

FLORIGENIC EFFECTS OF IAA FOR IMPROVING PISTILLATE AND STAMINATE FLOWERING IN SOME CUCURBITS UNDER PB STRESS

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Abstract

This study contributes to the enhancement of on knowledge about behaviour of cucurbits with heavy metal i.e., Pb (NO₃)₂ and/or their combination with growth hormones (IAA). Effects of Pb and IAA were studied on flowering in *Cucumis sativus* L. and *Momordica charantia* L. There is very little information on the effects of heavy metals on the flowering in plants particularly in cucurbits; this in itself makes the issue of interest apart from the interaction of heavy metals with hormones. Plant under the stress of heavy metals can be treated with growth hormones to improve growth parameters, to avoid delay in flowering and likewise quality of fruit can be improved. Applied 400 mg/l IAA caused precocious flowering, increasing the number of flowers in both the plants. Lead caused significant delay in flowering, consequently leading to reduction in the number of pistillate and staminate flowers. However, when IAA was applied with Pb (NO₃)₂, there was less decrease in staminate and pistillate flowers, revealing the dominant effect of IAA. Current study reveals that inhibitory effects of heavy metals on flowering were partially restored by IAA applications.

Introduction

Many structural changes in the tissues of green plants in response to Pb have been reported (Tomar *et al.*, 2000; Olivares *et al.*, 2002; Neculita *et al.*, 2005; Douchkov *et al.*, 2005). 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 & Khan, 2006; Pinchasov *et al.*, 2006; Zhang *et al.*, 2006; Ahluwalia & Goyal, 2007). They affect the growth and vitality of plants reducing cell wall metabolism, cell elongation as well as cellular volume (MacFarlane & Burchett, 2000). Heavy metals pollution from different sources like industrial or agricultural activities or motor vehicles has detrimental impact on surrounding areas (Arun *et al.*, 2005; Douchkov *et al.*, 2005; Liu *et al.*, 2007). Plants which adapt to growth in the presence of Pb (NO₃)₂ exhibit extensive morphological adaptations. Significant effects are delay in the onset of growth and cell division and numerous structural modifications associated with cell wall and cytoplasmic membrane synthesis and function (Mor *et al.*, 2002). Higher concentration of Pb in plants can interfere with important physiological functions and can cause imbalance of nutrients and have detrimental effects on synthesis and functioning of enzymes, vitamins and hormones (Luo & Rimmer, 1995). In the root tissues, heavy metals are accumulated in the cortex, vascular tissue and parenchyma cells surrounding the metaxylem vessels (Seregin *et al.*, 2004). Substitution of the central atom of chlorophyll, magnesium, by lead results in the breakdown of photosynthesis (Douchkov *et al.*, 2005; Neculita *et al.*, 2005). Furthermore, lead decreases the water translocation to leaves by reducing the number and radius of vessels due to partial blockage with cellular debris and gums (Chaudhry & Khan, 2006; Khan & 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. IAA (indole-3-acetic acid) is major auxin involved in many physiological processes in plants and stimulates cell elongation, differentiation of vascular cambium and promotes flowering (Achard, 2004; Wang *et al.*, 2007;). Okada *et al.*, 1991 reported similar results that in plants treated with IAA inhibitors, flowers developed aberrant architecture having long carpel with papillae at tip, suggesting the importance of IAA for normal flower development. The present report gives a record of florigenic effects of IAA for improving pistillate and staminate flowering in some cucurbits under PB stress.

Materials and Methods

Seeds of the *Cucumis sativus* L., and *M. charantia* L., were sown in pots (5-kg soil capacity) in the month of March which were earmarked according to their treatments. These plants were watered at regular intervals and maintained under the natural conditions of light, temperature and humidity. Control plants were cultivated at the same time. When cotyledonary leaves were opened, 27 μ l of both hormonal treatments was applied on the apical meristem of the plant after every 24 hours (Khan & Chaudhry, 2006). However, heavy metal treatment was applied directly through soil and was repeated twice a week (Chaudhry & Khan, 2006 & 2007).

Different Pb and IAA treatments applied were 100 mg/l Pb(NO₃)₂, 200 mg/l Pb(NO₃)₂, 200 mg/l IAA, 400 mg/l IAA, 100 mg/l Pb(NO₃)₂ + 200 mg/l IAA, 100 mg/l Pb(NO₃)₂ + 400 mg/l IAA, 200 mg/l Pb(NO₃)₂ + 200 mg/l IAA, 200mg/l Pb(NO₃)₂ + 400 mg/l IAA till forty five days. There were five replicates for each treatment.

