

EFFECT OF SOME GROWTH HORMONES (GA₃, IAA AND KINETIN) ON THE MORPHOLOGY AND EARLY OR DELAYED INITIATION OF BUD OF LENTIL (*LENS CULINARIS MEDIK*)

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Abstract

The effect of growth hormones on the morphology of shoot of lentil was examined. The hormones viz., 1.5 mM (500 mg L⁻¹) GA₃, 2.85 mM (500 mg L⁻¹) IAA and 0.14 mM (30 mg L⁻¹) kinetin were applied individually as well as in combination i.e., 1.5 mM GA₃ + 2.85 mM IAA, 1.5 mM GA₃ + 0.14 mM kinetin, 2.85 mM IAA + 0.14 mM kinetin and 1.5 mM GA₃ + 2.85 mM IAA + 0.14 mM kinetin. GA₃ showed a marked elongation in the length of shoot and increase in the number of internodes and compound leaves. Application of IAA showed a decrease in length of shoot and number of internodes. The increase in the diameter, area and number of leaves was also observed. IAA induced branching with lush green colour of leaves. Kinetin showed inhibition in length and in the number of internodes. Inhibition was associated with a significant expansion in diameter and an increase in area of leaves as well as their number. The combined dose of GA₃+IAA, GA₃+kinetin and GA₃+IAA+kinetin showed a significant increase in length and number of internodes as well as in the number of compound leaves. The colour of leaves was green and no branching was induced. However, the diameter of main stem showed inhibition. The dose of IAA+kinetin showed a decrease in length and number of internodes. However, expansion in the main stem diameter and increase in the number and area of leaves was also observed. The colour of leaves was lush green with more branches as compared to control. In GA₃ treated plants, early flowering with higher number of floral buds was recorded. Applied IAA caused late flowering and increased the number of floral buds, while kinetin showed no significant delay in flowering but number of floral buds was more as compared to control. The mixed doses of GA₃ with IAA and kinetin revealed early flowering alongwith nonsignificant increase in the number of flower buds. However, the dose of IAA+kinetin promoted late flowering with noticeable increase in number of floral buds.

Introduction

Plant growth regulators are the chemicals which influence the plant growth when applied in very minute quantity. There are many reports which indicate that application of growth regulators enhanced plant growth and crop yield (Hernandez, 1997; Ashraf *et al.*, 1987, 1989). Lee *et al.*, (1999) reported that GA₃ increased stem length and number of flower per plant. Kabar (1990) found that GA₃ accelerated bud development and stem elongation but the best results can be achieved if GA₃ is applied in combination with kinetin.

IAA exerts influence on plant growth by enlarging leaves and increasing photosynthetic activities in plants. It also activates the translocation of carbohydrates during their synthesis (Awan *et al.*, 1999; Ritenour *et al.*, 1996). Cytokinins enhanced the cell expansion in soybean (Makarova *et al.*, 1988) and increased stem thickness while kinetine reduced shoot

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length but increased the fresh weight by increasing stem diameter in morning glory (Kaul & Farooq, 1994) and in okra (Chaudhry & Khan, 2000). There are also some reports which indicate that kinetin in combination with GA₃ enhanced germination and seedling growth in chick pea (Kaur *et al.*, 1998).

Lentil, a very important legume crop, is a good source of protein (35-40 %) and very popular among the people of third world countries because the other protein sources like meat is very expensive in these countries. With the ever growing population of third world countries the demand of lentil increased, so it is the urgent need of the present age that the yield of this crop should be increased using different means. The use of growth regulators is becoming popular to enhance crop productivity and varieties of such substances are available in the market which are being utilized for crop production. Therefore, keeping in view the importance of different growth regulators in increasing crop growth, studies were carried out to compare the effect of GA₃, IAA, kinetin alone and in combination on the growth of lentil.

