http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 8 (4): 622-625, 2005
ISSN 1028-8880

Effect of Heavy Metals on the Developmental Stages of Ovule and Embryonic Sac in *Euphorbia cheiradenia*

Behrouz E. Malayeri, Abdolkarim Chehregani, Fariba Mohsenzade and Roghayeh Golmohammadi
Department of Biology, Bu-Ali Sina University, Hamedan, Iran

Abstract: The aim of this research was to elucidate some microscopic effects of heavy metals on the developmental stages of ovules in plants. We chose a large area in the near Malayer city as a polluted area. In this region amount of heavy metals is several times more than non-polluted area. Flowers and young pods removed from plants. The specimens fixed in FAA70 stored in 70% ethanol, embedded in paraffin and sectioned at 8 μm with microtome. Staining was carried out with Hematoxylin and developmental stages were compared in plants that collected from polluted and non-polluted area. The results of this research showed that heavy metals can affect some abnormality in during of ovule developmental process, in plants that collected from polluted area. These abnormalities include changes of ovule shape, quick degradation of embryonic sac's cell, quick growth of integuments, an increase in embryonic sac cytoplasm concentration, irregularity and even stopping the nuclear envelope formation. Finally reduction in number of ovules were seen in polluted plants.

Key words: Heavy metals, toxicity, embryonic sac development, pollution, *Euphorbia cheiradenia*

INTRODUCTION

Heavy metals make a significant contribution to environment as a result of human activities such as mining, smelting, electroplating, energy and fuel production, power transmission, intensive agriculture, sludge dumping and milting operations. Some heavy metals, e.g. Mn, Fe, Cu, Zn, Mo and Ni are essential or beneficial micronutrient for microorganisms, plants and animals[1]; others have no known biological or physiological function. All heavy metals at high concentrations have strong toxic effects and environmental pollutants[2]. Although plants adapt rather readily to chemical stress, they also may be very sensitive to an excess of particular trace element. Visible symptoms of toxicity vary for each plant species and even for individual plants, but most common and non specific symptoms of phytotoxicity are chlorotic or brown, stunted, coralloid roots[3]. Toxic effects of element excesses are changes in permeability of the cell membrane, reactions pf thiol groups with cations, damage to photosynthesis apparatus, occupation of sites for essential ions[3].

Pollen grains accumulate heavy metals, i.e. lead and cadmium, but also nitrate and other compounds[4-6]. The higher cadmium concentrations caused cyanobacterial cells to die, while the lower concentrations resulted in as light inhibition of growth[7].

We studied effects of heavy metals pollution on the ovule development and embryogenesis of *Euphorbia cheiradenia.* Most euphorbs have three-locular ovaries except *Crotonopsis* species which form a one-seeded urticle[8]. Ovules of *Euphorbia cheiradeni,* as well as other euphorbs are anatropous throughout all stages of development[8-10]. However, orthotropous and even hemianatropous ovules have been reported in some members of euphorbiaceae[8]. The crasinucellate and bitegumic nature of Euphorbia ovules appear to be universal features of Euphorbiaceae[8]. Embryo sac development have been described as *Polygonum* type (monosporic, seven called, eight nucleate embryo sac consisting of three antipodal cells; a large, binucleate central cell and an egg cell adjacent to two synergides) is considered to be the most common and most primitive[11]. Lyon[9], Weniger[12], Carminchael and Selbo[13] have reported that antipodal cells are ephemeral and appear to degenerate prior to embryogenesis. The two polar nuclei found in embryo sac of *Euphorbia* remain separate and distinc until the time at which a zygot is distinguished[12,13].

Although many researchers have been showed detrimental effects of environmental pollutants on the sexual reproductive organs[4,14-16], but we could not find any report about the heavy metal effects on the developmental stages of plant ovules and embryonic sac. These stages are most important on survival of plants.

MATERIALS AND METHODS

We chose a large area in the near Malayer city as a polluted area. This region is near the Lead main and amounts of heavy metals is several times more than

Corresponding Author: Abdolkarim Chehregani, Department of Biology, Bu-Ali Sina University, Hamedan, Iran
E-mail: chehregani@basu.ac.ir

