MORPHOGENETIC EFFECTS OF MERCURY IN *LAGENARIA SICERARIA* (MOL) STANDL AND THEIR PARTIAL REVERSAL BY EXOGENOUS AUXIN

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Summary. The effects of mercuric chloride ($HgCl_2$), indole-3-acetic acid (IAA) and their combination on the development of different cell types in the stem of *Lagenaria siceraria* (Mol) Standl. were studied. Mercury treatment resulted in a reduced stem diameter when compared to untreated control plants. This is attributed to a decrease in the diameter of fibre cells, sieve tube members, large xylem vessels and phloem cells as well as to the interference with cambium development. IAA treatment caused enhanced growth of cambial region, sieve tube members and xylem vessels both in transverse and longitudinal planes. Application of $HgCl_2$ in combination with IAA caused less reduction in growth parameters, thus suggesting that the inhibitory effect of mercury can be restored to some extent due to the application of IAA.

Keywords*:* Auxin; *Cucurbitaceae*; mercury; secondary growth; sieve tube members; xylem vessels.

Abbreviations*:* IVC - inner vascular cylinder; OVC - outer vascular cylinder.

INTRODUCTION

Normally in the environment plants are exposed to a range of abiotic stresses like osmotic, salinity, temperature and heavy metals toxicity, which affect their growth and other physiological processes (Levitt, 1980). Heavy metals cause irreversible damage to a number of vital metabolic constituents in plants (Tomar et al., 2000).

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Disorders in biochemical process which affect the growth and vitality of plants are often observed. Furthermore, cell wall metabolism, cell elongation as well as cellular volume are reduced (Olivares et al., 2002).

Mercury which occurs naturally in the environment is highly toxic (Thangavel et al., 1999) and exists in several forms, such as metallic mercury, inorganic and organic mercury. Diverse biochemical and structural changes in tissues of green plants in response to mercury have been reported (Neculita et al., 2005). Plants which adapt to growth in the presence of $HgCl_2$ exhibit extensive morphological abnormalities. Furthermore, mercury decreases the water translocation to leaves by reducing the radius of vessels and by partial blockage of cellular debris and gums (Lamoreaux and Chaney, 1977).

Auxins, gibberellins, cytokinins, ethylene and abscisic acid are well known plant hormones which play an integral role in controlling growth, development, metabolism and morphogenesis of higher plants (Taiz and Zeiger, 1991; Stoynova et al., 1996). IAA (indole-3-acetic acid) is the major auxin involved in many physiological processes in plants. It stimulates cell elongation, differentiation of xylem and phloem and controls cambial growth (Wang et al., 1997). IAA is involved in the initiation of lateral roots and usually it accumulates in high amounts in the pericycle (Blakesley et al., 1991).

MATERIALS AND METHODS

Seeds of *L. siceraria* (Mol.) Standl. were sown in pots (5 kg soil capacity). The plants were watered at regular intervals and were maintained under natural conditions of light, temperature and humidity. Different concentrations of $HgCl_2$ and IAA were applied either individually or in combinations as follows: 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. IAA was applied on the plant apical meristem 24h after cotyledonary leaves were opened. $HgCl_2$ treatment was applied through the soil three times in a week. Plants were grown for 45 days.

To study the internal morphology (Sanderson, 1994) plant material was firstly 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 using rotary microtome (Reichert- Jung, Nippon Optical Work, Japan). It was fixed on glass slides by using adhesive material prepared from equal amounts of egg albumin and glycerine. It was then passed through a descending series of alcohol kept in safranin followed by an ascending series of alcohol. Slides were dipped in fast green, passed through an ascending series of xylene and mounted in Canada balsam. All observations were subjected to statistical analysis (Steel and Torrie, 1981).

