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Morphogenetic Effect of Growth Hormones i.e., Indole-3-Acetic Acid, Gibberellic Acid and Heavy Metal i.e., Lead Nitrate on the External and Internal Morphology of Seedlings of *Cicer arietinum* L.

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Abstract: The present study on *Cicer arietinum* L., deals with the morphogenetic effects of growth hormones i.e., IAA, GA_3 and heavy metal i.e., $Pb(NO_3)_2$ on the external and internal morphology of the plant. Following concentrations of hormones and heavy metal were used individually; 200 ppm IAA, 200 ppm GA_3 and 50 ppm $Pb(NO_3)_2$. In combination 200 ppm IAA+200 ppm GA_3, 200 ppm IAA+50 ppm $Pb(NO_3)_2$, 200 ppm GA_3+50 ppm $Pb(NO_3)_2$, 200 ppm IAA+200 ppm GA_3+50 ppm $Pb(NO_3)_2$. Results were collected after 15 days and compared with control. Extraneous IAA inhibited elongation in root, hypocotyl and shoot, which was accompanied by increase in diameter. Contrarily, increase in length accompanied by inhibited diameter was observed with the doses of GA_3. Applied 50 ppm $Pb(NO_3)_2$ individually as well as in combination with GA_3 and in the mixture of all three doses inhibited length. Number of rootlets increased with the application of IAA and were inhibited with $Pb(NO_3)_2$. Branching was promoted by IAA and IAA+GA_3. Number of compound leaves increased with IAA, IAA+GA_3 treatment. Moreover number of leaflets in first three compound leaves decreased with $Pb(NO_3)_2$ and IAA+$Pb(NO_3)_2$. Leaflet area decreased with $Pb(NO_3)_2$ and GA_3+$Pb(NO_3)_2$ treatment. In the internal morphology of root, shoot and rachis epidermal cells remained more or less constant in treated plants. Width of cortical region, cortical cells, xylary region and pith of root and shoot showed expansion with IAA and inhibition with GA_3 applications. 200 ppm IAA showed expansion in width and early maturation of metaxylem elements. Width of rachis and ground tissue showed inhibition with GA_3, $Pb(NO_3)_2$ and GA_3+$Pb(NO_3)_2$ applications. Number of vascular bundles of rachis remained same except with $Pb(NO_3)_2$ and GA_3+$Pb(NO_3)_2$ which showed inhibition in number.

Key words: *Cicer arietinum* L., root-shoot, leaf cortex, xylary region, hormones, IAA, GA_3, heavy metal $Pb(NO_3)_2$

INTRODUCTION

Growth, metabolism and morphogenesis in higher plants are under specific chemical control, which are known as plant hormones[1]. The hormone mediated control of plant growth and development involves both synthesis and response[2]. Plant growth regulators i.e., auxins, gibberellins and kinetins exerts far reaching effects on the external morphology and internal structure of the plant. Auxins play an important role in root development through its life[3]. Auxin induced growth results in the lateral expansion of root and stem tissues[4]. According to Booker *et al.*[5] apically derived auxin is transported basipetally directly into the axillary buds where it inhibits their growth. Several morphological changes in the leaf shape and size are brought about by auxin. In *Cicer arietinum* L., increase in the size of leaf blade was highest with applied IAA[6]. The application of exogenous auxins to some trees significantly increased the fruit peduncle in diameter and development of vascular tissue was also increased[7].

Gibberellins have the ability to stimulate elongation of root and stem in certain dwarf plants. Modest *et al.*[8] reported that in Rangpurlime (*Citrus limonia* Osbeck) significant increase in plant height was observed with exogenous GA_3. According to Chaudhry[9,10] and Awan *et al.*[11] stem elongation is induced by applied GA_3. Cottrell *et al.*[12] reported that gibberellins are far more effective than auxins as far as length is concerned.

Heavy metals have some physiological toxic effects. Different types of deleterious effects are caused by heavy metals[13]. They have inhibitory effects on plant growth[14].

Heavy metals are said to cause inhibitory effects, whereas hormones are growth promoters. In the present

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study attempt has been made to see whether the inhibitory effect of $Pb(NO_3)_2$ can be reversed by extraneous IAA and GA_3.

MATERIALS AND METHODS

The present study is undertaken to observe the effects of growth hormones i.e., IAA, GA_3 and $Pb(NO_3)_2$ on the external and internal morphology of root, shoot and rachis of *Cicer arietinum* L. (Leguminosae). Growth hormones and heavy metals were used individually as well as in combinations. Individually 200 ppm IAA, 200 ppm GA_3, 50 ppm $Pb(NO_3)_2$ were used. In combination 200 ppm IAA+200 ppm GA_3, 200 ppm IAA+50 ppm $Pb(NO_3)_2$, 200 ppm GA_3+50 ppm $Pb(NO_3)_2$ and 200 ppm IAA+200 ppm GA_3+50 ppm $Pb(NO_3)_2$ were applied.

