LEAF AND NODULE SENESCENCE AND THE ROLE OF GROWTH REGULATORS IN CHICKPEA

By

SAHAR ALI JEBREEL ABU EQAB
(M.sc Biology)

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degree of Doctor of Philosophy

Department of Biological Sciences,
Quaid-i-Azam University,
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DEDICATED

To

My loving and dearest father and husband

DECLARATION

I hereby declare that the work presented in the following thesis is my own effort, except where otherwise acknowledged, and that the thesis is my own composition. No part of this thesis has previously been presented for any other degree.

Sahar Ali Abu Eqab

March- 2002

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CERTIFICATE

This thesis submitted by Sahar Ali Abu Eqab is accepted in its present form by the Department of Biological Sciences, Quaid-i- Azam University, Islamabad, as satisfying the requirements for the degree of Doctor of Philosophy in Biological sciences (Plant Physiology).

Supervisor ----------

Co-Supervisor
(NARC, Islamabad) ----------

External Examiner ----------

External Examiner ----------

Chairman ----------

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ABSTRACT

The present investigation was aimed at determining the effect of kinetin and Abscisic acid (ABA) on leaf and nodule senescence in chickpea (*Cicer arietinum* (L.)) cv. CM88. The changes in the endogenous level of ABA and indole-3-acetic acid (IAA) have also been determined. The seeds were soaked in aqueous solution of kinetin and Abscisic acid each at 10^{-5}M for 6h prior to sowing. Both the hormones were also applied during the vegetative phase between 1000 h-1200 h as foliar spray at 10^{-6}M. The plants were allowed to grow under natural conditions and the experiments were repeated for three consecutive years. The effects of the hormones were studied on young expanded leaves and fully expanded mature leaves at four developmental stages; vegetative stage, flowering stage, early pod filling stage and late pod filling stage. Increase in protein content in plant leaves coincided with the increase in nodule activity at both the flowering and early pod filling stages, after which senescence of the leaves became more evident. Degradation of chlorophyll and protein content become more pronounced in old leaves as compared to young leaves. Kinetin at 10^{-5}M was more effective treatment than kinetin at 10^{-6}M in delaying leaf and nodule senescence by its stimulatory effect on chlorophyll, protein and sugar content of young leaves and by increasing diameter of pink bacteroid tissue, nodule weight and nitrogenase activity per mm pink bacteroid tissue of the nodules at both the flowering and early pod filling stages. Both of the concentrations of ABA (10^{-5}M and 10^{-6}M) were effective in enhancing leaf and nodule senescence by degradation of chlorophyll, protein and sugar content of young leaves, and by decreasing diameter of

pink bacteroid tissue, nodule weight and nitrogenase activity of the nodules at both the flowering and early pod filling stages. Both the treatments of ABA caused early maturity resulting in significant decrease in plant growth and yield as compared to the control. The endogenous level of ABA was higher at late pod filling stage than that of the vegetative stage, whereas exogenous application of kinetin decreased the endogenous level of ABA in the treated plants. The IAA content of the root, increased significantly following kinetin treatments at the early pod filling stage. The effect of ABA in enhancement of leaf and nodule senescence is possibly mediated by the increased proline content of leaves as well as the antagonistic effect of ABA on the content of growth promoting hormones as IAA and kinetin. Nodule senescence appeared to be associated with leaf senescence and the former is appears to be correlated with the diameter of pink bacteroids tissue.

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Sahar Ali Abu Eqab

LIST OF ABBREVIATIONS

%	Percentage
<	Less than
>	More than
µl	Micro liter
ABA	Abscisic acid
ANOVA	Analysis of variance
ARA	Acetylene reduction activity
ATP	Adensine-5'-triphosphate
BA	Benzyl adenine
BHT	Butylated hydroxy toluene
BSA	Bovine serum albumin
C	Carbon
CK	Cytokinin
cpm	Count per minute
cv	Cultivar
DLS	Delay leaf senescence
DMRT	Duncan's multiple range test
DOA	Sodium deoxycholate
DW	Dry weight
FW	Fresh weight
g	gram
GA	Gibberellin

HPLC	High pressure liquid chromatography
IAA	Indole-3- acetic acid
K	Potassium
M	Molar
Max	Maximum
mg	milligram
Min	Minimum
ml	milliliter
mM	millimolar
mmol	millimole
mol	mole
mRNA	messenger RNA
N	Nitrogen
NARC	National Agricultural Research Center
°C	Degree centigrade
P	Phosphorus
P_5C	$<^{-1}$ Pyrroline-5 carboxylate
P_5CR	$<^{-1}$ Pyrroline-5 carboxylate reductase
P_5Cs	$<^{-1}$ Pyrroline-5 carboxylate synthetase
RFE	Rotatory thin film evaporator
RH	Relative humidity
rpm	Revolutions per minute
SDS	Sodium dodecyl sulphate
TBq	Tera Becquerel
TCA	Trichloroacitic acid

v/v	Volume over volume
w/v	Weight over volume
WANA	West Asia and North Africa
WAS	Weeks after sowing
Ψp	Water potential
Z	Zeatin

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INTRODUCTION

1.1 Chickpea:

Legumes have played a crucial role in agricultural production throughout history. These maintain soil fertility, particularly in dry land due to their capacity to fix nitrogen in association with *Rhizobia* by using solar energy collected through plant photosynthesis (Suzuki and Konno, 1982). A key strategy for improving production and maintaining soil fertility in the region of West Asia and North Africa (WANA) has been to encourage farmers to retain the traditional pulse legumes, chickpea and lentil as a basis for rotational cropping systems. Strategies aimed at conserving N and P involve internal remobilization of N and P, decreased growth rate (Schactman *et al.*, 1998; Ragothama, 1999), more growth per unit N or P taken up, and modified carbon/ nitrogen metabolism (Plaxton and Carswell, 1999). Legumes are ideal for crop management schemes that aim at enhancing sustainability and buffering against dependence on N and P fertilizes (Vance, 2001).

Chickpea is an annual winter legume that belongs to the family Leguminoceae, tribe ciceraea. It is a very good source of protein and carbohydrate, which constitute 80% of the total seed dry weight. The crude protein varies from 17-24%. It is also rich in minerals like calcium, iron and vitamins. that are essential elements of human diet. Moreover, the availability of iron as an essential element of human diet is 90 % (Crown *et al.*, 1967). Being one of the most important pulse crop in Pakistan, it is annually grown on an acreage of about 1.1 million hectares with a production of 0.7 million tonnes (Agric. Stat. of Pak., 1998/1999). Chickpea is also the most important pulse crops of dry land agriculture of the world and is widely

grown in Asia, Middle East, Africa and South and Central America (Nene, 1982; FAO, 1987). In the world, it is cultivated on an acreage of more than 10.4 million and is mainly grown in tropical regions of the world such as Turkey, Syria, Bangladesh, Pakistan, India, Sudan, and Burma and partly in regions of western world such as Australia and Canada. In Asia 13 countries grow chickpea, which account for 91% of the global area and 96% of world production.

In addition to providing high protein food and feed, Chickpea as a legume is reported to improve soil structure and stability, by increasing the content of soil organic matter (Greenland, 1971; Soon and Arshad, 1996). It also influences exchangeable N, P, and K in the soil. Exchangeable K decreases by cropping system that is rich in legume crop (Soon and Arshad, 1996). Leaf and nodule senescence is the basic factor that affects the productivity of plants and soil fertility. Leaf senescence of leaves of chickpea is of basic physiological interest and provides insight into such correlative controls, that govern the terminal phase of the plant development to retain the photosynthetically active leaves throughout fruit development. It is valuable in reducing the C deficiency that accompanies senescence.

Plant responses to environmental stimuli are integrated in the endogenous developmental programmes by a complex network, which is characterized by extensive ramification and redundancy (Hare *et al.*, 1999). Also plant hormones are critical factors in a complex molecular process responsible for directing the early development and fate of floral tissues (Metzger, 1987; Nooden 1988a). Where the concentration of hormones may be regulated by translocation, synthesis and release from bound forms and/or inactivation occurs through conjugation (Cohen and Bandurski, 1982).

The regulation of plant morphogenesis depends on environmental factors such as light, temperature and nutrient supply, the concentration and interaction of endogenous plant hormones and upon changing sensitivity of the tissue to these substances (Grossmann *et al.*, 1987). Grossmann and coworkers assumed that the strong manipulation of vegetative growth reflects a distinct effect on phytohormone level in the various parts of plants possibly as a cause for the growth regulation or as a side effect of the action of the growth retardants.

1.2 Aims and Objectives:

The present investigation was aimed at studying (1) the role of kinetin and Abscisic acid in leaf senescence, and in causing physiological and biochemical changes if any, during growth and maturation phases of chickpea plants. (2) physiological relationship between leaf and nodule senescence, as nodule growth and functioning is dependent on assimilates derived from leaves, and (3) changes in endogenous level of ABA in plant leaves; ABA being an important hormone in leaf and nodule senescence.

1.3 Leaf Senescence:

In common with all multi-cellular organisms, higher plants are mortal and the life of the individual plants is ultimately terminated by death. However, it is considered likely that prior to the death of the whole plant may have been earlier death of cells, tissues and a number of its organs (Thimann, 1980; Smart, 1994). As a result of the activity of the apical meristem, the upper part of the shoot shows prolonged embryonic conditions while at the same time senescence and death is occurring in the older lateral organs, notably in the leaves of *Phaseolus vulgaris* (Hensel *et al.*, 1993), parts of flowers and fruits (Miyoji, 1986).

Greening and senescence normally occur at opposite ends of the life span of the plant tissue (Sinclair, 1989; Chory *et al.*, 1994; Kusnetsov *et al.*, 1994). The combined action of several internal and external signals may be involved in the induction of senescence (Wollaston, 1997).

According to Samet and Sinclair (1980) leaf senescence is defined as the period after full expansion when declines in physiological activity can be observed thus, the decline in photosynthesis that comes with advancing of leaves age should be considered a part of the leaf senescence process, especially as this reduction might influence ultimate yield. Leaf area is reduced during leaf senescence and abscission (Turk *et al.*, 1980) accompanied by reduction of protein and chlorophyll content (Atkins *et al.*, 1984)

Available evidence suggests that leaf senescence is initiated at the time of full-leaf expansion and that subsequent course of senescence may be regulated by various metabolic and environmental conditions imposed on the plant measured as the rate of net exchange in dry matter accumulation (Brandner *et al.*, 1984a; Ticha *et al.*, 1985).

Based on the seasonal profile, chlorophyll and acetylene reduction activity are the limitations of senescence. Senescence seems to occur at the approximate time when chlorophyll concentration is higher in the upper canopy than in the lower canopy of leaves (Brandner *et al.*, 1984a). In addition a reduction in C assimilation rate occurs at a time when seed sink requirements are greatest as has been demonstrated for soybean (Whittenbach *et al.*, 1980, Okaton *et al.*, 1981, Teaney and Fuhrmann, 1992), *Festoca protensis* (Thomas, 1983) and red clover (Jacquard *et al.*, 1987).

1.4 The Signals of Senescence:

In many annual crops, leaf senescence during reproductive development leads to death of the parent plant following a single reproductive phase, and this has been termed as monocarpic senescence (Nooden, 1980; Nooden, 1988a; Nooden and Guiamet, 1989). The number of leaves that senesce during the reproductive phase is related to the number of fruit left to mature on the plant (Lindoo and Nooden, 1977). This relationship suggests that changes in the ratio of photosynthetic source size to reproductive sink may influence the course of monocarpic senescence (Sinclair and DeWit, 1975).

Senescence has been judged by changes in metabolites, levels of dry matter accumulation and by visual observation (Whittenback *et al.*, 1980, Okaton *et al.*, 1981, Steven *et al.*, 1984). It involves degradation of proteins, chlorophyll, nucleic acid and subsequent transport of some of the degradation products to other parts of the plant (Nooden, 1988a and Wollaston, 1997). There is also proteolysis and nucleic acid hydrolysis leading to redistribution of nitrogen and phosphorus from the degraded products to the developing organs (Wollaston, 1997 and Gan and Amasino, 1997). In

the bean plant, it has been suggested that senescence signals are transported basipetally (Imre *et al.*, 1981). In *Vigna unguiculata* L., carbohydrates are depleted in the stems of senescent plants, while starch and sucrose accumulate in stems of delay leaf senescence (DLS) plants, and total seed yield is higher as compared to the leaf senescent genotypes (Gwathmey *et al.*, 1992a).

1.5 Factors Affecting Plant Growth:

Senescence can be induced by environmental stresses. Endogenous factors, including leaf age and reproductive development, also trigger senescence (Smart, 1994 and Gan and Amasino, 1997). Partitioning of assimilated carbon among sink organs is a critical factor that controls the rate and pattern of plant growth (Geiger *et al.*, 1996). The differences in total proteins recovered at maturity in the seed, pod and stem, in general, reflect the differences in total plant dry matter. Where N demand for seed production in several legumes cannot be met by N uptake alone, the remaining N has to be obtained from vegetative tissues (Sinclair and DeWit, 1975 and Sanetra *et al.*, 1998).

