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GA_3 improves flower yield in some cucurbits treated with lead and mercury

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Present study reveals florigenic effects of growth hormones (i.e. gibberellic acid, GA_3) and heavy metals (i.e., $Pb(NO_3)_2$ and $HgCl_2$) in *Cucumis sativus* L. and *Momordica charantia* L. Applied GA_3 at 400 ppm caused precocious flowering, increasing the number of pistillate and staminate flowers in both plants. Application of $Pb(NO_3)_2$ and $HgCl_2$ caused significant delay in flowering, consequently leading to reduction in number of flowers. However, when GA_3 was applied with $Pb(NO_3)_2$ and $HgCl_2$, there was less decrease in staminate and pistillate flowers, revealing the dominant effect of GA_3. It can be concluded that inhibitory effects of heavy metals on flowering were partially restored by phytohormones.

Key words: Flowering, heavy metals, phytohormones.

INTRODUCTION

Plant hormones play an integral role in controlling the growth, development, metabolism and morphogenesis of higher plants (Taiz and Zeiger, 1991). Auxins, gibberellins, cytokinins, ethylene and abscisic acid are well known plant hormones. However, growth hormones differ considerably in their mode of actions (Saunder, 1991).

The gibberellins (GAs) are widespread and ubiquitous in flowering and non flowering plants. GAs form a large family of diterpenoid compounds, some of which are bioactive growth regulators, that control such diverse developmental processes as seed germination, stem elongation, leaf expansion, trichome development, and flower and fruit development (Davies, 1995). They are believed to be synthesized in young tissues of roots; however, leaves may also be a source of some biosynthesis. In the plant species examined, bioactive GAs or synthesizing activities are present mainly in rapidly developing tissues, such as the shoot tips, expanding leaves and petioles near elongating internodes, and developing seed (Aach et al., 1997). GAs has long been acknowledged as regulators of cellular division and elongation. GAs stimulate deseeded pea pericarp growth (length and fresh weight) and, together, synergistically enhance growth (Ozga and Reinecke, 1999). During germination, GA promotes embryo growth and/or reduces the physical restraint imposed by the endosperm and testa that allows radicle protrusion. In Arabidopsis seed, the primary role of GA seems to be to facilitate the breakage of the seed coat (Telfer et al., 1997). There are four types of gibberellins but gibberellic acid, GA_3 ($C_{19}H_{26}O_6$) is best known. Fresh and dry weights of shoots and roots, plant height and leaf area with gibberellic acid treatment caused a significant ameliorative effect with respect to these growth attributes in wheat plants (Ashraf et al., 2002). The contents of GA_3 and GA_{20} in seeds with high germination rate were twice and five times higher, respectively, than those from seeds with a low germination rate, indicating a possible role of gibberellins in dormancy release in this plant species (Perez et al., 2002). Excising the sepals reduced fresh and dry weights, as well as the length of buds and the peduncles, indicating that sepals may be a source of gibberellins during flower development in rose (Ganelevin and Zieslin, 2002). During Arabidopsis flower development, GA is essential for the development of stamens and petals (Koornneef and van der Veen, 1980). High concentration of GAs may have positive role on

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flower formation in olive during induction and initiation period (Ulger et al., 2004). In addition to acting in the anthers, GA synthesized in the anthers appears to control the development of other floral organs. In petunia, removal of the anthers blocked petal growth and the accumulation of pigments (Weiss and Halevy, 1989). The treatment of emasculated flowers with GA was sufficient to restore petal development. GA_3 prevents chlorophyll degradation in *Zantedeschia* leaves (Janowska and Jerzy, 2003). More flowers were produced in GA_3 treated plants than in the control plants by the 40th day of flowering through the end of flowering (Biles and Cothren, 2001). Gibberellic acid affects growth and flowering of *Philodendron* (Chen et al., 2003). However, GA_3 applications decreased levels of total phenolic substances in *Diospyros lotus* fruits. The results showed that GA_3 not only induced parthenocarpic fruit formation, but they also changed chemical content of the fruit. Therefore GA applications might be a good way to produce the high quality fruit in *D. lotus* (Kadioglu and Atalay, 2002).

Gibberellins are the most powerful of the growth promoters because they increase internode spacing, induce and promote flowering in many plants, and modify the flower sex expression in some plants (Davies, 1995). During germination, GA_3 promotes embryo growth and reduces the physical restraint imposed by the endosperm and testa that allows radicle protrusion. In *Arabidopsis* seed, the primary role of GA seems to be to facilitate the breakage of the seed coat (Neil et al., 2002). After germination, bioactive GA_3 promote stem elongation, leaf expansion, and root growth (Yaxley et al., 2001).