Under the controlled environmental conditions seeds were grown in Petri plates in growth chamber for 15 days during the month of November in Plant Anatomy Research Laboratory. After germination seeds were shifted in light chamber having 18 h light period. Then 10 ml of nutrient solution was given on alternate days. Hormones were given daily for 15 days and 50 ppm $Pb(NO_3)_2$ after six days. After 15 days length, diameter, fresh and dry weight of root and shoot were undertaken. In the case of root number of rootlets and in shoot number of branches were also calculated. For the study of compound leaf number of compound leaves, number of leaflets in first 3 compound leaves and area of leaflets of first compound leaf were studied. For the study of internal morphology 1 cm long portion of root, shoot and rachis was fixed in Corney's modified fluid. Material was dehydrated and cleared in the grades of tertiary butyl alcohol, infiltrated and embedded in paraffin wax. The embedded material was processed in transverse planes with rotary microtome (10-15 μm). Then material was passed through descending series of xylene and stained with safranine and fast green and mounted in Canada balsam. Material was observed under microscope and compared with control. All data was subjected to statistical analysis[15].

RESULTS

External morphology: The root of *Cicer arietinum* L., showed inhibited length with extraneous 200 ppm IAA, which was accompanied by expanded diameter and increased fresh and dry weight. In applied 200 ppm GA_3 21.28% increase in length was recorded with inhibited diameter (Fig. 1) and decreased fresh and dry weight. However, in the application of 50 ppm $Pb(NO_3)_2$ inhibition in length as well as decrease in fresh and dry weight was recorded. Treatments with mixed doses of 200 ppm IAA+50 ppm $Pb(NO_3)_2$ showed well marked decrease in length, which being 34.35%. The rest of the doses showed some increase in length (Table 1). Number of rootlets increased with applied 200 ppm IAA and 200 ppm IAA+200 ppm GA_3, 200 ppm IAA+50 ppm $Pb(NO_3)_2$ and 200 ppm IAA+200 ppm GA_3+50 ppm $Pb(NO_3)_2$. Inhibition in number was recorded with 50 ppm $Pb(NO_3)_2$, 200 ppm GA_3+50 ppm $Pb(NO_3)_2$. In shoot inhibition in length was recorded with applied 200 ppm IAA however this inhibition was accompanied by expansion in diameter. A remarkable increase in length was recorded with GA_3,

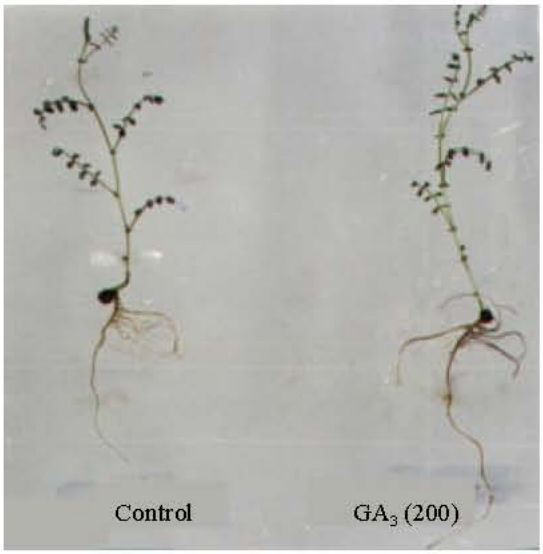

Fig. 1: Effect of 200 ppm GA_3 on the length of root and shoot

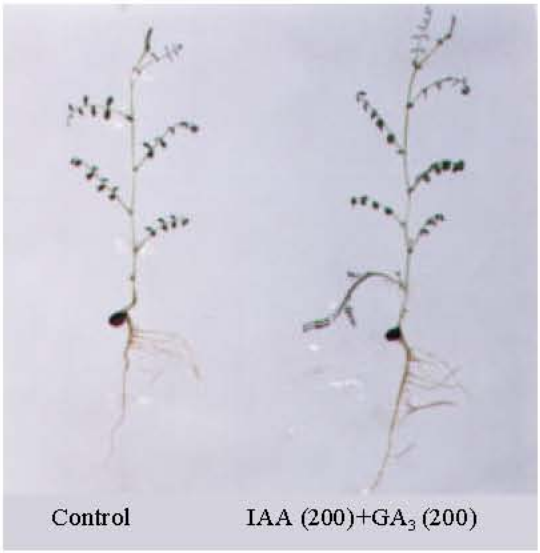

Fig. 2: Treatment with 200 ppm IAA+200 ppm GA_3 showing branching

Table 1: Effect of growth hormones i.e., IAA, GA_3 and heavy metal i.e., $Pb(NO_3)_2$ on the external morphology of root

Treatments	Length (cm)	Diameter (cm)	No. of rootlets	Fresh weight (g)	Dry weight (g)
Control	23.98±2.270	0.72±0.099	22.00±1.050	1.69±0.034	0.39±0.029
IAA	18.50±0.538	0.87±0.309	30.00±1.740	2.41±0.062	0.53±0.018
GA_3	29.21±0.970	0.63±0.065	21.00±0.784	1.64±1.020	0.35±0.010
Pb	22.70±0.210	0.77±0.762	17.00±0.630	1.75±0.024	0.42±0.051
IAA+GA_3	25.29±0.950	0.76±0.014	28.00±1.430	2.38±0.023	0.521±0.04
IAA+Pb	16.25±0.510	0.82±0.076	26.00±1.480	1.79±0.180	0.46±0.005
GA_3+Pb	25.79±0.347	0.67±0.032	16.00±1.152	1.63±0.070	0.35±0.006
IAA+GA_3+Pb	25.11±0.532	0.75±0.061	24.00±1.260	2.10±0.080	0.49±0.010

Table 2: Effect of growth hormones i.e., IAA, GA_3 and $Pb(NO_3)_2$ on the external morphology of shoot