Mineral nutrients taken up by the root are, as a rule, transported through the xylem to the shoot, and photoassimilates are transported in the phloem to the roots. According to the Thornby model of photosynthetic partitioning, nutrient deficiencies should favor photosynthetic partitioning to the roots (Marschner *et al.*, 1996). However, a stage is reached when the growth rate of the pods exactly equals that of the total tops. At this stage all of the increase dry matter in the tops is accounted for by the increase in pod dry matter, and there is no net storage of the assimilate in the vegetative components of the tops. Subsequent to this stage, pod growth rate exceeds the growth rate of the total tops, thus indicating mobilization and translocation of

previously stored assimilates from other plant parts into the pods (Lawn and Brun, 1974 and Grover *et al.*, 1985). Wollaston (1997) has reported that senescence is a complex and highly regulated development phase in the life of a leaf that results in the coordinated degradation of macromolecules and the subsequent mobilization of components to other parts of the plant. Leaf dry matter reaches a maximum at early pod development and then decreases rapidly due to leaf abscission and carbon translocation as has been noted in pigeon pea (Sanetra *et al.*, 1998).

1.5.1 Protein Content:

Staswick (1989) has reported that protein stored in soybean leaves can be rapidly synthesized or degraded according to the need for nutrients by other plant tissues. However, the mobilization of nitrogen involves degradation of proteins, since most of the nitrogen in leaves is in the form of soluble proteins (Grover *et al.*, 1985). The leaf senescence is assessed on the basis of loss in chlorophyll and protein as a result of drainage of leaf nitrogen and, perhaps, other nutrients that begin before leaf yellowing (Samet and Sinclair *et al.*, 1980). Proteins decline during senescence and recover during re-greening, most strongly in cytokinin application (Mancera *et al.*, 1999). Genes encoding degradative enzymes such as proteases and nucleases enzymes involved in lipid and carbohydrate metabolism and nitrogen mobilization have all been among the most detectable senescence-induced genes (Smart, 1994 and Wollaston, 1997), and during re-greening, protease activity declines and total protein level recovers (Mancera *et al.*, 1999). Where ABA has been shown to inhibit protein synthesis (Fam *et al.*, 1973 and Rock and Quatrano, 1996), regardless of the mechanisms through which plant growth regulators act, changes in cell wall extensibility might reasonably be expected to involve changes in protein synthesis

(Bensen *et al.*, 1988). In *Spirodela polyrrhiza* L. ABA rapidly induces elevated levels of mRNA transcript encoding a novel basic peroxidase (Chaloupkova and Smart, 1994).

1.5.2 Proline Content:

Proline is one of the free amino acids that shows the largest increase under stress (Qadar *et al.*, 1980 and Badapati *et al.*, 1992). The level of proline in the plant is regulated by proline synthesis. Proline is synthesized from L-glutamic acid as a precursor (Chauhan *et al.*, 1980 and Hare and Cress, 1997) via Δ'-pyrroline-5-carboxylate (P_5C) by two enzymes, P_5C synthetase (P_5Cs) and P_5C reductase (P_5CR). The other pathway is from ornithine, which predominates at high levels of available nitrogen (Delauney *and* Verma, 1993). Another important factor that controls the level of proline in plants during degradation or metabolism of proline is that it is oxidized to P_5C in plant mitochondria by proline dehydrogenase (Oxidase) and P_5C is converted to L-Glu by P_5C dehydrogenase (Elthon and Stewart, 1981). Such oxidation of proline is inhibited during the accumulation of proline under water stress and is activated in rehydrated plants (Stewart and Voetberg, 1985; Stewart et *al.*, 1986).

The roles of proline synthetic and degradation enzymes in stress-related responses must be superimposed upon the continuous requirements of plants for protein synthesis during growth (Zhang *et al.*, 1997a). The accumulation of proline in dehydrated plants is caused both by activation of biosynthesis of proline and by inactivation of the degradation of proteins. Traditionally, stress physiologists have considered proline to be a compatible solute, which stabilizes subcellular structures and attenuates water loss under hyperosmotic conditions even in the absence of stress

(Hare and Cress, 1997). Proline is thought to play an important protective role in plants subjected to several stresses (Hare *et al.*, 1999), including frost (Dörffling *et al.*, 1990 and Delauney and Verma, 1993), drought and salt stress (Kishor *et al.*, 1995, Hare and Cress, 1997 and Hare *et al.*, 1998). Several environmental stresses are also associated with proline-accumulation (Hare *et al.*, 1997). Moreover, it has been demonstrated that proline acts as an osmoprotectant and that overproduction of proline results in transgenic tobacco plants in increased tolerance to osmotic stress (Yoshiba *et al.*, 1997) through rehydration of protoplasm (Chauhan *et al.*, 1980). It also acts as a storage compound for carbon and nitrogen during moisture stress when starch and protein synthesis are inhibited. It has also been advocated that proline may function as a source of solute for intracellular osmotic adjustments (Flowers *et al.*, 1977). Considerable circumstantial evidence supports an important role for proline synthesis in regulating several physiological responses, including developmental transitions, even in the absence of stress (Hare and Cress, 1997). It has been suggested that sucrose positively affects proline accumulation. High level of sugar in leaves keeps proline high (Beevers, 1976b and Zhang *et al.*, 1995). Much circumstantial evidence implicates the importance of integrating proline synthesis with the energy assimilating capacity of the plant. A primary limitation to foul elucidation of the process that regulates free proline levels under both optimal and stressful conditions; is the realization that, the multitude of signals which regulate plant growth and development are not transduced via linear pathway operating in parallel.

1.5.3 Sugar Content:

Photoassimilates are transported from the sieve element to the recipient sink cells principally in the form of sucrose (Patrick and Offler, 1996). Sucrose is the

major export and storage form of photoassimilates in leaves (Sheem, 1994 and Koch, 1996). Several lines of evidence indicate that partitioning of photosynthesis between starch and sucrose is influenced by the relative concentration of inorganic phosphate (pi) in the cytosol and chloroplast (Steven and Brandner, 1992). The production and accumulation of sucrose within leaves determines the availability of carbon for export from the leaf (Foyer and Galtier, 1996 and Prioul, 1996). The rate of sucrose synthesis can also affect the rate of photosynthesis (Stitt, 1986).

In *Arabidopsis thaliana*, glucose is more effective in stimulating NO_3 uptake by intact soybean plants (Delhon *et al.*, 1996). Photosynthetic carbon fixation acts as a precursor required for amino acid biosynthesis or respiration to provide energy (Lewis *et al.*, 2000). The mobilization of nitrogen involves degradation of carbon in the form of sugars from leaves and it is a common feature and is not associated with senescence (Grover *et al.*, 1985). In *G. max*, carbohydrate supply to the nodules and the capacity of the plant tops to act as a sink for combined nitrogen; is simultaneously reduced at pod filling stage, while depletion of carbohydrates from stems is also occurring during pod fill (Streeter, 1972, Jeffrey *et al.*, 1984 and Brandner *et al.*, 1984a). Most of the $NH4^+$ excreted from mature bacteroids is assimilated into amino acids or ureides and is exported to the other parts of the plant resulting in increase in N level in the nodule and plant sap (Streeter, 1992).

The source-limited condition may lead to insufficient carbohydrate supply for maintenance of vegetative systems and to senescence of the whole plant.

Photosynthesis is inhibited when the production of photosynthesis exceeds the rate of utilization and carbohydrates accumulate in the leaves of *Arabidopsis thaliana* (Krapp *et al.*, 1991, 1993).

Duncan *et al.* (1981) have found that non-senescent sorghum maintains consistently higher basal stem sugar concentration from anthesis to maturity than the stems of delay leaf senescence (DLS) genotype of cowpea that exhibit delayed leaf senescence, while these carbohydrates are depleted from stems of senescence genotype (Gwathmey *et al.*, 1992b). A source-limited condition due to depletion of carbohydrate and sugar from stem of intact soybean during pod fill may lead to insufficient carbohydrate supply for maintenance of vegetative system and to senescence of the whole plant during mid pod set, which suggests that the supply of photoassimilate exceeds the combined demand during process of growth and maintenance of soybean (Brandner *et al.*, 1984a and Gwathmey *et al.*, 1992b). It is reasonable to expect that, delay in senescence and/or promotion in leaf production at the expense of flower production will increase yield perhaps by increasing crop longevity in cowpea (Gwathmey *et al.*, 1992a). This provided that the retained leaves do not lose more carbon by respiration than they gain by photosynthesis (Wilson, 1981).

1.5.4 Nutrients:

Even though N is among the most abundant elements on earth, it is the critical limiting element for the growth of most of the plants due to its unavailability (Smil, 1999, Socolow, 1999 and Garham and Vance, 2000). Production of high quality, protein-rich food is extremely dependent upon availability of sufficient N. Phosphorus is second only to N as the most limiting element for plant growth (Bieleski, 1973 and Vance *et al.*, 2000). The net photosynthesis, nitrogen assimilation, and phosphorous uptake cease at the same approximate time in soybean (Brandner *et al.*, 1984b), which suggests that a specific stage of pod development is a prerequisite for triggering leaf

senescence, and simple nutrient drain hypothesis would have to include an interaction mechanism depending upon the developing pod and seeds (Grover *et al.*, 1985). In annual grain legumes, monocarpic senescence results in the loss of functional leaf area during pod production, which may limit the availability of fixed C to support seed filling. Theoretical evidence indicates that carbon and nitrogen simultaneously limit increase in soybean yield (Sinclair, 1989).

Leaf senescence is accompanied by the remobilization of amino-N from leaf proteins and it's export to the filling seed, which further impairs C assimilation (Sinclair and DeWit, 1976). The rapid loss of assimilatory capacity during fruiting has been suggested as a yield-limiting factor in soybean (Wittenbach *et al.*, 1980). The withdrawal of nutrients (particularly nitrogen) from leaves during reproductive development plays an important role in the process of leaf senescence and has been studied intensively in several legumes-crops in relation to seed nutrient cumulation, biochemical changes in the leaves, or whole plant budgeting in soybean (Molisch, 1928, Sinclair and DeWit, 1975, Lindoo and Nooden 1976, Sesay and Shibles, 1980 and Mauk and Nooden, 1992) and in Chickpea (Hooda *et al.*, 1986 and Hooda, 1990).

In particular, N and P are also remobilized from the leaves during reproductive growth (Hanway and Weber 1971a, 1971b, Brandner *et al.*, 1984a and Dalling, 1985). Remobilization efficiency represents an important feature during the time of decreasing N_2 fixation (Sinclair and DeWit 1975, Nooden *et al.*, 1978 and Rao *et al.*, 1996). Plants dependent on N_2 require more P than plants using mineral N (France, 1977). This need reflects the vital role of P in energy transfer and the large quantity of energy required for the reduction of N_2 to NH_3 (Cassman *et al.*, 1981 and Freine, 1982). Phosphorus and reduced N have a similar remobilization pattern from leaves to pods in soybean (Brandner *et al.*, 1984b).

1.6 Role of Growth Regulators in Plant Development:

Plant growth and development are regulated by hormones, of which auxins are one of the five classical types that include ethylene, gibberellins, (+)- abscisic acid (ABA), and cytokinins (Kenede and Zeevaart, 1997). Phytohormones are implicated not only in the correlative control of plant development but also in environmental effects that are mediated through modulation of internal growth substance levels and distribution within the plant body (Naqvi, *et al.*, 1982). The correlative development of individual plant parts or organs depends on exchange of hormonal signals that may be common for both growth and senescence (Nooden, 1988b).

Physiological events occurring within the plant may initiate changes in the growth regulators, which then may control the speed of development. Evidence suggests that it may not be the concentration of a single hormone that is important in delaying or initiating the senescence process but, rather, the concentration of hormones relative to each other (Samet and Sinclair, 1980). In the soybean, such change is important in leaf senescence (Raschke, 1979 and Jewer and Incoll, 1980). Environmental stress also imbalances the endogenous hormonal level of plants (Azcon *et al.*, 1978).

Cytokinins and auxins generally promote growth processes. ABA has most often been considered as an inhibitory substance (Walton, 1980). Correlations between rates of ABA accumulation in major sinks have been found in pea (Browning, 1980) and bean (Quebedeaux *et al.*, 1976 and Hsu, 1979).

In the soybean, cytokinins affect seed growth by attracting nutrients to the developing fruit or indirectly through protein synthesis by regulation of seed growth or they could stimulate seed growth by triggering cell division (Davey and Van

Staden, 1978, Nesling and Morris 1979 and Hein *et al.*, 1986). Jeschke *et al.* (1997) have reported that it is possible that ABA is a shoot to root signal. Therefore shoot dry weight is slightly decreased by ABA application (Cho and Harper, 1993). ABA-treated plants show reductions in root and shoot dry weight and in leaf area per plant in *Faba beans* (Bano *et al.*, 1983). The fresh weight of root increases significantly following kinetin treatment. While kinetin increases the shoot and root length of lentil, no significant effect of phytohormone is observed on shoot dry weight (Bano and Raza, 1990).

