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Influence of Some Growth Regulators and Cations on Inhibition of Chlorophyll Biosynthesis by Lead in Maize

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Phytotoxic effects of Pb pollution are well established. In order to analyse the physiological basis of toxic symptoms and of reduced plant productivity, its effect on chlorophyll content has been examined in some plants. Thus, a decrease in total chlorophyll content during Pb supply has been observed in oats (Fiussello and Molinari 1973), cucumber (Burzynski 1985), dodder (Jana et al. 1987), mung bean (Prasad and Prasad, 1987), pea (Sinha et al. 1988) etc. The activity of delta aminolevulinic acid dehydratase, an important enzyme in the biosynthesis of heme pigments, is inhibited by Pb in mung bean (Prasad and Prasad, 1987) and several other species (Hampp and Ziegler, 1974). This observation may perhaps indicate that a reduction in chlorophyll content in the presence of lead is due to an inhibition of pigment synthesis. The effect of Pb on greening maize leaf segments in the presence of various precursors of chlorophyll has been studied in the present investigation to evaluate this hypothesis. The effect of some growth regulators and cations, which could otherwise modify chlorophyll biosynthesis, has been examined to see whether the toxic effects of Pb on photosynthetic pigments could also be modified by these effectors.

MATERIALS AND METHODS

Seeds of Zea mays L. GS-2 purchased from National Seed Corporation, New Delhi were surface sterilized with 1% $Ca(OCl)_2$ for 5 min and then washed thoroughly with glass distilled water before planting. Seedlings were grown in small plastic pots (20 x 10 cm) containing acid washed sand for 14 days in dark, at 25 ± 2°C. Seedlings were watered daily with half strength Hoagland's solution (Arditti and Dunn, 1969) containing no nitrogen.

The leaves were excised into pieces (ca 2 mm) and floated on 1/10 strength Hoagland's solution containing the desired nitrogenous salt with or without 0.1 mM lead acetate. The desired growth regulators and salts were dissolved in the Hoagland's solution, maintaining pH at 6.0. Incubation was for 12 h in continuous light (about 60 W m^2) at 25±2°C.

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Table 1. Effect of nitrogenous precursors on chlorophyll and carotenoid biosynthesis in greening leaf segments in the presence or absence of lead.

Nitrogenous compound in incubation medium	Pigment content µg (g fr. wt.)$^{-1}$			
	Chlorophyll		Carotenoid	
	-Pb	+Pb	-Pb	+Pb
None	53±1	30±1*(-43)	40±2	53±4(+32)ns
KNO_3 (10 mM)	113±3	30±3*(-73)	93±2	41±1*(-56)
NH_4Cl (10 mM)	88±4	27±0*(-69)	80±1	43±0*(-46)
Glutamine (5 mM)	148±3	30±4*(-80)	110±6	45±6*(-59)
Glycine (5 mM)	151±3	20±1*(-87)	115±1	43±6*(-63)
CD at 5%	5.26	4.11	13.58	7.33

Maize seedlings were grown with half strength Hoagland's solution containing no nitrogen in the dark for 14 days. Leaves were excised and floated on 1/10 strength Hoagland's solution containing desired nitrogenous compounds as the only nitrogen sources (pH 6.0) and no Pb or 0.1 mM Pb in light at 25±2°C for 12 h. The values in bracket indicate percent inhibition (-) or promotion (+) in the presence of Pb, as compared to the corresponding no Pb control.
ns= not significant; *$p < 0.05$.

Fresh leaf material (0.5 g) was extracted with cold 80% acetone and centrifuged at approximately 10,000 x g for 10 min. Extraction and centrifugation was repeated until the residue became colourless. Amount of the supernatant was made upto 20 ml with acetone. Absorbance of the supernatant was recorded at 663 and 645 nm for chlorophyll and at 440 nm for carotenoids. Chlorophyll and carotenoid contents were calculated by the formula of Strain and Svec (1966) and Ikan (1969) respectively.

The data presented in this paper are the average of three independent replicate and each experiment was repeated twice. Student 't' test was applied to test the significance of difference due to Pb treatment as compared to no Pb treatment (row wise). Analysis of variance was done and critical difference (CD) were calculated to test the significance of variation between treatments (column wise).

