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 6 (2): 157-163, 2003
ISSN 1028-8880

Effect of Growth Hormones i.e., IAA, Kinetin and Heavy Metal i.e., Lead Nitrate on the Internal Morphology of Leaf of *Phaseolus vulgaris* L.

N.Y.Chaudhry and Qurat-ul-Ain
Department of Botany, University of the Punjab, Quaid-e-Azam Campus, Lahore 54590, Pakistan

Abstract: Histomorphology of the leaves of *Phaseolus vulgaris* L. were studied following treatments with growth hormones i.e., IAA and Kinetin and heavy metal i.e., Pb $(NO_3)_2$. The data was collected after 15 days and compared with control. In 50 ppm IAA, 50 ppm Kinetin as well as 50 ppm IAA+50 ppm Kinetin 3 additional leaf primordia were observed when compared with control while in all other treatments five leaves were observed. Moreover, application of 50 ppm IAA and 50 ppm Kinetin also registered an increase in leaf area, consequently the fresh and dry weight of leaves were also increased. While application of 50 ppm Pb, 50 ppm IAA+50 ppm Pb and 50 ppm Kinetin+50 ppm Pb revealed decrease in the above parameter. SLA also showed increase. The growth hormones also registered increase in the number of stomata, size of guard cells, as well as stomatal pore, stomatal index and %age of open stomata. Whereas, 50 ppm Pb and 50 ppm Pb+50 ppm Kinetin showed remarkable decrease in the %age of open stomata. Moreover, the number and size of palisade cells, width of spongy cells, width of abaxial and adaxial epidermal cells, number of xylem elements and width of metaxylem elements also showed increase when treated with 50 ppm IAA, 50 ppm Kinetin individually as well as in combinations. While all the above parameters showed decrease with the application of 50 ppm Pb alone and in combination with 50 ppm IAA and 50 ppm Kinetin.

Key words: Leaf growth, hormones, indole-3-acetic acid, kinetin, heavy metal, lead nitrate

Introduction

Growth and development of plants is the net result of metabolic processes controlled by the natural as well as synthetic hormones. Appropriate concentration of growth hormones can stimulate the activity of key chemical substances and physiological processes, which are reflected by growth (Awan, 1999). Auxins bring about a number of morphological, genetical and physiological changes in leaf (Steward, 1972; Krishnamoorthy, 1981). Furthermore, Leopold and Kriedemann (1975) showed that application of IAA to plant tissues caused increase in the number of open stomata. The epidermal cells of leaves treated with IAA were smaller than control was reported by Keller (2001). Moreover, El-Aishy *et al.* (1976), Iqbal and Mahmood (1980), Chaudhry and Zahur (1992), Tuominen *et al.* (1997) and Awan *et al.* (1999) reported that IAA increased the leaf area. IAA also increased the dry weight of leaves (Kumar *et al.*, 1981). Cytokinins are involved in the control of numerous and important processes associated with plant growth and development (Brault *et al.*, 1999). Ferguson and Guinel (2001) reported that cytokinins are involved in chlorophyll stabilization and may have a role in the altered levels of chlorophyll found in leaves. Application of cytokinins promote the expansion of leaf tissue (Pozsar, 1967). Heavy metals are observed to be growth inhibitors. Application of heavy metals to the leaves show decrease in the number of open stomata and thereby O_2 deficiency in plant (Fitter and Hay, 1981). Heavy metals have direct physiological toxic effects was reported by Botkin and Keller (1995). Furthermore, Stoyanova (1998) reported that heavy metals bring about anatomical changes in primary leaves hence treatments induced changes in the shape of palisade cells. Pb dust from automobile exhaust which lands on the crops growing on roadsides result in deleterious effects when absorbed through leaves (Zaikovskaya, 1990). Furthermore, Pb binds the essential enzymes and cellular components and inactivates them (Cunningham and Saigo, 1995). Moreover, effects of Pb on plants include reduction in growth parameters (Dalal *et al.*, 1985; Baura *et al.*, 1986; Prasad and Prasad, 1987). Heavy metals also disrupt the metabolic processes of living organisms (Moran *et al.*, 1986).

