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Rootlets, Xylary Region and Abnormal Initiation of Cambium in the Root of *Cicer arietinum* L. Following Treatments with GA₃, IAA and Kinetin

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Abstract: Effect of growth hormones i.e., gibberellic acid (GA₃), indole-3-acetic acid (IAA) and Kinetin was studied on the root of *Cicer arietinum* L. after forty-five and sixty days of treatments and compared with control. The application of GA₃ alone and in combination with IAA or Kinetin or both caused extension growth and insignificant increase in the number of rootlets accompanied with decrease in the diameter of root. Applied IAA caused expansion in radial diameters and decreased the length of root. The application of IAA also registered an increase in number of rootlets. With 20 ppm Kinetin, a negligible increase in the length of root as well as in the number of rootlets was observed. With the application of IAA + Kinetin, a remarkable increase in diameter and number of rootlets was revealed. In the internal structure, the application of GA₃ alone had no effect on the secondary growth but in combination with IAA and Kinetin individually as well as in combinations, secondary growth was initiated earlier. When IAA and Kinetin were applied alone and even in the combination of the two, enhancement of the cambium was registered. One significant observation was the abnormal initiation of cambium around the pith region in the dose of 20 ppm Kinetin and in the mixed dose of IAA + Kinetin.

Key words: Cambium, Growth hormones, Gibberellic acid (GA₃), Indole-3-acetic acid, Kinetin

Introduction

In vascular plants natural as well as synthetic hormones bring about a number of morphological and physiological changes (Krishnamoorthy, 1981). Gibberellins have the ability to stimulate elongation of root and shoot accompanied by decrease in the diameter. Pain and Basu (1985) observed a general increase in length with GA₃. Torrey (1976) came out with the result that GA₃ appears to play no role in lateral root initiation. Wang *et al.* (1997) reported that GA₃ in combination with IAA controls the longitudinal and cambial growth in roots and shoots of *Pinus sylvestris*. Similarly, Awan *et al.* (1999) working on rice seedlings have reported that GA₃ controls cambial growth. Auxins have the ability to stimulate elongation and expansion of roots and shoots (Leopold and Kriedemann, 1975). Goodwin and Morris (1979) said that auxins increased the number of lateral roots while low levels of GA₃ resulted in decreased lateral root production. Jernstedt (1984) reported that IAA caused root contraction and differential cell expansion in hyacinth roots. Ghazarian *et al.* (1984) reported the inhibition activity in the roots of sunflower under the influence of IAA. Tuominen *et al.* (1997) observed the secondary xylem development in *Asper* by a radial concentration gradient of indole-3-acetic acid. Similarly, Ugglá *et al.* (1998) reported that IAA is related to secondary xylem development. Kinetin causes expansion of cells as observed by Kantharaj and Padmanabhan (1991). Kaul and Farooq (1994) indicated that Kinetin inhibited length was accompanied by fresh weight gain in hypocotyl segments. Soh *et al.* (1998) also suggested that cytokinins have stimulating effects on root formation from callus cultures of *Vigna unguiculata* that previously did not show rhizogenic activity on medium with auxin alone. Eames (1972) has reported that cambial development in roots lags behind that of stem after extension growth ceases. Ball (1944) reported that anomalous type of secondary growth can be observed with the application of growth hormones like auxins.

Materials and Methods

The effect of growth hormones i.e., GA₃, IAA and Kinetin on the root of *Cicer arietinum* Linn. after 45 and 60 days have been observed in the month of October. The following doses of growth hormones were used i.e., 100 ppm GA₃, 100 ppm IAA, 20 ppm kinetin, 100 ppm GA₃ + 100 ppm IAA, 100 ppm GA₃ + 20 ppm Kinetin, 100 ppm IAA + 20 ppm Kinetin and 100 ppm GA₃ + 100 ppm IAA + 20 ppm Kinetin.

Plants were grown in earthenware pots and 27 µl of each hormonal treatment was applied on apical meristems after every 24 hours till forty-five days for the first set and for 2nd set treatment was continued till sixty days. One set was taken as control. The rootlets were counted after forty-five and sixty days. In the internal morphology, 1 cm long pieces of roots were fixed in Corney's modified fluid. The air was removed. The material was dehydrated and cleared in tertiary butyl alcohol grades, infiltrated and embedded in paraffin wax. The embedded material was processed in transverse planes with rotary microtome (10-15 µm). The material was passed through the descending series of xylene. The material was stained with safranin and fast green and mounted in Canada balsam. The prepared slides were observed under microscope to see the internal structure of roots in all treatments and compared with control. All data was subjected to statistical analysis (Steel and Torrie, 1981).