RESULTS AND DISCUSSION

Supply of nitrogenous compounds increased chlorophyll contents in greening maize leaf segments, only in absence of Pb (Table 1). In the absence of Pb, very little chlorophyll was formed when there was no nitrogen source in the incubation medium. Maximum chlorophyll synthesis was recorded in glycine and glutamine treated leaf segments followed by that in KNO_3 and NH_4Cl in that order. When Pb was

Table 2. Effect of growth regulators on chlorophyll and carotenoid biosynthesis in greening leaf segments in the absence and presence of lead.

Growth regulators/ nitrate in the incubation medium	Pigment content µg (g fr. wt.)$^{-1}$			
	Chlorophyll		Carotenoid	
	-Pb	+Pb	-Pb	+Pb
KNO_3 (10 mM only)	107±5	30±0*(-72)	90±1	50±0*(-44)
GA (2 ppm)	125±3	63±5*(-50)	116±11	100±2(-14)ns
KNO_3 + GA	149±5	64±1*(-57)	115±0	80±2*(-30)
IAA (2 ppm)	149±7	41±1*(-72)	121±1	90±1*(-26)
KNO_3 + IAA	207±10	67±6*(-68)	131±3	80±1*(-39)
Salicylic acid (0.1 mM)	151±5	37±2*(-75)	120±7	100±10(-17)ns
KNO_3+Salicylic acid	205±2	70±2*(-66)	149±7	200±6(+34)*
CD at 5%	3.02	6.45	7.31	7.0

Details as in table 1. GA= Gibberellic acid, IAA= Indole-3-acetic acid. ns= not significant; *$p < 0.05$.

also supplied in the incubation medium the chlorophyll content declined drastically, and by almost similar magnitude in each nitrogen source.

Almost similar effects of nitrogenous compounds and of Pb were seen on carotenoid content of the leaves (Table 1).

Growth regulators, Gibberellic acid (GA), indole-3-acetic acid (IAA) and Salicylic acid (SA) when supplied to the excised leaf segments in the presence or absence of nitrogen (as nitrogen sources) increased chlorophyll and carotenoid contents to various degrees (Table 2). The maximum chlorophyll content was recorded in IAA + nitrate treated leaf segments. Supply of Pb, inhibited chlorophyll formation by almost similar magnitude (50-75%) in each case.

The growth regulators increased carotenoid contents and decreased the inhibitory effect of Pb to some extent (Table 2). In fact, in KNO_3+ SA treated leaf segments, Pb increased carotenoid content by 34%.

Supply of Ca^{2+} and Mg^{2+} increased chlorophyll content to some extent, while Mn^{2+}, Zn^{2+} and Cu^{2+} inhibited the same (Table 3). Supply of Pb had no effect on chlorophyll content, in the presence of these ions.

The carotenoid content of the leaf segments was unaffected by Ca^{2+} and Mg^{2+} but was decreased by Mn^{2+}, Zn^{2+} and Cu^{2+} supply (Table 3). The Pb supply inhibited carotenoid content, although it had no effect in the presence of Ca^{2+}, Mg^{2+} and Mn^{2+}. But the heavy metal increased carotenoid content in the presence of Zn^{2+} and Cu^{2+}.

Table 3. Effect of some divalent cations on chlorophyll and carotenoid biosynthesis in greening leaf segments in the absence and presence of lead.

Addition in the incubation medium	Pigment content $\mu g\ (g\ fr.\ wt.)^{-1}$			
	Chlorophyll		Carotenoid	
	-Pb	+Pb	-Pb	+Pb
KNO_3 (10 mM)	110±1	40±1*(-64)	93±1	60±0*(-35)
+ $CaCl_2$ (10 mM)	115±10	109±10(-5)ns	86±9	90±10(+5)ns
+ $MgCl_2$ (10 mM)	130±11	125±11(-4)ns	91±2	94±6(+3)ns
+ $MnCl_2$ (5 mM)	40±2	40±6(0)ns	25±1	30±5(+20)ns
+ $ZnCl_2$ (5 mM)	42±1	40±1(-5)ns	28±0	51±1*(+82)
+ $CuSO_4$ (5 mM)	38±3	36±2(-5)ns	23±1	46±2*(100)
CD at 5%	8.35	8.25	6.05	6.81

Details as in table- 1.
ns= not significant; *$p < 0.05$.

