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# Role of IAA in Flower Development in Cucurbits under Mercury Stress

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## ABSTRACT

This study contributes to enhance the knowledge about cucurbits' behaviour in relation to heavy metals, in particular mercury chloride ($HgCl_2$), and their combination with plant growth hormones (here IAA). There is very little information on the effects of heavy metals on flowering and fruit formation in plants, particularly in cucurbits; this in itself makes the issue of interest apart from the interaction of heavy metals with hormones. Changes in various morpho-anatomical traits indicated that IAA can partially alleviate the detrimental effect of mercury in Hg-treated cucurbits. Effects of Hg and IAA were studied on flowering in *Cucumis sativus* L. and *Momordica charantia* L. It is suggested that plants under the stress of heavy metals can be treated with growth hormones to improve growth parameters, to avoid delay in flowering and likewise the quality of fruit can be improved. The application of 400 mg/l IAA caused precocious flowering, leading to early fruit development. Mercury caused a significant delay in flowering, consequently leading to a reduction in the number of pistillate and staminate flowers. However, when IAA was applied with $HgCl_2$, there were fewer staminate and pistillate flowers, indicating the dominant effect of IAA. This study reveals that the inhibitory effect of heavy metals on flowering could be partially restored by phytohormones.

**Keywords:** auxin, *Cucurbitaceae*, flowering, pistillate, staminate

## INTRODUCTION

The term heavy metal refers to any metallic chemical element that has a relatively high density and is toxic or poisonous even at low concentrations (Neculita *et al*. 2005; Chaudhry and Khan 2006). They affect the growth and vitality of plants reducing cell wall metabolism, cell elongation and cellular volume (MacFarlane and Burchett 2000; Almeida *et al*. 2007). Plants in the environment are exposed to a range of abiotic stresses like osmotic, salinity, temperature and heavy metal toxicity, which affect their growth and various physiological processes (Chaudhry and Khan 2006; Pinchasov *et al*. 2006; Zhang *et al*. 2006). Heavy metal pollution from different sources like industrial or agricultural activities or motor vehicles has a detrimental impact on surrounding areas (Arun *et al*. 2005; Douchkov *et al*. 2005). Diverse biochemical and structural changes in the tissues of green plants in response to lead (Pb) have been reported (Neculita *et al*. 2005). Plants which adapt to growth in the presence of $Pb(NO_3)_2$ exhibit extensive morphological abnormalities. Significant effects include a delay in the onset of growth and cell division and numerous structural irregularities associated with cell wall and cytoplasmic membrane synthesis and function (Mor *et al*. 2002). Many structural changes in the tissues of green plants in response to mercury (Hg) have been reported (Olivares *et al*. 2002; Neculita *et al*. 2005; Douchkov *et al*. 2005; Almeida *et al*. 2007). It is known that a high concentration of Hg in plants can interfere with important physiological functions of plants can cause an imbalance of nutrients and have detrimental effects on synthesis and functioning of enzymes, vitamins and hormones (Luo and Rimmer 1995). In root tissues, heavy metals accumulate in the cortex, vascular tissue and parenchyma cells surrounding the metaxylem vessels (Seregin *et al*. 2004). Hg is a hazardous pollutant among the heavy metals and affects both the light and dark reactions of photosynthesis (Mor *et al*. 2002; Neculita *et al*. 2005). Substitution of the central atom of chlorophyll, magnesium, by Hg results in the breakdown of photosynthesis (Douchkov *et al*. 2005). Furthermore, Hg decreases the water translocation to leaves by reducing the number and radius of vessels due to partial blockage with cellular debris and gums (Chaudhry and Khan 2006; Khan and Chaudhry 2006).