Materials and Methods

The present study deals with the effects of growth hormones i.e., IAA and Kinetin and heavy metal i.e., Pb (lead nitrate) on the leaves of *Phaseolus vulgaris* L., of family Leguminosae (Fabaceae). Growth hormones and heavy metal were used individually as well as in combinations i.e., 50 ppm IAA, 50 ppm Kinetin, 50 ppm Pb, 50 ppm IAA+50 ppm Kinetin, 50 ppm IAA+50 ppm

Pb, 50 ppm Kinetin+50 ppm Pb, 50 ppm IAA+50 ppm Kinetin+50 ppm Pb.

Seeds were grown in petri plates under controlled environmental conditions in growth chambers in the month of March in Anatomy research lab for 15 days. Ten ml of each concentration of growth hormones as well as heavy metal were added individually as well as in combination (as mentioned above) in separate petri plates. They were shifted to the dark chamber until germination started. Then they were transferred to the chamber having 16 h light period and 8 h dark period. Then dose of 10 ml nutrient solution (Hewitt, 1963) and 10 ml of distilled water were applied on alternate days for 15 days. After 15 days the number of leaves, leaf area, fresh and dry weight and specific leaf area were calculated. In the internal morphology, 1 cm long x 1 cm wide leaf pieces were fixed in Corney's modified fluid. Material was dehydrated and cleared in tertiary butyl alcohol grades, infiltrated and embedded in paraffin wax. The embedded material was processed in transverse planes with rotary microtome (10-15 μm). The material was then passed through descending series of xylene and stained with Safranine and fast green and mounted in Canada balsam.

The material was observed under the microscope. Data obtained was compared with control as well as among themselves. All data was subjected to statistical analysis (Steel and Torrie, 1981).

Results

External morphology: In the control plants, after 15 days the number of leaves observed were five. In IAA and Kinetin treatments and in the mixed dose of IAA+Kinetin 3 additional leaf primordia were observed, while in all other treatments the number observed was the same as in control (Table 1).

Fresh weight of all the five leaves in control was 0.2416 g. An increase of 4.71 and 16.26% was observed with IAA and Kinetin treatments, whereas Pb treatments showed a well marked decrease i.e., 51.24%. Similarly, application of the mixed doses of IAA+Pb, Kinetin+Pb and IAA+Kinetin+ Pb registered decrease in fresh weight as compared to control (Table 1). On the other hand, the dry weight of leaves increased with the application of IAA and Kinetin, which being 14.15 and 39.73%, respectively. In Pb treatments as well as in the mixed doses of IAA+ Pb, Kinetin+Pb and IAA+Kinetin+Pb the decrease in dry weight was observed (Table 1). However, in the mixed dose of IAA+Kinetin the decrease was negligible.

The area of leaves in plants treated with IAA and Kinetin showed expansion (Fig. 2, 3). Contrarily Pb treatments showed significant decrease i.e., 44.54%. Moreover, all the mixed doses registered decrease in the area (Table 1).

Fig.1: Seedling of *Phaseolus vulgaris* in control.

Fig. 2: Plant treated with IAA.

Furthermore, the specific leaf area observed in control was 981.16%. Application of IAA and Kinetin showed decrease of 8.25% and 20.98%, whereas the maximum increase in SLA was observed in Pb and Kinetin+Pb treatments, which being 39.29% and 37.66%, respectively. All other mixed doses also registered increase as compared to control (Table 1).

Table 1: Effect of growth hormones i.e., IAA, Kinetin and heavy metal i.e., Pb on external morphology of leaves

Treatments	No. of leaves per plant	Fresh weight of leaves (g)	Dry weight of leaves (g)	Area of leaf (sq. cm)	Specific leaf area (SLA)
Control	5	0.2416±0.00004	0.04301±0.0001	42.2±15.36	981.16
50 ppm IAA	5+3 Primordia	0.2530±0.021	0.0491±0.0001	44.2±13.82	900.20
50 ppm Kinetin	5+3 Primordia	0.2802±0.00004	0.0601±0.023	46.6±15.13	775.38
50 ppm Pb	5	0.1178±0.021	0.0174±0.0002	23.4±7.40	1344.83
50 ppm IAA+50 ppm Kinetin	5+3 prim	0.2369±0.0005	0.0420±0.0001	42.0±14.10	1000.00
50 ppm Kinetin+50 ppm Pb	5	0.1270±0.00005	0.018±0.00009	24.6±7.59	1366.66
50 ppm IAA+50 ppm Pb	5	0.2117±0.0001	0.0379±0.046	41.8±17.81	1102.90
50 ppm IAA+50 ppm Kinetin+50 ppm Pb	5	0.1998±0.00008	0.034±0.023	39.6±18.31	1164.70