Results

Number of Rootlets: In the control plants, 48 rootlets were observed after forty-five days and 52.1 rootlets after sixty days. The number decreased with applied 100 ppm GA₃ i.e., 42 and 46 after forty-five and sixty days, respectively (Fig. 1). Extraneous IAA showed increase in the number of rootlets (Table 1, Fig. 1). Likewise, applied 20 ppm Kinetin increased the number but proved to be least effective when compared with IAA (Fig. 1). The mixed dose of 100 ppm IAA + 20 ppm Kinetin showed significant increase in the number of rootlets which being 59.1 and 64.2 after

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Table 1: Effect of growth hormon on the external and internal morphology of root of *Cicer arietinum* L.

Treatments	45 days					60 days				
	No. of rootlets	Diam. of xylem Strands (µm)	Diam. of metaxylem elements (µm)	Diam. of pith region	Diam. of pith cells (µm)	No. of rootlets	Diam. of xylem Strands (µm)	Diam. of metaxylem elements (µm)	Diam. of pith region	Diam. of pith cells (µm)
Control	48.0 ±	215.0 ±	42.5 ±	110.0 ±	25.5 ±	52.1 ±	23.0 ±	47.5 ±	145.0 ±	30.0 ±
GA ₃ (100 ppm)	00.017	000.0016	00.504	001.18	00.271	00.40	02.081	00.362	000.51	00.031
IAA (100 PPM)	42.0 ±	190.51 ±	34.0 ±	090.0 ±	23.3 ±	46.0 ±	210.0 ±	40.0 ±	125.0 ±	27.6 ±
Kinetin (20ppm)	00.04	000.152	00.939	000.134	00.118	00.061	001.034	00.263	000.47	00.05
GA ₃ + IAA (100 + 100 ppm)	57.0 ±	250.0 ±	46.66 ±	165.5 ±	36.0 ±	62.0 ±	256.6 ±	50.0 ±	200.4 ±	41.2 ±
	00.83	000.097	00.944	000.42	00.35	00.039	001.09	00.311	000.81	00.33
	52.0 ±	232.5 ±	45.0 ±	150.3 ±	31.6 ±	56.1 ±	246.0 ±	48.5 ±	190.0 ±	36.6 ±
	00.019	001.247	00.590	001.32	00.47	00.014	000.201	01.201	000.64	00.08
	51.0 ±	228.2 ±	41.0 ±	135.8 ±	30.6 ±	54.7 ±	238.7 ±	43.8 ±	170.3 ±	34.5 ±
	00.23	000.741	01.031	001.89	00.361	00.019	000.467	00.837	000.57	00.99
	49.9 ±	226.8 ±	40.0 ±	142.5 ±	27.5 ±	53.4 ±	233.3 ±	42.9 ±	156.0 ±	31.66 ±
	00.51	000.538	00.38	000.948	00.075	00.713	000.799	00.03	000.03	00.640
	59.1 ±	283.5 ±	49.0 ±	185.7 ±	40.3 ±	64.2 ±	290.0 ±	55.6 ±	210.4 ±	43.3 ±
	00.037	000.437	01.247	000.691	00.57	00.03	000.836	00.097	000.081	00.661
	52.0 ±	273.5 ±	47.0 ±	149.5 ±	34.6 ±	57.0 ±	281.8 ±	51.66 ±	185.7 ±	39.9 ±
	00.018	000.0081	01.11	001.76	00.10	00.08	000.199	00.921	000.66	00.453
L.S.D.	00.91	005.32	00.84	005.25	16.8	00.97	094.3	00.87	005.11	00.98

forty-five and sixty days, respectively when compared with control (Table 1, Fig. 1). The rest of the mixed doses showed a negligible increase in comparison to control (Table 1, Fig. 1).