Materials and Methods

To study the effects of growth hormones, the following composition of growth hormones viz., 1.5 mM (500 mg L⁻¹) GA₃, 2.85 mM (500 mg L⁻¹) IAA, 0.14 mM (30 mg L⁻¹) kinetin above and in combination 1.5 mM GA₃ + 2.85 mM IAA, 1.5 mM GA₃ + 0.14 mM kinetin, 2.85 mM IAA + 0.14 mM kinetin and 1.5 mM GA₃ + 2.85 mM IAA + 0.14 mM kinetin. The seeds of lentil were soaked in water for 24 h. Then they were sown in earthen pots in the month of October. The plants were watered at regular intervals. When the epicotyl emerged from soil and was oriented vertically to the hypocotyledonary axis, then 27 µl of each hormonal treatment i.e. 500 ppm GA₃, 500 mg L⁻¹ IAA, 30 mg L⁻¹ kinetin, 500 mg L⁻¹ GA₃ + 500 mg L⁻¹ IAA, 500 mg L⁻¹ GA₃ + 30 mg L⁻¹ kinetin, 500 mg L⁻¹ IAA + 30 mg L⁻¹ kinetin and 500 mg L⁻¹ GA₃ + 500 mg L⁻¹ IAA + 30 mg L⁻¹ kinetin, were applied on the apical meristem of the plant. The treatment was repeated after every 24 hours into 30 days. After that the plants were removed from the pots. The treatments were continued for the second set of pots into 60 days. Effect of different doses of GA₃, IAA and kinetin on the structural organization of the plants were observed and compared with control as well as among themselves. The collected data was statistically analysed and DMRT test was used to compare the treatment and varietal means (Steel & Torrie, 1982).

Results

The shoot of lentil showed remarkable increase where 1.5 mM GA₃ was applied thus increasing the length up to 77.9% and 62.4% after 30 and 60 days respectively over the control. This clearly shows the well marked effect of GA₃ where length is concerned (Fig.1 A). Contrary to 1.5 mM GA₃, 2.85 mM IAA showed an inhibition in length i.e. 11.72% after 30 days and an insignificant inhibition i.e. 3.8% after 60 days. Similarly, 30 mg L⁻¹ kinetin also showed an inhibition i.e., 21.4% after 30 days and nonsignificant decrease i.e. 9.1% after 60 days compared to control (Fig. 1a).