Plant growth is controlled by numerous hormonal and environmental stimuli that interact to regulate cell division and both the direction and rate of cell expansion. Plant biochemical regulators (plant growth and development involves the integration of many environmental and endogenous signals), together with the intrinsic genetic program, determine plant form. Plant hormones are signals that together with the intrinsic genetic program, determine plant form (Gray *et al*. 2001). A single hormone can regulate an amazingly diverse array of cellular and developmental processes, while at the same time multiple hormones often influence a single process (Gray *et al*. 2001). IAA (indole-3-acetic acid) is a major auxin involved in many physiological processes in plants and stimulates cell elongation, differentiation of vascular cambium and promotes flowering (Alam *et al*. 2002; Achard 2004; Wang *et al*. 2007; Tang *et al*. 2008). Indole auxins are natural while some like 2,4-D and 2,4,5-T are synthetic. Okada *et al*. (1991) reported similar results in which plants treated with IAA inhibitors developed an aberrant flower architecture having a long carpel with papillae at the tip, suggesting the importance of IAA for normal flower development.

The present work was conducted to observe various florigenic effects of Hg on the growth of some plants belonging to family Cucurbitaceae i.e., *Cucumis sativus* L., and *Momordica charantia* L. Moreover, selected plants were treated with IAA individually as well as in combination with Hg to see if there is any improvement in growth parameters in plants under Hg stress.

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Short Communication

## MATERIALS AND METHODS

### Plant material and treatments

Plants belonging to *Cucurbitaceae* i.e., *C. sativus* and *M. charantia* were selected for this study because they are important regional vegetables, easy to grow and can survive under stress conditions until the flowering stage. Seeds (Raja Seed Centre, Lahore) of both plants were sown in pots (5-kg soil capacity) in March. These plants were watered at regular intervals and maintained under the natural conditions of light, temperature and humidity. Untreated control plants were cultivated at the same time. When cotyledonary leaves opened, 27 µl of both hormonal treatments was applied to the apical meristem of plants every 24 hours (Khan and Chaudhry 2006). However, a $HgCl_2$ solution (Merck) was applied directly through soil and was repeated twice a week (Chaudhry and Khan 2006, 2007).

One set was taken as untreated control plants. $HgCl_2$ and IAA treatments were applied: 50 mg/l $HgCl_2$, 100 mg/l $HgCl_2$, 200 mg/l IAA, 400 mg/l IAA, 50 mg/l $HgCl_2$ + 200 mg/l IAA, 50 mg/l $HgCl_2$ + 400 mg/l IAA, 100 mg/l $HgCl_2$ + 200 mg/l IAA and 100 mg/l $HgCl_2$ + 400 mg/l IAA. There were five replicates per treatment. After 40 days, the plants were removed from pots.

### Growth measurements

Growth was expressed relative to control plants, and data was the average of at least three independent experiments ± standard error of the mean (SEM), calculated according to the following formula:

Relative growth inhibition (%) = (control – treatment) / control ×100.

### Statistical measurements

All the observations were compared with the control and subjected to statistical analysis. Data is presented as the means of five replicates. The results were analyzed by one-way ANOVA. All statistically significant differences were tested at the $P \leq 0.05$ level using the MINITAB v. 13.0 software.

## RESULTS

### *Cucumis sativus* L.

Mercury treatment caused a reduction in the number of pistillate as well as staminate flowers, although they were normal in appearance (Table 1). The 100 mg/l $HgCl_2$-treated plants showed significantly inhibited flowering compared with 50 mg/l $HgCl_2$-treated plants. Exogenous doses of 200 mg/l and 400 mg/l IAA caused a 20.55% and 25.26% increase in pistillate and staminate flowers, respectively over the control (Table 1). Fruit formation initiated on average after 31 days in IAA-treated plants compared with control where fruit were produced after 40 days. However, doses of 100 mg/l $HgCl_2$ + 200 mg/l IAA and 100 mg/l $HgCl_2$ + 400 mg/l IAA of heavy metals with IAA showed interesting results since IAA promoted both pistillate and staminate flowers compared to individual doses of $HgCl_2$ (**Table 1**).