Table 2: Effect of growth hormones i.e., IAA, Kinetin and heavy metal i.e., Pb on epidermis of leaves

Treatments	No. of stomata/ mm^2	No. of epidermal cells/ mm^2	Size of Guard cells	
			Length (µm)	Width (µm)
Control	15.28±0.34	45.85±0.54	100.66±0.54	30.66±0.27
50 ppm IAA	15.70±0.60	43.31±0.24	120.00±0.94	40.00±0.47
50 ppm Kinetin	20.80±0.24	40.76±0.23	100.88±0.72	29.33±0.42
50 ppm Pb	14.40±0.94	43.31±0.60	120.00±0.24	37.00±0.54
50 ppm IAA+50 ppm Kinetin	19.29±0.60	45.83±0.24	110.00±0.27	32.33±0.60
50 ppm Kinetin+50 ppm Pb	14.10±0.54	33.12±0.72	100.66±0.98	31.33±0.54
50 ppm IAA+50 ppm Pb	17.83±0.72	44.58±0.42	100.83±0.23	31.33±0.23
50 ppm IAA+50 ppm Kinetin+50 ppm Pb	19.10±0.42	40.76±0.24	119.66±0.60	36.33±0.72

	Size of stomatal pore		Stomatal index	% of open and closed stomata	
	Length (µm)	Width (µm)		Open (%)	Close (%)
Control	60.5±0.23	30.0±0.42	24.99	91.66	8.33
50 ppm IAA	79.00±0.54	36.66±0.27	26.00	92.66	7.33
50 ppm Kinetin	67.66±0.42	38.33±0.24	33.70	83.33	37.50
50 ppm Pb	83.33±0.72	35.00±0.60	24.95	57.14	42.18
50 ppm IAA+50 ppm Kinetin	72.00±0.026	31.66±0.72	29.62	81.81	18.18
50 ppm Kinetin+50 ppm Pb	62.66±0.54	32.33±0.24	28.72	37.40	62.60
50 ppm IAA+50 ppm Pb	60.00±0.27	29.00±0.42	28.56	44.00	56.00
50 ppm IAA+50 ppm Kinetin+50 ppm Pb	80.50±0.60	32.00±0.27	31.90	76.80	23.20

Table 3: Effect of growth hormones i.e., IAA, Kinetin and heavy metal i.e., Pb on epidermis and mid vein of leaves

Treatments (50 ppm)	Width of adaxial epidermal cells (µm)	Width of abaxial epidermal cells (µm)	Width of median vascular bundles (µm)	No. of xylem elements	Width of metaxylem elements (µm)
Control	30.00±0.89	22.00±1.09	304.00±0.61	31.00±0.45	32.00±0.33
IAA	29.20±0.81	21.00±0.96	331.00±0.54	33.80±1.27	34.00±0.90
Kinetin	32.20±0.67	20.20±0.47	329.00±0.54	30.90±0.24	31.80±0.90
Pb	28.60±1.09	19.20±0.36	268.00±0.81	26.40±0.96	28.20±0.24
IAA+Kinetin	38.40±1.20	26.40±0.47	357.00±1.47	38.80±1.34	36.00±0.96
IAA+Pb	26.20±0.80	18.60±2.00	296.00±1.50	30.20±0.67	28.80±0.54
Kinetin+Pb	27.40±0.67	17.80±1.90	312.00±0.72	24.40±0.89	25.80±0.28
IAA+Kin+Pb	29.00±1.09	16.20±1.30	320.00±1.86	26.60±0.45	28.60±0.45

Table 4: Effect of growth hormones i.e., IAA, Kinetin and heavy metal i.e., Pb on mesophyll tissue of leaves

Treatments (50 ppm)	No. of palisade cells in 100 µm	Layers of palisade cells	Length of palisade cells µm (x40)	Width of palisade cells µm (x40)	Width of spongy cells µm (x40)	Width of mesophyll tissue µm
Control	49.00±1.28	1	170.00±0.24	44.20±0.54	60.00±0.99	226.90±0.16
IAA	65.00±0.68	1	172.00±0.45	47.00±2.50	76.00±0.24	248.60±1.07
Kinetin	72.00±1.14	1	170.20±0.76	45.80±0.47	68.40±0.47	221.20±0.65
Pb	44.00±0.92	1	162.20±0.89	37.20±0.60	58.60±0.67	168.80±0.16
IAA+Kin	78.00±0.86	1	184.40±1.24	48.20±0.45	80.00±1.05	148.60±1.02
IAA+Pb	52.00±1.11	1	164.40±0.90	38.40±1.09	68.80±1.03	118.80±0.81
Kin+Pb	54.00±1.06	1	154.80±1.09	39.60±1.90	60.00±1.09	161.80±0.65
IAA+Kin+Pb	60.00±0.71	1	179.20±0.54	40.00±0.96	70.60±0.65	130.80±1.69