Lead (Pb) is one of the major heavy metals of the antiquity and has gained considerable importance as a potent environmental pollutant. Apart from the natural weathering processes, Pb contamination of the environment has resulted from mining and smelting activities, Pb containing paints, gasoline and explosives as well as from the disposal of municipal sewage sludges enriched in Pb (Chaney and Ryan, 1994). Pb causes multiple direct and indirect effects on plant growth and metabolism (Tomar et al., 2000). Root development is especially hampered (Salt et al., 1995). Root endodermis acts as a barrier to lead uptake to shoots (Sobotik et al., 1998). Germination of seeds is drastically affected at high Pb concentrations. Development and growth of root and shoot in seedling stage are also affected, with roots being more sensitive. Initiation of lateral roots is most sensitive (Fargasova, 1994). The extent to which Pb enters plants via the leaves depends on the ability of leaves to absorb Pb from aerial sources. However it is agreed that the bulk of the Pb taken up by plants remains in the roots (Kumar et al., 1995).

Mercury is highly toxic (Thangavel et al., 1999), occurs naturally in environment and exists in several forms such as metallic mercury, inorganic and organic mercury. Mercury is a hazardous pollutant among the heavy metals and affects both light and dark reactions of photosynthesis. Substitution of the central atom of chlorophyll, magnesium, by mercury results in breakdown of photosynthesis (Patra and Sharma, 2000; Neculita et al., 2005). It is known that high concentration of mercury can interfere with important physiological functions of plants leading to imbalance of nutrients with detrimental effects on synthesis and functioning of enzymes, vitamins and hormones (Luo and Rimmer, 1995). A part of mercury emitted from the source into the atmosphere is absorbed by plant leaves, and migrates to humus through fallen leaves. Airborne mercury thus seems to contribute significantly to the mercury content in crops (Mosbaek et al., 1998) . Higher mercury content was recorded in fruits of plants grown close to highly industrialized areas (Wojciechowska-Mazurek et al., 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 and cell division and numerous structural irregularities associated with cell wall and cytoplasmic membrane synthesis and function (Vaituzis et al., 1975). Plants absorb mercury through roots and volatilise it from through leaves (Kozuchowski and Johnson, 1978). Furthermore, mercury decrease 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).

MATERIALS AND METHODS

Seeds of *Cucumis sativus* L. and *Momordica charantia* L. were sown in earthenware pots (5- kg soil capacity) in the month of May 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. When the cotyledonary leaves were fully opened and oriented horizontally with respect to hypocotyledonary axis, 27 µl of hormonal treatment was applied on the apical meristem of the plant (Chaudhry and Khan, 2000) . This treatment was repeated after 24 h. $Pb(NO_3)_2$ and $HgCl_2$ were applied to the plants in the soil thrice the week. Following treatments were applied 400 ppm GA_3, 100 ppm $Pb(NO_3)_2$, 200 ppm $Pb(NO_3)_2$, 50 ppm $HgCl_2$, 100 ppm $HgCl_2$, 400 ppm GA_3 + 100 ppm $Pb(NO_3)_2$, 400 ppm GA_3 + 200 ppm $Pb(NO_3)_2$, 400 ppm GA_3 + 50 ppm $HgCl_2$ and 400 ppm GA_3 + 100 ppm $HgCl_2$. One set was taken as control. After forty five days, the plants were removed from the pots. All observations were subjected to statistical analysis (Steel and Torrie, 1981).

RESULTS

C. sativus

Applied 400 ppm GA_3 siginificantly enhanced the number of pistillate and staminate flowers after 45 days by 37.8% and 42.7%, respectively, in comparison with control (Table 1). Contrary to this, heavy metals treatments caused delay in flowering; however mercury showed more inhibitory effects. Both lead and mercury reduced the number of pistillate as well as staminate flowers,

Table 1. Effects of phytohormone (GA_3) and heavy metals ($Pb(NO_3)_2$ and $HgCl_2$) on flowering in *Cucumis sativus*.