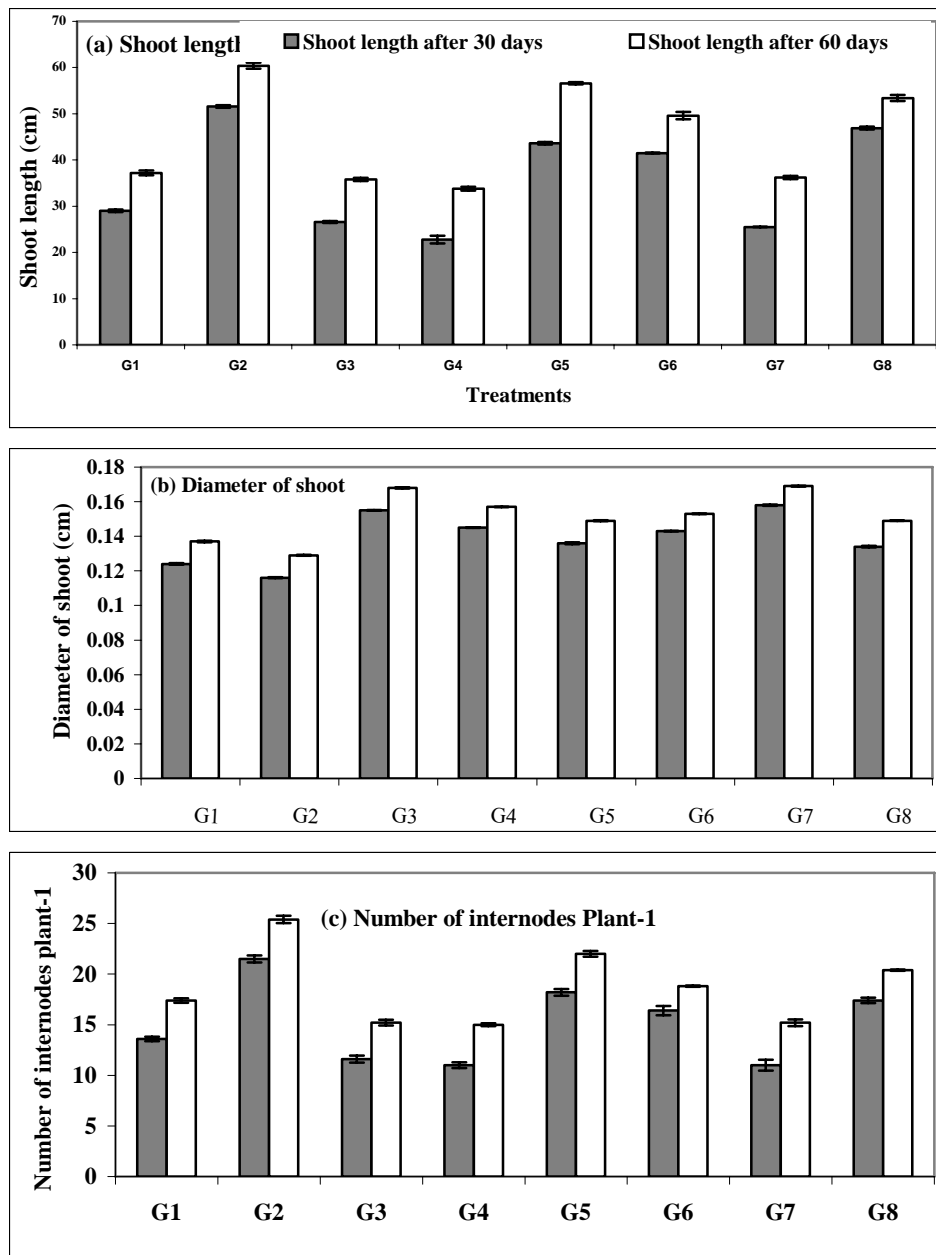


Fig. 1. Effect of growth hormones on plant morphology of lentil after 30 and 60 days of growth period.

G1 = Control
 G2 = 1.5 mM GA₃
 G3 = 2.85 mM IAA
 G4 = 0.14 mM Kinetin
 G5 = 1.5 mM GA₃ + 2.85 mM IAA
 G6 = 1.5 mM GA₃ + 0.14 mM Kinetin
 G7 = 2.85 mM IAA + 0.14 mM Kinetin
 G8 = 1.5 mM GA₃ + 2.85 mM IAA + 0.14 mM Kinetin

