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A CONTRIBUTION TO THE STUDY OF ROOT OF *LENS CULINARIS* L., ROOTLETS, XYLARY REGION, TRANSITIONAL REGION AND INITIATION OF VASCULAR CAMBIUM FOLLOWING TREATMENTS WITH IAA, GA_3 AND KINETIN

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Abstract

The action of growth hormones i.e., GA_3, IAA and Kinetin (individually as well as in combinations) on the external and internal morphology of root of *Lens culinaris* L., has been comprehensively reviewed The following concentrations of the three hormones applied individually 500 ppm GA_3, 500 ppm IAA and 30 ppm Kinetin. In combination concentrations used were 500 ppm GA_3 + 500 ppm IAA, 500 ppm GA_3 + 30 ppm Kinetin, 500 ppm IAA + 30 ppm Kinetin, 500 ppm GA_3 + 500 ppm IAA + 30 ppm Kinetin. In the external morphology applied GA_3 increased the length, however, a corresponding decrease in diameter was registered. Furthermore, the fresh and dry weight showed decrease when compared with control. Exogenous IAA showed decrease in length and expansion in diameter, the fresh and dry weight also increased. Kinetin revealed positive effect on all the above parameters. The number of rootlets increased with IAA and Kinetin. The mixed doses of GA_3 showed extension in growth IAA and Kinetin inhibited length. In the internal morphology cortical region and endodermis showed no significant response. The stellar region showed a well marked increase in the diameter of metaxylem elements as well as increase in the number and early maturation following treatments with IAA and Kinetin.

Introduction

Plant growth hormones initiate biochemical reactions and changes as chemical messengers and are responsible for the formation and growth of different plant organs (Leopald and Kriedman, 1975). Auxins, gibberellins and cytokinins etc., regulate various aspects of growth and differentiation Auxin induced growth results in lateral expansion of stem and root tissues (Abeles, 1973; Eisenger, 1983). This expansion is accompanied by inhibition in length (Bairathi and Nathawat, 1980). Applied GA_3 causes elongation in the stem (Chaudhry, 1995, 1997). This elongation is accompanied by inhibition in diameter of the stem. Kabar (1997) reported that GA_3 alone and in combination with Kinetin were equally effective in wheat and barley. Cytokinins have the ability to promote cell division in roots and stems. Kantharaj and Padmanabhan (1991) observed the molecular aspects of cytokinins stimulatory action on auxin mediated new root formation in the hypocotyls. Chaudhry and Rashid (2000) observed abnormal initiation of cambium in the root of *Cicer arietinurn* L., following hormonal treatments. The present study will help to elucidate the mode of action of the hormones i.e., GA_3, IAA and Kinetin.

Materials and methods

The following doses of growth hormones were used on the root of *Lens culinaris* L., i.e., 500 ppm GA_3, 500 ppm IAA and 30 ppm Kinetin and in combination they were 500 ppm GA_3 + 500 ppm IAA, 500 ppm GA_3 + 30 ppm

Kinetin, 500 ppm IAA + 30 ppm Kinetin and 500 ppm GA_3 + 500 ppm IAA + 30 ppm Kinetin.

In order to study the effect of growth hormones on external and internal morphology of roots the seeds were grown in November in earthenware pots and watered at regular intervals. 27 µl of each hormonal treatment was applied on the apical meristem of the plants. This treatment was repeated after 24 hours till 30 days for the first set and for second set treatment was continued till 60 days.

In the external morphology the following parameters were observed: length of root (cm), diameter of root (cm), number of rootlets, fresh and dry weight of root.

In order to study the internal morphology 1 cm long portions of roots were fixed in Corney's modified fluid, dehydrated and cleared in tertiary butyl alcohol grades infiltrated and embedded in paraffin wax then processed with the help of rotary microtome (10-15 µm), stained with safranin and fast green and mounted in Canada balsam. The parameters observed were number of cortical layers, protoxylem poles and metaxylem elements; diameter of cortical, endodermis, metaxylem elements and pith cells, early initiation or inhibition of cambium; initiation of pith following treatments; root shoot transition region.