RESULTS

Parameters studied in transection

The width of epidermal cells was 15.1μm in the control samples (Table I). This parameter differed significantly in the variants treated with IAA and $HgCl_2$ (Table I). $HgCl_2$ applied at a concentration of 100ppm caused 28.8% inhibition of the cortical region and 20.19% reduction of the sclerenchyma region compared to controls (Fig. 2). In contrast, treatment with 400 ppm IAA resulted in an expansion (by 8.9%) of the sclerenchyma region. Combined treatments influenced insignificantly the studied parameters regarding the sclerenchyma (Table I).

Vascular region

There are two types of vascular cylinders in transection of the internode of *L. siceraria* (Mol) Standl which are bicollateral. The rings of the large inner vascular cylinder (IVC) and the outer small vascular cylinder (OVC) are situated between the ridges of assimilation parenchyma (Fig.1).

Application of 50 ppm and 100 ppm $HgCl_2$ inhibited the size of the external and internal phloem regions in the external phloem of IVC (Table I, Fig. 1-3). Application of 400 ppm IAA enhanced both upper and lower phloem regions (Table I, II). Increased growth of cambial region was registered after 400 ppm IAA treatment. As a consequence, 9 upper and 4 lower cambial layers were observed (Fig. 4).

Vessels having a diameter above 50 μm were considered to be large-sized xylem vessels. Our results showed 18.4% and 24.9% inhibition of IVC in plants treated

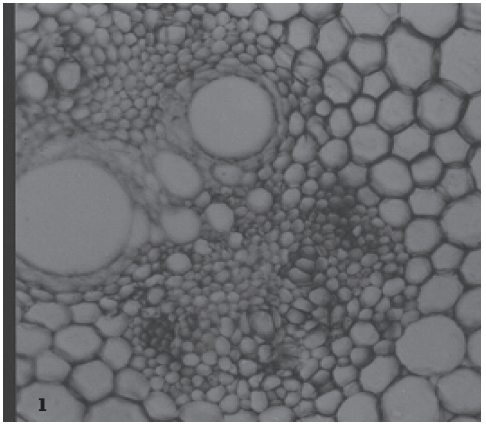

Figure 1. Transection of *Lagenaria siceraria* (Mol) Standl. internode

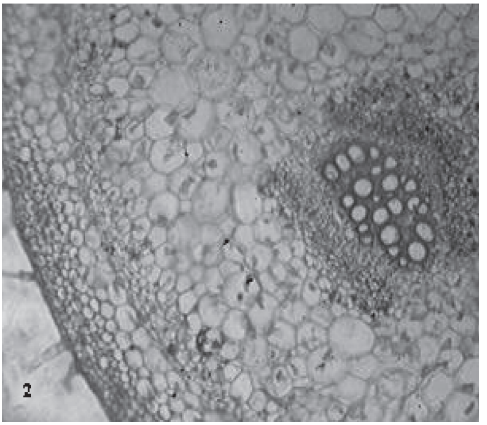

Figure 2. Internode treated with 50 ppm $HgCl_2$

Table I. Effects of $HgCl_2$ and IAA on the internode of inner vascular cylinder (IVC) of *Lagenaria siceraria* (Mol) Standl. in transection (results are means of five replicates).

