EFFECT OF LEAD ON LIPID METABOLISM IN MUSTARD SEEDS

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ABSTRACT

The present study has been undertaken with the objective to investigate the effect of lead (Pb) on lipid metabolism in mustard during seed development. The study has been divided into four sections namely: Effect of different levels of lead on lipid composition of developing mustard seeds, Effect of lead (Pb) level on fatty acid composition of total lipids in developing mustard seeds, Effect of different level of lead on fatty acid composition of non- polar lipids of developing mustard seeds, Effect of different level of lead on fatty acid composition of polar lipids of developing mustard seeds.

Keywords:- *polar lipids, DAF, methylate, iodometric*

INTRODUCTION

Heavy metals such as lead, cadmium, mercury, arsenic and selenium are an increasing environmental problems worldwide (Zhu *et al.*, 1999) .In contrasts to the organic contaminants, which can undergo biodegradation, heavy metals remain in the environment. Long-term deposition of metals in soil can lead to accumulation, transport and bio-toxicity caused by mobility and bio-availability of significant fraction of metals (Adriano *et al.*, 2004).

Heavy metals are toxic for plants as well as these depress growth and yield of crops. The primary toxicity mechanisms of heavy metals alter the catalytic function of enzymes, damage cellular membranes and inhibit root growth. These changes cause numerous secondary effects: inhibition of photosynthesis, hormonal imbalance and water-stress. The toxicity symptoms seen in the presence of excessive amounts of heavy metals may be due to a range of interactions at the cellular/molecular level. Toxicity may result from binding of metals to the sulphdryl groups in proteins, leading to an inhibition of activity or disruption of structure (Van Assche *et al.*, 1990). Plants possess a range of potential cellular mechanisms that may be involved in the detoxification of heavy metals and thus tolerance to metal stress (Hall, 2002).

Among heavy metals, Pb is a potential environmental pollutant. Lead is not essential for plant and is toxic even in low concentrations. The possible sources of lead entry into soil and plant are as an aerial deposit and in precipitation, irrigated water, mine drainages, leaf litter and ground dust blown in from elsewhere. Lead is added to the soil in certain fertilizers and pesticides. They also reach soil and plant from air .Aerial lead is generated mostly by combustion of gasoline in vehicles (Lagerwerff, 1967) due to fall out of large particles, roadside

concentration of lead in both soil and vegetation decrease away from the traffic (Lagerwerff and Specht, 1970) and are detected up to a distance of 100 m from the road-side in vegetation. Lead enters into plants by two possible pathways-uptakes by the foliage and uptake by the roots. Once inside the system, Pb (lead) seems to be retained by cell membranes, mitochondria and chloroplast (Sabins *et al.*, 1969). Heavy metals have been shown to affect a wide range of plant cellular activities like photosynthesis, respiration, mineral nutrition, membrane structure and properties. Many of these effects include alteration of lipid metabolism also. Heavy metals cause deleterious effects on fatty acid metabolism in plants.

Surface lipids are a layer of lipid molecule that protects plants from UV-irradiation, hydrophobic pollutants and diseases. Polymeric lipids such as cutins and suberins which contain phenolics and other metal-binding groups are also present as cell surface constituent in sphagnum mosses. Heavy metals can interact with the main lipid assembling pathway and so affect the distribution of flux between major products. This distribution is influenced by both the activity of enzymes and the availability of acyl-CoA substrates (Shtemenko, 1997).

Diverse biochemical changes in green plants in response to lead have been reported, such as rapid decrease in chlorophyll content (Kastori *et al.*, 1998), carotenoids, proteins, nitrate reductase activity.

The acetate of lead acetate is incorporated well into the acyl chains of complex lipids. This aspect tells that heavy metals have often been noted to change fatty acids biosynthesis in plants (Jones *et al.*, 1987; Maksymic *et al.*, 1992 and Harwood, 1995). Lead present as lead acetate resulted in increase in chlorophyll content and rate of photosynthesis in low concentration (200 mg/kg), medium concentrations showed no marked effects, whereas, high concentration (800 mg/kg) decreased the area of leaves, the content of green pigments in them, and the rate of photosynthesis in barley and oat (Kaznina, 2005).