1.6.1 Role of Growth Regulators in Leaf Senescence:

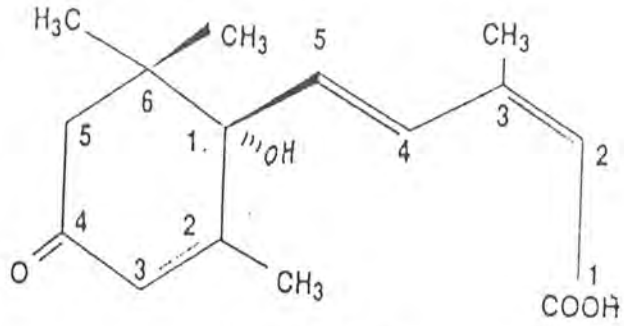
All five known plant hormones or groups of hormones have been implicated in foliar senescence; cytokinins delay senescence in excised leaves of *Xanthium* and many other species (Woolhouse, 1978). Exogenously applied ABA accelerates senescence in leaves of many species and excised leaves of oat, which may be involved in promoting rapid senescence (Gepstein and Thimann, 1980). Gepstein and Thimann have reported that ABA appears to block the translation of the GA-induced message- *in vivo*. Chang and Jacobs (1973) have reported that the reduction in the amount of extractable free IAA from ABA-treated petioles of *Coleus* may play a role in leaf senescence. A wide variety of studies have shown that leaf senescence is usually correlated with a decrease in cytokinin activity levels in the leaves and have implicated roots as the major source of cytokinins in mature leaves (Van Staden *et al.*, 1988). Evidence suggests that plant hormones are involved in leaf senescence, and that changes in hormonal concentrations might regulate the speed of the leaf aging process (Beevers, 1976a). Auxins, gibberellins, CKs, ethylene and ABA have all been shown to be capable of affecting cell wall extensibility (Cleland, 1986).

A decline in cyokinin production by the roots can account for the decrease in foliar cytokinin levels (Nooden and Letham, 1986 and Singh *et al.*, 1988), a fact that reflects that CK plays an important role in monocarpic senescence of soybean. This decline appears to be induced by the pods, where the pods suppress cytokinin production in the roots and inhibit root growth (Nooden, 1988a and Nooden and Guiamet 1989). This occurs quite early at full leaf extension or earlier. This signal may or may not be the same as the senescence-signal which is excreted on the leaves (Nooden *et al.*, 1990).

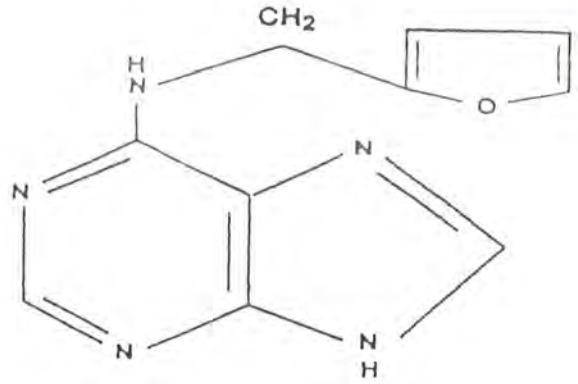
Senescing muskmelon ovaries probably import ABA and export IAA because free ABA accumulates only in detached ovaries (Dunlop and Rodbacker, 1990) that lead to suppression of IAA synthesis and stimulation of the esterification of both ABA and IAA.

1.6.2 Effect of Growth Regulators on Proline Content:

Proline accumulates in response to the applied ABA. Hare and Cress (1997) and Hare *et al.* (1999) have observed that exogenous application of ABA to unstressed plants can induce biochemical changes similar to those observed under stress. Such results have also been observed for barley (Stewart 1980, Stewart and Voetberg, 1985 and McDonnell *et al.*, 1995), pea (Hasson and Mayber, 1983) and *Zea mays* (Dallmier and Stewart, 1992). Treating plants with ABA frequently hardens them against stress. This suggests that ABA may normally be used to control at least certain aspects of acclimation to stress. It plays a role in conserving water, facilitating recovery of plants and its metabolism following stress relief (Naidu *et al.*, 1990). It is possible that ABA is involved in the redistribution of proline in maize root (Kenneth and Stewart, 1992). Such a causal link between ABA and proline accumulation has



Abscisic Acid



Kinetin

Structural formula of ABA and kinetin

been suggested for some but not for all plant species investigated (Xin and Li, 1993 and Savoure *et al.*, 1997). ABA acts by triggering the principal enzyme (P_5Cs) involved in proline biosynthesis (Savoure *et al.*, 1997) or by converging on the promoters of stress-related genes for proline synthesis (Xin and Browse, 1998). It is reduced by CK when applied simultaneously in Faba bean (treated with kinetin alone) and results in the maximum increase in shoot fresh weight and decreased proline content of the seedling (Bano, 1986).

Conflicting evidence regarding a role for exogenously applied cytokinin in inducing proline accumulation has been reviewed by Hare *et al.* (1997). A recent report by Peters *et al.* (1997) may contribute to the resolution of the seemingly paradoxical effects of enhanced cytokinin levels in stress response pathway in the leaves of *Mesembry anthemum* and *Cryrt allinum*. CK induces increases in free proline.

1.6.3 Cytokinin (CK):

Cytokinin can act as a powerful local promoter for transport of assimilations in stems (Takagi *et al.*, 1985) and can also augment the longer-distance effects of IAA (Garrison, 1984). Cytokinins are known to inhibit the activity of IAA oxidase and other peroxidases (Dilworth and Kende, 1974, Minchin and Pate, 1975, Bekki *et al.*, 1987, Garg, 1992 and Garg *et al.*, 1995).

The possible role of roots on cytokinin translocation from roots to shoot has been demonstrated by Sitton *et al.* (1967). Cytokinins are synthesized in the root (Skene, 1975), and have been implicated as root-to-shoot signals (Davies and Zhang, 1991 and Letham, 1994). High concentration of kinetin is needed to control the low receptor concentration in the mature tissue (Trewavas, 1982). Cytokinins are

generally considered to be antagonists of ABA. Kinetin not only totally inhibits the induction of turions by ABA but also alleviates ABA-induced growth inhibition (Chaloupkova and Smart, 1994). Carmi and Koller (1979) have found that the photosynthesis of leaves and the rate of leaf growth are regulated by distribution of factors that are carried from the root to the leaves in the transpiration stream. Seth and Wareing (1965) have suggested that the regulation of photosynthetic capacity and the delay in leaf senescence are controlled by an increased availability of cytokinin, which is transported from root to shoot when the plant reaches its maximum size. It has been reported that CKs can be synthesized in leaves (Carmi and Van Staden, 1983). In mungbean, kinetin-induced hypertrophied growth of the root and arrested leaf senescence, reproductive development and embryogenesis are believed to be regulated by cytokinin. Further more, it has been accepted that because cytokinins stimulate cell division they define in some way the "sink strength" of active meristem and establish relation in the plant (Syono *et al.*, 1976 and Jacqmand *et al.*, 1994). Cytokinins play a major role in the cellular differentiation of the moss protonema because they induce the formation of buds (Cove, 1992 and Reski, 1998).

Chory *et al.* (1994) and Kusnetsov *et al.* (1994, 1998) have reported that a common feature of greening and senescence is the influence of cytokinins, which promotes de-etiolating. Since in *Phaseolus vulgaris*, the stem has a higher CK content than both the leaves and the roots, this suggests that the stem could be an alternative source of CK to the leaves (Carmi and Van Staden, 1983).

1.6.3.1 Cytokinin and Senescnece

Previously, different theories attempted to illustrate the role of CK effect in plant senescence. Itai and Vaadial (1965) reported that spraying with CK prevents symptoms of senescence. Cytokinin also occurs in bean cuttings where a leaf treated with kinetin attracts metabolites from other plant parts. Its deficiency, result in loss of soybean potential to retain nutrients during pod development and leads to senescence (Nooden *et al.*, 1990). High concentration of cytokinin in the developing fruits may similarly direct the flow of assimilates from the leaves to fruits which may have lost some or most of their retention potential (Leopold and Kawase, 1964 and Sitton *et al*., 1967).

In *Glycine max*, foliar application of cytokinin and auxin are almost fully effective against leaf senescence. The total nitrogen and carbohydrate content of the leaves increases significantly during the spraying, which suggest that photosynthesis and N_2 fixation continue at unusually high rates in the plants (Nooden and Guiamet, 1989). Soijima *et al.* (1995) have reported that the conjugated Z is transported from the root to the shoot and causes slowdown of leaf senescence during the ripening stage of rice where, chlorophyll content is significantly correlated with the flux of total cytokinin per plant or per unit leaf area. Other theories have been proposed which would implicate cytokinins directly in the processes of regulation of transcription and translation (Crowell and Amasino, 1994), possibly by cytokinin binding proteins (Brinegar, 1994).

Cytokinin-transgenic technology is available for senescence autoregulation, cytokinin attenuates the promotion of senescence-induced proteases (Gan and Amasino, 1995). Cytokinin can induce re-greening in a range of plant species (Marek and Stewart, 1992). It has, therefore, been proposed that the senescence delaying

effects of this treatment may be due to increased availability of endogenous cytokinins, rather than to changed source-sink relationships (Colbert and Beever, 1981).

Although application of exogenous cytokinins alleviates some or all of the abscission inducing factors (Carlson *et al.*, 1987), application of cytokinins to the foliage for the purpose of preventing abscission has been ineffective (Kinet *et al.*, 1985 and Carlson *et al.*, 1987). Foliar-applied cytokinin analogs may have the potential to increase the number of pod and nodules subtending the treated leaves (Dyer *et al.*, 1987). Abscission of late flowers and pods in the mid to upper part of the canopy can depress cytokinin production by the roots at an early stage (Heindl and Brun, 1984), and this decrease in cytokinin production is required for monocarpic senescence and may then be explained by a decrease in the concentration of CKs in the xylem sap (Carlson *et al.*, 1987 and Nooden *et al.*, 1990).

Cytokinins play a major role in the regulation of pod set in legumes (Crosby *et al.*, 1981 and Clifford, 1981). The probability that a flower would initiate a pod is directly related to the concentration of total cytokinins present in the exudates when the flower opens in soybean (Nesling and Morris, 1979 and Carlson *et al.*, 1987) and in *Phaseolus* (Michael and Ketbitsch, 1972). Application of exogenous cytokinins has been shown to alter reproductive development in ways that could significantly enhance the harvest index of these already commercially valuable pulses (Atkins and Pigeaire, 1993).

Cytokinin appears to be a major senescence-retarding hormone in plants, and its role, particularly in leaves, is important (Van Staden *et al.*, 1988). It has been suggested that the effect of partial defoliation on the remaining leaves is mediated by an accumulation and greater availability of CKs (Skene, 1975, Wang *et al.*, 1977 and

Van Staden and Carmi, 1982) produced in the roots that delays senescence (Woolhouse, 1967), with accumulation of protein and chlorophyll (Carmi and Koller 1978 and Carmi and VanStaden, 1983). In barley, it has been found that kinetin can retard the characteristic loss of protein and pigment during leaf senescence (Cohen *et al.*, 1979).

1.6.4 Abscisic Acid (ABA):

The plant hormone ABA mediates many vital processes in plant growth and development, including seed dormancy, cell division, water use efficiency, and adaptation to drought, salinity and others (Hagenbeek *et al.*, 2000). Despite the complex multitude of data that implicate ABA in stress responses, the pathways that trigger them are largely unknown (Hetherington *et al.*, 1998). ABA signal in xylem stream of plants reflects the water status of the soil in the root zone (Zhang *et al.*, 1997b). It is well documented that ABA is not only synthesized in leaves but also in roots, and root-sourced ABA may play an important role in shoot growth and shoot physiology (Neales *et al.*, 1989). Although ABA is transported basipitally towards the elongation zone (Chanson and Pilet, 1982), Zhang and Davies, (1990) and Hartung and Davies (1991) have reported that, ABA could act as a root-shoot signal in the regulation of stomatal transpiration and cause reduction in growth rate and other mechanisms (Hartung and Davies 1991 and Davies and Zhang, 1991). The mature leaves of *Xanthium* produce ABA that is transported and accumulated in the younger leaves, whereas the youngest leaves are least capable of synthesizing ABA (Zeevart and Boyer, 1984). The combined effect of ABA and leaf senescence defoliates the crop more rapidly and terminally because of the pattern of acropetal leaf senescence. ABA has a potential to antagonize other growth hormones,

including IAA (Greg *et al.*, 1991) and cytokinin (Chaloupkova and Smart, 1994). It appears to be transported into the senescing ovary from an external source and alters the IAA metabolism in such a manner as to suppress the level of free IAA, while stimulating accumulation of the ester IAA in *Cucumis melo* L. (Dunlop and Rodbacker, 1990).

