pollen grains and fertile pollen were decreased in plants that treated by acid solutions. Each data represents the mean of 25-30 flowers

Different treated groups	Pollen grains number	Pollen fertility (%)
H_2SO_4, pH2	79	53
H_2SO_4, pH3	81	76
H_2SO_4, pH4	83	78
H_2SO_4, pH4.5	97	82
HNO_3, pH2	60	46
HNO_3, pH3	66	66
HNO_3, pH4	73	72
HNO_3, pH4.5	86	77
H_2SO_4 and HNO_3, pH2	50	43
H_2SO_4 and HNO_3, pH3	74	49
H_2SO_4 and HNO_3, pH4	81	54
H_2SO_4 and HNO_3, pH4.5	87	72
Control	122	97

DISCUSSION

Microscopic studies showed that developmental process of pollen grains in bean plants was taking to ordinary process in dicotyledonous plants. In bean plants

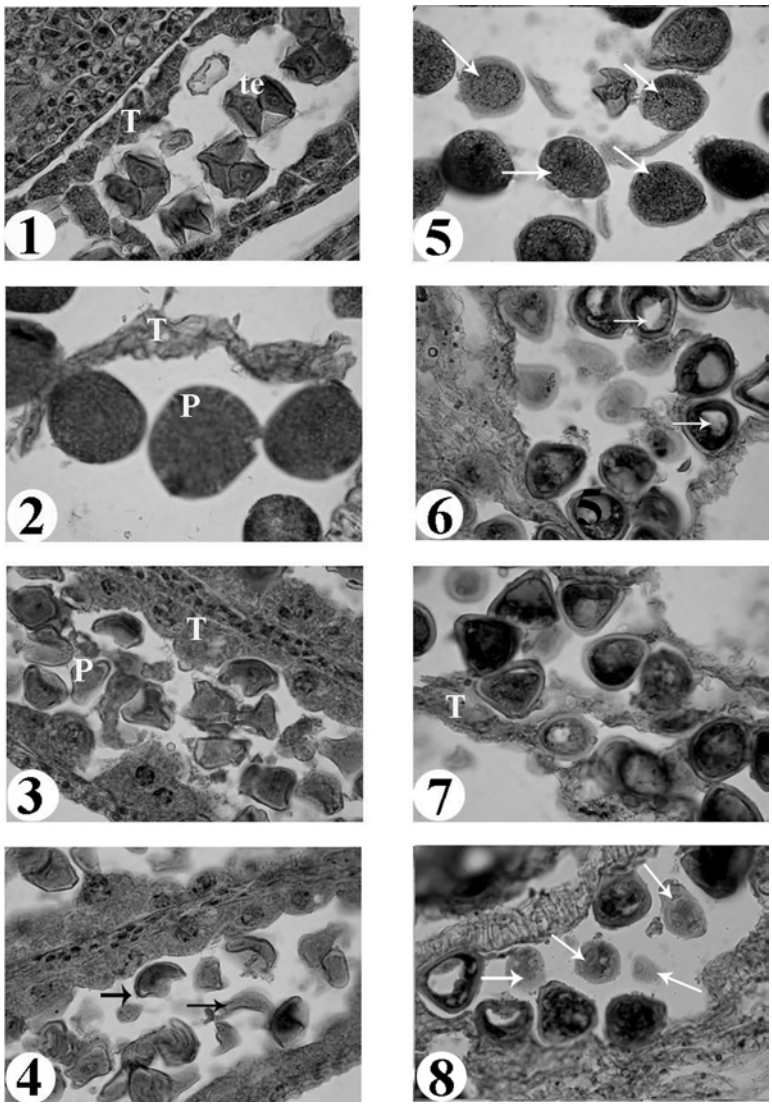

Fig. 1-8: Pollen grains development in *Phaseolus vulgaris* plants that treated by different acid solutions

Fig. 1: Longitudinal section through an anther and tetrads. Shape of tetrads was changed from spherical to polygonal in treated plants (X 400)

Fig. 2: Longitudinal section through an anther which shows the pollen grains. In normal plants, tapetum layer cells were degrade very soon and plasmodium results that fed developmental pollen grains (X 1000)

Fig. 3: Longitudinal section through a young anther that show development of pollen grains (X 600) Degradation of tapetum cells were take later in plants that treated by acid solutions.

Fig. 4: Longitudinal section through an anther that prepared from plants treated by acid solutions. (X 500) Shape of pollen grains is triangle in normal plants, but abnormal shapes including vibrate (↑) and asteroid shapes were seen in plants that treated by acid solutions

Fig. 5: Exine was not formed in some case of treated plants by acid solutions (X800)

Fig. 6: Many vesicles and vacuoles (↑) were seen in cytoplasm of pollen grains in treated plants, but few small vacuoles visible in control plants (X600)

Fig. 7: Acumolation of black particles in pollen grains is one of acid treatment results (X600)

Fig. 8: Unfertile pollen grains (↑) were increased considerably in plants that treated by acid solutions (X500) Abbreviations: T = Tapetum cells; te = tetrads; P = Pollen grains

that treated by different acid solution, some abnormality was seen during pollen developmental process (Fig. 1-7). Changing shape of tetrad from spherical to polygonal is a result of acid treatment that is evidence in treated plants (Fig. 1). It seems that acid treatments cause to change direction of cell division in experimental plants. Our previous reports (Chehregani *et al*., 2004a) indicate that environmental stresses cause to same results in plants that treated by SO_2 and environmental pollutants. Results showed that degradation of tapetum layer was take later in plants that treated by acid solutions (Fig. 3). This phenomena cause to deficiency in fading of developmental pollen grains.

Shape of pollen grains is spherical to triangle in normal plants (Fig. 4). It seems that dilation in degrading of tapetum cells and their inability to provide nutrients is reason of this change. Also, exine is not form in treated plants (Fig. 5). Formation of exine needs to a lot of precursors that should provided by tapetum layer. If tapetum cells can not degrades on time, some abnormalities are taken in during of pollen maturation (Chehregani *et al*., 2004b). Results showed that numbers of pollen grains were decreased in plants that treated by acid solutions. That is accordance with finding of some prior reports (Waldron and Cracker, 1989).

Pollen fertility was checked in both normal and treated plants. Results indicate that pollen fertility was decreased significantly in plants that treated by acid solutions. This is accordance with finding of Kratky *et al*. (1974) in tomato and Chehregani *et al*. (2004a) in *Zinnia elegans* plants that treated by polluted air.

Several reports showed that air pollution might affect pollen grains indirectly via stress on the growth on the plant or directly either through contamination of the anthers on the plant or during the flight of pollen grains through the air when it dispersed. Changes of the structure, ultra-structure, chemical compound and allergenicity of some pollen were reported in polluted areas (Chehregani *et al*., 2004b; Majd *et al*., 2004). Present results, in addition to, indicate that air pollution can induce several abnormalities during pollen development, through acid rain. In groups that treated by pH2 acid solutions and especially in HNO_3 solution, is more evidence than other groups.

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