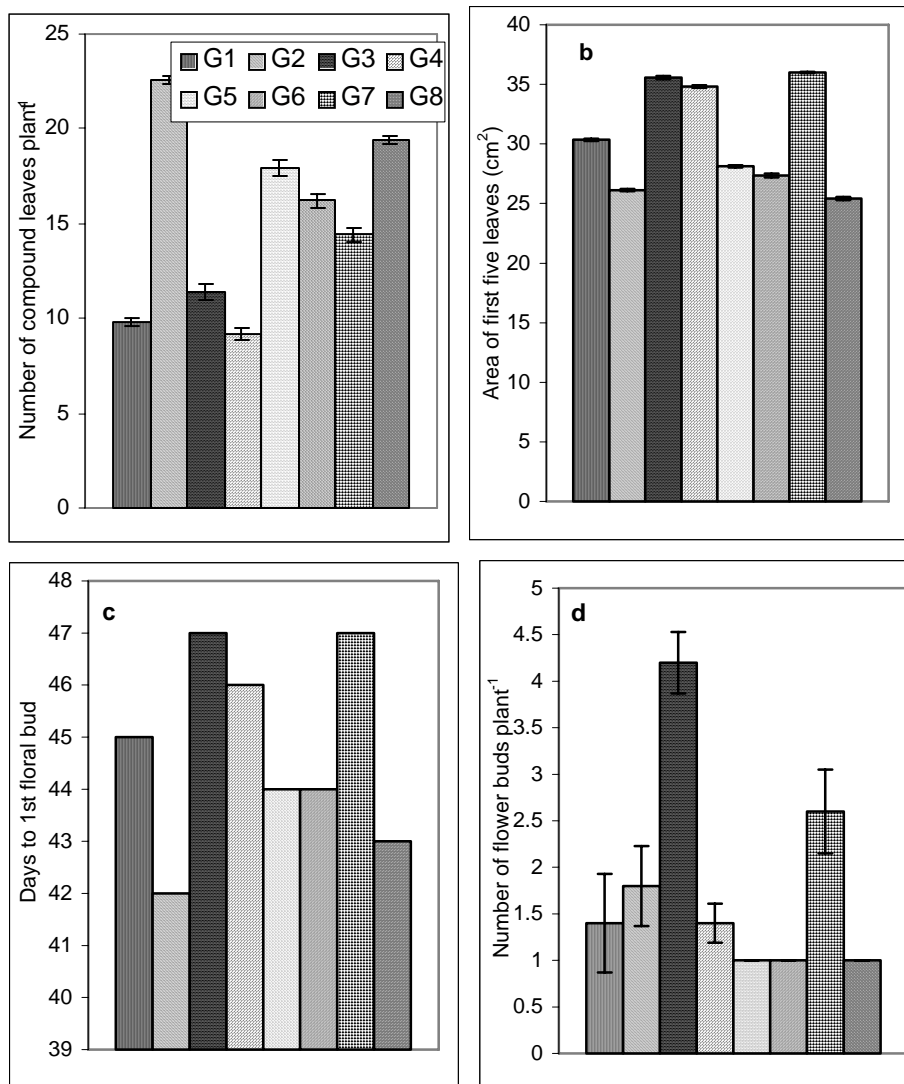


Fig. 2. Effect of growth hormones on plant morphology of lentil (number of compound leaves, leaf area, days for initiation of flowering and number of flower bud) after 30 and 60 days of growth period.

The application of mixed doses i.e., 1.5 mM GA₃ + 2.85 mM IAA showed as increase in length of shoot i.e., 50.34% after 30 days and 52.15% after 60 days over the control. This again shows the dominant effect of GA₃. Similarly, GA₃ + kinetin also showed an increase i.e., 43.10% and 33.33% after 30 and 60 days, respectively in comparison to control. It is noticeable that GA₃ + IAA was more effective as compared to GA₃ + kinetin. On the other hand, IAA + kinetin showed insignificant result when compared with control. When all the three hormones were applied in combination, i.e., GA₃ + IAA + kinetin a marked effect in increase of 61.72% and 43.54% was observed, after 30 and 60 days, respectively.

The shoot of lentil showed decrease in diameter with the application of GA₃ compared to control. In contrast, when IAA was applied, it showed an expansion in the diameter i.e., 25% after 30 days (Fig. 1b). After 60 days, the inhibition was 22.6% (Fig. 1b). Like-wise kinetin also showed an increase as compared to control. In the combined doses, the dominant effect of IAA was prominent and very clear when GA₃ + IAA was applied which increased the shoot diameter as compared to control i.e., 9.7% and 5.8% after 30 and 60 days respectively. Applied GA₃ + kinetin showed an expansion in diameter up to 15.3% after 30 days and 11.7% after 60 days. The mixed dose of IAA + kinetin proved to be the most effective thus increasing the diameter up to 27.41% after 30 days and 23.4% after 60 days (Fig. 1b). The concentration of all three hormones i.e., GA₃ + IAA + kinetin showed an increase in diameter.

The numbers of internodes observed in control plants were 13.6 after 30 days and 17.4 after 60 days. The GA₃ treated plants showed an increase in the number of internodes i.e. 21.6 after 30 days and 25.4 after 60 days. This may have been due to the increase in length. The extraneous IAA as well as kinetin showed no significant increase or decrease in the number of internodes thus showing no effect of the above mentioned hormones (Fig. 1c). In the mixed doses of GA₃ + IAA, an increase in the number of internodes was observed i.e., 18.2 and 22.0 after 30 and 60 days respectively. Similarly GA₃ + kinetin also showed increase in the number of internodes which being 16.4 and 18.8 after 30 and 60 days, respectively as compared to control. The IAA + kinetin showed an inhibition in the number of internodes i.e., 11.0 and 15.2 after 30 and 60 days, respectively when GA₃ + IAA + kinetin were applied. There was an increase in the number of internodes which being 17.4 after 30 days and 20.4 after 60 days as compared to control (Fig. 1c).

The number of compound leaves counted after 30 days showed that GA₃ was more effective in increasing the number of compound leaves i.e., 22.6 after 30 days (Fig. 2a) as compared to control. Similarly, IAA showed more number of compound leaves which being 11.4 after 30 days. Kinetin showed insignificant increase in the number of compound leaves as compared to control. In mixed dose of GA₃ + IAA and GA₃ + kinetin increase in the number of compound leaves was observed i.e., 17.9 and 16.2 after 30 days in comparison to control. Similarly, IAA + kinetin showed more number of compound leaves i.e., 14.4 after 30 days when compared with control. When all three hormones were applied i.e., GA₃ + IAA + kinetin, there was an increase in the number of compound leaves i.e., 19.4 after 30 days as compared to control (Fig. 2a).