Growth measurements: Growth was expressed relative to control plants, and data given is average of at least three independent experiments \pm standard error mean (SEM), calculated according to the following expression:

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

Statistical measurements: All the observations were compared with control and subjected to statistical analysis. The data are 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 software of MINITAB v 13.0.

Results

a. *Cucumis sativus* L.: Flowering in cucurbits normally starts in about 30-40 days after sowing depending upon the weather condition. In *C. sativus* and *M. charantia* floral buds initiated after 30- 35 days of sowing in control plants. There were average of 19- 22 staminate and 15 -18 pistillate flowers in control plants (Figs. 1 and 2). In cucurbits, there are more staminate than pistillate flowers and the ratio can be 6-10 staminate to 1 pistillate flower produced. However, staminate flowers will never produce fruit. Therefore, it is important to realize that an abundance of flowers will not necessarily translate into an equivalent number of fruit.

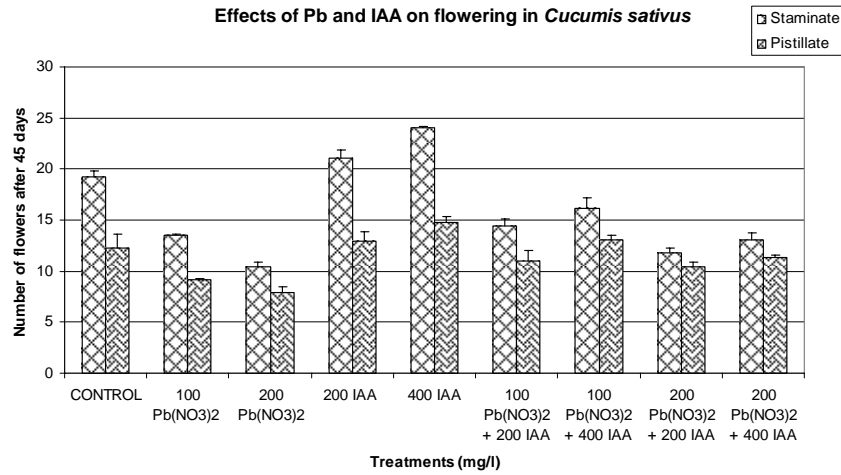


Fig. 1. Florigenic effects of IAA and Pb(NO₃)₂ on *Cucumis sativus* L. (readings are mean of five replicates, LSD at 0.05 for stamineate flowers = 2.152; LSD at 0.05 for pistillate flowers = 3.140)

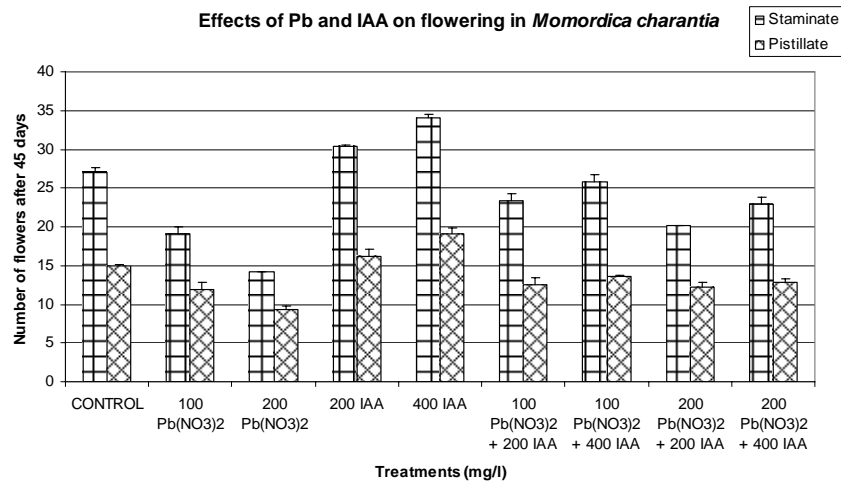


Fig. 2. Florigenic effects of IAA and Pb(NO₃)₂ on *Momordica charantia* L. (readings are mean of five replicates, LSD at 0.05 for stamineate flowers = 4.295, LSD at 0.05 for pistillate flowers = 5.032).