Treatments (ppm)	Width of epidermal cells(µm)	Diameter of cortical region (µm)	Diameter of sclerenchyma region (µm)	Diameter of external phloem region (µm)	Diameter of internal phloem region (µm)	Number of upper cambial layers	Number of lower cambial layers	Width of metaxylem vessel (µm)	Width of protoxylem elements (µm)
Control	15.1± 0.04	114.2± 0.17	84.2 ± 0.26	87.2± 0.36	62.6 ± 0.74	6± 0.02	3± 0.51	68.2 ± 0.68	53.8 ± 0.82
50 (ppm) $HgCl_2$	13.4 ± 0.28	92.3 ± 0 .57	73.4 ± 0.79	75.2 ± 0.48	54.9 ± 0.92	4± 0.71	3 ± 0.46	55.6 ± 0.19	45.1 ± 0.51
100 (ppm) $HgCl_2$	12.2 ± 1.38	81.2 ± 0.04	67.2 ± 0.93	64.3 ± 0..05	47.2 ± 0.58	4 ± 0.55	3 ± 0.89	51.2 ± 0.15	37.4 ± 0.02
400(ppm) IAA	14.6 ± 0.69	132.4 ± 0.51	91.7± 0.05	98.7 ± 0.69	77.5 ± 0.35	9.2 ± 0.01	4 ± 0.36	78.9 ± 0.73	63.2 ± 0.03
50(ppm) $HgCl_2$ + 400(ppm) IAA	14.9± 0.39	105.3 ± 0.35	78.9 ± 0.48	83.5 ± 0.23	59.5± 0.49	5.6 ± 0.36	3 ± 0.11	61.6 ± 0.17	50.7 ± 0.87
100 (ppm) $HgCl_2$+ 400 (ppm) IAA	14.0 ± 0.01	93.2 ± 0.48	74.3 ± 0.07	71.2± 0.17	53.2 ± 0.03	5.8 ± 0.24	3 ± 0.92	58.2 ± 0.83	44.6 ± 0.99
LSD at 0.05	**0.27**	**3.76**	**2.46**	**2.98**	**5.37**	**0.72**	**0.27**	**2.16**	**4.58**

with 50 ppm $HgCl_2$ and 100 ppm $HgCl_2$, respectively (Fig. 2, 3). However, application of IAA led to an increase (by 15.6%) of the xylem vessels (Fig. 4). Mixed doses caused an inhibition of the studied parameters compared to controls (Table I, II). Treatment with 50 ppm $HgCl_2$ and 100 ppm $HgCl_2$ caused an inhibition of protoxylem vessels in IVC and OVC (Fig. 2, 3).

Parameters studied in a longitudinal plane

Annular, spiral and helical thickenings of protoxylem elements were observed in *L. siceraria* (Mol) Standl (Fig 5). IAA promoted the diameter of spiral and helical pro-

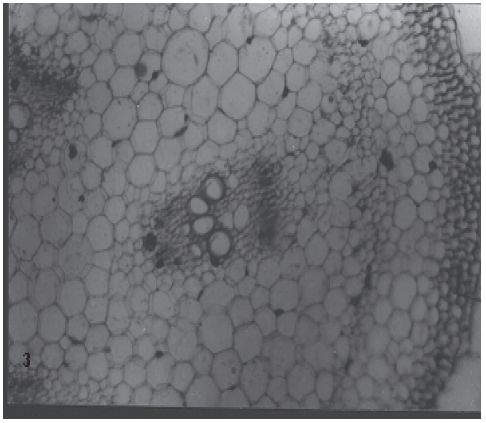

Figure 3. Effect of 100 ppm $HgCl_2$ on cambial growth and diameter of xylem vessels