Treatments	Length (cm)	Diameter (cm)	No. of Branches	Fresh weight (g)	Dry weight (g)
Control	30.34±2.470	0.52±0.036	0	1.68±0.120	0.347±0.069
IAA	22.60±1.103	0.59±0.055	2	2.01±0.040	0.54±0.0350
GA_3	38.50±1.110	0.49±0.020	0	1.66±0.830	0.32±0.0640
$Pb(NO_3)_2$	18.50±1.310	0.54±0.047	0	1.60±0.035	0.30±0.0380
IAA+GA_3	34.00±1.020	0.53±0.490	1	2.27±0.074	0.59±0.0390
IAA+$Pb(NO_3)_2$	19.52±0.370	0.55±0.044	0	1.65±0.067	0.32±0.0610
GA_3+$Pb(NO_3)_2$	32.60±1.520	0.51±0.020	0	1.64±0.063	0.314±0.037
IAA+GA_3+$Pb(NO_3)_2$	31.80±0.300	0.55±0.010	0	1.70±0.053	0.51±0.0360

Table 3: Effect of growth hormones i.e., IAA, GA_3 and heavy metal i.e., $Pb(NO_3)_2$ on the external morphology of compound leaf

Treatments	No. of compound leaves	No. of leaflets in first 3 compound leaves	Area of leaflets of first compound leaf
Control	6.00±0.28	21.00±1.23	22.06±1.02
IAA	8.00±0.63	21.00±0.85	24.10±1.04
GA_3	6.00±0.98	21.00±0.47	22.00±1.16
$Pb(NO_3)_2$	4.00±0.02	18.00±0.45	18.89±1.23
IAA+GA_3	9.00±0.39	21.00±1.94	22.48±1.68
IAA+$Pb(NO_3)_2$	5.00±0.26	19.00±0.58	22.00±1.03
GA_3+$Pb(NO_3)_2$	6.00±0.39	21.00±0.36	19.06±1.33
IAA+GA_3+$Pb(NO_3)_2$	6.00±0.56	21.00±0.67	21.80±0.63

Table 4: Effect of growth hormones i.e., IAA, GA_3 and heavy metal i.e., $Pb(NO_3)_2$ on the internal morphology of root

Treatments	Width of epidermal cells (μm)	Layer of cortical cells (μm)	Width of cortical region (μm)	Width of cortical cells (μm)	Width of xylem strand (μm)	Width of metaxylem elements (μm)	No. of metaxylem element	Width of pith cells (μm)
Control	23.96±0.21	11	475.70±2.75	61.31±1.38	110.20±2.09	63.27±0.93	7	215.11±1.38
IAA	24.19±0.35	13	548.31±2.37	72.91±0.72	117.46±2.73	67.90±1.37	9	240.74±0.99
GA_3	23.14±0.26	11	451.01±1.64	53.98±1.97	106.00±1.72	56.45±0.61	7	183.38±1.62
Pb	24.01±0.28	11	507.90±2.56	67.93±0.71	110.70±1.37	62.88±0.84	7	221.95±1.39
IAA+GA_3	24.21±0.75	12	509.91±1.52	66.98±2.73	115.80±1.50	68.28±1.85	9	234.67±0.76
IAA+Pb	24.07±0.69	11	526.01±2.91	71.85±1.61	114.20±1.37	66.31±1.44	7	239.89±0.85
GA_3+Pb	23.06±0.73	11	456.36±2.75	59.38±0.67	104.29±1.25	60.25±1.70	7	193.37±1.60
IAA+GA_3+Pb	24.10±0.81	16	519.62±2.76	69.75±1.29	115.86±1.37	65.99±0.26	8	236.00±1.31

Table 5: Effect of growth hormones i.e., IAA, GA_3 and heavy metal i.e., $Pb(NO_3)_2$ on the internal morphology of shoot

Treatments	Width of epidermal cells (μm)	Layers of cortical cells	Width of cortical region (μm)	Width of cortical cells (μm)	Width of vascular cylinder (μm)	Layers of of vascular cambium	Width of vascular cambium (μm)	No. of xylem elements in a strand	Width of metaxylem element (μm)	Width of pith region (μm)	Width of pith cells (μm)
Control	22.67 ±1.013	12	462.76 ±2.81	59.53 ±0.73	185.47 ±1.33	6	117.31 ±2.21	5	40.75 ±1.37	453.80 ±1.49	55.29 ±0.81
IAA	23.15 ±1.62	14	511.36 ±2.74	68.11 ±0.46	225.71 ±1.08	8	141.45 ±1.62	5	48.55 ±1.66	497.75 ±1.70	79.63 ±1.60
GA_3	21.72 ±1.28	12	439.15 ±1.69	51.64 ±1.37	169.52 ±1.61	6	101.92 ±1.92	5	36.84 ±1.58	442.92 ±1.06	40.97 ±1.73
Pb	23.01 ±0.64	12	488.64 ±2.75	63.22	192.43 ±1.02	6	118.02 ±2.21	5	41.00 ±1.67	476.35 ±1.29	63.50 ±0.97
IAA+GA_3	23.11 ±1.33	12	493.85 ±1.99	66.25	206.57 ±0.78	7	132.10 ±1.34	6	45.34 ±1.39	487.48 ±0.75	70.85 ±1.38
IAA+Pb	23.00 ±1.29	12	507.23 ±2.68	66.87	211.37 ±1.69	6	135.52 ±2.89	5	44.25 ±0.39	491.55 ±0.80	72.09 ±1.33
GA_3+Pb	22.38 ±1.29	12	452.49 ±2.51	56.46	176.84 ±1.39	5	112.01 ±1.26	5	36.15 ±1.06	438.22 ±2.71	37.99 ±1.70
IAA+GA_3+Pb	23.02 ±1.62	12	492.26 ±2.31	67.98	201.18 ±1.79	7	134.68 ±1.65	5	43.73 ±1.08	473.61 ±1.44	68.72 ±1.29