Diameter of xylem strands: In the internal morphology applied GA₃ registered inhibition in the diameter of xylem strands which being 11.62% after forty-five days and 8.6% after sixty days when compared with control (Fig. 1g, h). Contrary to GA₃, applied IAA showed an increase upto 16.26% after forty-five and 11.56% after sixty days in comparison to control. Applied dose of 20 ppm Kinetin was not as responsive as the IAA and the expansion in diameter was less than IAA (Table 1). The increase/decrease ratio in the mixed dose of 100 ppm GA₃ + 100 ppm IAA and 100 ppm GA₃ + 20 ppm Kinetin were insignificant when compared with control (Fig. 1k). Contrarily, the mixed dose of 100 ppm IAA + 20 ppm Kinetin showed a well marked increase which being 31.7% after forty-five days and 20.68% after sixty days. Likewise, in the combined dose of 100 ppm GA₃ + 100 ppm IAA + 20 ppm Kinetin, the increase was well marked i.e., 27.20% after forty-five days and 22.52% after sixty days thus showing the dominant effect of IAA and Kinetin (Table 1).

Diameter of metaxylem elements: Applied 100 ppm GA₃ registered inhibition in the diameter of metaxylem elements. The decrease being 20% after forty-five days and 15.78% after sixty days when compared with control (Fig. 1h). On the other hand, applied IAA showed increase after forty-five days and sixty days (Fig. 1i). Moreover, the increase was insignificant with applied 20 ppm Kinetin in comparison to control (Table 1). The mixed dose of GA₃ + IAA and GA₃ + Kinetin showed insignificant increase after forty-five and sixty days (Fig. 1k). The mixed dose of 100 ppm IAA + 20 ppm Kinetin proved to be the most effective among all the other doses and maximum expansion was revealed. The combined dose of all the three hormones registered an increase but it was less than that in IAA + Kinetin. Thus clearly showing the inhibitory effect of GA₃ (Table 1).

Diameter of pith region: The diameter of pith region decreased with the application of 100 ppm GA₃ (Fig. 1g, h).

On the other hand, applied 100 ppm IAA showed increase in diameter which was 50.45% after forty-five days and 38.1% after sixty days when compared with control (Fig. 1i). Likewise, applied Kinetin increased the diameter upto 36.6% and 31.0% after forty-five and sixty days respectively as compared to control. Anomalous type of secondary growth i.e., cambial layer around the pith was also observed in this treatment (Fig. 1j). The application of all the mixed doses showed a general increase in the diameter. For instance, in the mixed dose of 100 ppm GA₃ + 100 ppm IAA increase observed was 23.45% after forty-five and 17.44% after sixty days. In the mixed dose of GA₃ + Kinetin increase was 29.5% after forty-five days and it was insignificant after sixty days in comparison to control (Fig. 1k). The application of mixed dose of 100 ppm IAA + 20 ppm Kinetin showed a well-marked increase in diameter which being 68.8% after forty-five and 45.1% after sixty days. The cambium in the roots initiates in the pericycle, in the present work abnormal initiation of cambium was speculated with mixed dose of IAA + Kinetin whereby cambium was initiated around the pith (Fig. 1l). This abnormality was seen in the above mentioned dose as well as in Kinetin treatments whereas all the rest of the doses individually or in combinations registered no anomaly in the initiation of cambium. The mixture of all the three hormones increased the diameter upto 35.9 and 27.58% after forty-five and sixty days respectively in comparison to control (Table 1).

Discussion

Plant hormones play an important role in the plant growth through the mobilization of its nutrients and consequently play an essential role in plant growth and development. The root of *C. arietinum* L. showed decrease in the number of rootlets with applied GA₃. This decreased number of rootlets may be due to the deficiency of nutrients which are consumed in the elongation phase (Allsopp, 1959). Goodwin and Morris (1979) came out with the result that GA₃ appears to play no role in lateral root initiation. With applied IAA, the number of lateral roots showed well-marked increase after forty-five and sixty days in comparison to control. The effect of IAA in the initiation of lateral roots has been reported by Street (1966). With the