Results and discussion

External Morphology: The root of *Lens culinaris* L., showed a general increase in length with applied GA_3 after 30 and 60 days when compared with control (Table 1). Gibberellins promote and regulate growth of root (Mertz, 1966; Tanimoto, 1987, 1988). The increase in length may further be attributed to the rapid mobilization of food material, plasticity of cell wall or due to enhancement of cell size (Ahmad and Javaid,. 1996). This increase in length was accompanied by a decrease in the diameter (Tables 1, 2). Similar effects were observed by Chaudhry and Zahur (1992) and Chaudhry (1995) working on *Abelmoschus esculentus*. Furthermore, GA_3 increased the number of rootlets after 30 as well as 60 days (Table 1) in comparison to control, it also showed decrease in the fresh and dry weight of roots with and without rootlets (Tables 3, 4). Sengupta *et al.* (1977) and Singh and Singh (1979) observed increase in the dry weight of roots. The present results do not agree with the above mentioned authors. The application of IAA caused inhibition in the length of roots after 30 and 60 days as compared to control, that root elongation is inhibited by IAA has been reported by Tanimoto and Watanabe (1986), Miller and Gow (1989). This inhibition in length was accompanied by a corresponding increase in the diameter (Table 2). The expansion in diameter may be due to enzymatic activity of IAA, permeability, formation of ATP as well as wall plasticity (Strefford, 1973). Audus (1959) reported that IAA effects initiation of root meristem in plants and an increase in the number of rootlets. Similar effects have been registered in the present work (Fig.2). The fresh and dry weight also showed increase, this may be due to enhanced cell division. Kinetin showed insignificant increase in the length of root, however, a significant, expansion in diameter was observed after 30 and 60 days as compared to control (Tables 1, 2). Zadoo (1986) and Makarova *et al.* (1988) reported similar effects, Kinetin showed increase in the number of rootlets. Furthermore, fresh and dry weight also showed increase (Tables 3, 4). This may be due to enhanced cell division (Miller, 1961).

The mixed dose of GA_3 + IAA, GA_3 + Kinetin and GA_3 + IAA + Kinetin showed positive effect on all the parameters mentioned above (Tables 1, 2).

Although the fresh and dry weight showed increase after 30 days, it was negligible after 60 days. This may be due to mixed GA_3. The mixed dose of IAA+ Kinetin showed inhibition in length after 30 and 60 days (Table 2). The decrease in length was accompanied by a well marked expansion in diameter. This may be due to the activity of both IAA and Kinetin, which promote cell division. The number of rootlets also increased. This is also reported by Chaudhry and Rashid (2000). Likewise the fresh and dry weight also showed increase.

Internal Morphology: The root is triarch (Fig.3). The epidermis is mostly sloughed off. The cortical region revealed inhibition with applied GA_3. This was due to narrowing of cell diameters. In the external morphology remarkable increase in the length of root was observed, which led to inhibition in the diameter. The narrowing of cell diameters may be due to rapid extension growth. According to Allsopp (1965) the increased length decreases the available sugars thus causing inhibition in diameter. No cell division was promoted in the cortical region, thus the number of layers remaining totally constant. The application of IAA caused expansion in the cortical region (Table 5, Fig.4). Similar results have been reported by Bairathi and Nathawart (1980). No cell division was observed in the cortical region, the layers remained constant. Applied Kinetin showed similar effects as observed for IAA (Tables 5, 6).

The mixed dose of GA_3 + IAA and GA_3 + Kinetin showed no significant effect (Tables 5, 6). Contrarily the mixed dose of IAA + Kinetin showed a well marked increase in diameter along with increase in the number of layers after 30 and 60 days. Similar reports are given by Phillips (1971). The application of all the three hormones simultaneously showed no significant increase, thus showing the antagonistic effect of GA_3.

The endodermis showed no positive response (Table 5). The stellar region showed inhibition with GA_3 (Fig.5) and expansion with IAA and Kinetin. Makarova *et al.* (1988) and Chaudhry (1995) observed similar effects. With the application of GA_3 + IAA the magnitude of expansion was found to be statistically insignificant. Likewise GA_3 + Kinetin and GA_3 + IAA + Kinetin registered similar effects as mentioned for GA_3 + IAA. However, IAA + Kinetin revealed expansion growth (Tables 5, 6). The metaxylem elements showed inhibition along with decrease in the number of metaxylem elements with applied GA_3. Thus showing no effect on transverse growth (Morris and Arthur, 1985). Applied IAA caused increase in the diameter as well as in the number (Tables 5, 6). The application of auxins produced wider vessels (Chaudhiy, 1997). The cell division was enhanced (Uggla *et al.*, 1998). Kinetin also enhanced cell division (Thimann, 1977). The GA_3 mixed doses more or less showed no cell division (Tables 5, 6). However, IAA + Kinetin showed promotion of cell division. The transitional region as afore-mentioned showed no response with GA_3, however, IAA and Kinetin showed increased cell division resulting in more metaxylem elements when compared with control (Tables 5, 6, Fig.6). Conclusively the mixed doses registered no regular pattern and need further investigation.