ABA plays a dual role in plant embryo development. Exogenous application of ABA on cotton, soybean and wheat, increases or maintains the expression of specific protein and characteristics of embryo maturation, while it suppresses precocious germination (Eisenberg and Mascarenhas, 1985), enhances water flux through roots (Glinka, 1980) and increases root/shoot ratios (Watis *et al.*, 1981). Applied ABA has been shown to result in stomatal closure (Jones *et al.*, 1984 and Trejo *et al.*, 1993). In soybean, it causes slower growth rates by decreasing ψp and/or by increasing cellular resistance to expansion (Bensen *et al.*, 1988) by reduced leaf growth, decreased hypocotyls growth (Quarrie and Jones, 1977 and Ray and Latoraya, 1984) and dry weight accumulation (Watis *et al.*, 1981 and Biddington and Dearmon, 1982).

ABA appears to be capable of influencing sink strength and photosynthetic accumulation in reproductive structures (Ackerson 1985 and Brenner, 1995). It is well documented that treatment of leaves and whole plants with ABA inhibits photosynthesis (Mawson *et al.*, 1981). Imre and Carol, (1981) have reported that ABA enhanced leaf senescence in bean. The suggestion that ABA may serve as the correlative signal of senescence is based on the rise in endogenous ABA level in senescing leaves (Lindoo and Nooden, 1978). In soybean, Samet and Sinclair (1980) have suggested that ABA concentrations increase significantly at the end of the season when the leaves had start to turn yellow, and after total soluble protein and

chlorophyll had started to decline. Older leaves at lower nodes abscised first show significantly higher ABA levels than leaves from nodes further up on the plants (Samet and Sinclair, 1980) and the concentration of ABA tends to be higher with further advanced degree of leaf yellowing.

Phillips (1971) was the first to demonstrate the inhibitory effect of exogenous ABA on the development of nitrogen fixing root nodules in *Pisum sativum*. The lower leaves of ABA-treated plants show early senescence because the lower leaves are regarded as the active donor of photosynthetic products to the nodules.

1.7 Root Nodulation:

1.7.1 Nodule Initiation and Development:

Under condition of nitrogen limitation, rhizobium bacteroids infect leguminous plants then form root nodules, which is governed by both internal and environmental factors. However, the most economically significant advantage is the symbiotic assimilation with N_2 fixing rhizobia, which provides the crop with N and precludes the need for costly additions of N fertilizer (McNeill *et al.*, 1990).

Organogenesis of symbiotic root nodules involves the induction of multiple developmental processes that are initiated by host-endosymbiont interaction and that results in functional exchange of nitrogen and carbon that supports both the host and the endosymbiont. Both legume and actinorhizal, hosts share common aspects of nodule development, including the infection process and nitrogen assimilatory pathways (Hirsch and LaRue, 1997 and Pawlowski, 1997). In this symbiotic activity, which is a specific process, the bacteria present in the nodules (as bacteroids differentiated form of the bacteria) fix atmospheric N and provide more than 80 kg N ha^{-1} (Sutton, 1983, Poi *et al.*, 1989 and Sindhu *et al.*, 1992) and convert it into

ammonia which is used in turn as a nitrogen source by the plant. The early events in nodulation, such as host recognition and specific adsorption of rhizobia to the root hairs, have been reviewed by many researchers (Carlson, 1987). The rhizobium-legume interaction usually leads to the development of highly organized root nodule tissue (Vance, 1983).

Soil microbial population, plays a key role in grassland ecosystems by regulating the dynamics of organic matter decomposition and plant nutrient availability (Brussard *et al.*, 1990 and Ragland and Theil, 1993). Mycorrhizal association helps the plant to withstand stress (Hirrel and Gerdemann, 1979 and Levy and Krikuhan, 1980). There is a close relationship between legume and Rhizobium, and the environment governs N_2-fixation by the symbiotic association. If the requirements of the host and of bacteria are not satisfied, the extent of fixation and productivity of the plant are reduced (Frcine, 1982).

Nodule carries out at least three major functions. The first of these is glucose catabolism, giving energy reducing-power and carbon skeleton for the plant cell cytoplasm. The second is the metabolism of the bacteroid compartment, nitrogen fixation, ammonia excretion and respiration. The third is ammonia assimilation in the plant cytoplasm, giving a net conversion of carbon skeletons and ammonia to the amino acids and/or ureides, which are then exported from the nodules.

Nodule development commences when the initial Rhizobial infection of plant epidermal cell has been completed, Maturation may be regarded as complete when the rate of nitrogen fixation per weight of nodule tissue reaches its maximum level (Sutton, 1983). In most chickpea growing areas light pink nodules can clearly be seen in 15 days. Initially, the nodules are spherical but later their tips become branched to give rise to multi-looped structures. An individual nodule can grow to 2 to 3 cm.

Variable size of the nodules can be seen on a given plant, suggesting that they are of different ages. The lobes on an individual nodule in a well nodulated plant can be fused together making nodule counting difficult (Saxena, 1988). In many legumes, the nodule mass is better correlated with biological nitrogen fixation than with nodule number per plant (Pate, 1958). After onset of flowering, the ARA decreases and is generally associated with reduced nodule mass.

1.7.2 Nodule Activity:

Root nodules are the site of a beneficial symbiotic association between legume plants and certain soil bacteria of the *Rhizobium* and *Bradyrhizobium* genera. In determinant symbiosis, an assessment of the requirements for a particular enzyme for the support of symbiotic nitrogen fixation can be inferred by the correlation of that enzyme activity which induces wide variation in N_2 fixation (Bano and Raza, 1990 and Smith *et al.*, 1994) and is related to the growth of plants (Guor *et al.*, 1991). N_2 fixation is enhanced with enhanced nodule occupancy (Champion *et al.*, 1992) wherein a direct incorporation of fixation products by bacteroids may be a critical feature in the establishment and continued growth of effective symbiosis in cowpea seedling (Atkins *et al.*, 1984).

All substances entering the nodule must pass through either the nodule cortex or the plant vascular system. In addition, all substances entering the maturing bacteroids must pass through the host cell membrane and the peribacteroid membrane.

In chickpea the amount of nitrogen fixed could be predicted from the knowledge of plant dry matter production alone (Pilbeam *et al.*, 1997), where N supply increases in proportion to dry matter production of shoot. The plant host provides the major source of nitrogen for the biosphere (Waters *et al.*, 1998).

Wherein, when the supply of nitrogen from the rhizosphere falls below the critical level required to meet the demand of young tissue, senescence is induced and N is mobilized from mature leaves of *Lolium lemulentum* (Thomas, 1983). In soybean, less than 80 Kg N ha^{-1} is taken up by early pod filling stage (Torres *et al.*, 1988).

Total acetylene reduction activity per plant increases in soybean during the flowering period, reaches a maximum near the end of flowering period and then declines markedly during the early pod filling stage.

1.7.3 Nodule Metabolism:

Although the metabolism of nodules is far from being fully understood, nitrogen fixation increases in symbiosis during early pod filling as a result of inadequate assimilate supply to the nodules. The increase in total nitrogenase activity per plant during flowering is associated with an increase in specific activity, nodule number and nodule fresh weight per plant (Evans, 1975).

The onset of reproductive growth is clearly a major factor in restricting the nodule's activity (ARA) in *P. sativum* and *G. max* and other legumes (LaRue and Kurz, 1973, Thomas and Stoddart,1980 and Bethlenfalvay *et al.*,1978). The reduction in nodule dry weight results in a net reduction of 46 percent in nodule"non-structural" carbohydrate per plant (Schweitzer and Harper, 1980). A possible explanation of activity loss by nodules during early pod filling is that the supply of photosynthetic assimilates to the roots is limited by competition from the developing pod and seed (Thomas and Stoddart,1980). Wheeler (1971) has reported a marked diurnal variation in nitrogen fixation in several species, indicating that the process is quite sensitive to the supply of photosynthetic assimilate.

Soybean nodules are certainly an active potential sink. The nodules appear to have low 'priority' for C (Vessey *et al.*, 1988). However, the requirement of the measurement method for tracer flow to be unidirectional from the source to the sink is in principle not true for soybean nodules, since there is export of carbon from the nodule to the shoot, other as amino acids or organic acids formed from the imported carbon, or as organic acids synthesized from carbon dioxide released by respiration (Walsh, 1995).

1.7.4 Nodule Longevity:

The longevity of nodules may be defined as the time that elapses between the first appearance of C_2H_2-reduction activity and the final disintegration of symbiotic tissue (Sutton, 1983). In a number of annual legumes, nodule senescence has been reported to coincide with the pod-filling stage of reproductive growth (Lawn and Brun, 1974). This suggests that the ontogeny of the host plant will also control nodule longevity (Wagner, 1987), as well as, a sudden reduction in the supply of photosynthate to the nodules may also induce premature nodule senescence.

There are undoubtedly many regulatory factors that operate directly or indirectly to influence nodule maturation (Vinect, 1980). Development may be slowed down or terminated by environmental changes such as variation in temperature, combined nitrogen, or light intensity (Phillips, 1971).

1.7.5 The Role of Growth Regulators on Nodule Development and Senescence:

Atkins *et al.* (1984) have suggested that in cowpea, a possible role for plant growth regulators produced by either symbiotic partner in promoting or retarding

nodule senescence cannot be overlooked. Libbenga and Bogers (1974) have found that during nodulation, chemicals such as IAA decrease following subsequent increase in the endogenous level of growth-retarding chemicals. Phytohormones are also implicated in morphogenesis, and the nodule is known to contain much higher levels of CKs than the root tissue (Jones *et al.*, 1987). It has been reported that root nodules of legumes (Henson & Wheeler, 1977) contain levels of CK which are higher relative to the parent root. Syono *et al.* (1976) found that CK of *P. sativum* root nodule declined with age. In the legume-*Rhizobium* symbiosis, there is well-documented evidence for bacterial production of CK and it's transfer to the host plant in certain strains of Pigeon pea (Upadhyaya *et al.*, 1991), where kinetin induces increase in net improvement in nodule dry weight, as well as nitrogenase activity (Dayal and Bharti, 1991 and Garg *et al.*, 1995).

ABA content, on the other hand, increases in parallel with growth of soybean nodule (Mizukami *et al.*, 1991). Mizukami *et al.* (1991) have also proposed that autoregulation of the nodules is related to an alteration of ABA level in the shoot. In addition, Phillips (1971) and Cho and Harper (1993) have reported that exogenous ABA application into root medium decreases nodulation in pea by inhibiting the cortical cell divisions required for root nodule formation and by inducing root dormancy, nodule senescence or premature senescence (Moro *et al.*, 1992), thereby, ABA temporarily reduces specific nitrogenase activity in Faba bean (Bano *et al.*, 1983). Thus, there may be a common response among the legumes in respect to the effect of ABA on nodulation. ABA application affects the plant response directly and not through the bacteria (Cho and Harper, 1993). Cho and Harper have reported that the degree of inhibition by ABA is concentration-dependent whereby nodule number

is inhibited, nodule weight is reduced but dry weight of root is not significantly affected.

Initiation of senescence is accompanied by a decrease in total protein and progressive degradation of the nodules at the subcellular level (Atkins *et al.*, 1984). Root nodules of the ABA-treated plants are about 50% smaller than those of the controls, and this is reflected in similar reductions in the volume of bacterial tissue per nodule in Faba bean (Bano *et al.*, 1983). The identity, source and fate of cytokinins in the nodules and the contribution of the nodules to the cytokinin economy of the whole plant is not known. Nodule CK may accumulate in excess of the quantities in the parent root and move from the nodule to the root and then to the shoot from where it is carried through the xylem into the leaves along the transpiration stream (Jones *et al.*, 1984 and Van Staden *et al.*, 1988).

1.7.5.1 Cytokinin:

Legume nodules contain high concentration of all three major groups of plant growth promoting hormones; they play important roles in early nodule development (Libbenga and Bogers, 1974). Dangors and Basu (1987) have reported that higher amount of plant growth substances such as IAA, CK, GA and ABA are obtained from mature root nodules in the leguminous tree, *Pterocarpus mursupium*.

Henson and Wheeler (1977) and Jones *et al.* (1987) have reported positive presence of CK and auxins in the nodules of leguminous plants where they are involved in infection, initiation and nodule development.

In mungbean, high concentration of kinetin has two distinct effects on nodule differentiation. Kinetin stimulates the size of the nodule, augments the bacteroids tissue production and its pigmentation, and induces branching of the nodules.

Moreover, it also produces indeterminate elongated nodules having distinct epidermal and meristimatic zones of the tissue with favorable effect on the efficiency and the longevity of the nodules (Bano, 1986).