Table 6: Effect of growth hormones i.e., IAA, GA_3 and heavy metal i.e., $Pb(NO_3)_2$ on the internal morphology of rachis

Treatments	Width of rachis (µm)	Width of epidermal cells (µm)	Width of parenchyma cells (µm)	Width of metaxylem elements of main bundle (µm)	No. of vascular bundles
Control	501.30±2.01	23.79±1.21	63.37±2.37	39.25±1.23	4
IAA	581.55±2.25	24.05±1.68	70.92±2.65	41.77±1.69	5
GA_3	488.09±2.65	22.74±1.39	60.35±2.81	38.35±0.78	4
Pb	458.61±2.68	24.08±1.85	63.97±1.65	38.10±0.59	3
IAA+GA_3	565.01±2.23	24.14±1.34	69.97±1.64	39.96±1.68	5
IAA+Pb	557.49±2.02	23.97±1.65	68.64±1.85	41.31±0.74	4
GA_3+Pb	455.00±2.98	22.16±1.33	59.47±1.91	38.82±0.65	3
IAA+GA_3+Pb	539.40±2.65	24.00±1.65	70.74±1.16	41.80±0.70	4

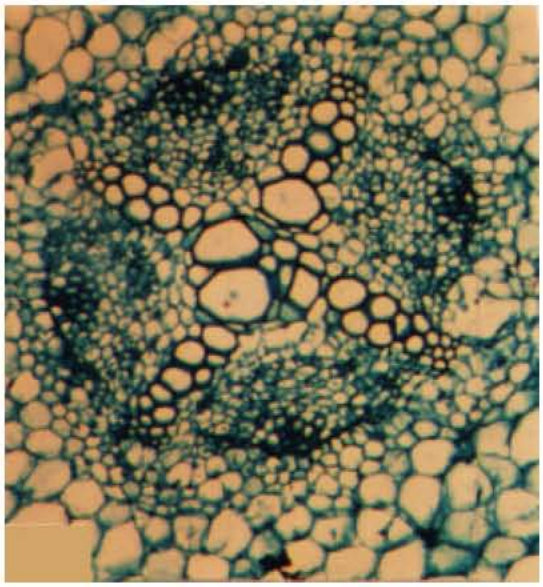

Fig. 3: T.S. of root showing tetrarch condition (control)

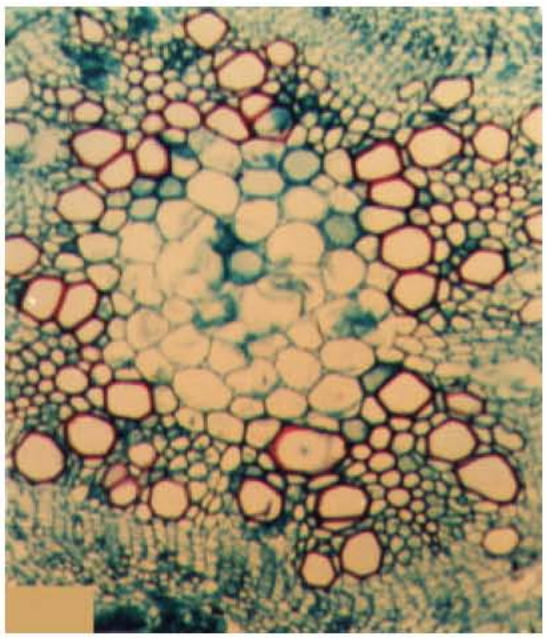

Fig. 4: Effect of 200 ppm IAA on the metaxylem elements and pith of root

which being 25.51%. However, this increase was accompanied by inhibited diameter as well as decreased fresh and dry weight (Table 2). Applied mixed doses showed increase in length except with 200 ppm IAA+50 ppm $Pb(NO_3)_2$ where inhibition in length, however, decrease in fresh and dry weight was observed accompanied by increase in diameter was recorded, when 200 ppm IAA was applied in combination with other doses expanded diameter as well as increased fresh and dry weight was recorded (Table 2). The applied 200 ppm IAA and 200 ppm IAA+200 ppm GA_3 showed increased branching consequently increasing the number of compound leaves (Fig. 2). Number of leaflets in first 3 compound leaves remained constant except with applied 50 ppm $Pb(NO_3)_2$ and 200 ppm IAA+50 ppm $Pb(NO_3)_2$, which decreased the number. Area of leaflets of first compound leaf was decreased by applied 50 ppm $Pb(NO_3)_2$ and 200 ppm GA_3+50 ppm $Pb(NO_3)_2$ (Table 3).

