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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 3 (8): 1263-1266, 2000

Effect of Growth Hormones i.e., GA_3, IAA and Kinetin on 1. Length and Diameter of Shoot, 2. Early Initiation of Cambium and Maturation of Metaxylem Elements in *Cicer arietinum* L.

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Abstract: Effects of growth regulators i.e., GA_3, IAA and Kinetin were studied on the shoot of *Cicer arietinum* L. after 45 and 60 days of treatment and was compared with control as well as among themselves. The following concentrations of growth hormones were applied individually 100 ppm GA_3, 100 ppm IAA and 20 ppm Kinetin. In combinations the concentrations used were 100 ppm GA_3 + 100 ppm IAA, 100 ppm GA_3 + 20 ppm Kinetin, 100 ppm IAA + 20 ppm Kinetin and 100 ppm GA_3 + 100 ppm IAA + 20 ppm Kinetin. Applied GA_3 increased the length of shoot significantly but in the internal morphology GA_3 revealed no positive effect in cambium enhancement and maturation of metaxylem elements. Applied IAA and Kinetin more or less promoted expansion in diameter but decreased the extension growth. IAA and Kinetin promoted initiation of cambium and maturity of metaxylem elements. In the mixed doses of GA_3 + IAA and GA_3 + Kinetin, increase in length as well as in the diameter was observed. The diameter of cambial region also revealed some expansion. In GA_3 + IAA + Kinetin no definite pattern was observed with regard to length, diameter and cambium.

Key words: Cambium, metaxylem elements, growth hormones, gibberellic acid (GA_3), indole-3-acetic acid, kinetin

Introduction

The prospect of employment of gibberellins as general stimulants of plant growth has been explored, mostly the increased height of shoot has been observed. However, auxins have not stimulated the increase in length. GA_3 causes remarkable elongation has been reported by a number of workers (Brian and Hemming, 1958; Phinney, 1956; Singh *et al*., 1979; Almeida and Pereira, 1997). Inhibition in length with IAA has been reported by Bairathi and Nathawart (1980) and Pilet and Saugy (1985).

Gibberellins either promote or inhibit cambial growth in plants. Stimulation of cambial growth by gibberellins has been reported in shoots of Pinus sylvestris by Wang *et al.* (1997). More recently Awan *et al*. (1999) have reported that GA_3 promotes cambial growth. Contrarily, inhibition of cambial region by applying GA_3 has been reported by Kaufman (1965) and Morris and Arthur (1985). Similarly, Ozeki and Komamine (1986) reported no effect of GA_3 on cell division in carrot suspension cultures. Acceleration of cell division in cambial region with auxins (IAA) has been reported by a number of workers. Tuominen *et al*. (1997) reported that a radial concentration gradient of IAA is related to secondary xylem development in Hybrid Aspen. IAA also promotes the diameter of metaxylem elements (Wareing and Roberts, 1956). The increase in metaxylem elements by applying IAA can be attributed to increased cell division in cambial region (Miller *et al*., 1955). More recently, Uggla *et al*. (1998) also reported that IAA controls cambial growth and acts as positive morphogen in cambial development. Cytokinins are also known to promote cell division in cambial region (Makarova *et al*., 1988).

Materials and Methods

The motif of the present work is to study the effect of growth hormones i.e., GA_3, IAA and Kinetin on the external and internal morphology of shoot of *C. arietinum*. Individually the following composition of growth hormones was used 100 ppm GA_3, 100 ppm IAA and 20 ppm Kinetin. In combination the concentrations used were 100 ppm GA_3 + 100 ppm IAA, 100 ppm GA_3 + 20 ppm Kinetin, 100 ppm IAA + 20 ppm Kinetin and 100 ppm GA_3 + 100 ppm IAA + 20 ppm Kinetin.

The effect of growth hormones i.e., GA_3, IAA and Kinetin after 45 and 60 days was observed in the month of October, 1998 in the glasshouse of Botany Department. In external morphology, the following parameters were studied (i) length and diameter of shoot, (ii) number of internodes and leaflets per shoot. In order to study the effect of growth hormones on the internal morphology, 1 cm long portions of first internodes of control as well as all the treatments were fixed in Corney's Modified Fluid. After the removal of air, the fixed material was first dehydrated in an ascending series of water ethyl alcohol mixture, cleared in tertiary butyl alcohol, infilterated and embedded in paraffin wax. The embedded material was processed with a rotary microtome in the usual manner and transverse sections were cut (10-15 μm). These sections were stained with safranine and fast green, washed in clove oil to remove excess stain and mounted in Canada balsam. In the internal morphology, the cells of cortical, xylem and pith regions were studied. Data of treated plants were recorded and compared with control as well as among themselves. Emphasis was mainly on the promotion or inhibition of cell division, following the treatments. All observations were subjected to statistical analysis (Steel and Torrie, 1981).