Table 1. Effect of growth hormones on the external morphology of root after 30 days.

Treatments	Length of root (cm)	Diameter of root (cm)	No. of rootlets
Control	14.8±0.27	0.14±0.01	10.3±0.20
GA_3 (500 ppm)	17.5±0.3	0.12±0.001	16.25±0.25
IAA (500 ppm)	13.6±0.2	0.22±0.001	25.0±0.23
Kinetin (30 ppm)	15.5±0.07	0.196±0.001	28.3±0.26
GA_3+IAA (500+500 ppm)	16.6±0.2	0.18±0.001	24.3±0.21
GA_3+Kinetin (500+30 ppm)	19.0±0.5	0.17±0.01	30.6±0.3
IAA+Kinetin (500+30 ppm)	13.2±0.1	0.24±0.02	34.3±0.1
GA_3+IAA+Kinetin (500+500+30 ppm)	15.4±0.2	0.22±0.02	31.6±0.1

Table 2. Effect of growth hormones on the external morphology of root after 60 days.

Treatments	Length of root (cm)	Diameter of root (cm)	No. of rootlets
Control	16.6±0.1	0.20±0.01	20.0±0.02
GA_3 (500 ppm)	19.8±0.4	0.18±0.02	24.0±0.02
IAA (500 ppm)	14.2±0.25	0.30±0.001	23.4±0.2
Kinetin (30 ppm)	16.2±0.3	0.34±0.01	34.4±0.15
GA_3+IAA (500+500 ppm)	18.5±0.25	0.25±0.001	28.0±0.1
GA_3+Kinetin (500+30 ppm)	18.2±0.1	0.26±0.001	33.1±0.3
IAA+Kinetin (500+30 ppm)	13.9±0.30	0.35±0.02	40.4±0.2
GA_3+IAA+Kinetin (500+500+30 ppm)	16.7±0.23	0.30±0.01	33.6±0.20

Table 3. Effect of growth hormones on the external morphology of fresh and dry weights of roots with and without rootlets, after 30 days.

Treatments	Fresh wt. of roots with rootlets	Fresh wt. of roots without rootlets	Dry wt. of roots with rootlets	Dry wt. of roots without rootlets
Control	1.22±0.02	0.657±0.01	0.65±0.01	0.52±0.01
GA_3 (500 ppm)	0.91±0.01	0.52±0.02	0.59±0.03	0.43±0.1
IAA (500 ppm)	1.9±0.01	0.88±0.01	0.65±0.1	0.59±0.2
Kinetin (30 ppm)	3.05±0.02	1.18±0.1	0.74±0.02	0.63±0.02
GA_3+IAA (500+500 ppm)	17.3±0.01	1.12±0.01	0.65±0.02	0.59±0.01
GA_3+Kinetin (500+30 ppm)	1.89±0.02	1.117±0.02	0.69±0.02	0.58±0.1
IAA+Kinetin (500+30 ppm)	2.37±0.01	1.35±0.2	0.67±0.01	0.62±0.01
GA_3+IAA+Kinetin (500+500+30 ppm)	1.07±0.01	0.676±0.02	0.55±0.1	0.52±0.1

Table 4. Effect of growth hormones on the external morphology of fresh and dry weights of roots with and without rootlets, after 60 days.

Treatments	Fresh wt. of roots with rootlets	Fresh wt. of roots without rootlets	Dry wt. of roots with rootlets	Dry wt. Of roots without rootlets
Control	0.583±0.01	0.254±0.1	0.163±0.1	0.101±0.01
GA_3 (500 ppm)	0.663±0.2	0.206±0.01	0.137±0.3	0.107±0.01
IAA (500 ppm)	1.05±0.01	0.228±0.01	0.130±0.02	0.109±0.2
Kinetin (30 ppm)	1.060±0.2	0.341±0.02	0.154±0.1	0.112±0.02
GA_3+IAA (500+500 ppm)	1.122±0.2	0.217±0.2	0.117±0.01	0.080±0.1
GA_3+Kinetin (500+30 ppm)	0.634±0.2	0.183±0.02	0.127±0.02	0.116±0.1
IAA+Kinetin (500+30 ppm)	0.478±0.1	0.200±0.2	0.213±0.2	0.144±0.1
GA_3+IAA+Kinetin (500+500+30 ppm)	0.451±0.01	0.209±0.1	0.123±0.1	0.119±0.1

Table 5. Effect of growth hormones on the internal morphology of root, after 30 days.