Internal morphology: In the study of internal morphology of root, shoot and rachis epidermal cells did not respond well with the application of all the treatments. In the root tetrarch condition remained constant with all the doses (Fig. 3). Increase in the number of cortical layers was registered with 200 ppm IAA, 200 ppm IAA+200 ppm GA_3 and 200 ppm IAA+200 ppm GA_3+50 ppm $Pb(NO_3)_2$. The layers remained the same with the rest of the doses (Table 4). Width of cortical region, cortical cells, xylem strand and metaxylem elements showed expansion with applied 200 ppm IAA, 200 ppm IAA+200 ppm GA_3, 200 ppm IAA+50 ppm $Pb(NO_3)_2$ as well as in the mixture of all three doses. Contrarily, 200 ppm GA_3 and 200 ppm GA_3+50 ppm $Pb(NO_3)_2$ showed inhibition. Moreover, no significant effect was recorded on the width of xylem strands as well as on the width of metaxylem elements with 50 ppm $Pb(NO_3)_2$. With the application of 200 ppm IAA, 200 ppm IAA+200 ppm GA_3 and 200 ppm IAA+200 ppm GA_3+50 ppm $Pb(NO_3)_2$ early maturation of metaxylem elements was recorded and consequently increase in the number of these elements. The number remained constant with other doses. Expansion in the width of pith region and pith cells was observed with the doses of IAA (Fig. 4). In 200 ppm GA_3 application a well marked inhibition was registered. Furthermore, some

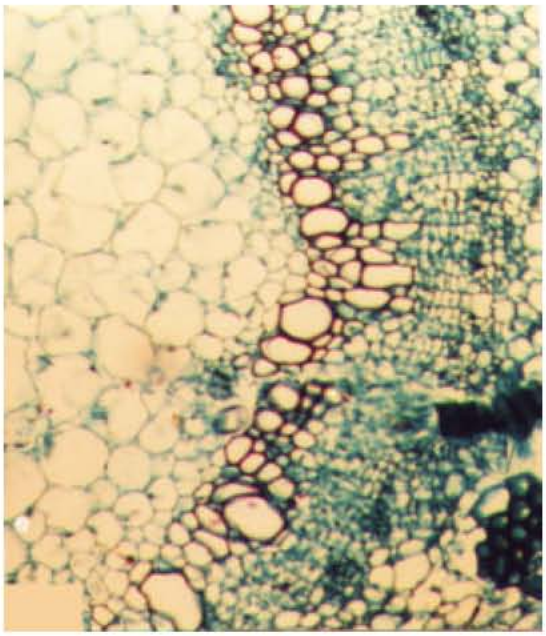

Fig. 5: 200 ppm IAA treatment showing cambium, xylem elements and pith of shoot

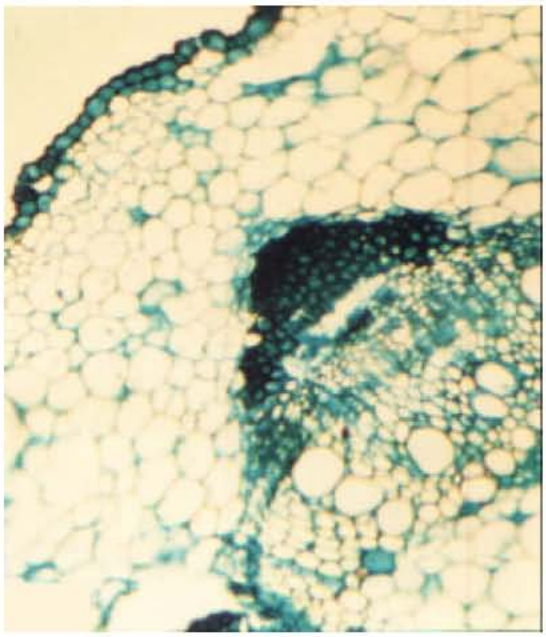

Fig. 6: 200 ppm GA_3 treatment showing epidermis, cortical region and xylem elements

inhibition was recorded with 200 ppm GA_3+50 ppm $Pb(NO_3)_2$. In the study of shoot extraneous 200 ppm IAA showed increase in the number of cortical layers, however, layers remained constant with the rest of the doses. Expansion in the cortical region as well as cortical cells was recorded with the doses of IAA. In applied

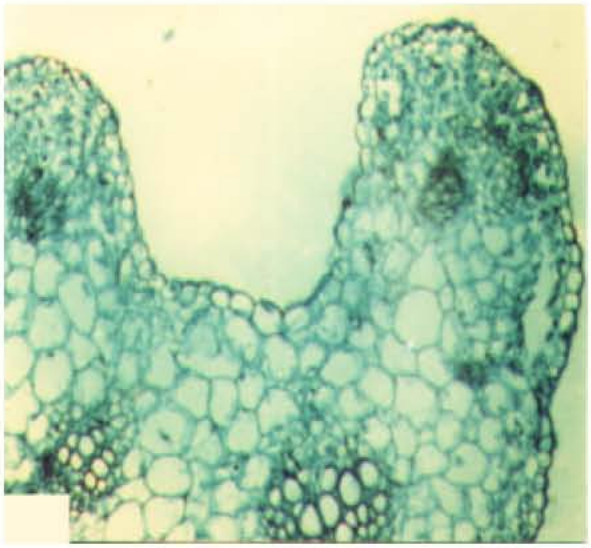

Fig. 7: Rachis showing increased number of vascular bundles with 200 ppm IAA

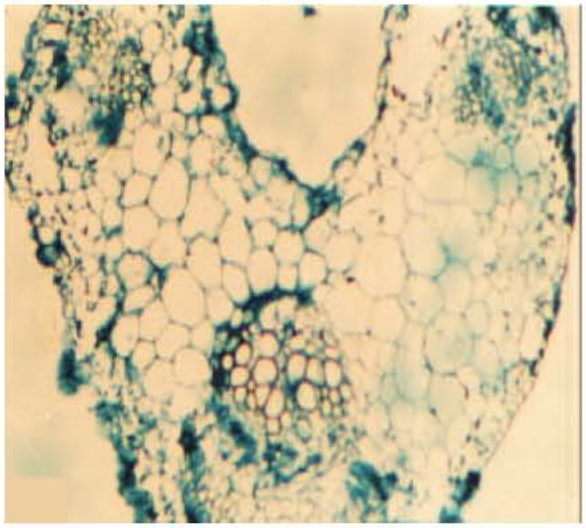

Fig. 8: Effect of 50 ppm $Pb(NO_3)_2$ on the width of rachis and number of vascular bundles