Internal morphology

Type of stomata: In the present study anisocytic as well as paracystic stomata were observed in the epidermis of *P. vulgaris*. However, anisocytic type was dominant i.e., 75% anisocytic and 25% paracystic type was observed (Fig.5).

In the internal morphology, the number of stomata/mm^2 showed nonsignificant increase with IAA treatments in comparison with control (Table 2). All other treatments registered increase except Kinetin+Pb which exhibited decrease in the number i.e., 14.10 (Table 2). On the other hand, the number of epidermal cells/mm^2 decreased with

Fig. 3: Kinetin treated plant.

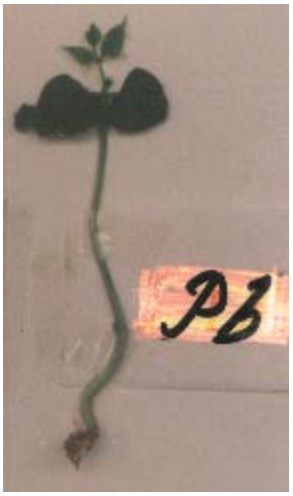

Fig. 4: Plant treated with lead nitrate.

all treatments. However, the decrease with IAA+Kinetin was negligible. The length and width of guard cells showed increase with applied IAA and Pb, whereas Kinetin treatments showed insignificant decrease. Moreover, the effects of the mixed doses of Kinetin+Pb and IAA+Pb are more or less similar to the control (Table 2). Application of the mixed doses of IAA+Kinetin and IAA+Kinetin+Pb showed increase in both the length and width. However, the size of stomatal pore was increased with IAA and Pb, while Kinetin treatments showed some inhibition in size (Table 2). An increase in the length and width was also observed with the mixed

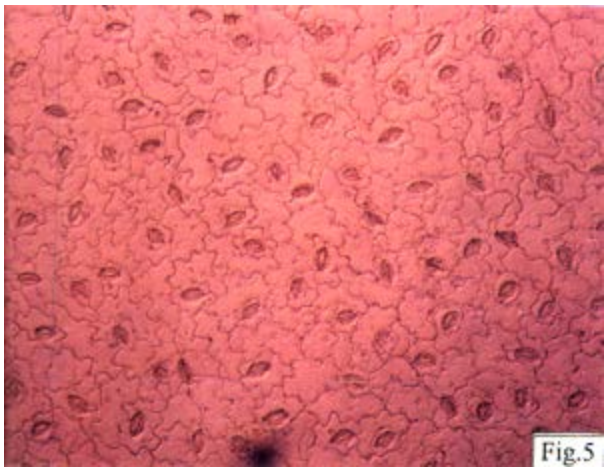

Fig. 5: Epidermis showing anisocytic and paracystic type of stomata.

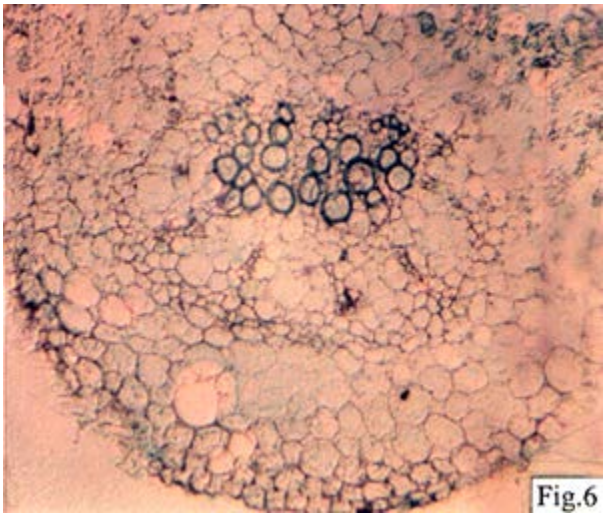

Fig. 6: Leaf of *Phaseolus vulgaris* treated with IAA showing mid vein.

doses of IAA+Kinetin, Kinetin+Pb and IAA+Kinetin+Pb (Table 2).

