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ROLE OF MERCURY AND EXOGENOUS IAA ON XYLEM VESSELS AND SIEVE ELEMENTS IN *CUCUMIS SATIVUS* L.

NAJMA YAQUB CHAUDHRY[1*] AND AISHA SALEEM KHAN[2]

Punjab University, Botany Department, Lahore, Pakistan
[1]Tel: 042-5889564, [2]Tel: 042-7514264

Abstract

High concentration of Hg i.e., 50 ppm and 100 ppm $HgCl_2$ was used to find the deleterious effects in internode of *Cucumis sativus* L. Xylem vessels and sieve tube members showed inhibition with mercury in both transverse and longitudinal planes. All data was compared with control plants grown under same conditions. When Hg was applied with IAA, there was less inhibition in growth as compared to plants treated with Hg alone, revealing the dominant role of IAA. Therefore, IAA can be beneficial in reducing the inhibition caused by mercury stress. The arrangement of vascular tissues within the bundle is bicollateral and hence the development of phloem region on both sides was studied. Xylem vessels and phloem cells showed more inhibition with Hg in large inner vascular cylinder (IVC) as compared with cells in smaller outer vascular cylinder (OVC).

Introduction

Heavy metals may influence developmental times or block particular stage altogether. Threats are associated mostly with lead, mercury and cadmium. Disorders in biochemical process are observed which affect the growth and vitality of plants as observed by Olivares *et al*., (2002). Mercury is highly toxic, occurs naturally in environment and exists in several forms such as metallic mercury, inorganic and organic mercury (Thangavel *et al*., 1999). It is known that high concentration of mercury in plants can interfere with important physiological functions of plants, can cause imbalance of nutrients and have detrimental effects on synthesis and functioning of enzymes, vitamins and hormones (Luo & Rimmer, 1995). Plants which adapt to growth in the presence of $HgCl_2$ exhibit extensive morphological abnormalities. Significant effects are delay in the onset of growth, cell division and numerous structural irregularities associated with cell wall and cytoplasmic membrane synthesis and function (Vaituzis *et al*., 1975). Furthermore, mercury decreases the water translocation to leaves by reducing the number and radius of vessels and tracheids and by partial blockage with cellular debris and gums (Barcelo *et al*., 1988).

Auxins, gibberellins, cytokinins, ethylene and absscisic acid are well known plant hormones. Alam *et al*., (2002) reported that IAA (indole-3-acetic acid) is major auxin involved in many physiological processes in plants and stimulates cell elongation, differentiation of xylem and phloem and promotes flowering. Indole auxins are natural while some like 2, 4-D and 2, 4, 5-T are synthetic. Auxin transport is important for the establishment of embryo symmetry as reported by Liu *et al*. (1993). Experiments were carried out to study the role of mercury and exogenous IAA on xylem vessels and sieve elements in *Cucumis sativus* L.

[*]Correspondence E-mail: dr.najmayaqoob@hotmail.com, aishasaleemkhan@hotmail.com

Materials and Methods

Seeds of *C. sativus* L., were sown in pots (5-kg soil capacity) in the month of March 2005 which were earmarked according to their treatments. These plants were watered at regular intervals and were maintained under natural conditions of light and air temperature and humidity. $HgCl_2$ was applied through the soil and 27 µl of hormonal treatment was applied on the apical meristem of the plant after 24 hours. Following treatments were applied. 50 ppm $HgCl_2$, 100 ppm $HgCl_2$, 400 ppm IAA, 50 ppm $HgCl_2$ + 400 ppm IAA and 100 ppm $HgCl_2$ + 400 ppm IAA. There were five replicates for each treatment. Control plants were cultivated at the same time. After 40 days, the plants were removed from the pots. 1cm long portions of internode were cut. The material was fixed in Corney's modified fluid. After the removal of air, the fixed material was first dehydrated in an ascending series of water, ethyl alcohol and tertiary butyl alcohol mixture, infiltered and embedded in paraffin wax. The embedded material was processed with the help of rotary microtome (Reichert- Jung, Nippon optical work, Japan). Sections were cut at 5 µm. The material was fixed on glass slides by using adhesive (egg albumin and glycerine in equal amount). It was passed through descending series of alcohol, kept in safranin and then passed through ascending series of alcohol. It was dipped in fast green and passed through ascending series of xylene and mounted in canada balsam.

Results

Growth measurements: Growth was expressed relative to control plants and data given were the average of at least three independent experiments ± standard deviation, calculated according to the following expression.

$$\text{Relative growth inhibition (\%)} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \text{ X } 100$$

Statistical analysis: The data are presented as the means of at least five replicates. All statistically significant differences were tested (Steel & Torrie, 1981).

Vascular region: In the internode of *C. sativus* L., two types of vascular cylinders are seen in transections which are bicollateral (Fig. 1). The ring of large inner vascular cylinder (IVC) and outer small vascular cylinder (OVC) is situated between the ridges of assimilation parenchyma.