200 ppm GA_3 alone, however, inhibition was registered in the width of this region as well as in cortical cells (Table 5 and Fig. 6). With applied 50 ppm $Pb(NO_3)_2$ some expansion was observed. Contrarily, 200 ppm GA_3+50 ppm $Pb(NO_3)_2$ showed inhibition in these parameters.

Vascular cylinder showed maximum expansion with applied 200 ppm IAA and some expansion in the mixture of 200 ppm IAA with other doses showing the dominating effects of hormones whereas in the combination of 200 ppm GA_3 and 200 ppm GA_3+50 ppm $Pb(NO_3)_2$ some inhibition in comparison to control was registered (Table 5). Treatment with 200 ppm IAA, showed early

initiation of vascular cambium and the number of cambial layers increased (Fig. 5). On the other hand, number remained constant with applied 200 ppm GA_3 (Fig. 6) and 50 ppm $Pb(NO_3)_2$. Application of 200 ppm IAA+200 ppm GA_3, 200 ppm IAA+200 ppm GA_3+50 ppm $Pb(NO_3)_2$ showed increase. With applied IAA+$Pb(NO_3)_2$ number remained same, however, it decreased with 200 ppm GA_3+50 ppm $Pb(NO_3)_2$. Early enlargement of cambial cells was recorded with the doses of 200 ppm IAA (Fig. 5), however, this effect was partially nullified with the presence of 200 ppm GA_3 in the mixture of doses. In the application of 200 ppm GA_3 some inhibition was registered (Fig. 6), whereas no significant effect was registered with 50 ppm $Pb(NO_3)_2$. In the mixture of doses 200 ppm GA_3+50 ppm $Pb(NO_3)_2$ showed inhibition (Table 5).

Treatment with 200 ppm IAA+200 ppm GA_3 showed increase in the number of xylem elements in a strand, which may be attributed to early differentiation of xylem, while in other cases number remained constant (Table 5). Width of metaxylem elements of shoot showed expansion with IAA, inhibition with GA_3 and no significant effect with $Pb(NO_3)_2$. In mixed doses expansion was observed with doses of IAA and inhibition with GA_3+$Pb(NO_3)_2$. The pith region and pith cells showed expansion with applied IAA and inhibition with GA_3 and GA_3+$Pb(NO_3)_2$. In the rachis increase in the width was observed with applied 200 ppm IAA, which being 16.10% whereas inhibition was recorded with 200 ppm GA_3 and 50 ppm $Pb(NO_3)_2$ (Fig. 8) and 200 ppm GA_3+50 ppm $Pb(NO_3)_2$ (Table 6). The rest of mixed doses showed expansion in comparison to control, however expansion was less with 200 ppm IAA alone. Likewise, expansion in the width of metaxylem element of main vascular bundle was registered with 200 ppm IAA and 200 ppm IAA+200 ppm GA_3 and combination of three doses. More or less similar results were registered with the other doses. Another important observation was the increase in the number of vascular bundles with applied 200 ppm IAA (Table 6 and Fig. 7) and 200 ppm IAA+200 ppm GA_3. On the other hand, 50 ppm $Pb(NO_3)_2$ (Fig. 8) and 200 ppm GA_3+50 ppm $Pb(NO_3)_2$ showed decrease in number. In applied 200 ppm GA_3, 200 ppm IAA+50 ppm $Pb(NO_3)_2$ and 200 ppm IAA+200 ppm GA_3+50 ppm $Pb(NO_3)_2$ number of vascular bundles remained constant in comparison to control (Table 6).