Application of Pb(NO₃)₂ doses caused reduction in the number of pistillate as well as stamineate flowers, although they were normal in appearance (Fig. 1). However, the applied dose of 200 mg/l Pb(NO₃)₂ showed more inhibition in number of flowers than 100 mg/l Pb(NO₃)₂, consequently, reducing the number of both stamineate and pistillate flowers. Contrarily, exogenous doses of 200 mg/l and 400 mg/l IAA caused 20.55% and 25.26% increase in pistillate and stamineate flowers respectively over the control (Fig. 1). However, Pb and IAA when applied in combination showed interesting results as IAA promoted both pistillate and stamineate flowers as compared to individual doses of Pb(NO₃)₂.

b. *Momordica charantia* L.: The applied dose of 200 mg/l $\text{Pb}(\text{NO}_3)_2$ registered more inhibition than 100 mg/l $\text{Pb}(\text{NO}_3)_2$ i.e., 37.3% in pistillate and 47.2% in staminate flowers when compared with control (Fig. 2). However, the 400 mg/l-IAA treated plants stimulated the number of both pistillate and staminate flowers (Fig. 2). On the other hand, the 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 (Fig. 2). However, the combined doses of Pb with IAA showed interesting results as IAA promoted both pistillate and staminate flowers as compared to individual doses of $\text{Pb}(\text{NO}_3)_2$. The application of IAA partially reversed the effects of $\text{Pb}(\text{NO}_3)_2$ because there was less reduction in number of flowers as compared with individual doses of $\text{Pb}(\text{NO}_3)_2$ (Fig. 2).

Discussion

Higher dose of lead caused significant reduction in number of flowers as compared with lower dose, showing higher metal concentrations accumulated in the different plant parts induced more toxic effects. Accordingly, internal metal concentrations in the plants are correlated to toxic effects (Gothberg *et al.*, 2004; Ahluwalia & Goyal, 2007). Heavy metals stress caused delay in flowering as flower formation is related to environmental conditions (Tooke *et al.*, 2005). Our results support above findings as reduced flowering in metals-treated plants can be attributed to initiation of disruption in biological processes (Van Assce & Clijsters, 1990). Environmental conditions can also affect flower production (Luo & Rimmer 1995; Thangavel *et al.*, 1999; Neculita *et al.*, 2005) in cucurbits as reported in current work. In both the treated plants lead treatments caused significant delay in flowering consequently leading to reduced number of staminate and pistillate flowers.

Application of IAA significantly enhanced pistillate flowering leading to precocious fruit development. Initiation of floral buds started on 21-26 days of treatment in IAA treated plants. Flowers were normal in appearance and male flowers produced pollen in all treated plants. 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 florigenic effect (Okada *et al.*, 1991). Exogenous doses of IAA i.e., 200 mg/l and 400 mg/l played positive role in early flower initiation accompanied by increase in number of pistillate flowers as compared with staminate flowers in all the treated plant (Wolbang *et al.*, 2004). Okada *et al.*, (1991) reported similar results that in plants treated with IAA inhibitors, flowers developed aberrant architecture having long carpel with papillae at tip, suggesting the importance of IAA for normal flower development. However, *M. charantia* registered maximum response with IAA treatments than *C. sativus* (Fig. 2). These species studied exhibited differential sensitivity to lead 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 IAA stimulated the formation of pistillate flowers in the treated plants which could otherwise produce male flowers. Young plants and those growing in poor environment produce staminate flowers, whereas those growing in good environment produce 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 & Khan, 2006). Present study supports above findings as there was significant increase in pistillate flowers with applied IAA.

The mechanism of auxin perception and action is intriguing; however it is known that gibberellins exert their effects by altering gene transcription. Flower development involves a complex interaction of molecular, biochemical and structural changes. However, little information is available on the physiology of early flower development, on the molecular aspects of fruit development in general, and on how flower development is coordinated with auxin action.

Interesting results were seen when heavy metals were applied with IAA. It was concluded that IAA partially reversed the inhibitory effects of $\text{Pb}(\text{NO}_3)_2$ in treated plants. Mixed dose of 50 mg/l $\text{Pb}(\text{NO}_3)_2$ + 400 mg/l IAA had inhibitory effects on flowering but this inhibition was less as compared with plants treated with 50 mg/l $\text{Pb}(\text{NO}_3)_2$ alone. High concentration of IAA may have positive role on flower formation during induction and initiation period (Wolbang *et al.*, 2004). Application of mixed doses of Pb with IAA showed enhancement in the number of pistillate and staminate flowers when compared with the individual doses of Pb which might be attributed to the presence of IAA and its florigenic effects (Farooqi, *et al.*, 2005). In present work, increased rate of flowering in metal treated plants can be attributed due to florigenic effects of IAA in reducing the inhibitory effects of plants under heavy metal stress.