Upper Phloem region was 143µm in control in IVC (Table 1). Application of 50 and 100 ppm $HgCl_2$ registered inhibition in growth of phloem cells (Figs. 2 and 3). Contrary to this, 400 ppm IAA caused expansion i.e., 10.6% in comparison with control. When IAA was applied with $HgCl_2$, less inhibition was observed over the control (Table 1). Similarly, growth of OVC was reduced with both doses of $HgCl_2$, which was partially restored with IAA as observed in mixed doses (Table 2).

Lower phloem was 124µm in control in IVC. 100 ppm $HgCl_2$ caused 16.1% growth reduction when compared with control (Table 1). In mixed doses IAA played major role (Fig. 5) as there was less reduction as compared with individual treatments of $HgCl_2$ in both IVC and OVC (Table 1 and 2).

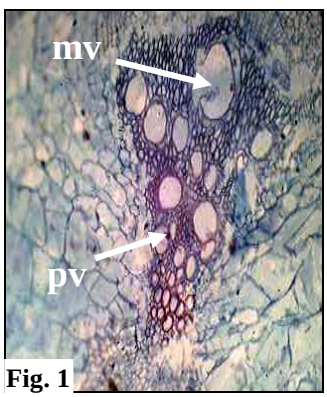

Fig. 1

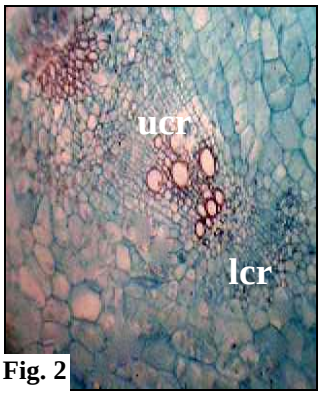

Fig. 2

Fig. 3

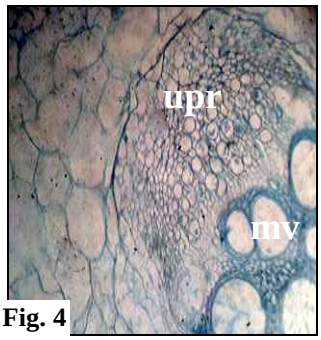

Fig. 4

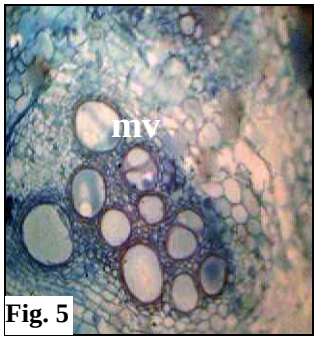

Fig. 5

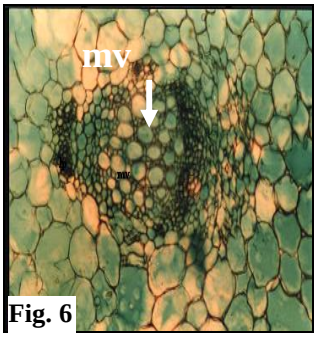

Fig. 6

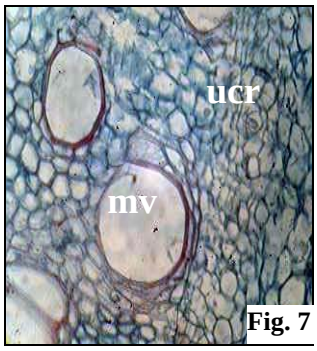

Fig. 7

Fig. 1. *Cucumis sativus* L., internode in transection showing metaxylem vessels (mv) and protoxylem vessels (pv) (10x).

Fig. 2. Effect of 50 ppm $HgCl_2$ on vascular region with ucr (upper cambial region) and lcr (lower cambial region) (10x).

Fig. 3. Inhibition by 100 ppm $HgCl_2$ on vascular region (10x).

Fig. 4. Stimulatory effect of 200 ppm IAA showing sieve cells and vascular cambium (10x).

Fig. 5. Effect of 200 ppm IAA on lower cambial development (10x).

Fig. 6. Effect of mixed dose of 100 ppm $HgCl_2$ + 400 ppm IAA on vascular bundle (10x).

Fig. 7. Effect of 400 ppm IAA on lower cambial development (40x).

Cambial layers: In control, number of upper cambial layers were 6 and lower cambial layers were 3 in IVC (Table 1). With 100 ppm $HgCl_2$, 4 upper and 2 lower cambial layers were observed. IAA applied @ 400 ppm enhanced the growth of cambial region and consequently 9 upper and 5 lower cambial layers were seen (Figs. 4 and 7). In the mixed dose of 100 ppm $HgCl_2$ + 400 ppm IAA, 5 upper and 3 lower cambial layers were produced .In OVC, 400 ppm IAA promoted cambial growth leading to 7 upper and 3 lower cambial layers. In mixed doses there was less reduction in cambium due to IAA (Table 2).

Metaxylem vessels were 83µm in control in IVC. Vessels having diameter above 80µm were considered to be large sized xylem vessels. $HgCl_2$ applied @ 50 ppm and 100 ppm showed 19.1% and 25.8% inhibition respectively over the control. However, the effect of IAA was very prominent on the xylem vessels i.e., 10.9% increase was observed over the control (Fig 4). Mixed dose of 100 ppm $HgCl_2$ + 400 ppm IAA caused 19.13% decrease when compared with control (Fig. 6). In OVC, IAA increased 9.17% increase in xylem vessels (Table 2).