The stomatal index in control plants was observed to be 24.99, which showed increase with all treatments. However, the %age of open and close stomata registered nonsignificant increase with the application of IAA. In Kinetin treatments 83.33% open and 37.5% close stomata were observed, while lead (Pb) treatments showed increase in the number of closed stomata up to 42.18% and decreased the number of open stomata up to 57.14%. The maximum number of closed stomata was observed in the leaves treated with Kinetin+Pb. In the rest of the mixed doses the number of open stomata showed decrease, while the number of closed stomata increased (Table 2).

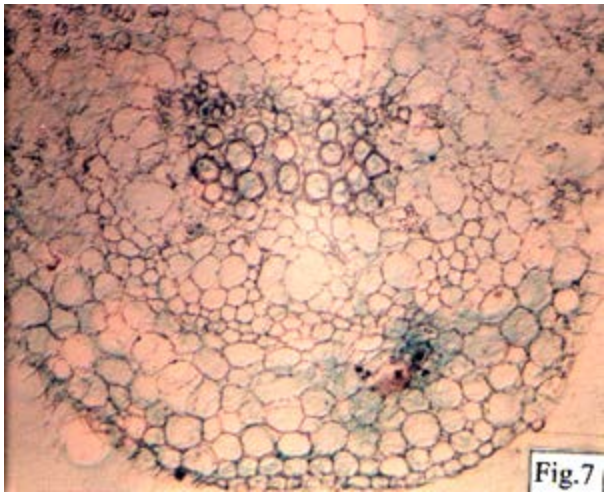

Fig. 7: Transaction of leaf treated with kinetin showing mid vein.

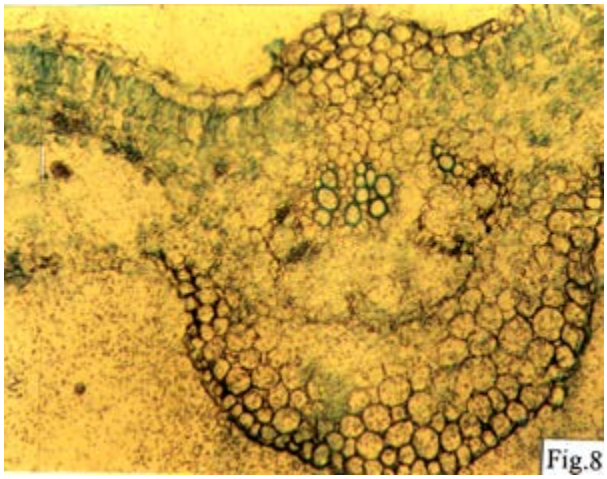

Fig. 8: Leaf treated with lead nitrate.

The width of adaxial epidermal cells showed negligible decrease with the application of IAA, whereas, the width was increased with Kinetin treatments, while Pb proved to be inhibitory (Table 3). The mixed dose of IAA and Kinetin showed increase, which being 28% as compared with control. Contrarily the application of the mixed doses of IAA+Pb, Kinetin+Pb and IAA+Kinetin+Pb showed decrease. In the abaxial epidermal cells a small decrease I-e; 4.5% was observed with applied IAA, while Kinetin and Pb treatments showed 8.18 and 12.72% decrease in width. The mixed dose of IAA+Kinetin increased the width, while other mixed doses showed inhibition (Table 3). The median vascular bundle showed increase in width with IAA and Kinetin treatments (Fig. 6, 7) whereas lead (Pb) showed decrease as compared with control (Fig. 8). A well marked increase of 17.43% was observed in width, with the application of IAA+Kinetin treatments. However, IAA+Pb treatments registered inhibition. Kinetin+Pb and IAA+Kinetin+Pb treatments showed negligible increase. The number of xylem elements showed increase with the application of IAA, whereas Kinetin showed negligible decrease (Table 3). Pb showed significant inhibition in the number i.e., 26.40 as compared with control (Fig.8). Application of the mixed dose of IAA+Kinetin exhibited increase in number up to 38.8, while all other mixed doses decreased the number (Table 3).

The width of metaxylem elements showed increase with IAA (Fig.6), no effect with Kinetin and inhibition with lead. Mixed doses also revealed decrease except for IAA+Kinetin, which showed expansion (Table 3).