Results

In the present work, the shoot of *C. arietinum* showed a significant increase in length with applied GA_3 i.e., the 100 ppm dose showed 66.11% increase after 45 days and 88.94% after 60 days. On the other hand, it registered inhibition in diameter of shoot. Contrary to GA_3, applied IAA registered inhibitory effect on length e.g. the dose of 100 ppm showed 16.66% decrease after 45 days and

9.54% after 60 days. However, it showed a well-marked increase in diameter which being 30% after 45 days and 22.72% after 60 days when compared with control. The 20 ppm kinetin dose registered an increase in length i.e., 19.44 and 18.09% after 45 and 60 days respectively. The increase in diameter being 20% after 45 days and 13.63% after 60 days in comparison to control (Table 1, Fig. 1).

The mixed dose of 100 ppm GA_3 + 100 ppm IAA showed 42.77% increase in length after 45 days and 58.29% after 60 days, this increase in length was accompanied by increase in diameter of shoot as well. The most interesting results were observed with a mixed dose of 100 ppm GA_3 + 20 ppm Kinetin, thus increasing the length upto 80.50% after 45 days and 114.82% after 60 days over the control (Table 1). The dose of 100 ppm IAA + 20 ppm kinetin showed 29.16% inhibition in length after 45 days and 23.36% after 60 days in comparison to control. The diameter increased significantly from control as well as from all other treatments i.e., 35% after 45 days and 31.81% after 60 days. The combined dose of 100 ppm GA_3 + 100 ppm IAA + 20 ppm kinetin revealed increase in length as well expansion in diameter (Table 1, Fig. 1).

In the internal morphology applied GA_3 registered inhibition in cambial region after 45 as well as 60 days when compared with control. The number of metaxylem elements also decreased with applied GA_3 i.e., 78 after 45 days and 98 after 60 days in comparison to control. Similarly single cambial strand also showed inhibition with applied GA_3. Contrary to GA_3 applied IAA showed 7.1% increase in cambial region after 45 days and 14.28% after 60 days. The number of metaxylem elements also increased i.e., 95 after 45 days and 120 after 60 days. The 20 ppm kinetin also registered increase when compared with control. The number of metaxylem elements were 98 after 45 days and 124 after 60 days (Table 1, Fig. 1).

The equally mixed dose of GA_3 + IAA showed 4.83% increase in cambial region after 45 days and 11.42% after 60 days, thus showing the dominant effect of IAA. The number of metaxylem elements increased upto 95 after 45 days and 108 after 60 days. The mixed dose of 100 ppm GA_3 + 20 ppm Kinetin showed 9.87% increase in cambium after 45 days and 12.5% after 60 days. The number of metaxylem elements observed after 45 days were 88 and after 60 days they were 119 in comparison to control. The mixed dose of 100 ppm IAA + 20 ppm kinetin showed a significant increase in cambium i.e., 13.54% after 45 days and 18.57% after 60 days when compared with control. The metaxylem elements showed maximum response i.e., 105 after 45 days and 125 after 60 days when compared with control. The combined dose of all three hormones showed no significant effect after 45 days, however, after 60 days it increased to 12.85%. The metaxylem elements also showed increase but this increase was not well marked when compared with control, this may be attributed to mixed GA_3 dose (Table1, Fig. 1).

Discussion

In the external morphology, application of GA_3 showed significant increase in the length of shoot after 45 and 60 days. GA_3 causes remarkable elongation has been reported by a number of workers (Brian and Hemming, 1958; Phinney, 1956; Lang, 1970; Singh *et al.*, 1979; Almeida and Pereira, 1997). The obvious effect being increase in the number of internodes (Thompson and Guttridge, 1959; Basford, 1961; Adams and Ross, 1983; Hernandez, 1997). This may be attributed to the intact cell elongation (Awan *et al.*, 1999). The marvellous increase in length on one hand was accompanied by inhibition in the diameter of shoot on the other hand. This may be attributed to the extra amount of sugars consumed during the rapid elongation of internodes, thus affecting the girth (Allsopp, 1959). The mixed doses of GA_3 + IAA and GA_3 + Kinetin showed significant increase in length after 45 and 60 days (Table 1). Thus revealing the dominant effect of GA_3 where length is concerned (Frankland and Wareing, 1960; Brian *et al.*, 1964). The diameter of shoot also increased significantly with these mixed doses (Table 1), which may be attributed to the activity of IAA and Kinetin (Eisinger, 1983). Applied IAA caused inhibition in length after 45 and 60 days (Table 1, Fig. 2). Similar results have been reported by Bairathi and Nathawart (1980) and Pilet and Saugy (1985). However, the diameter of shoot increased significantly with IAA (Table 1). The mixed dose of IAA + Kinetin registered inhibition in length accompanied by a well-marked increase in diameter. Applied kinetin showed significant increase in length and diameter after 45 and 60 days (Table 1, Fig. 3, 4). Similar results have been reported by Makarova *et al.* (1988).