Protoxylem elements were 52µm in control in IVC (Fig. 1). $HgCl_2$ applied @ 50 ppm and 100 ppm reduced protoxylem elements in comparison with control in both IVC and OVC (Table 1 and 2). Applied IAA enhanced the diameter of small xylem elements. In the mixed doses, IAA played major role as comparatively less inhibition was observed over the control.

Discussion

Application of $HgCl_2$ caused significant reduction in growth parameters. However $HgCl_2$ 100 ppm revealed more inhibition in the treated tissues as compared with 50 ppm $HgCl_2$ showing that the effects of metal are more pronounced at high concentration (Gothberg *et al*., 2004).

Auxin is the major controlling factor for induction of vascular differentiation as reported in present work (Aloni *et al*., 1987)**.** They not only stimulate cambial cells to begin mitosis but also cause new daughter cells to differentiate into xylem cells as a result wider vessels were produced with extraneous IAA (Alam *et al*., 2002). Similar is the case with *C. sativus* treated with IAA. IAA applied 400 ppm registered expansion in cambial region (Figs. 4 and 7). Both xylem and phloem development was enhanced by IAA application. IAA exerts influence on plant growth in many ways including cell growth and also enhances cell division (Liu *et al*., 1993). Similar results have been reported by many workers. Auxins are key signals in secondary xylem formation (Alam *et al*., 2002). In the present study similar results were observed as xylem vessels showed enhanced growth with applied IAA accompanied by increased cambial growth and wide sieve plates.

Exogenous auxin increases mechanical extensibility of cell wall (Cosgrove, 1993). Plant cells elongate irreversibly only when load-bearing bonds in the walls are cleaved. Auxin causes the elongation of stem and coleoptile (Rayle & Cleland, 2000). In the present work increase in cell expansion due to IAA application can be attributed to cell wall loosening and increased cell wall plasticity.

IAA partially reversed the toxic effects of $HgCl_2$ when applied with Hg as antagonistic effects of $HgCl_2$ were less effective. Application of 100 ppm $HgCl_2$ + 400 ppm IAA had inhibitory effects on width of cambium, xylem vessels and phloem region but this inhibition was less as compared to plants treated with 100 ppm $HgCl_2$ alone (Table 1). This can be attributed to the presence of IAA and its well known effects on cell division, vascular differentiation and parenchyma cell formation (Touminen *et al*., 1997).

References

Alam, S.A., A. Shereen and M.A. Khan. 2002. Growth and response of wheat cultivars to NAA and Ethrel. *Pak J. Bot*., 34: 135-137.

Aloni, R. 1987. Differentiation of vascular tissues. *Ann. Rev. Plant Physiol*.**,** 38: 179-204.

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

Barcelo, J., M.D. Vazquez and C.H. Poschenrieder. 1988. Cadmium-induced structural and ultra structural changes in the vascular system of bush bean stems. *Botanica Acta*., 101: 254-261.

Cosgrove, D.J. 1993. Wall extensibility, its nature, measurement, and relation to plant cell growth. *New Phytol*., 124: 1-23.

Gothberg, M., K.H. Greger and B.E. Bengtson. 2004. Influence of nutrient level on uptake and effects of mercury, cadmium and lead in water spinach. *J. Environ. Quality*, 33: 1247- 1255.

Liu, C.M., Z.H. Xu and N.H. Chua. 1993. Auxin polar transport is essential for the establishment of bilateral symmetry during early plant embryogenesis. *Plant Cell*., 5: 621-630.

Luo, Y. and D. Rimmer. 1995. Zinc-copper interaction affecting plant growth on a metal-contaminated soil. *Environ. Pollut*., 88: 79-83.

Olivares, E., E. Pena and G. Aguiar. 2002. Metals and oxalate in *Tithonia diversifolia* (Asteraceae): concentrations in plants growing in contrasting soils and Al induction of oxalate exudation by roots. *J. Plant Physiol*., 159: 743-749.

Rayle, D.L. and R.E. Cleland. 2000. The acid growth theory of auxin-induced cell elongation is alive and well. *Plant Physiol*., 99: 1271-1274.

Steel, T. and J.H. Torrie. 1981. *Principles and Procedures of Statistics*. New York.

Thangavel, P., A.S. Sulthana and V. Subburam. 1999. Interactive effects of selenium and mercury on the restoration potential of leaves of medicinal plants *Portulaca oleracea* L. *Sci Total Environ*., 1-8: 243-244.

Touminen, H., I. Puech, S. Fink and B. Sundberg. 1997. A radial concentration gradient of IAA is related to secondary xylem development in hybrid aspen. *Plant Physiol*., 155: 577-585.

Vaituzis, Z., J.D. Nelson, L.W. Wan and R. Colwell. 1975. Effects of mercuric chloride and morphology of selected strains of mercury resistant bacteria. *Applied Microbiol*., 29: 275-286.

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