Applied IAA and Kinetin registered increase in the number of palisade cells in 100 μm. However, Pb treatments showed inhibition. Application of the mixed doses also showed increase in the number. Moreover, the maximum number was observed in IAA+Kinetin doses i.e., 78 palisade cells in 100 μm. The length of palisade cells was observed to increase with IAA treatments when compared with control (Table 4). Applied Kinetin also showed increase but it was nonsignificant, contrarily lead (Pb) inhibited the length. Application of the mixed doses of IAA+Kinetin and IAA+Kinetin+Pb showed increase up to 8.47 and 6.14%. Contrarily IAA+Pb and Kinetin+Pb exhibited decrease i.e., 3.92 and 8.94%, respectively. The width of palisade cells registered some increase with IAA and Kinetin treatments, while lead (Pb) showed inhibition in width i.e., 15.83% (Fig. 8).

Contrarily the mixed dose of IAA+Kinetin showed an increase which being 12.66%. Decrease of 13.12, 10.40 and 9.50% was registered with the mixed doses of IAA+Pb, Kinetin+Pb and IAA+Kinetin+Pb, respectively. The width of spongy cells increased with IAA and Kinetin treatments as compared with control (Table 4), while Pb treatments showed negligible inhibition. However, the mixed dose of IAA+Kinetin registered well marked increase i.e., 33.33%, while all the rest of the mixed doses showed negative effect (Table 4). The width of mesophyll tissue as a whole showed increase with IAA treatments, while applied Kinetin and Pb reduced the width. Moreover, all the mixed doses also registered inhibition (Table 4).

Discussion

In the present study applied IAA and Kinetin showed increased number of leaves, this increase may be attributed to the enhancement of apical meristemic activity (Gordon and Buess, 1973). Contrarily applied lead nitrate showed inhibition in growth parameters. Dalal *et al.* (1985), Baura *et al.* (1986) and Prasad and Prasad (1987)

came out with the conclusion that lead has inhibitory effect. Moreover, applied IAA and kinetin showed expansion in leaf area (Table 1; Fig. 2, 3). This may be due to the wall loosening enzymes which were activated by the application of hormones (Keller and Christopher, 2001) whereas Pb treatments showed negligible decrease in area (Fig. 4). Begonia *et al.* (1998) working on Indian mustard observed similar effects. The mixed doses of Pb more or less showed same effects (Table 1).

In the internal morphology, applied IAA, kinetin and IAA+kinetin showed increase in the width of both the adaxial and abaxial epidermal cells. This increase may be attributed to the expansion of leaf blade (Pozsar *et al.*, 1967; El-Aishy *et al.*, 1976; Iqbal and Mahmood, 1980; Chaudhry and Zahur, 1992; Tuominen *et al.*, 1997; Awan *et al.*, 1999). The individual and mixed doses of Pb showed some decrease which may be due to the inhibitory effects of Pb as aforementioned (Magdalena and Poskuta, 1998). In the present study, anisocytic as well as paracystic stomata were observed (Fig. 5) in the epidermal layer. However, anisocytic type was dominant i.e., 75% anisocytic and 25% paracystic. According to Guyot (1971) plants may have more than one type of stomata but a particular type is always dominant. Similar results were observed in this study. The number of stomata/mm^2 registered increase with the application of growth hormones individually and in mixed doses, while a decrease in the number of epidermal cells/mm^2 was observed (Table 2). However, Pb and kinetin+Pb treatments showed decrease. This decrease may be due to the inhibitory effect of Pb (Magdalena and Poskuta, 1998). The length of guard cells increased in IAA, IAA+kinetin and IAA+kinetin+Pb treatments. This increase in length may be due to increase in the enzymatic activity which in turn controls cell division as well as cell enlargement (Torrey, 1967). However, in IAA+Pb and kinetin+Pb treatments reduction in length was observed. This may be due to Pb which binds cellular compartments and inactivates them (Cunningham and Saigo, 1995). Width of guard cells increased in all treatments. On the other hand, size of stomatal pore increased with all treatments except IAA+Pb (Table 2). Furthermore, the stomatal index in all the treatments registered increase. This may be due to the change in the number of stomata/mm^2 and number of epidermal cells/mm^2. These morphological changes are in turn due to the activity of growth hormones (Krishnamoorthy, 1981).