The effect of various concentrations of $MgCl_2$ on chlorophyll and carotenoid content was examined (Table 4). In the absence of Pb, 5 and 10 mM Mg^{2+} increased chlorophyll, while 20 mM of the Mg^{2+} inhibited it. Supply of Pb inhibited chlorophyll content, only in the absence of Mg^{2+}, and had no effect in the presence of 5 to 20 mM $MgCl_2$. Almost similar effects of Mg^{2+} and of Pb were observed on carotenoids also.

A lower level of pigment contents in the leaves in the presence of heavy metal may be either due to their reduced synthesis or to accelerated degradation. In greening leaves, there is net synthesis of the pigment, and no degradation and hence an effect observed during this phase (Table 1), may suggest that the effect of heavy metal is on synthesis of the pigment. Chlorophyll is a nitrogenous molecule, and one of the possible mechanisms of inhibition of chlorophyll biosynthesis, may be through the inhibition of nitrate assimilation by the leaf segments (Sinha et al. 1988). Although, the supply of inorganic nitrogen, and their assimilatory products glycine and glutamine, which are also the amino acid precursors of chlorophyll (Beale, 1978), increased chlorophyll formation, they could not alleviate Pb toxicity. Thus, the effect of Pb on chlorophyll formation seems to be independent of its effects on nitrogen assimilation or on incorporation of nitrogenous precursors into chlorophyll molecules. The effect seems to be on a more generalized metabolic process, as the formation of non-nitrogenous pigment, carotenoid was also inhibited by the heavy metal (Table 1). Ultrastructural changes in the chloroplast has been observed in Ceratophyllum by Rebechini and Hanzely (1974).

One of the possible mechanisms of Pb effects may be through growth regulators, which otherwise influence various physiological and metabolic processes including the functional development of chloroplasts. Among the three growth regulators tested, salicylic acid and IAA

Table 4. Effect of different concentration of $MgCl_2$ on chlorophyll and carotenoid contents of greening leaf segments in the presence or absence of lead.

Addition in incubation mixture	Pigment content µg (g fr. wt.)$^{-1}$			
	Chlorophyll		Carotenoid	
	-Pb	+Pb	-Pb	+Pb
KNO_3 (10 mM)	110±2	51±2*(-40)	94±1	60±1*(-36)
+ $MgCl_2$ (5 mM)	122±1	115±1(-6)ns	98±0	95±2(-3)ns
+ $MgCl_2$ (10 mM)	130±1	120±2(-8)ns	101±2	100±1(-1)ns
+ $MgCl_2$ (20 mM)	71±2	62±0 (-13)ns	49±2	48±1(-2)ns
CD at 5%	1.15	1.92	1.62	1.00

Details as in table- 1.
ns= not significant; *$p < 0.05$.

increased chlorophyll and carotenoid contents to some extent, but they also did not alleviate the inhibitory effects of Pb on chlorophyll and carotenoid. Increase in chlorophyll biosynthesis by SA has been observed in earlier studies also (Mishra and Srivastava 1983). Among the cations tested, Ca^{2+} and Mg^{2+} not only increased the chlorophyll biosynthesis but they alleviated the toxic effects of Pb as well (Table 3). Although the exact mechanism of antagonistic action of these ions may not be discerned at the moment, it is likely that they interact with the Pb, as far as its absorption and internal transport is concerned. Lime application in soil is known to inhibit the absorption of Pb by lettuce plants (Bassuk 1986). Magnesium may have some specific role in the biosynthesis of chlorophyll and in alleviating the toxic effects of Pb. Since, at 20 mM of it, the inhibition of chlorophyll formation by Pb, was not completely reversed (Table 4), there seems to be some physiological process involved in the antagonism between Pb and Mg rather than a simple ionic counteraction in the environment (floating medium) of the leaf segments. Magnesium is essential for biosynthesis of chlorophylls and it is required as a cofactor for delta aminolevulenic acid dehydratase (Tamai et al. 1979, Liedgens et al. 1980).

The effects of various modulators on inhibition of carotenoid formation by Pb, was different to that of chlorophyll in many cases and thus the mechanism of effect of Pb on two pigments seems to be independent. The increase in carotenoid by Pb, in the presence of SA or Zn or Cu is not understood at present. However, an increase in cytosolic pigment anthocyanin by Pb has been reported in Acer rubrum (Davis and Barnes, 1973).

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