DISCUSSION

External morphology: Effect of IAA as length inhibitor has been reported by Gerrit *et al.*[16]. However, inhibition in length was accompanied by expansion in diameter, which may be due to expansion of cells as reported by Chaudhry[10]. Marked increase in the length was observed with GA_3 in the above mentioned parameters (Fig. 1) which was followed by inhibition in diameter[9]. Decrease in diameter may be attributed to the decrease in the concentration of available sugars[17]. The dose of $Pb(NO_3)_2$ showed reduction in length which shows, the inhibitory effects of $Pb(NO_3)_2$ (Table 1). Another important observation was expansion in diameter. According to Sobotik *et al.*[18] roots of barley showed no significant response with $Pb(NO_3)_2$ but in the present work inhibition in length and increase in diameter revealed some response. In the combination of hormones i.e., IAA+GA_3 increase in length was observed in comparison to control (Fig. 2). However, increase in length was less than GA_3 alone. This may be due to the antagonistic effects of IAA so far as length is concerned. Expansion in diameter was recorded showing the effect of IAA, which was also less than IAA alone thus showing the combined effect of both hormones. More or less similar reports have been given by Chaudhry and Khan[19]. Reduction in length was registered thus revealing the inhibitory effects of IAA and $Pb(NO_3)_2$ as far as length is concerned. Chaudhry and Qurat-ul-Ain[20] showed similar results. The doses of GA_3+$Pb(NO_3)_2$ and IAA+GA_3+$Pb(NO_3)_2$ showed some increase in length, which may be due to length promoting effect of GA_3[21]. However, increase in length was less than with GA_3 alone. Fresh and dry weight of both root and shoot showed increase with the doses of IAA. According to Kumar *et al.*[21] IAA increased the dry weight of plant. GA_3 applications showed decrease, which may be due to inhibition in diameter. Likewise, $Pb(NO_3)_2$ and GA_3+$Pb(NO_3)_2$ showed decrease. This may be attributed to inhibitory effect of $Pb(NO_3)_2$, as mentioned earlier. Number of rootlets increased with extraneous IAA. However, Tory *et al.*[22] have reported that auxins have no effect on lateral root formation. Contrarily with applied GA_3 number remained constant. However, decrease in number was registered with $Pb(NO_3)_2$ revealing $Pb(NO_3)_2$ as growth inhibitor[23]. Equally mixed doses of IAA+GA_3 showed increased number of rootlets. This revealed the dominating effect of IAA over GA_3. According to Naqvi and Sexton[24] GA_3 and IAA enhance auxin transport. With IAA+$Pb(NO_3)_2$ and in the combination of all three doses increase was recorded. On the other hand, mixed GA_3+$Pb(NO_3)_2$ showed decrease. In shoot applied IAA and IAA+GA_3 showed increased number of branches (Table 2 and Fig. 2). This shows the accelerated bud development[25]. Number remained constant with remaining doses. Applied IAA alone and its mixture with GA_3 showed increase in the number of compound leaves, which may be due to increased number of branches (Table 3). However, in the applied GA_3 no significant effect was observed. In the $Pb(NO_3)_2$ and IAA+$Pb(NO_3)_2$

decrease was recorded, which may be due to inhibitory effect of $Pb(NO_3)_2$ on growth parameter[26]. Number of leaflets in first three compound leaves remained constant with all doses except with $Pb(NO_3)_2$ and IAA+$Pb(NO_3)_2$ in which decrease was observed. Applied IAA showed increase in leaflet area (Table 3). Jaiwal and Bhambie[6] working on *Cicer arietinum* L., observed similar results. Leaflet area remained same with GA_3. These results are contrary to the reports given by Chaudhry[10]. According to this worker applied GA_3 showed inhibition in leaf area. With the application of $Pb(NO_3)_2$ decrease was registered. In the mixed doses there was no significant effect with all doses except with GA_3+$Pb(NO_3)_2$ that showed decrease.

Internal morphology: Root of *Cicer arietinum* L., is tetrarch. The tetrarch condition of the root remained totally undisturbed in treated plants in comparison to control (Fig. 3). Epidermal cells of the root, shoot and rachis showed no significant effects with all treatments. According to Wardlaw[27] the epidermal cells are the most unresponsive. The number of cortical layers in root increased in comparison to control with applied IAA, which may be due to enhanced cell division as reported by Cleland[28]. However, the number remained constant with applied GA_3. According to Ozeki and Komamine[29] there is no promotion of cell division with applied GA_3. Likewise, no significant effect was recorded with $Pb(NO_3)_2$. $Pb(NO_3)_2$. Increase in number was observed with the doses of IAA thus showing the dominating effect of IAA except with 200 ppm IAA+50 ppm $Pb(NO_3)_2$ in which number remained constant. This may be due to the inhibitory effects of $Pb(NO_3)_2$. Mixed GA_3+$Pb(NO_3)_2$ also showed no effect on cortical layers. Bairathi and Nathawart[30] have reported more or less similar effects. Expansion in cortical region and cortical cells was observed with the application of IAA alone and its combination with GA_3 and $Pb(NO_3)_2$ and mixture of all three doses (Table 4). This revealed the dominating effects of IAA over GA_3 and heavy metal. According to Keller[31] auxin induced growth is apparently mediated by cell wall loosening or extensibility. Application of GA_3 showed inhibition was recorded in both parameters, which may be attributed to decrease in diameter as observed in external morphology (Table 4). Some expansion was observed with $Pb(NO_3)_2$ treatment. This may be due to some physiological activities[13]. Maximum expansion in the width of xylem strand was recorded with the application of IAA, it is well known for promoting expansion in vascular tissues and in loosening the cell walls[32]. However, inhibition has been observed with GA_3. Similar reports have been given by Chaudhry and Zahur[33]. No significant effect was registered with $Pb(NO_3)_2$. According to Acativinei[34] in the root of *Secale cereale* L., cellular divisions were hindered by the application of $Pb(NO_3)_2$. Applied mixed doses like IAA+GA_3, IAA+$Pb(NO_3)_2$ and IAA+GA_3+$Pb(NO_3)_2$ showed expansion, which may be attributed to the presence of IAA as mentioned elsewhere. However, the expansion was less than with IAA alone, which shows the antagonistic effects of GA_3 and $Pb(NO_3)_2$. In the study of metaxylem elements width was found to be expanded with IAA (Fig. 4). These results are in harmony with the reports given by Eckardt[35]. An insignificant inhibition with applied GA_3 was observed. A significant effect was observed with applied $Pb(NO_3)_2$. Width of metaxylem elements was found to be decreased with GA_3+$Pb(NO_3)_2$. Increase in the number of metaxylem elements in a strand was revealed with IAA. According to Fukuda[36] applied IAA causes early vascular differentiation. The number remained constant with remaining doses except with IAA+GA_3 and IAA+GA_3+$Pb(NO_3)_2$ in which increase was registered (Table 4).