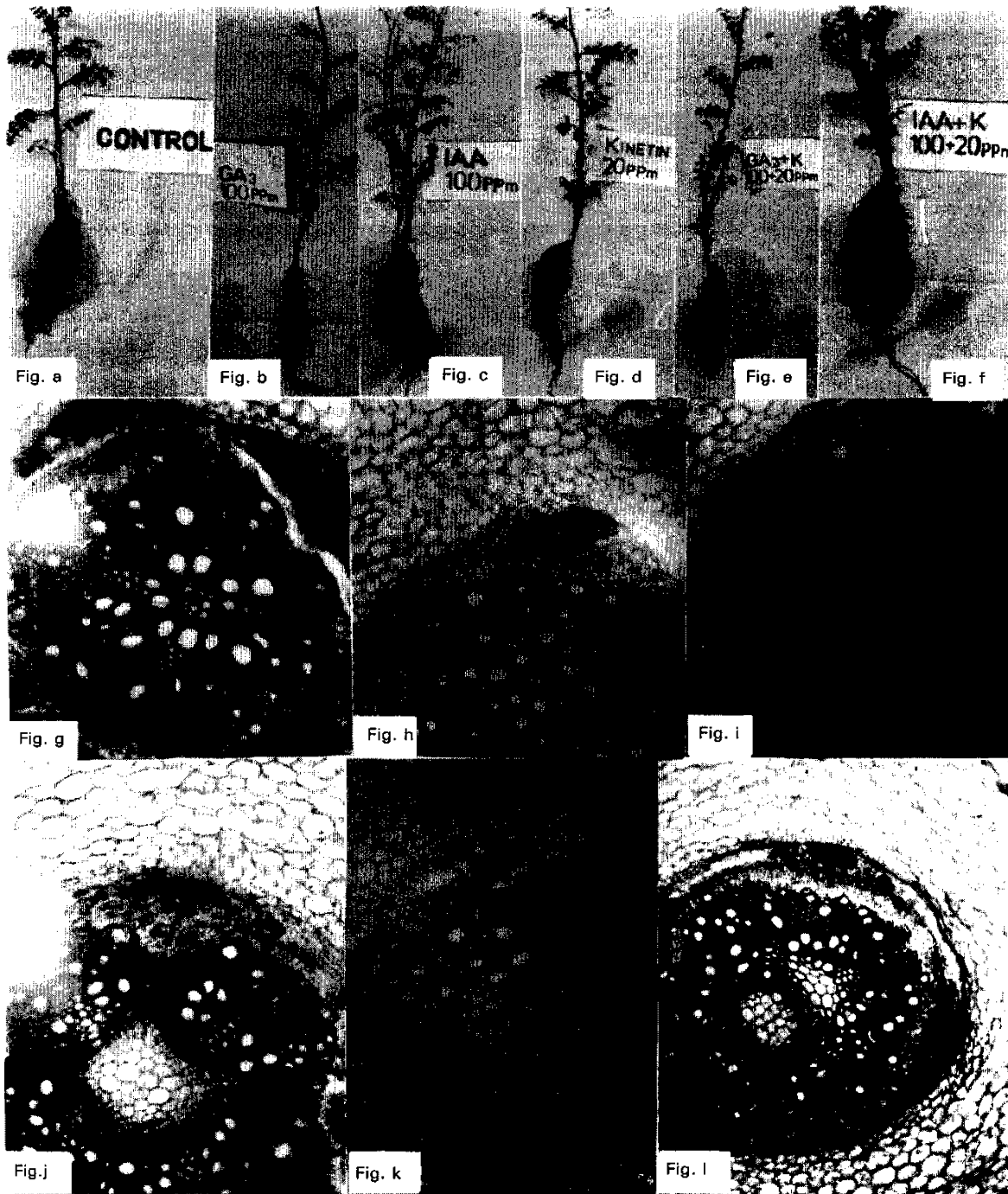


Fig. a: *Cicer arietinum* root and rootlets in control

Fig. c: Effect of IAA on the rootlets

Fig. e: Effect of mixed dose of GA₃ + Kinetin on the number of rootlets

Fig. g: Root of *Cicer arietinum* L. in transsection showing stelar and pith region in control (10X)

Fig. i: Effect of IAA on metaxylem elements and pith region (10X)

Fig. j: Effect of Kinetin showing increased pith region and abnormal initiation of cambium around the pith (10X)

Fig. l: Effect of IAA + Kinetin showing the anomalous initiation of cambium around the pith (5X)

Fig. b: Effect of GA₃ on the rootlets

Fig. d: Applied Kinetin showing number of rootlets

Fig. f: Effect of IAA + Kinetin showing the increased number of rootlets

Fig. h: Effect of GA₃ on the diameter of xylem strands, metaxylem elements and pith region (10X)

Fig. k: Effect of GA₃ + Kinetin on the stelar and pith region (10X)