There are numerous investigations on the influence of heavy metals on the plant metabolism, but the data for the effect of Pb on the fatty acids composition of the various lipids classes in Brassica during seed development are very limited. Brassica oilseed species now hold the third position among oilseed crops and are an important source of vegetable oil. The high amount of linoleic acid in mustard oil makes it good quality edible oil. Emphasis on the advantages of polyunsaturated fats in human diet has extended the use of mustard oil in edible fats and processed food. It is, therefore, important to know the effect of environmental pollutant Pb on the oil quality of mustard. It has also been shown that lipid changes in *Brassica juncea*, the well known heavy metal hyperaccumulator species, revealed a more stability of its cellular membranes to Pb-stress as compared to *Brassica napus*. Therefore in order to investigate the effect of heavy metal on lipid metabolism in *Brassica juncea* seems to be better than *Brassica napus*. In view of the above, the present investigation has been undertaken with the objective to study the effect of lead (Pb) on lipid metabolism in mustard (*Brassica juncea*) during seed development.

MATERIALS & METHODS

Plant material

The seeds of mustard (*Brassica juncea* L.) were procured from the Department of Plant Breeding, CCS Haryana Agricultural University, Hisar.

Chemicals and reagents

The chemicals and reagents used during the present investigation were of analytical grade.

Raising of crop and treatments

Mustard crop was raised in earthen pots filled with sandy loam soil in the screen house and the pots were lined with polyethylene bags to avoid contamination. A recommended dose of nitrogen (60 ppm) and phosphorus (30 ppm) was given in the form of urea and potassium dihydrogen orthophosphate. A basal dose of micronutrients Zn, Mn, Cu and Fe at the rate of 5, 2.5, 2.5 and 10 ppm, respectively was also supplied. The lead in the form of lead acetate was applied at the rate of 0 (control), 10, 20, 30 and 40 ppm (μg g^{-1} soil).After the emergence of seedlings, the number of plants were thinned to three plants per pot. Nitrogen supplied by $(NH_4)_2SO_4$ was taken in account and remaining nitrogen was supplied through urea in order to keep the level of basal dose of nitrogen equal in all the treatments. The pots were kept under natural conditions in the screen house. Deionized water was used for irrigation.

Sampling

Plants were tagged at initiation of flowering and one hundred siliquae were collected in triplicate from control, 20, 40, 60 days after initiation of flowering and at maturity which were further separated into siliquae wall and seeds. Seeds samples were taken for further analysis. After drying seed samples in hot air oven maintained at 60 °C, the seeds were grinded in a micro Willay mill and these samples were stored in the polyethylene bags for the estimation of various biochemical constituents.

BIOCHEMICAL ANALYSIS

Extraction and estimation of total lipids

Total lipids were extracted according to the method of Folch *et al.* (1957). Five hundred mg of sample was taken and crushed thoroughly in a pestle and mortar with 0.5 g anhydrous sodium sulphate. To this 100 ml of chloroform: methanol (2: 1 v/v) was added and the whole material was transferred in air tight glass stoppered iodometric flask. The contents of the flask were shaken for one hour on an electric shaker and filtered through the glass sintered funnel. The extraction was repeated twice. All the extractions were pooled and solvent was distilled off under

vacuum from the filtrate till lipids were completely free of the solvents. To remove water soluble impurities, the crude lipid sample was weighed. To this, 20 volumes of chloroform: methanol (2: 1 v/v) mixture and 5 volumes of 1 per cent sodium chloride solution were added. The whole contents were put in a separating funnel, shaken and allowed to stand for 5 minutes. The pure lipid fraction came in the lower chloroform layer, while soaps glycerols and other water soluble impurities remained in the upper layer. The lower layer was recovered and the upper layer was again treated with 5-10 ml of chloroform: methanol mixture to obtain the residual lipids if any. This operation was repeated thrice until all the lipids were completely recovered. To remove moisture from the pure fat, anhydrous sodium sulphate was added. The excess solvent was removed under vacuum and the recovered fat was taken up in 10 ml of chloroform and stored in the deep freezer until further use. Total lipids were determined gravimetrically.