Treatments	No. of cortical layers	Diameter of cortical region (µm)	Diameter of endodermis (µm)	Diameter of stellar region (µm)	Diameter of metaxylem elements (µm)	No. of metaxylem elements	No. of larger metaxylem elements in transitional region
Control	8	200±2.38	20±0.01	650±3.30	42±0.9	28	35
GA_3 (500 ppm)	8	150±0.57	18±0.7	500±4.1	40±0.49	25	30
IAA (500 ppm)	8	250±1.28	24±1.12	650±2.20	50±1.03	34	40
Kinetin (30 ppm)	8	270±1.19	23±2.1	660±1.29	44±1.76	33	38
GA_3+IAA (500+500 ppm)	7	210±2.57	20±1.2	600±2.29	38±0.69	30	35
GA_3+Kinetin (500+30 ppm)	7	190±2.20	20±0.07	580±1.20	40±1.12	30	32
IAA+Kinetin (500+30 ppm)	8	280±1.95	27±0.9	850±0.07	50±1.10	35	65
GA_3+IAA+Kinetin (500+500+30 ppm)	7	270±2.37	18±1.102	700±1.37	40±0.5	32	50

Table 6. Effect of growth hormones on the internal morphology of root, after 60 days.

Treatments	No. of cortical layers	Diameter of cortical region (µn)	Diameter of endoderm is (µm)	Diameter of stellar region (µm)	Diameter of metaxylem elements (µm)	No. of metaxylem elements	No. of larger metaxylem elements in transitional region
Control	8	210±2 54	20±1 9	700±2 08	47±0 34	34	49
GA_3 (500 ppm)	7	150±1 27	18±0 8	540±0 26	46±0 69	30	40
IAA (500 ppm)	8	200±1 23	24±1 12	900±0 46	54±0 8	40	50
Kinetin (30 ppm)	7	200±1 10	24±0 6	850±1 92	45±1 18	40	55
GA_3+IAA (500+500 ppm)	6	215±0 70	20±2 1	850±0 921	43±1 15	32	50
GA_3+Kinetin (500+30 ppm)	6	190±0 8	20±1 02	800±0 34	42 5±2 30	36	48
IAA+Kinetin (500+30 ppm)	9	300±2 82	27±1 23	900±0 702	56±0 39	44	80
GA_3+IAA+Kinetin (500+500+30 ppm)	7	180±0 27	18±0 07	700±2 54	44±0 82	38	66