References

- Achard, P., A. Herr, D.C. Baulcombe and N.P. Harberd. 2004. Modulation of floral development by a gibberellin-regulated microRNA. *Development*, 131: 3357-336.
- Ahluwalia, S.S. and D. Goyal. 2007. Microbial and plant derived biomass for removal of heavy metals from wastewater. *Biores. Technol.*, 98: 2243-2257.
- Arun, K., T.C. Shankera, C. Cervantesb and H.S. Loza-tavera. 2005. Chromium toxicity in plants. *Environ. Int.*, 31: 739- 753.
- Chaudhry, N.Y. and A.S. Khan. 2006. Improvement of pistillate flowers yield with GA_3 . *Plant Grow Regul.*, 50: 211-217.
- Chaudhry, N.Y. and Khan, A.S. 2007. Role of mercury and exogenous IAA on xylem elements and sieve cells in *Cucumis sativus* L. *Pak J. Bot.*, 39: 135-140.
- Douchkov, D., C. Gryczka, U.W. Stephan and H. Baumlein. 2005. Ectopic expression of nicotianamine synthase genes result in improved iron accumulation and increased nickel tolerance in transgenic tobacco. *Plant Cell Environ.*, 28: 365-368.
- Farooqi, H.A., S. Fatima, A. Khan and S. Sharma. 2005. Ameliorative effect of Chlormequat Chloride and IAA on drought stressed plants of *Cymbopogon Martinii* and *C. Winterianus*. *Plant Grow Regul.*, 46: 277-284.
- Gothberg, A., M. Greger, K. Holm and B.E. Bengtson. 2004. Influence level on uptake and effects of mercury, cadmium and lead in water spinach. *J. Environ. Qual.*, 33: 1247-1255.
- Khan, A.S. and N.Y. Chaudhry. 2006. Auxins partially restores the cambial activity in heavy metals treated plants. *Luffa cylindrica* L. (Cucurbitaceae) under mercury stress. *J. Food Agri. Environ.*, 4: 276-281.
- Liu, Y.J., Y.G., Zhu and H. Ding. 2007. Lead and cadmium in leaves of deciduous trees in Beijing, China: development of a metal accumulation index (MAI). *Environ. Poll.*, 145: 387-390.
- Luo, Y and D. Rimmer. 1995. Zinc-copper interaction affecting plant growth on a metal-contaminated soil. *Environ. Poll.*, 88: 79-83.
- Ma, T.H., Z. Xu, C. Xu, H. McConnell, E.V. Rabago, G.A. Arreola and H. Zhang. 1995. The improved *Allium/Vicia* root tip micronucleus assay for clastogenicity of environmental pollutants. *Mut. Res.*, 334: 185-195.
- Macfarlane, G.R and M.D. Burchett. 2000. Cellular distribution of copper, lead and zinc in the grey mangrove, *Avicennia marina* (Forsk.) Vierh. *Aquat Bot.*, 68: 45-59.

- Mor, I.R., S.J. Gokani and S.V. Chanda. 2002. Effect of mercury toxicity on hypocotyl elongation and cell wall loosening in *Phaseolus* seedlings. *J. Plant Nutr.*, 2: 843-860.
- Neculita, C.M., J.Z. Gerald and D. Louis. 2005. Mercury speciation in highly contaminated soils from Chlor- Alkali plants using chemical extractions. *J. Environ.*, 34: 255-262.
- Olivares, E., E. Pena and G. Anguiar. 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.
- Pinchasov, Y., T. Berner and Z.H. Dubinsky. 2006. The effect of lead on photosynthesis, as determined by Photoacoustics in *Synechococcus leopoliensis* (Cyanobacteria). *Water Air Soil Poll.*, 175: 117-125.
- Seregin, I.V., L.K. Shpigun and V.B. Ivanov. 2004. Distribution and toxic effects of cadmium and lead on maize roots. *Russ. J. Plant Physiol.*, 51: 525-533.
- 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.*, 243-244: 1-8.
- Tomar, M., I. Kaur, N. Bhatnagar and A.K Bhatnagar. 2000. Effect of enhanced lead in soil on growth and development of *Vigna radiata* (L). *Wilczek. Indian J. Plant Physiol.*, 5: 13-18.
- Tooke, F., M. Ordidge, T. Chiurugwi and N. Battey. 2005. Mechanisms and function of flower and inflorescence reversion. *J. Exper. Bot.*, 56: 2587-2599.
- Van Assche F. and H. Clijsters. 1990. Effects of heavy metals on enzyme activity in plants. *Plant Cell Environ.*, 13: 195-206.
- Wang, D., Q. Li, C. Ming and Y. Zhu. 2007. Gibberellin is Involved in the regulation of Cell Death-mediated Apical Senescence in G2 Pea. *J. Int. plant Biol.*, 49: 1627-1633.
- Wolbang, M.C., M.P. Chandler, M.M. Smith and J.J Ross. 2004. Auxin from the developing inflorescence is required for the biosynthesis of active gibberellins in barley stem. *Plant Physiol.*, 134:769-776.
- Zhang, R.Q., R.C. Tang and S.Z. Wen. 2006. Advances in Research on Genetically Engineered Plants for Metal Resistance. *J. Int. Plant Biol.*, 48: 1257-1265.

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