Libbenga and Bogers (1974) and Torres *et al.* (1988) have suggested that shifts in endogenous hormone balance occur in developed nodules and act as a major factor for nodule senescence. Richard *et al.* (1982) have reported that the translocation of C to the total root system is slightly increased by increasing N incorporation in the shoot. A role of CK in nodule senescence has been supported by the work of Syono *et al.* (1976). It is possible that the preferential survival of the nodule meristem and the early senescence of the tissue at the nodule base are caused by CK-directed translocation. As soon as older nodules cease to produce cytokinin, they become vulnerable to competition for photosynthate from younger nodules and from other meristimatic tissue (Sutton ,1983).

1.7.6 Nodule Senescence:

The host plant provides the bacterium with carbon (Lugtenberg, 1992). This symbiosis ends with nodule senescence (Hodgson and Stacey, 1986). Nodule senescence has three fold practical importance. Firstly, the amount of fixed nitrogen made available to the host plant obviously depends on the duration as well as the volume of activity of the symbiotic tissue (Pate, 1977). Secondly, nodule degeneration may allow the release of at least some host-specific rhizobia back into the rhizosphere and the microsymbionts up to a threshold level due to bacteroid degradation during nodule senescence (Jimenez and Casedesus, 1989 and Mizukami *et al.*, 1991). Thirdly, nodule decay may result in an underground transfer of combined nitrogen from legumes to other plants in the ecosystem whether they are pasture grasses or a

following crop (Butter and Bothurst, 1956). So delay of nodule senescence is one of the factors that contribute significantly to nitrogen fixation in legumes (Hardy, 1977). The benefits of nodule senescence, generally, are that the host legumes can enrich their immediate soil environment with *Rhizobia* through rhizosphere effects.

It is reported that mRNA, protein concentration and cytokinin content of the nodule is high in early nodule ontogeny and decreases as the nodules grow and total nodule activity develops (Heindl and Brun, 1984 and Cho and Harper, 1993). Nodule senescence includes a general loss in cytosolic protein (Sutton, 1983) especially the leghaemoglobins (Pfeiffer *et al.*, 1983) whose function is to mediate oxygen transport to the nitrogen fixing symbiotic bacteroids (Appleby, 1984).

Chickpea nodules start greening at the proximal end and about three weeks after flowering. Here the green portion increases in the nodules when the supply of photosynthate to the roots is known to be limited (Lawn and Brun, 1974), and due to destruction of the haeme porphyrin ring and denaturation of proteins, which increase with time until the late podfill stage when nodule is fully green (Pfeiffer *et al.*, 1983). This structural breakdown seen during nodule senescence has often been described as 'autolytic' (Bergersen *et al.*, 1985). Nodule senescence of soybean is characterized by a decline in the N_2 fixing activity and in the content of cytosolic proteins due to enhanced proteolytic activity (Wagner and Sarath, 1987).

With the senescence of the leaves of the plants, the nodule shrivels and dries but generally remains attached to the roots. Dark brown and bright pink nodules have occasionally been seen in some regions but not in others.

1.8 Nodulation and Plant Growth

General relationship between photosynthesis and symbiotic nitrogen fixation have been developed at length in the 1930s, and later by Bethlenfalvay and Phillips (1978), Hardy and Havelka (1975) and Sesay and Shibles (1980). It has been reported that the over all effects on nodulation are a function of carbohydrate-nitrogen relationship. Whereas the increased level of carbohydrate may delay nodule senescence, it does not prevent it, as suggested by Wilson *et al.* (1978). Most of the assimilate reaching the roots appears to come from the lower leaves on the plant.

The pivotal role of photosynthesis in nitrogen fixation is well established in soybean. The decrease in nitrogenase activity is closely related to the decrease in photosynthate, which in turn decreases the energy charge of the nodules (Patterson *et al.*, 1979) and changes the photosynthetic pool size (Finn and Brun, 1980, Vessey *et al.*, 1988, Denison *et al.*, 1992 and DeLima *et al.*, 1994), such results found in *Cajanus cajan* (Grover *et al.*, 1985) and chickpea (Sinha, 1974).

The shoot development in cowpea plants relying solely on symbiotically fixed N is probably limited by N supply (Atkins *et al.*, 1984), and there is a greater proportional contribution of cotyledon N to protein synthesis of plant cells and higher proportional investment of newly fixed N bacteroids, "Carbohydrate supply" or "Carbohydrate-nitrogen relationship" as being essential determinants of nodule senescence (Sutton, 1983). Nitrogen left in the canopy at harvest is considered to be not fully utilized by the plant, unless the assimilate supply of such leaves with delayed leaf senescence supports the maintenance of nodule N_2-fixation (Abu-Shakra *et al.*, 1978), which can indeed lead to significantly higher yields as reported for soybean.

In the senescent nodule stage, the supply of carbohydrate to the nodule reduces, nitrogenase activity and the leghaemoglobin content also declines (Sutton

,1983). In soybean, N_2 fixation is enhanced with increased nodule occupancy by superior variants due to more effective interaction of strain-cultivars (Champion *et al.*, 1992). The crop's nitrogen metabolism has also been found to improve leaf protein yield (Imsande, 1989). In white clover (*Trifolium repens*), N content of root and stolon are remobilized to support the re-growth of leaves. Nitrogen in regrowing leaves comes mainly from N reserves of the organs remaining after defoliation (Corre *et al.*, 1996).

1.9 The Correlation Between Leaf and Nodule Development:

N-fixation is an extremely important process since next to water, nitrogen is the second most important compound that limits world crop production (Lugtenburg, 1992). Gresshoff (1988) has proposed that outregulation of nodulation involves the synthesis of shoot-derived inhibitors in response to initial nodule formation, which are translocated to the root and function to arrest the development of the infected subepidermal cells into nodules. The plant supplies the bacteria with an energy source and, in return, the bacteria reduce (fix) the atmospheric N_2 gas to $NH4^+$ providing it to the plant for assimilation into amino acids, proteins and other essential nitrogenous compounds (Hunt and Layzell, 1993). According to this structural relationship, the plant can influence the development of bacteroids by selective transport of substrates and controlling factors (Sutton, 1983). N-fixation requires about 10 kg of carbohydrates for each 1 kg of fixed N_2 and the equivalent of 25-28 molecule of ATP for each molecules of N_2 fixed (Havelka *et al.*, 1982). In the soybean, there is a parallel increase seed protein with the greater N accumulation resulting from N-fixation (Leffel *et al.*, 1992). Nitrogen fixation generally reaches a peak at early pod fill and declines during the late reproductive phases (Imsande, 1989). The nodulation-

nitrogen-fixation process may consume about 10 to 20% of the photosynthetic output of a tropical grain legume. Enhanced N-fixation during pod fill increases the supply of usable plant nitrogen. This, in turn, stimulates the photosynthetic output and accordingly increases the seed size. These results suggest that enhanced nitrogen fixation during pod fill significantly increases seed yield (Imsande, 1988).

Senescence of soybean root nodules is functionally defined as an irreversible decline in whole plant N_2-fixation that sets at about the time of pod development. Ontologically, senescence is the final stage of plant growth following anthesis and the onset of reproductive development (Atkins, 1980). In time, the seeds develop and eventually the nodules slough from the root.

CHAPTER- 2

MATERIALS AND METHODS

2.1 Plant Material and Growing Conditions:

Seeds of chickpea, *Cicer arientinum* (L.) cv. CM88, were obtained from National Agricultural Research Centre (NARC), Islamabad, Pakistan. Uniform seeds were surface sterilized with colorox and sown in pots measuring 25x18 cm^2. Every pot was filled with 10 Kg of soil and organic matter in the ratio as 2:1. The seeds were sown by mid November and harvested in the first week of May. Five plants per pot, twenty pots per treatment per year were maintained during the course of experiments.

The experiments had a completely randomized design. Standard agronomic and irrigation practices were followed throughout the course of the study. Half strength Hoagland's nutrient solution (Hoagland and Arnon, 1950) was applied to the plants once a week. The pots were kept under natural conditions. Seventy days after sowing, the control plants were sprayed with distilled water and the experimental plants with ABA (10^{-6}M) and kinetin (10^{-6}M), using a plastic hand sprayer. The hormone treated plants were sprayed once.

The climatic data, including the minimum and maximum temperature, monthly rainfall and relative humidity were collected from the Meteorological Department, Islamabad (Table 1).

Table 1: The climatic conditions according to the Meteorological Department, Islamabad.

Month / Year	Max. Temp. (°C)	Min. Temp. (°C)	R.H.(%) 0800-1400 h		Rain fall (mm)
1997-1998					
November	22.2	7.70	91.80	49.30	22.10
December	16.10	4.60	92.5 0	61.10	23.50
January	16.80	2.60	91.60	51.00	43.50
February	18.80	5.50	88.10	54.10	236.50
March	22.20	8.20	84.30	45.10	85.90
April	30.00	14.90	73.10	44.60	121.10
May	35.60	19.20	60.60	35.70	24.90
1998-1999					
November	26.45	6.68	85.37	45.37	0.00
December	20.64	1.75	86.35	39.52	0.00
January	16.10	4.16	88.81	62.00	108.80
February	20.30	6.90	86.21	51.21	28.20
March	23.80	9.20	81.65	49.00	59.60
April	33.10	13.40	63.10	42.2	1.30
May	37.40	19.08	62.94	32.84	13.30
1999-2000					
November	24.60	8.40	90.70	42.3	22.50
December	22.00	2.20	87.00	38.0	0.00
January	16.90	3.20	91.00	56.00	135.90
February	18.40	3.60	91 .00	52.00	51.00
March	24.10	12.70	75.00	52.00	18.80
April	33.20	14.00	55.50	31.50	4.50
May	39.50	21.80	48.00	25.50	8.30

Each value represents monthly average of maximum and minimum temperature, rainfall and relative humidity.

2.2 Preparation of Growth Regulators:

The solutions of abscisic acid (ABA) and kinetin were prepared by dissolving the requisite amount of ABA-mixed synthetic isomer (Sigma) in 1ml methanol and that of kinetin in 0.1% HCL. Finally, the volume of all these solution was made up to 100 ml with distilled water. From these stock solutions further dilutions were made as required.

2.3 Application of Growth Regulators:

Aqueous solutions of kinetin and Abscisic acid were applied separately, each at 10^{-5} M for seed soaking, while 10^{-6}M was applied as foliar spray. The percentage of uptake of applied hormone from the soaked seeds, and the percentage of hormone accumulated by the seeds for use during plant growth may affect the actual concentration of the hormones necessary for action. Hence, the hormones may or may not be available in their original concentration to exhibit their effect, while the plant has achieved a certain age. For this reason higher concentrations were applied for seed soaking and ten fold lower concentrations were applied as foliar spray. The seeds were soaked for 6 hours in 10^{-5} M solution of either hormone. Control seeds were soaked in distilled water. Foliar spray of each hormone was carried out 70 days after sowing at the vegetative stage. The control plants were sprayed with distilled water.

2.4 Methods of Inoculation:

Before sowing, the seeds (1kg) were moistened in sugar solution (48%). Thereafter carrier-based inoculums of *Rhizobium*, strain leguminosarum TAL 620, (obtained from Soil Biology of Biochemistry section of NARC, Islamabad) was spread over the seeds at 16g /Kg seeds, and mixed thoroughly to a uniform coating prior to sowing. (inoculant's density used was at least 10^4 *Rhizobia* per seed).

2.4 The Following Treatments Were Used:

Kinetin (10^{-5}M), seed soaking

Kinetin (10^{-6}M), foliar spray

ABA (10^{-5}M), seed soaking

ABA (10^{-6}M), foliar spray

2.6 Parameters Investigated:

Prior to sowing and after the harvest, composite soil samples (10 g) from 3-4 pots per treatment were taken to determine their N content. Six plants from different pots per treatment, per experiment were (pooled tissues) tested for each analysis.

2.6.1 Nitrate-Nitrogen Content of Soil:

A sample of 10g soil and 20 ml of the AB-DTPA, (ammonium bicarbonate di-ethylene triamine Penta acetic acid) extracting solution were taken in a 250 ml Erlenmeyer flask and shaken in a reciprocating shaker for 15 min. The suspension was filtered through filter paper (whatman No. 42) along with a blank. To 1 ml of the above filtrate, 3 ml Cu SO_4 (1.5 N) and 2 ml of hydrazine sulfate were added carefully and placed in a water bath for 20 minutes at 38°C. Later, 3 ml color reagent was added to each sample. The contents were mixed again and left for 20 minutes for full color development. The readings standards and samples were read at 540nm using spectronic 21 (Winkleman *et al.*, 1985).

Color reagent:

Color reagent comprised 5 g sulfanilamide 0.25g NC, 1-nephthyl-ethylendiamine dihydrochloride in 300ml distilled water and was prepared by stirring and adding to 50 ml H_3PO_4. The reagent was diluted to 500 ml volume.