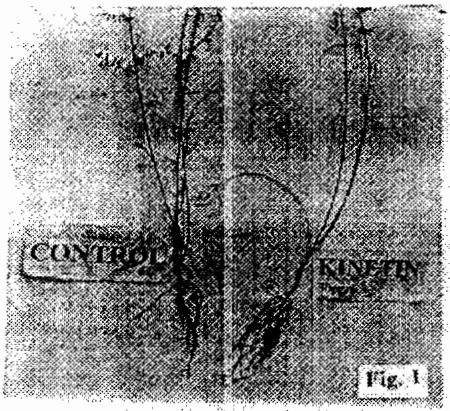

Fig. 1

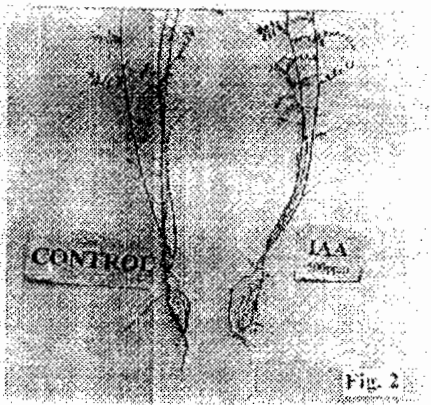

Fig. 2

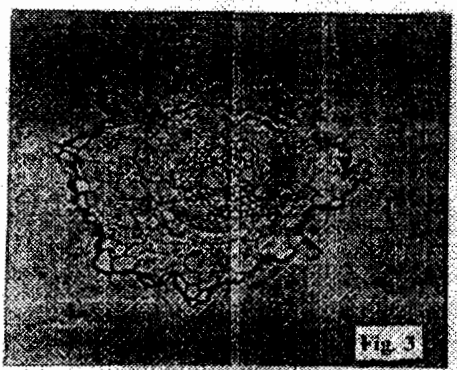
Fig. 3

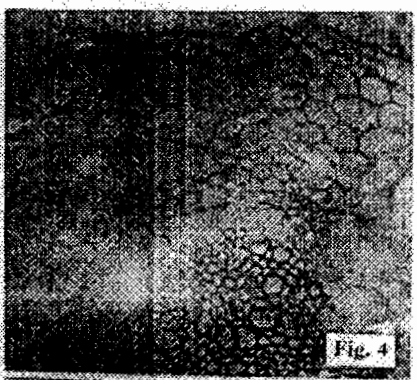
Fig. 4

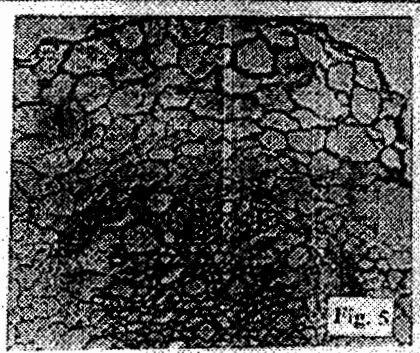
Fig. 5

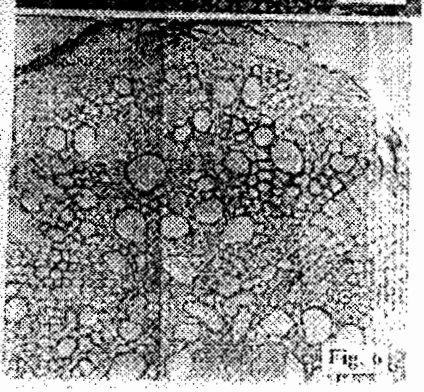
Fig. 6

Legend to figures

Figure 1. Kinetin treated plants showing increased rootlets.
Figure 2. IAA treated plants.
Figure 3. Triarch root (5x).
Figure 4. IAA treatments showing cortical region and endodermis & stellar region (10x).
Figure 5. GA_3 treatments showing cortical & stellar region (10x).
Figure 6. Kinetin treated plants showing transitional region (10x).

References

Abeles, F.B., 1973. *Ethylene in plant biology*. Academic Press, New York.

Ahmad, Q. and A. Javaid,.1996. Effect of IAA, GA_3 and Coumarine on growth of wheat seedlings. *Sci. Int. (Lahore)*, 8(4): 369-372.

Allsopp, A., 1965. Effect of gibberellic acid on the juvenility in *Marselia* and certain other plants. *Nature*, 184: 1575-1576.

Audus, L.J., 1959. *Plant growth substances*. Leonard Hill (book) Limited, London.

Bairathi, M.K. and G.S. Nathawart. 1980. Effect of IAA and 2,4-D on root apical organization and tissue differentiation in sennhemp (*Crotolaria juncea* L.). *Flora*, 169: 336-350.

Chaudhry, N.Y., 1995. Effect of growth regulators i.e., IAA and GA_3 on the external morphology of hypocotyl and stem internodes of *Abelmoschus esculentus* (Linn.) Moench. *Acta Sci.*, 5(1): 17-40.

Chaudhry, N.Y., 1997. Effect of growth regulators i.e., IAA and GA_3 on *Abelmoschus esculentus* (Linn.) Moench. Internal structure of root. *Acta Sci.*, 7(2): 109-124.

Chaudhry, N.Y. and A. Rashid.2000. Rootlets, xylary region and abnormal initiation of cambium in the root of *Cicer arietinum* L., following treatments with GA_3, IAA and Kinetin. *Pakistan J. Biol. Sci.*, 3(8): 1255-1259.

Chaudhry, N.Y. and M.S. Zahur.1992. Effect of growth regulators i.e., IAA and GA_3 on *Abelmoschus esculentus* L. Internal structure of hypocotyl and stem internodes. *Biologia*, 37(2): 217-244.

Eisenger, W., 1983. Regulation of pea internode expansion by ethylene. *Annu. Rev. Plant Physiol.*, 34: 225-240.

Goodwin, P.B. and S.C. Morris.1979. Application of phytohormones to pea roots after removal of the apex. Effects of lateral root production. *Aust. J. Plant Physiol.*, 6: 195-200.

Kabar, K., 1997. Comparison of reversal of abscisic acid induced inhibition of seed germination and seedling growth of some Gramineae and Liliaceae members by Kinetin and Gibberellic acid. *Tr. J. Bot.*, 21: 203-210.