The average increase in the area of first five leaves was recorded in treated plants. Narrowing of leaves was observed with 500 mg L⁻¹ GA₃ i.e., 13.9% as compared to control after 30 days (Fig. 2b). After 60 days first 6-9 leaves abscised and no observation was carried out. In contrast IAA applied @ 500 mg L⁻¹ showed an increase in the area i.e., 17.16% over the control. Similarly, applied 500 mg L⁻¹ kinetin also showed an increase which being 14.72% after 30 days, however, leaves abscised after 60 days. The combined dose of GA₃ + IAA showed a decrease in the area i.e., 7.34% after 30 days. Likewise an insignificant decrease was observed in GA₃ + kinetin in comparison to control which being 9.91% after 30 days. IAA + kinetin showed an increase in the area i.e., 18.68% after 30 days (Fig. 2b). Thus maximum expansion was observed in this dose. Applied GA₃ + IAA + kinetin showed an increase upto 17.24% after 30 days as compared to control.

Number of floral buds were observed in control as well as in treated plants. In control

the first floral bud appeared on 45th day and the number of floral bud was 1.4 after 60 days (Fig. 2 c). In GA₃ the first floral bud appeared on 42nd day, which shows early flowering as compared to control. The number of floral buds increased up to 1.8 after 60 days in comparison to control. In IAA the first flower bud appeared on 47th day, which reveals late flowering. It showed maximum number of floral buds i.e. 4.2 after 60 days as compared to control. Similarly, in kinetin first flower bud appeared on 46th day and there was no significant decrease or increase in number of floral buds after 60 days as compared to control.

In the mixed doses of GA₃+ IAA and GA₃ kinetin first floral bud appeared on 44th days thus showing early flowering. However, the decrease in the number of floral buds was observed. In IAA + kinetin late flowering was observed, as the first floral bud appeared on 47th day (Fig. 2c). The number of floral buds increased after 60 days i.e., 2.6 as compared to control (Fig. 2d). In contrast early flowering was initiated in GA₃ + IAA + kinetin and first floral bud appeared on 43rd day. There was no increase / decrease in the number of floral buds over the control (Fig. 2d).

In control plants the number of branches recorded were 2 after 30 days. However, GA₃ revealed no branching after 30 days i.e., a single main branch was only present. In 500 mg L⁻¹ IAA the number of branches increased up to 4 after 30 days. Kinetin showed no increase or decrease in the number of branches as compared to control. In mixed dose of GA₃ + IAA GA₃ + kinetin branching was delayed and only one branch was observed. On the other hand IAA + kinetin had more number of branches i.e., 4 after 30 days as compared to control. The combined effect of GA₃ + IAA + kinetin showed insignificant increase as compared to control.

Discussion

Plant hormones exert far reaching effects on plant growth, the precise action depending on the concentrations of the substances present and the sensitivity of the organ concerned. The shoot of lentil showed marked increase in length with applied GA₃. Similar results were observed by Chaudhary (1995 and 1997) and Modesto *et al.*, (1999). The increase in length was accompanied by inhibition in the diameter. Allospp (1965) is of the view that the increase in length with GA₃ decreased the concentration of available sugars, which lead to the narrowing of diameter of shoot. Furthermore, Chaudhry & Zahur (1992) working on *Abelmoschus esculentus* L., and Chaudhry & Khan (2000) working on *Cicer arietinum*. reported similar effects. Increase in number of internodes were also observed in a number of crops (Koukourikou & Porlings, 1997; Hernadez, 1997; Bagatharia & Chanda, 1998). The present observations are in harmony with the result of these workers.

Applied IAA showed decrease in length of shoot after 30 and 60 days. It was significantly less than GA₃ treatments. The decrease in length with IAA was earlier reported by Pilot & Saugy (1985). Lee *et al.*, (2000) working on *Zinnia* cultures reported that IAA causes increase in length. However, the present observations do not agree with these workers. The decrease in length was accompanied by expansion in diameter of shoot.

When Kinetin was applied there was an inhibition in the length of shoot accompanied by the expansion in diameter. Cytokinins promote growth by swelling rather than elongation in soybean (Fatima & Bano, 1998). Zadoo (1986) confirmed that cytokinin induced expansion of growth in hypocotyl segments of morning glory and inhibited the extension growth. Kinetin revealed no increase or decrease in the number of internodes thus showing no effect

on the initiation of internodes.