In the pith region of root maximum expansion was observed with IAA alone (Fig. 4). These results are in agreement with the reports given by Chaudhry and Rashid[37]. On the other hand, inhibition was recorded with GA_3, which may be due to inhibition in cell division[38]. An important observation was expansion in the width of pith region and pith cells with $Pb(NO_3)_2$. In the IAA+GA_3, IAA+$Pb(NO_3)_2$ and IAA+GA_3+$Pb(NO_3)_2$ expansion was observed in the pith of root. In fact the inhibitory effects of heavy metal and GA_3 was nullified by the presence of IAA (Table 4).

In the shoot cortical layers increased with IAA. It may be due to accelerated cell divisions[35]. However, the number of cortical layers remained constant with the rest of the doses. Width of cortical region and cortical cells showed more or less similar results with all treatments as in the case of root (Table 4, 5 and Fig. 6). Vascular cylinder showed expansion in the width with applied IAA (Table 4). This increase in width may be due to differentiation of vascular tissues[7]. Treatment with GA_3 showed inhibition. GA_3 is well known for cell inhibition as reported by Chaudhry and Khan[19]. Combination of IAA+GA_3 showed expansion, which may be due to the positive effects of IAA in the vascular region[39]. In the rest of doses of IAA expansion was observed, however, GA_3+Pb $(NO_3)_2$ showed inhibition. The vascular cambium showed increase in the number of cambial layers (Table 5 and Fig. 5) with applied IAA, which causes secondary xylem development by radial concentration gradient[40]. No significant effect was observed with GA_3 and $Pb(NO_3)_2$ (Fig. 6). In the mixture of doses i.e., IAA+GA_3 and IAA+GA_3+$Pb(NO_3)_2$ increase in the number

of cambial layers was registered, thus showing the dominant effect of IAA. Uggla[41] has reported increase in the number of secondary xylem development with applied IAA. IAA+$Pb(NO_3)_2$ showed no effect thus showing the antagonistic effect of heavy metal. However, in the combination of GA_3+$Pb(NO_3)_2$ decrease in number was registered, which shows the inhibitory effect so far as cell division is concerned[10]. Vascular cambium showed maximum expansion with applied IAA. Moreover, IAA produces softening of cell wall and due to the activity of IAA one or more new enzymes act on the cell wall to increase its plasticity[42]. Inhibition in cambial region was observed with GA_3. These results are further confirmed by the reports given by Moris and Aurthur[43]. Mixed IAA with GA_3 showed expansion. Mixed IAA+$Pb(NO_3)_2$ and IAA+GA_3+$Pb(NO_3)_2$ expansion was revealed, which was less than with IAA alone and IAA+GA_3. This may be due to $Pb(NO_3)_2$ because movement of hormones may be hindered by the presence of heavy metal[27] and expansion may be less. Number of xylem elements in a strand were found to be constant with all doses except with applied IAA+GA_3 in which increase was recorded. This may be due to IAA as mentioned earlier. Width of metaxylem elements was found to be expanded with IAA (Fig. 5). These results are further confirmed by the reports given by Chaudhry and Rasheed[32]. Inhibition was revealed with GA_3. According to Inada and Shimmen[44] GA_3 caused decrease in the width of cells. No significant effect was registered with $Pb(NO_3)_2$. The pith of shoot responded almost in the same manner as was observed in the pith of root (Table 4 and 5). Width of rachis showed maximum expansion with IAA (Table 6 and Fig. 7). IAA is well known for cell expansion. Similar reports have been given by Chaudhry and Rasheed[32]. However, treatment with GA_3 showed inhibition as reported earlier. Marked inhibition was recorded with $Pb(NO_3)_2$ (Fig. 8). According to Ning *et al.*[45] heavy metals block essential enzyme components. Mixed doses like IAA+GA_3, IAA+$Pb(NO_3)_2$ and mixture of all three doses showed expansion, however, GA_3+$Pb(NO_3)_2$ showed inhibition in the width of rachis. In the study of ground tissue expansion was registered with IAA and its doses and inhibition with GA_3 and $Pb(NO_3)_2$. Wider metaxylem elements were recorded with IAA. Similar reports have been given by Tuominen *et al.*[40], Uggla *et al.*[41] and Chaudhry and Qurat-ul-Ain[20]. Applied GA_3 and $Pb(NO_3)_2$ showed inhibition. In the mixture of doses some expansion was revealed except with the mixed dose of GA_3+$Pb(NO_3)_2$ in which maximum inhibition was registered. According to Takano *et al.*[46] mixture of GA_3+$Pb(NO_3)_2$ causes inhibition. Application of IAA and IAA+GA_3 showed increase in the number of vascular bundles (Table 6 and Fig. 7). According to Carlos *et al.*[7] IAA increases the development of vascular tissues. Decrease in the number of vascular bundle was observed with $Pb(NO_3)_2$ and GA_3+$Pb(NO_3)_2$ (Table 6 and Fig. 8), thus showing $Pb(NO_3)_2$ as growth inhibitor as far as vascular tissue is concerned[20]. In the application of GA_3, IAA+$Pb(NO_3)_2$ and IAA+GA_3+$Pb(NO_3)_2$ the number of vascular bundle remained constant (Table 6).

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