In the internal morphology, applied GA_3 registered inhibition in cambial region after 45 and 60 days (Table 1, Fig. 5). Similar reports have been given by Morris and Arthur (1985) and Ozeki and Komamine (1986). Wang *et al.* (1997) and Awan *et al.* (1999) reported that GA_3 controls cambial growth but in present work, this hormone failed to reveal the above mentioned effect. Moreover, the number of metaxylem elements showed more or less no effect, with GA_3. Likewise no effect on cell division (Jones and Moll, 1983). However, the mixed doses of GA_3 + IAA and GA_3 + Kinetin showed a significant increase in cambial region after 45 and 60 days (Table 1) accompanied by increase in the number of metaxylem elements as well. These observations are in harmony with the work of Kabar (1997) and Uggla *et al.* (1998). Application of extraneous IAA causes remarkable increase in cambial region (Wang *et al.*, 1997; Uggla *et al.*, 1998). Tuominen *et al.* (1997) reported that a radial concentration gradient of IAA is related to secondary xylem development in Hybrid Aspen, these reports are in harmony with the present work (Fig. 6). The number of metaxylem elements also increased with IAA, thus showing increased cell division with this hormone (Wareing and Roberts, 1956). One significant observation was the combined effect of IAA and Kinetin. Although these two hormones individually showed increase in cambial region as well as in metaxylem elements but the combined effect was remarkable thus enhancing cambial activity and maturation of metaxylem elements above all the other hormones (Table 1, Fig. 1). The stimulation of cambial activity observed in the present work with these hormones is further confirmed by Miller *et al.* (1955), Phillips (1971) and Tuominen *et al.* (1997). Applied Kinetin registered a significant increase in cambial region after 45 and 60 days. However, this increase in diameter was less than with IAA + Kinetin thus showing that IAA and Kinetin in combinations enhance cell division (Fig. 7, 8). The number of metaxylem elements also registered increase with the above mentioned hormone, thus showing promotion of cell division (Miller *et al.*, 1955; Phillips, 1971). When all the three hormones were applied simultaneously, the cambial region as well as number of metaxylem elements showed