### *Momordica charantia* L.

The 100 mg/l-dose of $HgCl_2$ showed remarkable inhibition i.e., 60.3% in staminate and 66.2% in pistillate flowers respectively over the control (Table 2). However, the 400 mg/l IAA-treated plants stimulated the number of both pistillate and staminate flowers. Exogenous doses of 200 mg/l and 400 mg/l IAA caused 26.24% and 27.47% increase in pistillate and staminate flowers, respectively over the control (**Table 2**).

The mixed doses of both heavy metals with IAA showed interesting results as IAA promoted both pistillate and staminate flowers compared to individual doses of $HgCl_2$ (**Table 2**). The application of IAA partially reversed the effects of $HgCl_2$ because there was less reduction in the number of flowers compared with individual doses of $HgCl_2$.

**Table 1** Florigenic effects of IAA and $HgCl_2$ on *Cucumis sativus* L.

| | Staminate | Pistillate |
|---|---|---|
| Control | 19.20 ± 0.474 | 12.26 ± 0.181 |
| 50 $HgCl_2$ | 12.92 ± 0.623* | 6.02 ± 0.013* |
| 100 $HgCl_2$ | 7.01 ± 0.513* | 5.45 ± 1.069* |
| 200 IAA | 21.03 ± 0.623* | 12.93 ± 0.068* |
| 400 IAA | 24.05 ± 0.104* | 14.78 ± 0.002* |
| 50 $HgCl_2$ + 200 IAA | 14.00 ± 0.510* | 8.02 ± 0.843* |
| 50 $HgCl_2$ + 400 IAA | 16.02 ± 0. 623 | 9.74 ± 0.078* |
| 100 $HgCl_2$ + 200 IAA | 12.04 ± 0.295* | 7.92 ± 0.182* |
| 100 $HgCl_2$ + 400 IAA | 12.82 ± 0.069* | 8.45 ± 0.167* |

*Treatments significantly different from control at $p<0.05$ according to one-way ANOVA.
All values are mean of five replicates, ± SEM i.e., standard error of the mean.

**Table 2** Florigenic effects of IAA and $HgCl_2$ on *Momordica charantia* L.

| | Staminate | Pistillate |
|---|---|---|
| Control | 27.01 ± 0.048 | 14.92 ± 0.056 |
| 50 $HgCl_2$ | 10.37 ± 0.034* | 7.02 ± 0.178* |
| 100 $HgCl_2$ | 9.12 ± 0.912* | 5.91 ± 0.140* |
| 200 IAA | 30.45 ± 0.045* | 16.35 ± 0.491* |
| 400 IAA | 34.10 ± 0.184* | 19.03 ± 0.389* |
| 50 $HgCl_2$ + 200 IAA | 14.92 ± 0.950* | 10.03 ± 0.193* |
| 50 $HgCl_2$ + 400 IAA | 17.67 ± 0.023* | 12.92 ± 0.145* |
| 100 $HgCl_2$ + 200 IAA | 13.32 ± 0.945* | 9.76 ± 1.623* |
| 100 $HgCl_2$ + 400 IAA | 11.87 ± 0.034* | 12.34 ± 0.056* |

*Treatments significantly different from control at $p<0.05$ according to one-way ANOVA.
All values are mean of five replicates, ± SEM i.e., standard error of the mean.

## DISCUSSION

The objectives of this work were to determine the florigenic effects of IAA and delay in flowering caused by heavy metal pollution, specifically by mercury, during early flower development and to determine how hormonal signals alter these growth parameters. Although staminate and pistillate flowers are reproductive features of the *Cucurbitaceae* family, there are however, in general, more staminate than pistillate flowers and the ratio can be 6-10 staminate to 1 pistillate flower produced. Pistillate flowers are responseble for fruit production. Therefore, it is important to realize that an abundance of flowers will not necessarily translate into an equivalent number of fruit. Flowering in cucurbits normally starts in about 30-40 days after sowing depending upon the weather conditions (Khan and Chaudhry 2006). In *C. sativus* and *M. charantia* floral buds initiated after 30-35 days of sowing in control plants. There was a range of 19-22 staminate and 15-18 pistillate flowers in control plants (**Tables 1, 2**).