Kantharaj, G.R. and G. Padmanabhan. 1991. Molecular aspects of cytokinin's stymied action on auxin mediated new root formation in the hypocotyls of *Phaseolus vulgaris* L., horticulture, new technologies and application. *Proc. Int. Sem. New Frontiers in Horticulture*, organized by Indo-American Hybrid Seeds, Bangalore, India, Nov. 25-28 (1990) (Edited by Prakash, J. and Pierik, R.L., 1991). *Curr. Plant Sci. Biotechnol. Agri.*, 12: 131-139.

Kaul, K. and , S. Farooq.1994. Kinetin induced changes in extension growth and activity of some enzyme in morning glory hypocotyl segments. *Indian J. Plant Physiol.*, 4: 214-216.

Krishnamoorthy, H.N., 1981. *Plant growth substances*. Tata McGraw Hill Pub. Co. Ltd., New Delhi.

Leopold, A.C. and , P.E. Kriedemann.1975. *Plants' growth and development* (2nd ed.). Tata McGraw Hill Publishing Co. Ltd., Delhi.

Makarova, R.V., , E.P. Baes., F. Martinish., P. Sanches.and K. Ranavira. 1988. The action of 6-benzylaminopurine on the growth of soy cotyledons and hypocotyls. *Biol. Nauki (Mosc.)*, 0: 81-84.

Mertz, D., 1966. Hormonal control of root growth. I. *Plant Cell Physiol.*, 7: 125-135.

Miller, A.L. and N.A.R. Gow., 1989. Correlation between root generated ionic currents, pH, fusicoccin, indole acetic acid and growth of the primary root of *Zea mays*. *Plant Physiol.*, 89: 1198-1206.

Miller, H.A., 1961. Kinetin and related compounds in plant growth. *Annu. Rev. Plant Physiol.*, 12: 395-408.

Morris, D.A. and E.D. Arthur. 1985. Effects of gibberellic acid on patterns of carbohydrate distribution and acid invertase activity in *Phaseolus vulgaris*. *Physiol. Plant.*, 65: 257-262.

Phillips, I.D.J., 1971. *Introduction to Biochemistry & Physiology of Plant Growth Hormones*. McGraw Hill Book Company, New York.

Sengupta, U.K., G. Kain. and Pokroyal, 1977. Effect of growth regulators on seed germination in groundnut. *Plant Physiol.*, 20: 91-96.

Singh, R.D. and L. Singh.1979. Effects of GA_3 and cycocel on growth and seed germination of soybean, *Glycine max*. *Bangladesh J. Bot.*, 8½: 79-90.

Steel, R.G. and J.H. Torrie.1981. *Principles and procedures of statistics*. A biometrical approach, 2nd ed. McGraw Hill International Book Company.

Strefford, 1973. Plant growth regulators. In: *Plant Physiology* (Pandey, S.N. and Sinha, B.K., eds.), pp.398. Vikas Publishing House, Delhi.

Tanimoto, E., 1987. Gibberellin-dependent root elongation in *Lactuca sativa*: recovery from growth retardant-suppressed elongation with thickening by low concentration of GA_3. *Plant Cell Physiol.*, 28: 269-280.

Tanimoto, E., 1988. Gibberellin regulation of root growth with change in galactose contents of cell walls in *Pisum sativum*. *Plant Cell Physiol.*, 29: 269-280.

Tanimoto, E. and , J. Watanabe.1986. Automated recording of lettuce root elongation as affected by auxin and acid pH in a new rhizometer with minimum mechanical contact to roots. *Plant Cell Physiol.*, 27: 1475-1487.

Thimann, K.V., 1969. The auxins. In: *The Physiology of Plant Growth and Development* (Vilkins, M.D., ed.), pp.3-45. McGraw Hill, New York.

Uggla, C.E., J. Mullerowicz,, and B. Sundberg. 1998. Indole-3-acetic acid controls cambial growth in Scots pine by positional signaling. *Plant Physiol.*, 117: 113-121.

Wang, Q., C.H.A. Little, and P.C. Oden.1997. Control of longitudinal and cambial growth by Gibberellic acid and Indole-3-acetic acid in shoots of *Pinus sylvestris*. *Physiologia Plantarum*, 95: 187-194.

Young, S.W., C. P. Son, and C. D. Yee. 1998. Effects of cytokinins on adventitious roots formation in callus culture of *Vigna bunguiculata* Linn. *In vitro Cell Dev. Biol. Plant*, 34: 189-195.

Zadoo, K., 1986. *Effect of some plant growth regulators on the growth and metabolism of isolated cotyledons*