Treatment (ppm)	Number of pistillate flowers	Number of staminate flowers
Control	12.26 ± 0.02	19.2 ± 0.12
400 GA_3	16.9 ± 0.87	28.01 ± 0.99
100 $Pb(NO_3)_2$	9.21 ± 0.93	13.54 ± 0.35
200 $Pb(NO_3)_2$	7.85 ± 0.07	10 .38 ± 0.04
50 $HgCl_2$	6.02 ± 0.46	12.92 ± 0.17
100 $HgCl_2$	5.45 ± 0.82	7.01 ± 0.16
400 GA_3+ 100 $Pb(NO_3)_2$	10.83 ± 0.79	17.76 ± 0.68
400 GA_3+ 200 $Pb(NO_3)_2$	10.09 ± 0.24	15.62 ± 0.04
400 GA_3 + 50 $HgCl_2$	9.25 ± 0.01	17.23 ± 0.93
200 GA_3 + 100 $HgCl_2$	8.92 ± 0.05	12.05 ± 0.37
LSD at 0.05	2.54	3.67

Readings are mean of four replicates.

Table 2. Effects of phytohormone (GA_3) and heavy metals ($Pb(NO_3)_2$ and $HgCl_2$) on flowering in *Momordica charantia*.

Treatment (ppm)	Number of pistillate flowers	Number of staminate flowers
Control	14.92 ± 0.05	27.01 ± 0.48
400 GA_3	23.76 ± 0.47	38.92 ± 0.03
100 $Pb(NO_3)_2$	11.87 ± 0.34	19.04± 0.94
200 $Pb(NO_3)_2$	9.35 ± 0.17	14.25 ± 0.06
50 $HgCl_2$	7.02 ± 0.03	10.37 ± 0.96
100 $HgCl_2$	5.91 ± 0.15	9.12 ± 0.27
400 GA_3+ 100 $Pb(NO_3)_2$	13.25 ± 0.96	24.73 ± 0.22
400 GA_3+ 200 $Pb(NO_3)_2$	11.56 ± 0.75	20.85 ± 0.98
400 GA_3 + 50 $HgCl_2$	10.21 ± 0.36	16.76 ± 0.07
400 GA_3 + 100 $HgCl_2$	8.46 ± 0.17	14.98 ± 0.95
LSD at 0.05	6.92	9.34

Readings are mean of four replicates.

although they were normal in appearance. When 100 ppm $Pb(NO_3)_2$ was applied, it registered 24.8% decrease in pistillate and 29.4% decrease in staminate flowers when compared with control (Table 1). Applied 200 ppm $Pb(NO_3)_2$ showed even more inhibitory effects, reducing the number of both flowers. In the mixed doses of GA_3 with 100 ppm $Pb(NO_3)_2$ and 200 ppm $Pb(NO_3)_2$, GA_3 revealed dominant effect as compared to individual doses because there was less inhibition in number of flowers (Table 1).

Applied 100 ppm $HgCl_2$ registered significant inhibition in flowering as compared with 50 ppm $HgCl_2$ (Table 1). However, mixed doses of $HgCl_2$ with 400 ppm GA_3 showed interesting results as GA_3 promoted both pistillate and staminate flowers as compared to individual doses of $HgCl_2$ (Table 1). In the mixed dose of 400 ppm GA_3 + 50 ppm $HgCl_2$, 53.6% increase in pistillate and 38.4% in staminate flowers was recorded as compared with 50 ppm $HgCl_2$ (Table 1). Applied 400 ppm GA_3 +100 ppm $HgCl_2$ caused 63.3% enhancement in pistillate and 71.8 % in staminate flowers in comparison with 100 ppm $HgCl_2$ (Table 1).

M. charantia

Application of 400 ppm GA_3 stimulated the number of both pistillate and staminate flowers (Table 2). Applied 200 ppm $Pb(NO_3)_2$ registered more inhibition than 100 ppm $Pb(NO_3)_2$ i.e., 37.3% in pistillate and 47.2% in staminate flowers when compared with control (Table 2). Mixed doses of GA_3 with 100 ppm $Pb(NO_3)_2$ and 200 ppm $Pb(NO_3)_2$, showed less inhibition in flowering due to florigenic effects of GA_3. Applied dose of 400 ppm GA_3 +100 ppm $Pb(NO_3)_2$ showed 11.6% increase in pisillate and 29.8% in staminate flowers when compared with 100 ppm $Pb(NO_3)_2$ (Table 2).