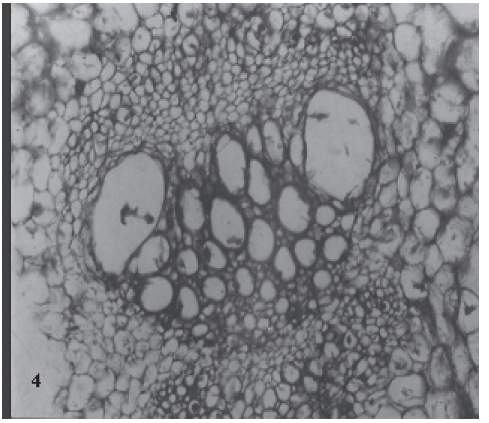

Figure 4. Enhanced cell division with 400 ppm IAA

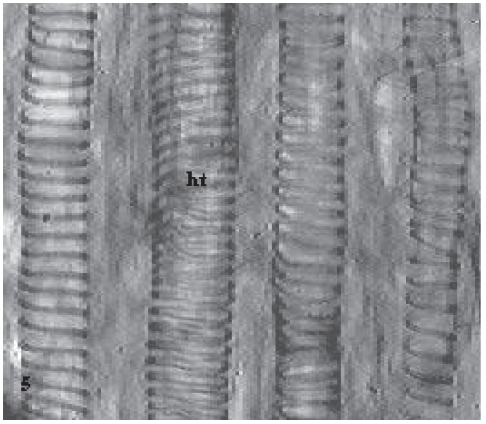

Figure 5. Helical thickenings (ht) of protoxylem vessels in control

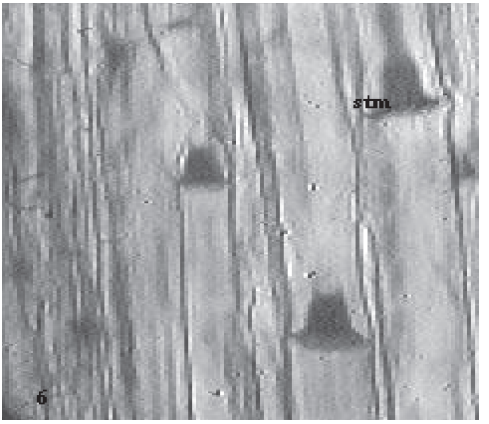

Figure 6. Sieve tube members (stm) in control

Table II. Effects of $HgCl_2$ and IAA on the internode of outer vascular cylinder (OVC) of *Lagenaria siceraria* (Mol) Standl. in transection (results are means of five replicates)

Treatments (ppm)	Width of metaxylem vessels (µm)	Width of protoxylem elements (µm)	Diameter of external phloem region (µm)	Diameter of internal phloem region (µm)	Number of upper cambial layers	Number of lower cambial-layers	Diameter of cellular pith region (µm)	Diameter of fistular pith region (µm)
Control	44.6 ± 0.14	31.2 ± 0.65	76.7 ± 0.03	38.2 ± 0.05	5 ± 0.17	4 ± 0.39	98.3 ± 0.29	243.2 ± 0.18
50 (ppm) $HgCl_2$	38.8 ± 0.12	25.3 ± 0.29	71.5 ± 0.58	33.4 ± 0.92	3 ± 0.04	2 ± 0.18	84.6 ± 0.73	254.9 ± 0.57
100 (ppm) $HgCl_2$	32.4 ± 0.17	21.1 ± 0.85	63.1± 0.97	27.4 ± 0.39	3 ± 0.18	2 ± 0.06	75.7 ± 0.92	261.5 ± 0.09
400 (ppm) IAA	57.3 ± 0.76	40.4 ± 0.64	82.5 ± 0.08	46.5 ± 0.19	9 ± 0.13	3 ± 0.12	103.5 ± 0.87	225.1± 0.06
50 (ppm) $HgCl_2$ + 400 (ppm) IAA	41.9 ± 0.45	29.5 ± 0.06	74.2 ± 0.16	37.2 ± 0.97	5 ± 0.04	3 ± 0..15	87.9 ± 0.72	236.5 ± 0.34
100(ppm) $HgCl_2$ + 400 (ppm) IAA	43.8 ± 0.56	27.4± 0.22	67.8 ± 0.67	32.1 ± 0.11	4± 0.36	2 ± 0.06	82.4 ± 0.27	254.8 ± 0.28
LSD at 0.05	**6.24**	**2.18**	**5.17**	**2.96**	**1.82**	**1.26**	**6.72**	**5.28**

Table III. Effects of $HgCl_2$ and IAA on *Lagenaria siceraria* (Mol) Standl. internode in longitudinal view (results are means of five replicates)