The mixed dose of GA₃ + IAA revealed significant results in the length of shoot as well as expansion in the diameter. The increase in length showed the dominant effect of GA₃. Similar observations were made on okra by Chaudhry & Zahur (1992). One significant observation was the increase in the number of internodes. The combined dose of GA₃ + kinetin showed dramatic increase in length of shoot accompanied by the expansion in diameter as well as increased number of internodes. Kabar (1997) reported that GA₃ alone and GA₃ + kinetin are equally effective. In the present work GA₃ alone showed inhibition in diameter as compared to control. Applied mixed dose IAA + kinetin showed inhibition in the length accompanied by marked increase in diameter. The combined effect of GA₃ + IAA + kinetin caused increase in length along with expansion in the diameter of shoot. This further proves the effect of GA₃ on extension growth because IAA and kinetin both inhibit extension growth. It was reported earlier that GA₃ can modify plant growth through an increase in volume of individual cells. The increase in length may be attributed to the above cause. The number of internodes also increased when compared with control.

Number of compound leaves were more in treated plants as compared to control. Application of GA₃ showed remarkable increase in the number of compound leaves. Furthermore leaves treated with GA₃ were light green, however, IAA treatment showed healthy lush green leaves with increase in the number of compound leaves. Qadeer (1996) reported similar results on wheat seedlings.

Applied kinetin showed increase in the number of leaves, which were dark green. Stetler & Laetsch (1965) reported that cytokinin stimulated the conversion of proplastid into chloroplast with grana, thus giving lush green colour to the leaves. The mixed dose of GA₃ + IAA and GA₃ + kinetin showed increase in the number of leaves. Moreover, they were light green. In the dose of IAA + kinetin, a significant increase was observed in the number of compound leaves, the colour of leaves was bright green. When all the three hormones were applied simultaneously they showed more compound leaves which were green as compared to control. The increase may be due to cell division and enhancing activity of apical meristem which may be promoted by the growth hormones.

The area of first leaves showed average decrease with applied GA₃, whereas applied IAA increased the area of leaves. Tuominen *et al.*, (1997) observed similar increase in leaf area with IAA. The dose of kinetin caused an increase in leaf area.

The dose of GA₃ + IAA showed an increase in the area of leaves, but the increase was less than IAA alone. The mixed dose of GA₃ + kinetin also showed increase in the area of leaves. The mixed dose of IAA + kinetin revealed significant increase in area of leaves. Chattapadhyay & Ghosh (1980) observed that the fast rate of leaf growth may be due to the formation of hormones as by product in leaves, which may lead to the expansion of leaves. The combined effect of three hormones i.e., GA₃ + IAA + kinetin showed an increase in the area of leaves as compared to control.

Applied exogenous GA₃ showed early flowering. Early flowering was accompanied by more number of flower buds. GA₃ had stimulatory effect on floral stem length and number of flowers in rice Awan *et al.*, (1999) and *Lilium* Lee *et al.*, (1999). Application of IAA showed no delay in flowering. In applied kinetin, there was no delay in flowering time as well as no significant increase in flower buds was observed.

In the mixed dose of GA₃ + IAA and GA₃ + kinetin early flowering was initiated,

however, decrease in the number of flower buds was observed. IAA + kinetin showed insignificant delay in flowering. Early flowering with decrease in number of flower buds was observed in the combined dose of GA₃ + IAA + kinetin.

Application of GA₃ showed a single main branch after 30 days. More number of branches were observed in IAA treatment as compared to control. Applied kinetin induced more branching in lentil Malik *et al.*, (1992) observed multiple shoot formation by applying cytokinin in *Pisum sativum*. The mixed dose of GA₃ + IAA and GA₃ + kinetin showed decrease in the number of branches. However, IAA + kinetin exhibited more number of branches. When all the three hormones were applied, a significant decrease was observed in the number of branches as compared to control.