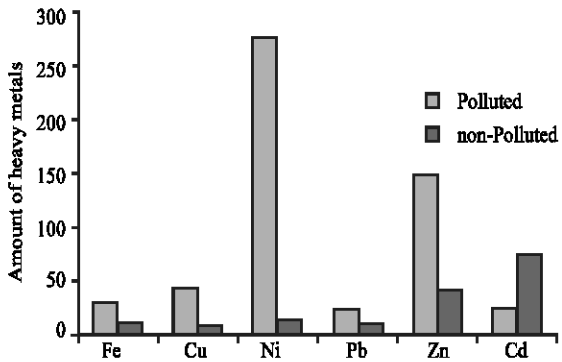

Fig. 1: Comparative of some heavy metals in the near of Lead main as a polluted area and out of this area as non-polluted area. Each column represent means of 8 sampling sides in polluted and non-polluted area. Data showed that amount of Zn and Ni in polluted area is several times more than non-polluted area

non-polluted area (Fig. 1). *Euphorbia cheiradenia* belonging to *Euphorbiaceae* is a common plants in that area and used as an accumulator plant for this study. Flowers and young buds from field plants had removed from polluted and non-polluted areas separately. The flowers were fixed in FAA70 (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 microtom. Staining was carried out with PAS (Periodic Acid Schiff) according to the protocol suggested by Yeung[17] and contrasted with Meyer's Hematoxilin. Several sections were studied under a light microscope Zeiss Axiostar Plus for each embryonic sac and ovule developmental stages. Developmental stages of polluted and non-polluted samples were compared. At each developmental stage, at least 20 flowers were studied and differentiations between polluted and non-polluted plants were analyzed.

RESULTS

Results of microscopic studies showed that each ovary of *Euphorbia cheiradenia* Boiss. consist of three carpals with a single ovule formed per carpel. The three ovules display relatively synchronous development, are initiated very early during flower development and occupy the majority of each locular chamber. Ovules have a relatively short funicle, are anatropous at inception and remain in that orientation throughout seed maturation (Fig. 2). Ovules are bitegumic, with the outer integument possessing a specialized Elaisome (caruncle). The micropyle consists of both the inner and outer integuments, with the outer integument completely surrounding the inner integument (Fig. 3). The inner and outer integuments also do not co-align and thus a zig-zag micropyle is formed. Embryo sac formation conforms to the polygonum type. The mature embryo sac consists of two polar nuclei and a well-defined egg cell located between two synergids. Prior to fertilization, the egg cell is densely cytoplasmic and contains numerous organelles (putative plastids). Synergids are characterized by a filiform apparatus, although it is not always easily discernible. Although antipodals are formed, they are short lived and appear to degenerate prior to embryo sac maturation. The two polar nuclei remain separate and distinct throughout embryo sac maturation and appear to fuse only after formation of a zygote (Fig. 4).

In plants that collected from polluted area some abnormalities were seen during ovule development. It was observed that the number of ovules decrease significantly in polluted plants. Ovular integuments are more condensed (Fig. 5). Nucellus cells are irregular and vacuolated considerably (Fig. 5). Degradation of nucellus tissue and embryo sac were observed in some ovules (Fig. 6). In plants that collected from polluted area, some nucleus in embryonic sac show irregularity and in these cases nuclear envelop did not formed after mitotic divisions (Fig. 7).

DISCUSSION

This report is the first to provide a detailed ovule and embryo sac development in *Euphorbia cheiradeni*. The results presented indicate that many sexual reproductive features expressed in *Euphorbia cheiradenia* are similar to those of other members of the Euphorbiaceae, but several unique structures are evident in plants that contaminated by heavy metals.

Ovaries of *Euphorbia cheiradenia* exhibited three-locular carpels and an ovules with each carpels (Fig. 2). Present observations showed that patterns of embryo sac development are evidence as standard *polygonum* type (monosporic, seven celled, eight nucleate embryo sac consisting of three antipodal cells; a large binucleate central cells; and an egg cell adjacent to two synergids) that accordance with findings of other researchers about other members of Euphorbia[11,13]. The mature embryo sac