RESULTS & DISCUSSION

The present study was carried out on mustard to investigate the effect of lead (Pb) on lipid metabolism during seed development. The results obtained are presented in this section under the following heads:

- Effect of different levels of lead on lipid composition of developing mustard seeds.
- Effect of different levels of lead on fatty acid composition of total lipids in developing mustard seeds.
- Effect of different level of lead on fatty acid composition of non- polar lipids of developing mustard seeds.
- Effect of different level of lead on fatty acid composition of polar lipids of developing mustard seeds.

Effect of different levels of lead on lipid composition of developing mustard seeds

The results of effect of different levels of lead on lipid composition of developing mustard seeds has been given in Table (1-4).

Table 1 depicts that the lipid composition affected appreciably by lead doses. At 20 days after initiation of flowering, total and non-polar lipids decreased regularly by increasing doses of lead at 10,20,30 and 40 ppm. But a reverse trend was observed in case of polar lipids. Polar lipids at lead concentration of 10, 20, 30 ppm were higher than the control. However at 40 ppm concentration, there was a slight decrease in the polar lipid concentration but still it is higher than control. Similar observations were recorded at 40 DAF, 60 DAF and at maturity (Table 2-4). These results are in agreements with the findings of Aggarwal et al (1995); who studied the effect of Cd^{2+} on lipid components of sunflower seeds. They also reported that total lipid contents in sunflower seeds decreased with increasing concentration of applied Cd^{2+}.

Polar lipids which form the major proportion of membrane lipids provide fluid matrix to the membrane. Pb cation at higher concentrations exhibit a quenching effect on lipid surface charge consequently decreases the polarity of the membrane lipids. The cells apparently restore the polarity by increasing synthesis of polar lipids (Demel, 1987).

Table 1: Effect of different levels of lead on lipid composition of developing mustard seeds (mg/g dry weight) at 20 DAF

Lead conc. (ppm)	Total lipids	Polar lipids	Non Polar lipids
Control	51.4±1.13	30.9±0.48	19.7±0.74
10	49.2±0.46	31.6±0.89	18.4±0.24
20	49.1±0.18	34.7±0.60	15.3±0.36
30	46.6±1.19	35.2±0.72	11.6±0.83
40	44.3±0.74	34.2±0.46	10.8±0.79

± indicates S.D.
DAF = Days after initiation of flowering

Table 2: Effect of different levels of lead on lipid composition of developing mustard seeds (mg/g dry weight) at 40 DAF

Lead conc. (ppm)	Total	Polar lipids	Non Polar lipids
Control	161.8±1.13	21.3±0.48	140.3±1.17
10	157.4±0.89	22.6±0.48	135.8±0.69
20	152.6±0.49	24.9±1.28	129.1±1.23
30	144.6±1.19	25.2±0.72	118.6±1.12
40	139.3±1.06	29.2±0.62	110.8±1.02

± indicates S.D.
DAF = Days after initiation of flowering

Table 3: Effect of different levels of lead on lipid composition of developing mustard seeds (mg/g dry weight) at 60 DAF

Lead conc. (ppm)	Total lipids	Polar lipids	Non Polar lipids
Control	380.5±4.11	30.9±0.58	348.4±3.89
10	374.6±2.46	32.6±0.89	340.4±1.24
20	357.4.1±1.18	35.7±0.82	322.7±1.36
30	342.6±1.19	38.2±0.72	303.6±1.83
40	329.3±1.74	42.7±0.76	289.8±1.79

± indicates S.D.
DAF = Days after initiation of flowering

Table 4: Effect of different levels of lead on lipid composition of developing mustard seeds (mg/g dry weight) at maturity

Lead conc. (ppm)	Total lipids	Polar lipids	Non Polar lipids
Control	397.4±5.13	20.9±0.48	376.7±3.74
10	391.2±1.46	22.6±0.89	369.4±1.24
20	376.1±1.18	25.7±0.86	351.3±1.36
30	354.6±1.19	32.2±0.77	322.6±0.83
40	342.3±1.74	37.2±0.46	303.8±1.79

± indicates S.D.

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