In the present study maximum number of open stomata were observed with applied IAA, Leopold and Kriedemann (1975) reported that applied IAA to plant tissues result in rapid and considerable increase in the respiratory rate hence maximum number of open stomata were observed, whereas in Pb treatments the number of open stomata decreased and the number of closed stomata increased which may be due to the oxygen deficiency which is reported by Fitter and Hay (1981).

The vascular bundle showed expansion with growth hormones. Kantharaj and Padmanabhan (1991) reported that growth hormones are very effective in causing expansion growth (Fig. 6, 7). In the present study similar effect was observed. Stoyanova (1998) reported some anatomical changes in primary leaves when treated with heavy metals. In the present study no anatomical changes were observed. However, the increase and decrease in the width of mesophyll and metaxylem elements was seen (Table 3). However, IAA and kinetin showed the most positive effect and the maximum cell enlargement was observed. Similarly, Tuominen *et al.* (1997) and Uggla *et al.* (1998) reported wider metaxylem vessels with IAA treatments. This may be due to the increase in cell wall plasticity. Contrarily Pb and its mixed doses showed inhibition in width. This observation is in confirmation with the report of Botkin and Keller (1995) that the heavy metals become incorporated in living tissues sometimes permanently and have direct toxic effects. Application of IAA and kinetin and in combination with Pb registered increase in the number of palisade cells in 100 μm (Table 4). This may be due to increase in cell division caused by IAA and kinetin (Jablonski and Skoog, 1954). The application of growth hormones caused the expansion and elongation of palisade cells. Ingram *et al.* (1986) reported similar effects. Similarly, when IAA, kinetin and lead were applied simultaneously some inhibition was observed (Table 4) thus showing the antagonistic effect of lead nitrate.

Spongy cells registered increase in the width with the application of IAA. Keller (2001) reported that auxin induced growth is apparently mediated by cell wall loosening or extensibility. Kinetin treatments also showed increase (Table 4). Leopold and Kriedemann (1975) observed more or less similar effects. However, Zaikovskaya (1990) reported the deleterious effects of heavy metals on plant. The width of spongy cells showed inhibition with Pb and its mixed doses (Fig. 8). In the present work the antagonistic effect of lead nitrate on the leaves was observed. Although extraneous hormones i.e., IAA and Kin and having metal Pb nitrate showed their clear effects with individual applications but no generalized pattern was observed with the combination of hormones and heavy metal.

References

Awan, I.U., M.S. Baloch, N.S. Sadozai and M.Z. Sulemani, 1999. Stimulatory effect of IAA and GA_3 on ripening process, kernel development and quality of rice. Pak. J. Biol. Sci., 2: 410-412.

Baura, I., M. Basalie, H. Jana Sasadhar and K. Gupta, 1986. Effect of heavy metal on germination and seedling growth of gram, *Cicer arietinum*. Environ. Ecol., 4: 300-303.

Begonia, G.B., C.D. Daves, M.F.T. Begonia and C.N. Gray, 1998. Bull. Environ. Contam. Toxicol., 61: 38-43.

Botkin, D.B. and E.A. Keller, 1995. Environmental Health and Technology in Environmental Sciences. John Wiley and Sons, Inc., USA, pp: 278.

Brault, Mathias, Maldincy and Regis, 1999. Laboratria de physiologie du development des plantes, Universsite Pierre-et-Marie-curie. Plant Physiol. Biochem. (Paris), 37: 403-412.

Chaudhry, N.Y. and M.S. Zahur, 1992. Effect of growth regulators i.e., IAA and GA_3 on *Abelmoschus esculentus* L. Internal structure of hypocotyls and stem internodes. Biologia, 37: 217-244.

Cunningham, W.P. and B.W. Saigo, 1995. Air pollution in environmental sciences, 3rd ed. WMC Brown Publishers, U.S.A, pp: 347.

Dalal, K., R. Tinkari and P. Baigain, 1985. Effect of mercury, assenic and lead on germination and seedling growth of two jute varieties. Environ. Ecol., 3: 403-407.

El-Aishy, S.M., S.A. Abd-Alla and M.S. El-Keredy, 1976. Effect of growth substances in rice seedlings grown from seeds irradiated with gamma rays. Environ. Exper. Bot., 16: 69-75.

Ferguson, B.J. and F.C. Guinel, 2001. Cytokinin in R50 or a pleiotropic low nodulating mutant of *Pisum sativum*. Poster: Root biology. Plant Physiol.

Fitter, A.H. and R.K.M. Hay, 1981. Environmental Physiology of Plants. Subsidiary of Harcourt Brace Joranovich Publishers.