Standard for NO3-N:

The stock solution was prepared by dissolving 3.609 g dried KNO_3 (F.W.101.108) in about 800 ml distilled water. It was mixed well and the volume was made to 1 liter.

2.6.2 Plant Growth Parameters Investigated:

1. Fresh weight of shoot and root
2. Fresh weight of nodules
3. Dry weight of shoot and root
4. Dry weight of nodules
5. Diameter of pink bacteriod tissue
6. Number of pods per plant
7. Weight of 100 seeds
8. Grain weight

Dry weight was determined after 48 hours of drying in an oven at 80°C. The number of pods plant^{-1}, weight of 100 seeds, and grain yield were recorded at harvest (140 days after sowing, at edible pod stage).

2.6.3 Determination of Chlorophyll Content of Leaves:

The chlorophyll content of young and old leaves at four different growth stages was determined by the method of Arnon (1949) as modified by Kirk (1968). Crude preparation of leaves (1ml) was mixed with 4ml of 80% (v/v) acetone and allowed to stand in the dark at room temperature for 10 min. It was then centrifuged at 2000 rpm for 5 minutes. Supernatant, which contained soluble pigments, was used for the determination of chlorophyll. Absorbance of the solution was read at 645nm (chlorophyll-A) and 663nm (chlorophyll-B) on spectrophotometer (Model UV-120-01 Shimadzu) against 80% (v/v) acetone blank. Total chlorophyll content was determined according to the formula of Arnon (1949).

Total chlorophyll (mg/l)= (20.2 x A645)+ (8.02 x B663)

2.6.4 Estimation of Protein Content of Leaves:

Protein content of young and old leaves of chickpea was assayed at four different growth stages according to the methods of Peterson (1977), which is a modification of the protein assay method of Lowry *et al.* (1951). Protein samples (containing approximately 5-100 µg of protein) were brought up to 1ml with distilled water. An aliquot of 100 µl of 0.15% (w/v) sodium deoxycholate (DOC) was added, mixed and allowed to stand for 10 minutes at room temperature. Another aliquot of 100 µl of 72% (w/v) trichloroacetic acid (TCA) was then added, mixed and the mixture was centrifuged at 3,000g for 15 minutes using a bench microfuge. The pellet was dissolved in 1ml of reagent A (equal parts of copper tartarate carbonate (0.1% w/v), copper sulphate (penta hydrate), 0.2% (w/v) potassium tartarate; 10% (w/v) sodium carbonate; 0.8M sodium hydroxide; 10% (w/v) SDS and water), mixed and allowed to stand for 10 minutes at room temperature. A 500 µl volume of reagent B (1 volume of folin-ciocalteu phenol reagent and 5 volumes of distilled water) was added, mixed immediately and left for 30 minutes at room temperature. Absorbance was read at 750 nm using a spectrophotometer. A standard curve was plotted using triplicate samples of the known concentrations of BSA versus their average absorbance at 750 nm. This was used to determine the concentration of protein in the sample.

2.6.5 Determination of Proline Content of Leaves:

Proline content of the young and old leaves of chickpea was determined at four different growth stages according to the method of Bates *et al.* (1973). A 0.5 g sample of fresh plant material was ground in 10ml of 3% sulphocalicyclic acid and the homogenate was filtered through glass wool. The filtrate (2 ml) was mixed with 2ml of reagent A (1.25 g ninhydrine, 20 ml glacial acetic acid and 20ml orthophosphoric acid (6M) and 2 ml of acetic acid) in a propylene tube. The mixture was then incubated in a water bath for one hour at >90°C. The tubes were then transferred to an ice bath and cooled. Toluene (4 ml) was then added and proline concentration was measured at 520 nm. A calibration series of 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 μg of proline (DL) was also run and a standard curve was plotted.

2.6.6 Determination of Sugar Content in Leaves:

Estimation of sugar content of the fresh young and old leaves of chickpea was made at four different growth stages following the method of Dubo *et al.* (1956) as modified by Johnson *et al.* (1966). Fresh leaf material (0.5g) was homogenized in 10 ml of distilled water in a clean mortar and was centrifuged at 3000 rpm for 3 minutes. To the supernatant was added 0.1ml concentrated H_2SO_4. After 1 hour of incubation, the absorbance of each sample was recorded at 420 nm.The concentration of the unknown sample was calculated with reference to the standard curve made by using glucose.

2.6.7 Extraction and Purification of ABA From Leaves:

Extraction and purification of the endogenous ABA at the vegetative stage (8WAS) and late pod filling stage (20WAS) was made from fully expanded leaves of the control and the treated plants according to the method of Kettner and Dörffling (1995). The leaves were freeze dried (Virtis, Gardiner. N.Y. 12525; UNDP, Model No.10-145MR-BA).

Leaves (1g) were homogenized in 80% methanol with 10mg l^{-1} butylated hydroxy toluene (BHT) used at as antioxidant. The extraction was over a 72 h period. The extract was filtered through a Buchner funnel, and reduced to aqueous phase using a rotary thin film evaporator at 35 °C. The aqueous phase was adjusted to pH 9 with 0.1N (NaOH) and partitioned 3x with 1/3 volume of ethyl acetate to remove basic and neutral compounds. The organic phase was discarded. The aqueous phase was readjusted to pH 2.5-3 using 0.1N HCL and partitioned 3x with 1/3 volume of ethyl acetate. The sample was dried on RFE at 35 °C, the residue was dissolved in methanol (100%), dried under oxygen-free nitrogen, and then re-dissolved in 100 % methanol. Each sample received during crushing 10µl of 2000 cpm ABA (DL cis trans – [H^3] ABA); specific activity 237 TBq/mmol $^{-1}$; (Radio chemical Centre, Amersham International Buckinghamshine UK) as internal standard to maintain the extraction of leaves. ABA analysis was carried out by mobile gradient HPLC (Model UNICAM 200, England) using a Particil-5 (P5-4659) column, Acetonitrile and methanol (70:30) solvent system, at a flow rate of 0.8 ml/minute and read at 254 nm, with a UV detector. The remaining samples were counted for radioactivity by liquid scintillation spectrometry (Beckman LS 1801,U.S.A.). Results were expressed as % of radioactivity in each treatment relative to the total activity applied to the plants at the time of extraction. The purification efficiency was between 60-70%.

2.6.8 IAA Content of Roots:

IAA content of roots was measured at four different growth stages by estimating residual IAA with Salkowskis's reagent (Malik and Singh, 1980). For this purpose, the reaction mixture was prepared by adding 1ml of solution containing 10mg IAA / 100ml distilled water, 0.5mM of $MnCl_2$, 0.25ml of 0.1mM 2-4, dichlorophenol, 4ml of 0.05mM phosphate buffer (pH 6.5) and 1ml root extract. The reaction mixture was incubated at 28-30°C for 1 hour in dark and 2ml of this reaction mixture was separated in a test tube containing 2ml of Salkowski's reagent (1ml 0.5M ferric chloride + 50ml 35% perchloric acid) in 1:50 ratio. After 20 minutes of incubation when color had developed, absorbance was measured spectrophotometrically at 530 nm.

The IAA left after oxidation by the enzyme was calculated using the standard curve that was plotted using triplicate samples of known concentrations of IAA.

2.6.9 Diameter of Pink Bacteroid Tissue:

Fresh nodules were collected at four different growth stages. Thin sections of the nodules (5μ) were made with the help of a razor blade and diameter of the pink bacteroid tissues containing leghaemoglobin in the nodule cortex was measured under a light microscope (Nikon research Microscope, optiphat with HFX-II Camera) under 4X objective lens (Gretchen, 1967).

2.6.10 Nitrogenase Activity:

The absorbance for acetylene reduction activity of the plant roots was measured at four different growth stages following the method of LaRue and Kurz (1973) by incubating nodules (1g) in 30 ml McCartiny's stoppered vials. Two ml of air was removed from the vials with a syringe and 2ml of C_2H_2 was injected. The plant nodules were incubated with 7% C_2H_2 for 90 minutes at 22°C. Therefore, 2ml of the gas phase was removed and injected in McCartiny's vials, which contained 1.5 ml of oxidant solution (40 ml of 0.05M $NaIO_4$, 5 ml of 0.005 M $KMnO_4$, pH adjusted to 7.5 with KOH and diluted to 100 ml). The vials were agitated vigorously on a rotatory shaker (OSK 6439 universal shaker, OGAMA Seiki Co. Ltd., Model V-SN, frequency

200-290 –340 S. P. M., amplitude 40mm, 100 V 200W 50/60Hz MOTOR, CMFG No. 1261006) at 300 rpm for 90 minutes at 22°C. The mixture received 4N $NaAsO_2$ (1/4 ml), 4N H_2SO_4 (1/4 ml), 1.6 ml Nash reagent (75g of ammonium acetate, 1.5ml of acetate acids, 1ml of acetyl acetone diluted to 500ml) and absorbance for C_2H_4 was determined at 412 nm after 60 minutes using a spectrophotometer (UV-120-01, Cat. No. 204-0001-01, serial No.129223, Shimadzu). A blank was prepared taking all the reagents, incubated and mixed for the same period and absorbance value of the sample was subtracted from that of the blank. A calibration series of different volumes of ethylene gas was also run and standard curve was plotted.

2.7 Statistical Analysis:

Data were analyzed statistically by Analysis of Variance (completely Randomized factorial Design) and treatment means were compared using Duncan's Multiple Range test (DMRT) using MSTAT-C version 1.4.2.

RESULTS

All the parameters were conducted at four stages; vegetative stage (8 WAS), flowering stage (16 WAS), early pod filling stage (18 WAS) and late pod filling stage (20 WAS). Symptoms of leaf senescence appeared after flowering, became conspicuous at early pod filling stage and persisted until late pod filling stage when all the old leaves abscised. The second old leaves from the bottom were then used for further studies. The readings for sugar content in leaves, fresh weight and biomass of shoot, root, and nodules were taken in the last year of the research work.

3.1 Shoot Fresh Weight and Dry Weight:

Data presented in figure 1 and 2 reveal that fresh and dry weight of shoot was maximum at early pod filling stage (18 WAS) followed by a significant decrease at late pod filling stage (20 WAS). The fresh weight and dry weight of shoot were taken without pods. The increase in fresh shoot weight and dry weight was linear until early pod filling stage. Fresh shoot weight and dry weight decreased by 50% at the late pod filling stage.

The interaction between the treatments and growth stages was highly significant ($P< 0.001$, see appendix II, Table 21). Application of kinetin showed a significant increase in fresh weight and dry weight of shoot as compared to the controls at all growth stages. Kinetin at 10^{-5}M (applied as seed soaking) was more effective in increasing fresh weight of shoots as compared to 10^{-6}M kinetin at all growth stages, while its effect on the shoot dry weight was significant only at later stages as compared to 10^{-6}M kinetin (applied as foliar spray). Kinetin (10^{-5}M) increased fresh weight of shoot at the vegetative stage and dry weight at flowering

stage by 300%. A further 50% increase occurred up to maturity (20WAS). During the plant growth, 15% and 8% in increase in fresh weight and dry weight of shoot was caused respectively in kinetin (10^{-6}M) treated and control groups.

ABA treatments showed a slight but non significant decrease in fresh shoot weight and dry weight as compared to the control at all growth stages except at the late pod filling stage. The most significant decrease caused by ABA on fresh shoot weight and dry weight was at the early pod filling stage. Higher concentration of ABA (10^{-5}M) was more (non significant) effective in decreasing fresh and dry shoot weight as compared to the lower concentration (10^{-6}M). The maximum decrease in fresh shoot weight and dry weight caused by 10^{-5}M ABA (applied as seed soaking) was 30% and 28%, respectively (see appendix I, Tables 8, 9).

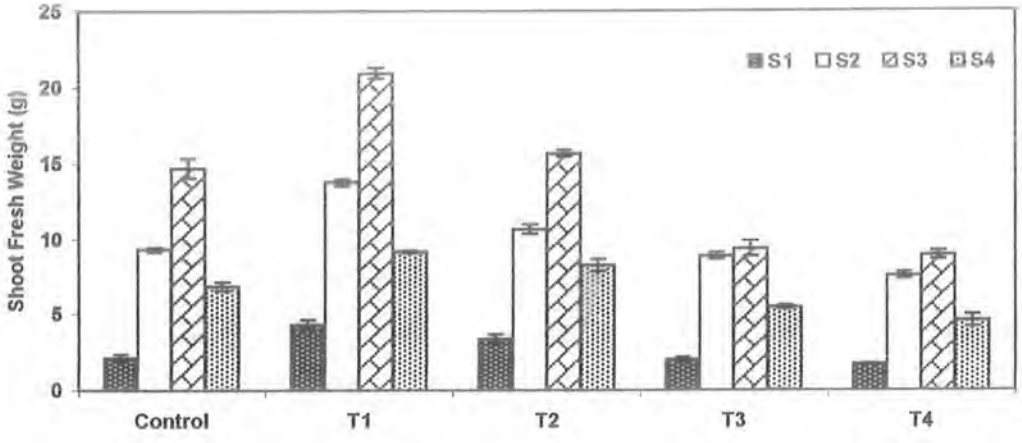


Figure 1: Fresh shoot weight (g) in *Cicer arietinum* L. cv. CM88 at different growth stages and the effect of growth regulators (year 2000). Vertical bars showed the standard error.