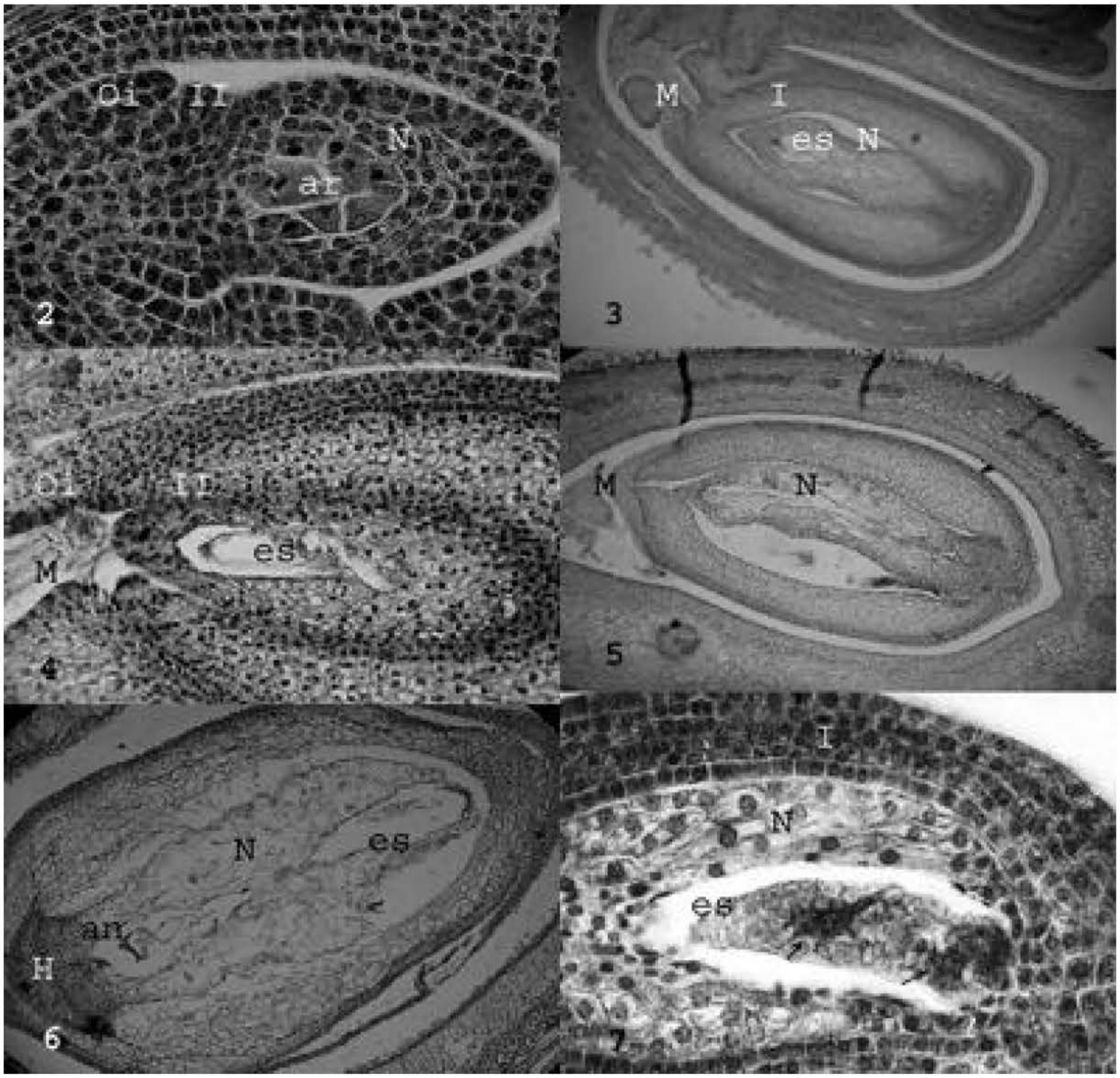

Fig. 2-7: Ovule development in *Euphorbia cheiradenia*

Fig. 2: Longitudinal section through an ovary and distinct young ovule. Outer and inner integuments have initiated. Nucells cell and archeospores were seem in this stage (X 400)

Fig. 3: Longitudinal section through a young ovule which shows the integuments and zigzag nature of micropyle. Ovule is anatropus with two integuments and a zigzag micropyle (X 400)

Fig. 4: Longitudinal section through a young ovule that shows development of embryonic sac (X 600)

Fig. 5: Longitudinal section through an ovule that prepared from plants collected from polluted area. Integuments are condensed and nucells show irregularity and degradation (X 500)

Fig. 6: Antipodal cells are formed in embryonic sac but they are died and disappear very soon. Nucells tissue show irregularity and degradation in plants that collected from polluted are (X 600)

Fig. 7: Longitudinal section through an ovule that prepared from plants collected from polluted area. The arrow designates the irregularity of nucleus in embryonic sac. It seems that nuclear envelop has been not formed after mitotic division in the embryonic sac development (X 600). Oi, Outer integument; II, inner integument; N, nucellus cells; ar, archeosporal cells; M, micropyle; es, embryonic sac; I, integuments; Arrow show nucleus of embryonic

consist of two polar nuclei and a well-defined egg cell located between two synergids (Fig. 4). The micropylar region of nucellus extends into the micropyle and thus represents a nucellar beak (Fig. 3). Although antipodals are formed (Fig. 4) they short lived and appears to degenerate prior to embryo sac maturation[13].