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application of Kinetin, no significant increase in the number of rootlets was observed in comparison to control. Blakely *et al.* (1972) have observed that cytokinins play no role in lateral root initiation. In the mixed dose of GA₃ + IAA, increase in the number of lateral roots was observed when compared with control. As mentioned earlier, IAA causes initiation of lateral roots. In the mixed dose of GA₃ + Kinetin, insignificant increase in number of rootlets was registered. Both GA₃ and Kinetin play no part in lateral root initiation (Goodwin and Morris, 1979). Applied IAA + Kinetin showed a well-marked increase in number of rootlets (Fig. 1f). This remarkable increase was due to the activity of both IAA and Kinetin. Soh *et al.* (1998) has reported that Kinetin in the presence of IAA enhanced the lateral root growth. Moreover, when all the 3 hormones were applied simultaneously the formation of lateral roots was enhanced. In the internal morphology, the application of GA₃ registered an insignificant inhibition in the diameter of xylem strands and metaxylem elements after forty-five and sixty days in comparison to control thus showing no effect of GA₃ on secondary growth (Chaudhry and Zahur, 1992). However, Wang *et al.* (1997) and Awan *et al.* (1999) observed that GA₃ controls the cambial growth but in the present work, this hormone more or less revealed no effect on cambium. Contrary to GA₃, the diameter the xylem strands and metaxylem elements showed expansion with applied IAA when compared with control. Tuominen *et al.* (1997) and Uggla *et al.* (1998) reported wider metaxylem vessels with the application of auxins. In the present work, results are in accordance with the observation of these workers. Applied Kinetin increased the diameter of xylary region and metaxylem elements after forty-five and sixty days in comparison to control. This increase in diameter of cambial region may be attributed to increased cell-division with extraneous Kinetin (Makarova *et al.*, 1988). The equal mixed dose of GA₃ + IAA did not show any significant increase in the diameter of xylary region and metaxylem elements, thus the magnitude of expansion was found to be statistically insignificant (Kazama and Katsumi, 1974). This shows the antagonistic effect of mixed GA₃. In the combination of GA₃ and Kinetin increase in the diameter of xylary region as well as metaxylem elements was observed after forty-five and sixty days. This shows the dominant effect of Kinetin which not only caused cell expansion but also enhanced cell-division (Kabar and Baltepe, 1990; Uggla *et al.*, 1998). When IAA + Kinetin was applied, a well-marked increase in diameter of xylem strands and metaxylem elements was recorded (Table 1). As aforementioned IAA and Kinetin both are very effective in causing expansion growth (Kantharaj and Padmanabhan, 1991). When all the three hormones were applied in combination, increase in the diameter of xylary region as well as in metaxylem elements was observed which may again be attributed to the activity of IAA and Kinetin (Wang *et al.*, 1997; Uggla *et al.*, 1998) because GA₃ fails to cause expansion growth (Awan *et al.*, 1999).

The pith region registered an inhibition in diameter with applied GA₃ after forty-five and sixty days in comparison to control. Gordon and Letham (1975) working on immature radish cotyledons have reported that gibberellins play no part in causing cell expansion. Applied IAA showed a significant increase in the diameter of pith region when compared with control (Fig. 1i). This increase in the pith may be due to the addition of new cell-wall materials

caused by IAA (Cleland, 1970). With the application of Kinetin, increase in the number of pith cells and in diameter of pith region was observed (Fig. 1j). This increase in the number may be attributed to enhanced cell-division (Phillips, 1971). In the equal mixed dose of GA₃ + IAA, increase in the diameter of pith region was observed which was accompanied by expansion in pith cells in comparison to control. However, this expansion was less than that observed in IAA treatments alone which clearly shows that GA₃ reduces the effect of IAA. Similar results have been reported by Kazama and Katsumi (1974). Moreover, mixed dose of GA₃ + Kinetin showed same results as mentioned above.

In the combination of IAA + Kinetin, a well-marked increase in the diameter of pith cells and pith region was observed. Thimann (1977) reported that in tobacco pith cultures extraneous IAA caused cell expansion when applied alone but when applied with Kinetin it enhanced cell division. In the mixed dose of all the three hormones, the diameter of pith region as well as pith cells showed an increase after forty-five and sixty days. One very important observation was the formation of cambial layer around the pith in this treatment. Ball (1944) and Eames (1972) have reported that growth hormones like auxins may cause abnormal secondary growth. It is speculative that in the present work, anomalous growth of cambium around the pith was observed. The cambium in the root arises in the pericyclic region but in this mixed dose it was initiated around the pith. Thus it can be concluded that the initiation of this cambium is a deviation from the normal tissue formation, which can only be attributed to the extraneous use of the hormones.

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