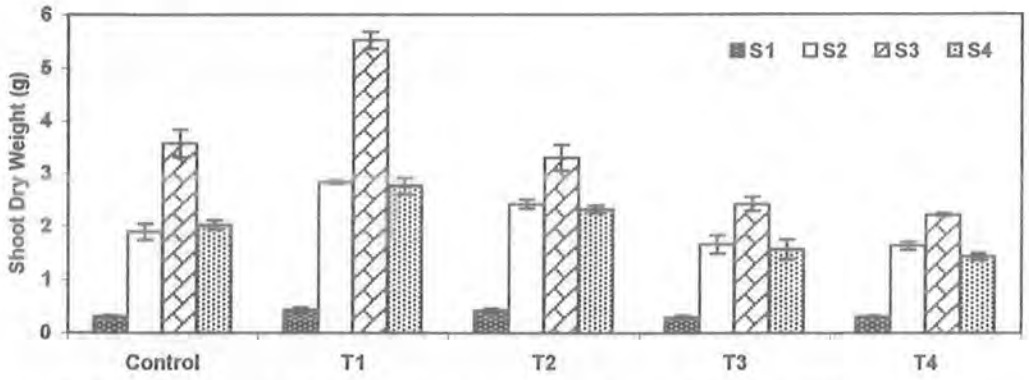


Figure 2: Dry shoot weight (g) in *Cicer arietinum* L. cv. CM88 at different growth stages and the effect of growth regulators (year 2000). Vertical bars showed the standard error.

Where:

Kinetin5 =T1= Kinetin 10^{-5}M (applied as seed soaking)
Kinetin6=T2= Kinetin 10^{-6}M (applied as foliar spray)
ABA6= T3= ABA 10^{-6}M (applied as foliar spray)
ABA5= T4= ABA 10^{-5}M (applied as seed soaking)

3.2 Root Fresh Weight and Dry Weight:

The interaction between treatments and plant growth stages was highly significant ($P< 0.001$, see appendix II, Tables 33, 34). The results showed an increase in fresh root weight and dry weight during different stages. At the flowering stage and at the early pod filling stage (16&18 WAS), fresh root weight and dry weight was significantly higher after which a significant decrease was noted at the late pod filling stage, Figure 3 and 4.

Kinetin significantly increased fresh root weight and dry weight as compared to the control. During the growing season and irrespective of the stages of plant growth, 10^{-5}M kinetin increased fresh root weight and dry weight over the control by 52% and 34%, respectively. Kinetin at 10^{-5}M concentration showed significant increase in fresh root weight and dry weight at the flowering stage and at the early pod filling stage but, 10^{-6}M kinetin was less effective in increasing the fresh root weight and dry weight during the growing season. Kinetin at 10^{-6}M showed insignificant increase in fresh root weight over the control at all growth stages, while it significantly increased dry root weight by 40%, only at the early and late pod filling stage.

ABA decreased fresh and dry weight of root as compared to the control at all growth stages. At 10^{-5}M, it decreased the fresh root weight and dry weight during the growing season by 15% and 18%, respectively. ABA at 10^{-6}M, applied as foliar spray, was more effective in decreasing fresh and dry weight of root by 22% and 27%, respectively. The highest decrease caused by ABA in root fresh weight and dry weight (50%), was at the late pod filling stage (20WAS). The ABA at 10^{-5}M non significantly decreased the fresh root weight as compared to the control at all growth stages, whereas its effect on dry root weight was significant as compared to the

control at almost all growth stages. With 10^{-5}M ABA, fresh root weight was slightly higher but not significantly different as compared to the effect of 10^{-6}M. The later decreased the dry root weight significantly at all growth stages as compared to 10^{-5}M ABA (see appendix I, Tables 10, 11).

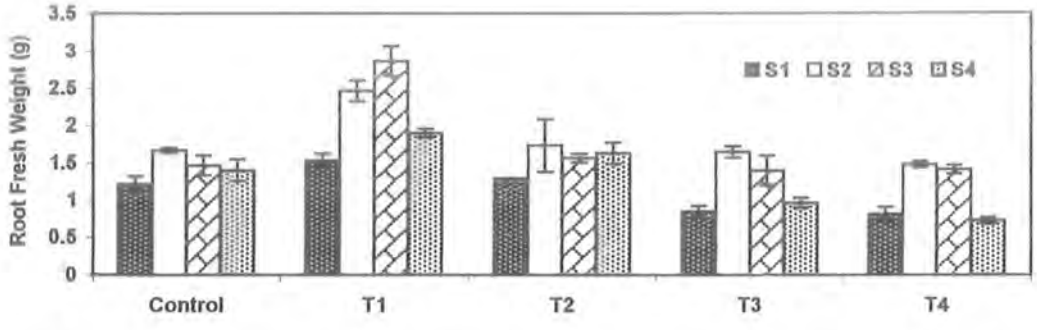


Figure 3: Fresh root weight (g) in *Cicer arietinum* L. cv. CM88 at different growth stages and the effect of growth regulators (year 2000). Vertical bars showed the standard error.

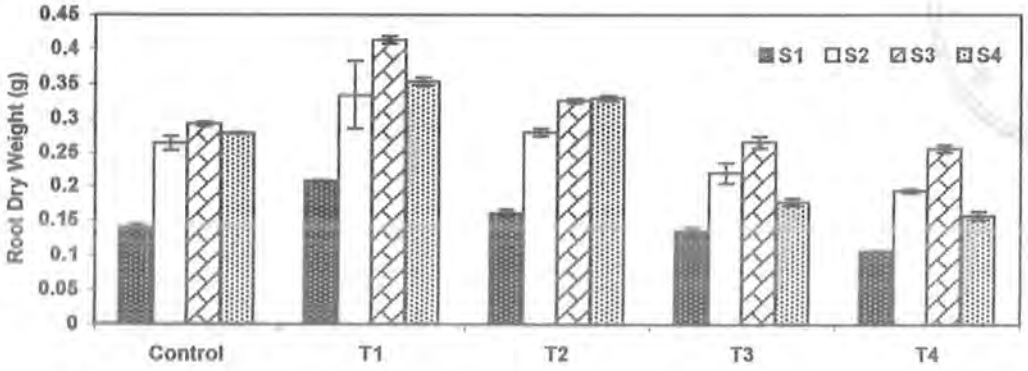


Figure 4: Dry root weight in *Cicer arietinum* L. cv. CM88 at different growth stages and the effect of growth regulators (year 2000). Vertical bars showed the standard error.

3.3 Chlorophyll Content:

The results presented in figure 5 show that chlorophyll content of chickpea decreased during plant growth in both young and old leaves. However, the young leaves showed higher chlorophyll content as compared to the old leaves. Subsequent to vegetative stage, a significant decrease in chlorophyll content was noted at the flowering and early pod filling stages in both the young and the old leaves. Young leaves had higher chlorophyll content as compared to the old leaves at all growth stages. The decrease in chlorophyll content in the old leaves was greater as compared to the young leaves in 1998 and 2000.

The stages of plant growth, treatments and leaf age, interacted significantly ($P<0.001$, see appendix II, Tables 36-37) and affected the chlorophyll content, whereas the interactions between the previous three factors were non significant.

Kinetin increased the chlorophyll content in the young and the old leaves at all stages of plant growth during all three years. At 10^{-5}M concentration it increased the chlorophyll content over the control by 10%, 20% and 5%, respectively, in the three consecutive years. However, the increase in chlorophyll content at 10^{-6}M concentration was 4%, 14% and 1%, respectively, in the three consecutive years.

At the flowering stage, 10^{-5}M kinetin showed the lowest decrease in chlorophyll content but the decrease in the young leaves at the early pod filling stage was minimum with kinetin treatments. The decrease in chlorophyll content in the old leaves at the same mentioned stages was several times higher than in the young leaves.

ABA decreased the chlorophyll content non-significantly in both the young and the old leaves at all growth stages. ABA at 10^{-5}M concentration was more

effective in decreasing the chlorophyll content as compared to the control. It showed 10%, 11%, and 3% decrease, respectively, in the three consecutive years. On the other hand, 10^{-6}M ABA decreased the chlorophyll content by 3%, 9% and 2%, respectively, in the three consecutive years. The highest significant decrease in chlorophyll content was noted with ABA (10^{-5}M) treatment at the early pod filling stage and in old leaves as compared to the young leaves.

The pattern of changes in the chlorophyll content of the leaves due to application of growth regulators was similar during the three consecutive years (see appendix I, Tables 12-14).

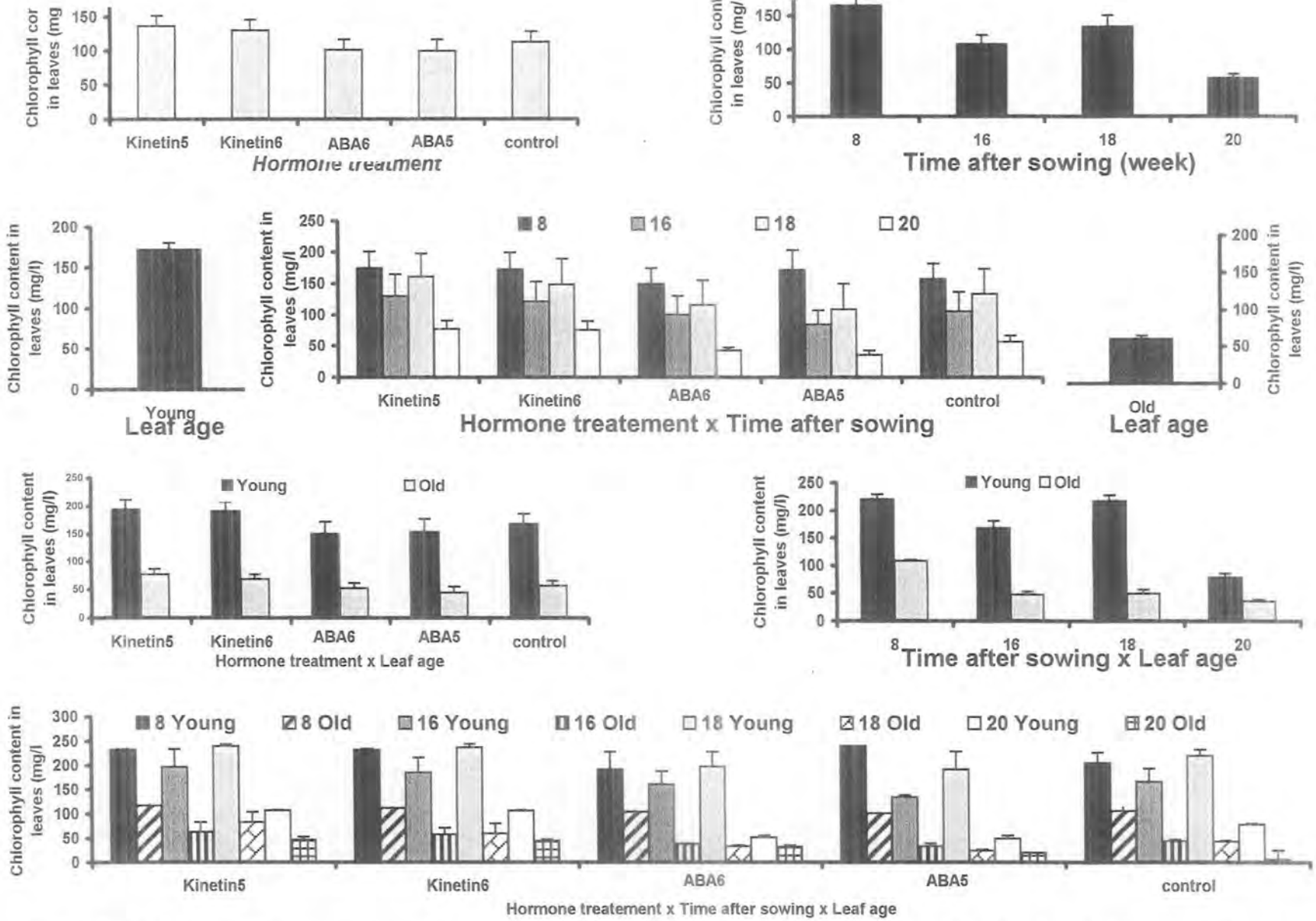


Figure 5: The effect of interactions between treatments, plant growth stages and leaf age on chlorophyll content (mg/l) in young and old leaves of *Cicer arietinum* L. cv. CM88 (year 1999).