Gordon, S.A. and E.M. Buess, 1973. Effects of auxin on the radiation induced changes in DNA, RNA metabolism and rootings. Rad. Bot., 13: 283-285.

Guyot, M., 1971. Phylogenetic and systematic value of stomata of Umbelliferae. Bot. J. Linn. Soc., 64: 199-214.

Hewitt, E.J., 1963. Mineral nutrition in pets culture media. In: Plant Physiology (Steward, F.E., Ed.), pp: 97-133. Academic Press, New York.

Ingram, T.J., J.B. Reid and J. MacMillan, 1986. The quantitative relationship between gibberellin A, and internode growth in *Pisum sativum* L. Planta, 168: 414-420.

Iqbal, J. and S. Mahmood, 1980. Germination and growth of *Capsicum annuum* L., seedling grown from gibberellic acid treated gamma-irradiated seeds. Biologia, 26: 173-181.

Jablonski, J.R. and F. Skoog, 1954. Cell enlargement and cell division in excised tobacco pith tissue. Physiol. Plant., 7: 17-24.

Kantharaj, G.R. and G. Padmanabhan, 1991. Molecular aspects of cytokinin's stymied action on auxin mediated new root formation in the hypocotyls of *Phaseolus vulgaris* L. Horticulture, new technologies and application. Proceedings of the International Seminar on new frontiers in the Horticulture, organized by Indo-American Hybrid seeds, Bangalore, India, Nov. 25-28, 1990. Edited by Prakash, J. and R.L. Pierik, 1991. Current Plants Science and Biotechnology in Agriculture, 12: 131-139

Keller, C.P., 2001. Auxin control of leaf expansion in common bean (*Phaseolus vulgaris*). Poster: Growth regulators/hormones. Plant Biol. Kell.

Krishnamoorthy, H.N., 1981. Plant growth substances. Tata McGraw Hill Publishers Co. Ltd., New Delhi.

Kumar, P., A.D. Rao and B.D. Baijal, 1981. Effect of some growth regulators on plant growth. Tuber initiation yield and chemical composition of potato (*Solanum tuberosum*). Pak. J. Bot., 13: 69-75.

Leopold, A.C. and P.E., Kriedemann, 1975. Plant Growth and Development (2nd ed.). Tata McGraw Hill Pub. Co. Ltd., New Delhi, pp: 128-129

Magdalena, L. and W.J. Poskuta, 1998. Development of photosynthetic apparatus and respiration in pea seedlings during greening as influenced by toxic concentration of lead. Acta Physiol. Plant, 20: 35-40.

Moran, J.M., M.D. Morgan and J.H. Wiersme, 1986. Water pollution. In: Introduction to Environmental Sciences, pp: 226. Freeman, W.H. and Co., New York.

Pozsar, B.I., M.E.L. Hammady and Z. Kiraly, 1967. Cytokinins effect of benzyl-adenine increase of nucleic acid and protein synthesis in bean leaves. Nature, 214: 273-274.

Prasad, D.P.H. and A.R.K. Prasad, 1987. Effects of lead and mercury on chlorophyll synthesis in mungbean seedlings. Phytochemistry, 26: 881-884.

Steel, R.G. and J.H. Torrie, 1981. Principles and Proceedings of Statistics. A Biometrical Approach, 2nd ed. McGraw Hill Int. Book Co.

Steward, F.C., 1972. Plant physiology. A treatise. Vol. 6B. Physiology of development. The hormones. Academic Press, New York.

Stoyanova, D., 1998. Effects of Acid Rain on the anatomy of the leaves of *Phaseolus vulgaris* L. Biol. Plant., 40: 587-588.

Torrey, J.G., 1967. Development in Flowering Plants. I. New York: McMillan, pp: 138-139.

Tuominen, H., L. Puech, S. Frink and B. Sunberg, 1997. A radial concentration gradient of Indole-3-acetic acid in relation to secondary xylem development in hybrid Aspen. Plant Physiol., 115: 577-585.

Uggla, C., E.J. Mellerowicz and B. Sundberg, 1998. Indole-3-acetic acid controls cambial growth in scots pine by positional signaling. Plant Physiol., 117: 113-121.

Zaikovskaya, E.A., 1990. Lead accumulation by urban plants in areas with high automobile traffic. Vestin Leniger Univ. Bio., 3: 29-37.