Applied 100 ppm $HgCl_2$ showed remarkable inhibition i.e., 60.3% in staminate and 66.2% in pistillate flowers. However, application of GA_3 partially reversed the effects of $HgCl_2$ because there was less reduction in number of flowers as compared with individual doses of $HgCl_2$ i.e., 400 ppm GA_3 +50 ppm $HgCl_2$ showed 45.4% and 61.6% increase in pistillate and staminate flowers, respectively, as compared with 50 ppm $HgCl_2$ (Table 2). Likewise 400 ppm GA_3 + 100 ppm $HgCl_2$ showed 43.1% increase in pistillate and 64.2% in staminate flowers when compared with 100 ppm $HgCl_2$.

DISCUSSION

The objectives of this study were to determine florigenic effects of GA_3 and delay in flowering caused by heavy metals pollution during early flower development and to determine how hormonal signals alter these growth parameters. Applied GA_3 significantly enhanced flowering leading to precocious fruit development. Initiation of floral buds started on 21^{th} days of treatment. Flowers were normal in appearance and male flowers produced pollen. This might be due to well known effects of GA_3 on early floral initiation (Ganelevin and Zieslin, 2002) as GA_3 is mandatory for the shift from vegetative to reproductive stage, revealing florigenic effect (Naor et al., 2004). GAs can replace the requirement of growing cells for maintenance of cellular division during early flower deve-

lopment. 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 hormonal action.

Plants under heavy metals stress caused delay in flowering as flower formation is related to environmental conditions (Tooke et al., 2005). Both lead and mercury inhibited flowering which can be attributed to initiation of disruption in biological processes due to heavy metals (Van Assce and Clijsters, 1990). The toxic threshold level of metal in the tissue is defined by stress point for metal toxicity and beyond this level physiological state of cell is irreversibily damaged. Application of 200 ppm $Pb(NO_3)_2$ caused significant reduction in number of flowers, as compared with 100 ppm $Pb(NO_3)_2$; thus, the lower dose was less effective. Higher metal concentrations that accumulated in the different plant parts induce higher toxic effects. Accordingly, internal metal concentrations in the plants were correlated to toxic effects (Gothberg et al., 2004).

Interesting results were seen when heavy metal was applied in combination with GA_3. It can be concluded that GA_3 has partially reversed the toxic effects of $Pb(NO_3)_2$ and $HgCl_2$. Formation of star shaped abnormalitity indesepalled flowers which is common phenomenon in rose flowers exposed to external ethylene is completely prevented by applying GA_3, suggesting the function of gibberellin in reducing the sensitivity of rose flower organs to ethylene (Ganelevin and Zieslin, 2002). Cheng et al. (2001) reported that absence of gibberellin in vegetative corms and its presence in corms at early floral initiation and flower development stages suggesting that gibberellin is a causal factor in inducing floral initiation in *Polianthes. tuberosa*. In the present work, increased rate of flowering in metal treated plants can be attributed due to role of GA_3 in reducing the inhibitory effects of plants under heavy metal stress.

Application of 100 ppm $Pb(NO_3)_2$ + 400 ppm GA_3 had inhibitory effects on flowering but this inhibition was less as compared with plants treated with 100 ppm $Pb(NO_3)_2$ alone as floral initiation and floral organ development are both regulated by the phytohormone gibberellin (Achard et al., 2004,). When 200 ppm $Pb(NO_3)_2$ was applied with 400 ppm GA_3, more inhibition in flowering was observed as compared with dose of 100 ppm $Pb(NO_3)_2$ + 400 ppm GA_3 (Tables 1 and 2). However, the number of flowers was less as compared with plants treated with 100 ppm $HgCl_2$. This is due to increasing concentration of $HgCl_2$ as effects of mercury toxicity differ with concentrations (Gothberg et al., 2004). However, this inhibition was less than that of 100 ppm $Pb(NO_3)_2$, as GA_3 has partially reversed the inhibitory effect of $Pb(NO_3)_2$. Mixed dose of 50 ppm $HgCl_2$ + 400 ppm GA_3 had inhibitory effects on flowering but this inhibition was less as compared with plants treated with 50 ppm $HgCl_2$ alone (Tables 1 and 2).

When 100 ppm $HgCl_2$ was applied with 400 ppm GA_3, more inhibition in flowering was observed as compared with dose of 50 ppm $HgCl_2$ + 400 ppm GA_3, revealing the dominant effect of GA_3.

In conclusion, application of plant growth hormones can be beneficial in the reduction of inhibition caused by heavy metals. 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

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