In both the treated plants, mercury treatments caused a significant delay in flowering consequently leading to reduced number of staminate and pistillate flowers (**Tables 1, 2**). Environmental conditions can affect flower production in cucurbits, as I reported in this study. Luo and Rimmer (1995), Thangavel *et al*. (1999), Neculita *et al*. (2005) and Zhang *et al*. (2006) reported that heavy metals negatively affect physiological processes in curcubits. Application of a higher dose (100 mg/l) of Hg caused a significant reduction in the number of flowers compared with a lower dose (50 mg/l) showing that higher heavy metal concentrations in the different plant parts induced more toxic effects. Tooke *et al*. (2005) observed that Hg stress caused a delay in flowering. Our results support these findings as reduced flowering in Hg-treated plants can be attributed to the initiation of disruption of floral initiation (van Assce and Clijsters 1990).

Initiation of floral buds started on 21-26 days after treatment in IAA-treated plants. Flowers were normal in appearance and male flowers produced pollen in all treated plants (**Tables 1, 2**). Application of IAA significantly enhanced pistillate flowering leading to precocious fruit development. This might be due to well known effects of IAA on early floral initiation (Wolbang *et al*. 2004) as IAA is mandatory for the shift from vegetative to reproductive stage, revealing

its florigenic effect (Okada *et al*. 1991). Young plants and those growing under environmental stress produce staminate flowers, whereas those growing in a normal environment produce more carpellate flowers. If fertilization occurs, the carpels develop into large fruits, and only a healthy, robust plant can afford to this. A young or poorly growing plant cannot supply enough carbohydrate and protein for fruit development, but it can supply enough to produce pollen (Chaudhry and Khan 2006). Our current results support our previous findings.

Exogenous doses of IAA i.e., 200 mg/l and 400 mg/l played a positive role in early flower initiation accompanied by an increase in the number of pistillate flowers compared with staminate flowers in *C. sativus* and *M. charantia* (Wolbang *et al*. 2004). Okada *et al*. (1991) reported similar results, i.e., that in plants treated with IAA inhibitors, flowers developed aberrant architecture having a long carpel with papillae at the tip, suggesting the importance of IAA for normal flower development. However, *M. charantia* registered a higher response with IAA treatments than *C. sativus*. These species studied exhibited differential sensitivity to Pb which might be due to the difference in the number of diploid chromosomes, total length of the diploid complement and the number of metacentric chromosomes of plant species as reported by Ma *et al*. (1995). Application of mixed doses of Hg with IAA showed enhancement in the number of pistillate and staminate flowers when compared with the individual doses of Hg which might be attributed to the presence of IAA and its florigenic effects (Farooqi *et al*. 2005). Thus IAA improves plant properties which are considered vital to stressed plants, osmotic adjustment and turgor maintenance (Marchant *et al*. 2002).

Simultaneous application of $HgCl_2$ with IAA showed that growth reduction imparted by Hg could be counteracted to some extent by IAA (**Tables 1, 2**). It was concluded that IAA partially reversed the inhibitory effects of $HgCl_2$ in treated plants. A mixed dose of 50 mg/l $HgCl_2$ + 400 mg/l IAA inhibited flowering but this inhibition was less than in plants treated with 50 mg/l $HgCl_2$ alone. When 100 mg/l $HgCl_2$ was applied with 400 mg/l IAA, flowering was more inhibited than at 50 mg/l $HgCl_2$ + 400 mg/l IAA, revealing the dominant effect of IAA. A high concentration of IAA may play a positive role on flower formation during the induction and initiation periods (Wolbang *et al*. 2004; Tang *et al*. 2008). In this study, an increased rate of flowering in Hg-treated plants can be attributed to the role of IAA in reducing the inhibitory effects of plants under heavy metal stress and due to the induction of development of early floral initiation.

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