Treatment (ppm)	Width of helical thickening of protoxylem vessel (µm)	Width of spiral thickening of protoxylem vessel (µm)	Width of sieve tube members (µm)	Width of pitted metaxylem vessel (µm)	Width of fusiform cambial cells (µm)
Control	54.2 ± 0.02	43.5 ± 0.31	36.4 ± 0.19	221.4 ± 0.37	9.5 ± 0.45
50 $HgCl_2$	45.1 ± 0.37	37.2 ± 0.04	28.7 ± 0.07	211.3 ± 0.43	7.21 ± 0.38
100 $HgCl_2$	41.2 ± 0.93	33.8 ± 0.67	23.6 ± 0.15	204.4 ± 0.82	7.11± 0.95
400 IAA	62.4 ± 0.24	47.1± 0.46	42.5 ± 0.24	237.7 ± 0.74	14.7 ± 0.26
50 $HgCl_2$ + 400 IAA	50.2 ± 0.36	41.6 ± 0.09	32.2 ± 0.72	217.5 ± 0.06	9.3 ± 0.45
100 $HgCl_2$ + 400 IAA	46.3 ± 0.93	39.5 ± 0.28	28.6 ± 0.36	212.8 ± 0.82	8.4 ± 0.29
LSD at 0.05	**3.28**	**2.47**	**2.15**	**5.06**	**1.21**

toxylem elements (Table III). Treatment with IAA applied at a concentration of 400 ppm stimulated growth in diameter of metaxylem vessels and sieve tube members (Fig 6, 7, 9 and 10). Application of different $HgCl_2$ concentrations inhibited significantly the helical thickenings, fusiform initials and width of pitted metaxylem vessels (Fig 8, Table III).

DISCUSSION

In the present study, epidermal cells of the internode showed negligible response to all treatments. Similar results describing the unresponsiveness of epidermal cells

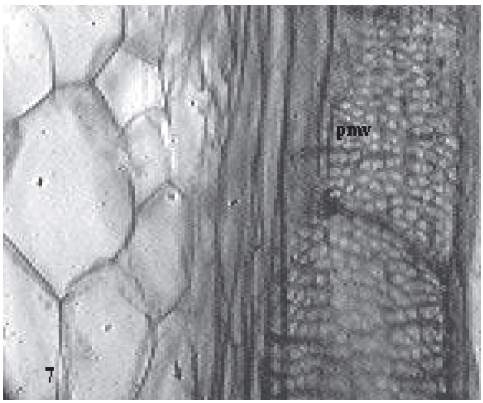

Figure 7. Pitted metaxylem vessels (pmv) in control

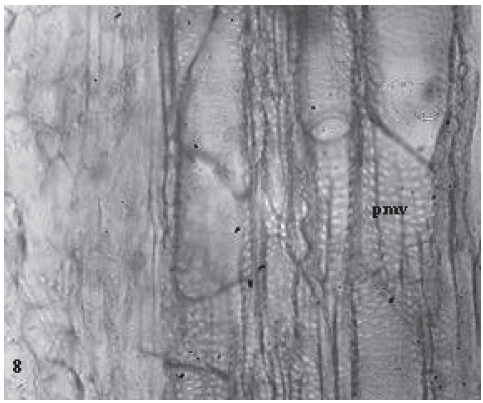

Figure 8. Inhibitory effect of 100 ppm $HgCl_2$ on pitted metaxylem vessel (pmv)

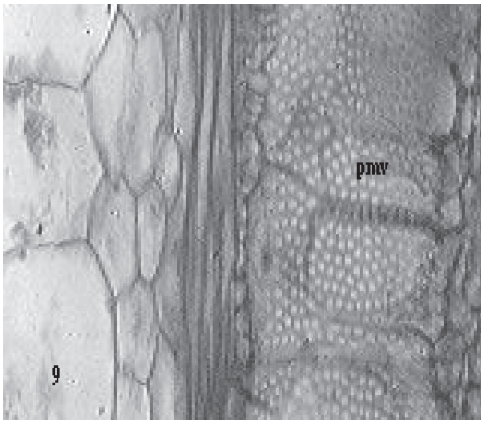

Figure 9. 400 ppm IAA promoted pitted metaxylem vessel (pmv)