References

- Ashraf, M.Y., N.A. Baig and F. Baig. 1989. Response of wheat (*Triticum aestivum* L.). Treated with cycocel under water stress conditions. *Acta Agron. Hung.*, 38(3-4): 265-269.
- Ashraf, M.Y., N.A. Baig and S.M. Alam. 1987. The influence of chlormequat on growth of raya (*Brassica juncea*). *Pak. J. Bot.*, 19(2): 259-262.
- Awan, I.U., M.S. Baloch, N.S. Sadozai, and M.Z. Sulemani. 1999. Stimulatory effect of GA₃ and IAA on ripening process, kernel development and quality of rice. *Pak. J. Biol. Sci.*, 2(2): 410-412.
- Bagatharia, S.B and S.V. Chanda. 1998. Modification of cell wall polysaccharides during cell elongation in *Phaseolus vulgaris* hypocotyls. *Acta Physiol. Plant.*, 20(1):15-18.
- Chatta-Padhyay, P.K. and S.P. Ghosh. 1980. Studies on the internal structure of lemon leaves as influenced by plant growth substance. *Curr. Sci.*, 49: 60-61.
- Chaudhry, N.Y. 1997 Effects of growth regulators i.e., IAA and GA₃ on petiole and leaves of *Abelmoschus esculentus* L. *Acta Sci.*, 7(1):91-102.
- Chaudhry, N.Y. and M.S. Zahur. 1992. Effect of growth regulators i.e., IAA and GA₃ on *Abelmoschus esculentus* L. internal structure of hypocotyls and stem internodes. *Biol. Sci.*, 37(2): 217-244.
- Chudhary, N.Y. and A. Khan. 2000. Effect of growth hormones i.e., A₃, IAA and kinetin on shoot of *Cicer arietinum* L. *Pak J. Biol. Sci.*, 3(8): 1263-1266.
- Fatima, Z. and A. Bano. 1998. Effect of seed treatment with growth hormones and *Rhizobium* on the oil contents, nitrogen fixation and yield of soybean. *Pak. J. Bot.*, 30(1): 83-86.
- Hernandez, P. 1997. Morphogenesis in sunflower as affected by exogenous application of plant growth regulators. *Agriscientia*, 13:3-11.
- Kabar, K. 1990. Comparison of kinetin and gibberellic acid effects on seed germination under saline conditions. *Phyton* (Horn., Austria), 30(2): 291-298.
- 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.
- Kaul, K. and S. Farooq. 1994. Kinetin induced changes in extension growth activity of some enzymes in morning glory hypocotyls segments. *Indian J. Plant Physiol.*, 4: 214-216.
- Kaur, S., A.K. Gupta and N. Kaur. 1998. Gibberellic acids and kinetin partially reverse the effect of water stress on germination and seedling growth in chick pea. *Plant Growth Regul.*, 25(1):29-33.
- Lee, J., K. T. Joung, K. H. Hayain and L. S. Hee. 1999. Effect of chilling and growth regulators in seedling stage on flowering of *Lilium formolongi*. *Hangut Wanye Hakcheochi.*, 40 (2): 248-252.
- Lee, S., B.J. Woffenden, E. Beers, P. Roberts and W. Alison. 2000. Expansion of cultured Zinnia mesophyll cells in response to hormones and light. *Physiol. Plant.*, 108: (2): 216-222.
- Malik, K.A. and P.K. Saxena. 1992. Thidiazuran induces high frequency shoot regeneration in chick pea and lentil. *Aust. J. Plant Physiol.*, 19: 6731-740.

- Makarova, R.V., E.P. Baes, F. Martinish, P. Sanches and K. Ranavira. 1988. The action of 6-benzylaminopurine on the growth of soybean cotyledons and hypocotyls. *Biol. Nauki. (Mosc.)*, 5: 81-84.
- Pilot, P.E. and M. Saugy. 1985. Effect of applied and endogenous IAA and maize root growth. *Planta*. 164: 254-258.
- Qadeer A. 1996. Effect of IAA, and GA₃ on growth of wheat seedlings. *Sci. Int. (Lahore)*, 8(4): 369-372.
- Ritenour, M.A., E.G. Sutter, D.M. William and M.E. Saltveit. 1996. IAA content and auxiliary bud development in relation to russet spotting in harvested Iceberg lettuce. *J. A. Soci. Hort. Sci.*, 121(3): 543-547.
- Stetler, D.A. and W.M. Laetsch. 1965. Kinetin induced chloroplast maturation in cultures of tobacco tissues. *Sciences*, 149: 1387-1388.
- Touminen, H., L. Puech, S. Fink and B. Sundberg. 1997. A radial concentration gradient of indole-3-acetic acid is related to secondary xylem development in Hybrid Aspen. *Plant Physiol.*, 115(2): 557-585.
- Zadoo, K. 1986. *Effect of some plant growth regulators on the growth and metabolism of isolated cotyledons and hypocotyl segments*. Ph.D. Thesis, Kashmir University, Srinagar, Kaskmir.

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