Our observations showed that developmental stages of ovules and embryo sac in plants that collected from polluted area were similar with those of non-polluted area. But there are some differences between polluted and non-polluted plants. In plants that collected from polluted area some abnormalities were seen during ovule development. It was observed that the number of ovules decrease significantly in polluted plants that is accordance with findings of Majd and Chehregani[14] in plants that treated by SO_2. Ovular integuments are more condensed (Fig. 5). Present findings (Fig. 5) showed that nucellus cells are irregular and vacuolated considerably in plants that collected from polluted area. Degradation of nucellus tissue and embryo sac were observed in some ovules of polluted plants (Fig. 6). In plants that collected from polluted area, some nucleuses in embryonic sac show irregularity and in these cases nuclear envelop did not formed after mitotic divisions (Fig. 7). These findings are accordance with reporting of some prior researchers about effects of other environmental pollutants such as SO_2[14], air pollutants[4] but this is the first report about effects of heavy metal pollution on the embryo sac development. It seems heavy metal pollutions could affect the same of other environmental pollutants and induce some abnormality in the during of developmental stages. Damaging of nuclear envelop is the common phenomena in plants that contaminated by environmental pollutants.

REFERENCES

1. Welch, R. and M. Micro, 1995. Nutrition of plants. Crit. Rev. Plant Sci., 14: 49-82.
2. Nedelkoska, T.V. and P.M. Doran, 2000, Characteristics of heavy metal uptake by plant species with potential for phytoremediation and phytomining. Mineral Engineering, 13: 549-561.
3. Alina, K.P., 2001. Trace Elements in Soil and Plants. 3rd Edn. CRC Press, NW Corporate Blud, Boca Raton, Fl 33431.
4. Behrendt, H., W.M. Becher, K.H. Friedichs, U. Darsow and R. Tomingas, 1992. Intraction between aeroallergens and airborne particulate mater. Intl. Arch. Allergy Immunol., 99: 425-428.
5. Sawidis, T., 1997. Accumulation and effects of heavy metals in Lilium pollen. Acta Horticult., 437: 153-158.
6. Sawidis, T. and H.D. Reiss, 1995. Effects of heavy metals on pollen tube growth and tube ultrastracture. Protoplasma, 185: 113-122.
7. Upatham, E.S., E.S. Rangsayatorn, M. Kruatrachue, P. Pokethitiyook and G.R. Lansa, 2002. Phytoremediation potential of spirulina (Arthrospira) platensis: biosorption and toxicity studies of cadmium. Environmental Pollution, 119: 45-53.
8. Davis, G.L., 1966. Systematic Embryology of the Angiosperms. John Wiley and Sons, New york.
9. Lyon, F.M., 1898. A contribution to the life history of Euphorbia corollata. Bot. Gaz., 25: 418-431.
10. Landes, M., 1940. Seed development in *Acalypha rhomboidea* and some other Ephorbiacea. Am. J. Bot., 33: 562-568.
11. Stebbins, G.L., 1974. Flowering Plants: Evolution Above the Species Level. Harvard University Press, Cambridge, Mass.
12. Weniger, 1917. Development of embryo sac and embryo in *Euphorbia preslii* and *E. spendens*. Bot. Gaz., 63: 266-281.
13. Carmichael and Selbo, 1999. Ovule, embryo sac, embryo and endosperm development in leafy spurge (*Ephorbia esula*). Canadian J. Bot., 77: 599-610.
14. Majd, A. and A. Chehregani, 1992. Studies on developmental processes in ovules of Soja (*Glycine Max*) plants and effects of certain toxins and envirimental pollutants. Acta Horticultyrae, 319: 431-436.
15. Chehregani, A., A. Majd, M. Moin, M. Gholami and M.A. Shariatzade, 2004. Increasing allergy potency of pollen grains in polluted areas. Ecotoxicology and Environmental Safety, 58: 267-272.
16. Chehregani, A., A. Majd, M. Moin, M. Gholami, M.A. Shariatzade and F. Mohsenzade, 2004. Effect of air pollution on some cytogenetic characteristics, structure, viability and proteins of *Zinnia elegans* pollen grains. Pak. J. Biol. Sci., 7:118-122.
17. Yeung, E.C., 1984. Histological and Histochemical Staining Procedures. In: Cell Culture and Somatic Cell Genetics of Plants. (Ed.) Vasil, I.K., Academic Press, Orlando, Fla, pp: 686-697.