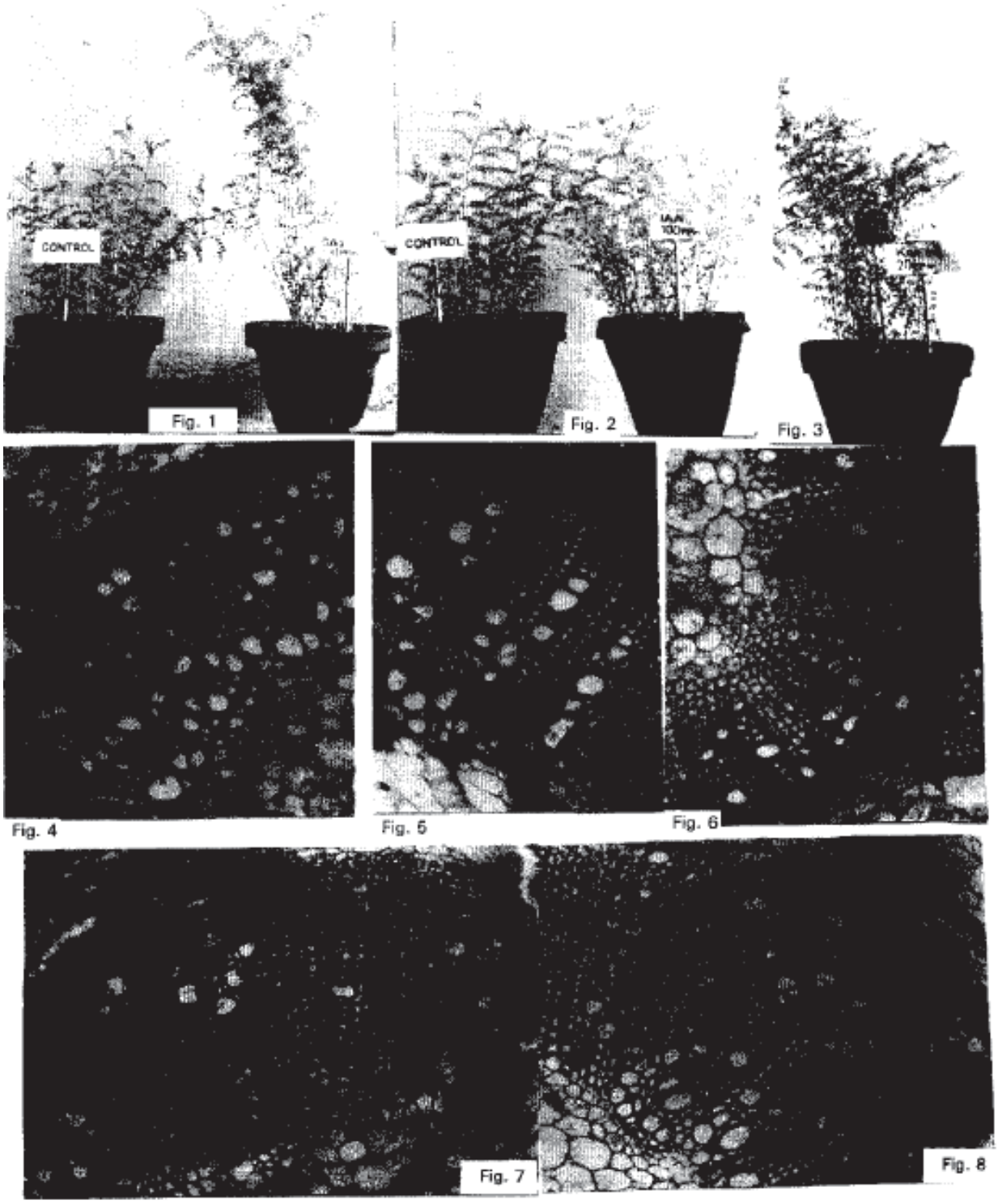

Fig. 1: Effect of 100 ppm GA3 on the shoot of *Cicer arietinum*
Fig. 2: Effect of 100 ppm IAA on the shoot
Fig. 3: Effect of 20 ppm Kinetin on the shoot
Fig. 4: Shoot of *C. arietinum* in transection (X10)
Fig. 5: Efect of 100 ppm GA3 in transection (x10) showing cambial region
Fig. 6: Effect of 100 ppm IAA in transection (X10) showing cambial region
Fig. 7: Effect of 20 ppm Kinetin in transection (X10) showing cambial region
Fig. 8: Effect of 100 ppm IAA + 20 ppm Kinetin in transection (X10) showing cambial region

Table 1: Effect of growth hormones on the external and internal morphology of stem after 45 and 60 days

Treatment (ppm)	45 days				60 Days			
	Length of shoot (cm)	Diameter of first internode (cm)	Diameter of xylem strand (µm)	Numer of metaxylem elements	Length of shoot (cm)	Diameter of first internode (µm)	Diameter of xylem strand	Numer of metaxylem elements
Control	36.0±0.57	0.20±0.013	310.0±0.35	105.0±0.28	39.8±0.32	0.22±0.008	350.0±0.12	105.0±0.28
100_{GA3}	59.8±0.79	0.19±0.0	300.0±0.03	98.0±0.31	75.2±0.88	0.21±0.05	335.0±0.18	98.0±0.31
100 IAA	30.02±0.88	0.26±0.005	332.0±0.75	120.0±0.61	36.0±0.57	0.27±0.05	400.0±0.23	120.0±0.61
20 Kinetin	43.0±0.57	0.24±0.0	350.0±0.28	124.0±0.73	47.0±0.52	0.25±0.03	410.0±0.74	124.0±0.73
100_{GA3}+100 IAA	51.4±0.328	0.22±0.006	325.0±0.24	108.0±0.43	63.0±0.57	0.23±0.138	390.0±0.75	108.0±0.43
100_{GA3}+20 Kinetin	65.0±0.835	0.21±0.003	340.0±0.30	119.0±0.22	85.5±0.61	0.23±0.012	395.0±0.39	119.0±0.22
100_{GA3}=20 Kinetin	25.5±0.66	0.27±0.02	352.0±0.28	125.0±0.28	30.5±0.529	0.29±0.011	415.0±0.39	125.0±1.22
100_{GA3}+100 IAA +20 Kinetin	45.5±0.08	0.24±0.003	315.0±0.19	112.0±0.21	49.3±0.502	0.25±0.015	395.0±0.97	112.0±0.21
L.S.D.	13.122	0.026	17.59	7.809	18.22	0.024	26.509	9.022

increase (Table 1). From all the above observations, it can be concluded that IAA, Kinetin and their combinations have a positive effect on cell division when compared with control as well as GA_3.

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