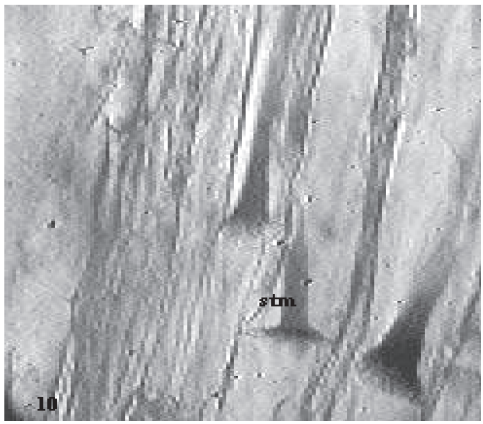

Figure 10. 400 ppm IAA promoted growth of fascinating bells (sieve tube members)

after various applications have been previously reported (Wardlaw, 1957). Application of $HgCl_2$ hampered the growth of fibre cells and cortical region. The higher dose of 100 ppm was found to inhibit stronger the studied parameters when compared with 50 ppm $HgCl_2$. These results support previously reported observations that higher metal concentrations which accumulate in different plant parts induce higher toxic effects (Gothberg et al., 2004).

Xylem vessels (including metaxylem and protoxylem elements) showed reduced growth when treated with $HgCl_2$ in both transverse and longitudinal planes (Table I, II and III). This can be due to a reduction of vessels radius caused by mercury application, thus leading to partial blockage with cellular debris and gums (Lamoreaux and Chaney, 1977). The growth of sieve tube members and cambial region was inhibited after 100 ppm $HgCl_2$ treatment (Barcelo et al., 1988). Plants adapted to grow in the presence of $HgCl_2$ exhibit extensive morphological abnormalities (Vaituzis et al., 1975). Our results support the above findings as reduced cell division was reported in vascular region in plants submitted to $HgCl_2$ treatment (Table I and II).

IAA applied at a concentration of 400 ppm caused expansion in cortical, sclerenchyma and cambial regions. Both xylem and phloem development was enhanced by IAA application (Reed, 2001). Auxins are key signals in secondary xylem formation (Wang et al., 1997). Similar results were observed in the present study as large xylem vessels and phloem region showed enhanced growth after IAA treatment and this was accompanied by increased cambial growth (Table I and II). They not only stimulated cambial cells mitosis, but also caused new daughter cells to differentiate to xylem cells. As a result of exogenous IAA treatment wider vessels were produced (Wareing and Roberts, 1956).

IAA affects plant growth in many ways including cell growth expansion in the vascular cambium (Awan et al., 1999). Similar was the observation made in *L. siceraria* treated with IAA. Plant cells elongate irreversibly only when load-bearing bonds in the walls are cleaved. Usually auxins cause the elongation of stem and coleoptile cells by promoting wall loosening via cleavage of these bonds (Rayle and Cleland, 1992). Increased cell expansion due to IAA application observed in the present study can be attributed to cell wall loosening and increased cell wall plasticity.

Simultaneous application of $HgCl_2$ with IAA showed that growth reduction imparted by mercury could be counteracted to some extent by IAA. Application of 50 ppm $HgCl_2$ + 400 ppm IAA reduced growth of fibre cells, cortical and cambial regions. However, this effect was weaker than in plants treated with 50 ppm $HgCl_2$ alone (Table I). This was due to the presence of IAA and particularly to its well-known effects on vascular differentiation (Alam et al., 2002). Similarly in plants treated with the mixture of 100 ppm $HgCl_2$ and 400 ppm IAA, the growth of the cambial region was inhibited to a higher extent when compared to 50 ppm $HgCl_2$ +

400 ppm IAA-treated plants. This was due to the increased $HgCl_2$ concentration (Gothberg et al., 2004). The present study suggests that IAA treatment can partially restore cambial growth in plants under mercury stress.

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