3.4 Protein Content:

The results presented in figure 6 indicate that the protein content of leaf tissues increased during the plant growth, being highest at the flowering stage (16 WAS) in the young as well as in the old leaves during 1998. Subsequent to the flowering stage, a significant decrease in protein content was noted in the young as well as the old leaves. During 1998 and 2000, the decrease was significantly higher at the early pod filling stage as compared to the flowering stage, whereas the decrease was higher in the old leaves at the late pod filling stage during in 1999.

The protein content in both the young and the old leaves was almost similar at the first two stages (i.e., 8WAS and 16WAS), while it was significantly higher in the young leaves as compared to the old leaves at later stages (i.e., 18 WAS and 20WAS). In the young leaves, the protein content was higher at 18 WAS (50%) than in the late pod filling stage (20WAS). It is noted that the increase in protein content in the young leaves at the late pod filling stage was almost equal to the decrease in protein content in the old leaves at same stage. The results showed that at the late pod filling stage (20WAS), the old leaves and the young leaves had almost the same protein content.

The stages of plant growth and leaf age had significant effect on protein content in plant leaves; their interaction with different treatments was highly significant at $P<0.001$ (see appendix II, Tables 38-40).

The application of kinetin showed slight but non significant increase in the protein content in the young and the old leaves of chickpea at almost all growth stages. During the course of plant growth, 10^{-5}M kinetin caused significantly higher protein content as compared to the control (10%, 34%, 14%, respectively) in the three

consecutive years. Kinetin-treated (10^{-6}M) had non-significantly higher protein content than in the control (6%, 15%, 6%, respectively) in the three consecutive years. The highest increase in protein content at the flowering stage in the young and the old leaves was caused by kinetin, whereas it was higher in the young leaves at the late pod filling stage in 1999 and 2000. Kinetin also increased the protein content in the old leaves as compared to the control at the late pod filling stage. On the average, the effect of kinetin (10^{-5}M) was higher but non significantly different as compared to the effect of 10^{-6}M kinetin in increasing the protein content in the young and the old leaves.

ABA treatment decreased the protein content in the young and the old leaves as compared to the control. The higher concentration of ABA (10^{-5}M) was more significant in decreasing the protein content over the control (20%, 25%, 16%, respectively) in the three consecutive years as compared to a non-significant decrease caused by 10^{-6}M ABA (8%, 14%, 7%, respectively) in the three consecutive years.

The effect of ABA was more pronounced in the old leaves and at the late pod filling stage. The ABA treatments showed decrease in protein content by 60% in the old leaves at 20 WAS, whereas the young leaves had higher protein content with the same treatment (80%) as compared to the old leaves (see appendix I, Tables 15-17).

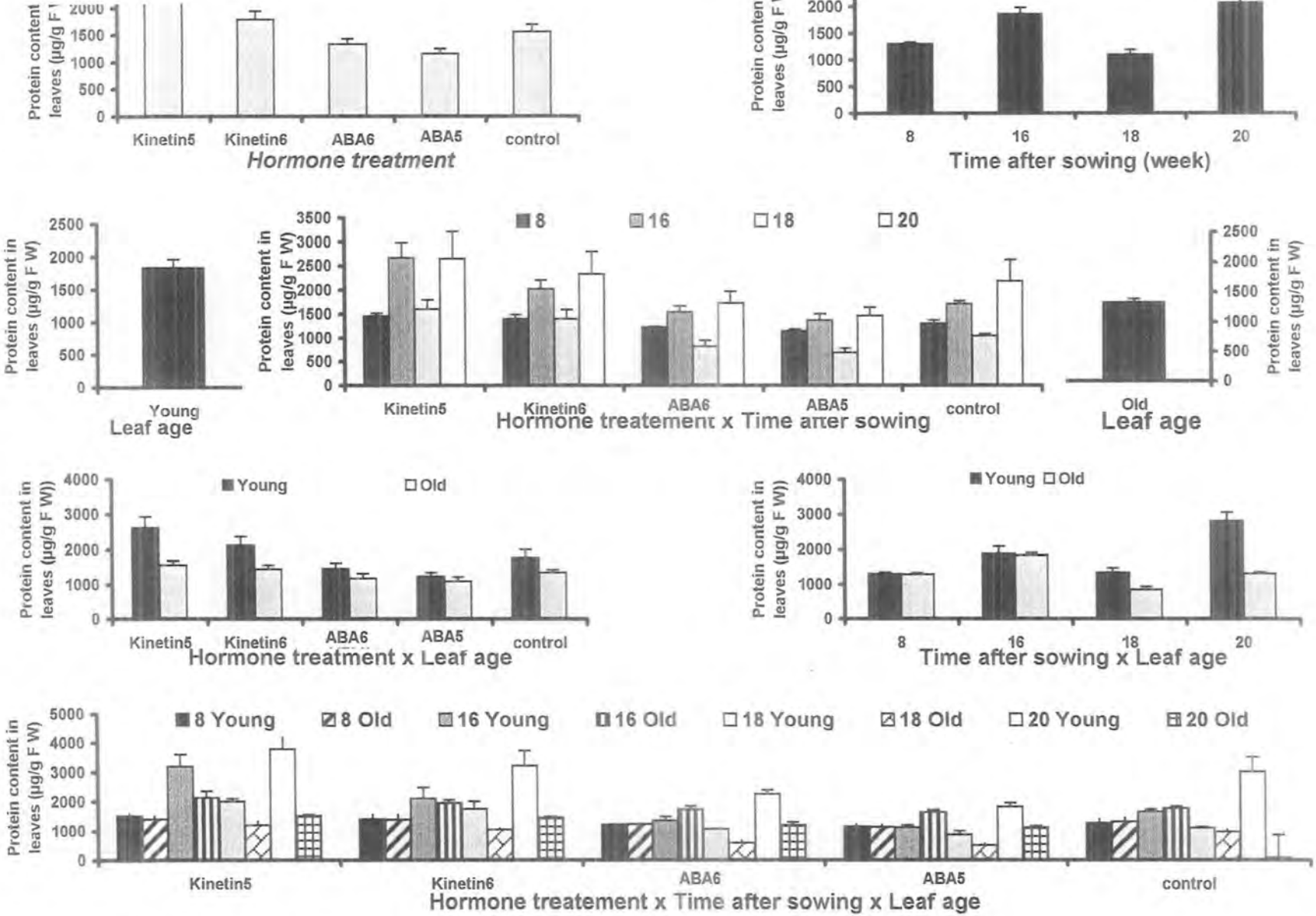


Figure 6: The effect of interactions between treatments, plant growth stages and leaf age on protein content (µg/g F W) in young and old leaves of *Cicer arietinum* L. cv. CM88 (year1999).

3.5 Proline Content:

The results presented in figure 7 indicate that proline content continuously increased starting from vegetative stage up to early pod filling stage (18 WAS) after which it decreased significantly. Proline content was higher in the old leaves as compared to the young leaves at all growth stages except the late pod filling stage. At the vegetative stage, all the treatments were non significantly different from each other for the young as well as the old leaves. The effect of treatments became significant at the flowering and at the early pod filling stages. The reduction in proline content at the late pod filling stage was greater in the old leaves as compared to the young leaves.

The interaction of plant growth stages and leaf age with different treatments was highly significant ($P<0.001$), whereas the interaction of the three factors was non significant (see appendix II, Tables 41,42).

The application of kinetin reduced the proline content in the young and the old leaves at all growth stages. With kinetin (10^{-5} M) treatment, the decrease in proline content was 27% and 22%, respectively, in the two consecutive years, whereas at 10^{-6} M kinetin the decrease in proline content was 13% in both years. The effect of kinetin in decreasing the proline content was more obvious at the early pod filling stage (18 WAS) in the young leaves as compared to the old leaves, whereas it was greater in the young leaves as compared to the old leaves at the late pod filling stage. The decrease in proline content in kinetin treated plants at the late pod filling stage was greater as compared to the control and was greater in the old leave than in the young leaves.

ABA increased the proline content in the young and the old leaves at all growth stages. With 10^{-5}M ABA, proline content increased by 43% and 42%,

respectively, in the two consecutive years, whereas with 10^{-6} M the proline content increased by 22% and 9%, respectively, in the two consecutive years. The effect of ABA was more obvious in old leaves as compared to young leaves.

At the flowering stage, the proline content was significantly increased in the ABA treated plants. The old leaves at this stage had higher proline content, which was nonsignificantly different from that in the young leaves. At the early pod filling stage, the proline content was increased significantly in the ABA treated plants, where the old leaves showed significant higher proline content than did the young leaves. ABA treatments also showed a decrease in proline content at the late pod filling stage in the young and the old leaves but the young leaves had non-significantly higher proline as compared to the old leaves. The pattern of changes due to hormone applications was same in the two years and even, the magnitude of the effect was almost similar (see appendix I, Tables 18,19).

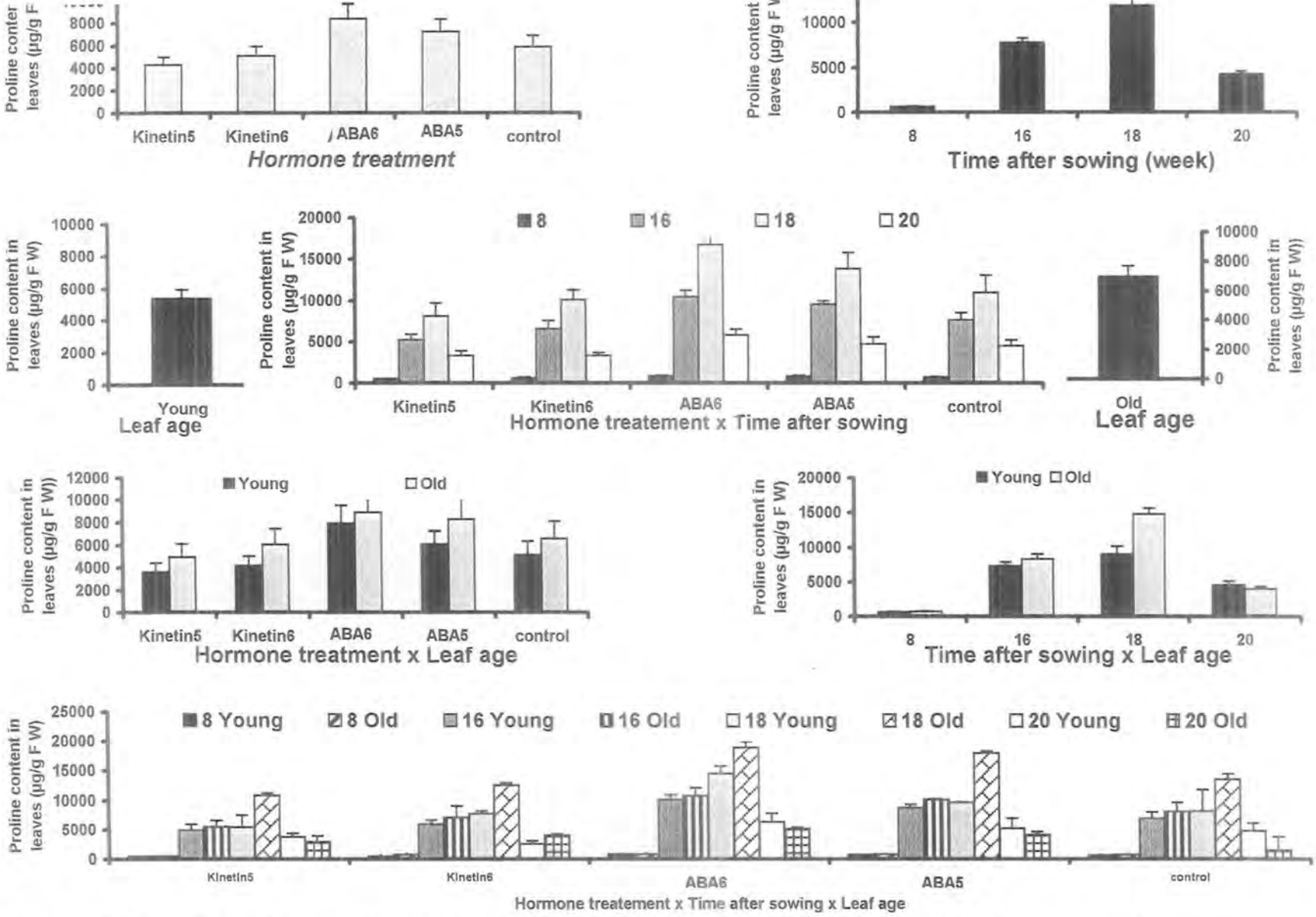


Figure 7: The effect of interactions between treatments, plant growth stages and leaf age on proline content (µg/g F W) in young and old leaves of *Cicer arietinum* L. cv. CM88 (year 1999).

3.6 Sugar Content:

The results presented in figure 8 indicate that in both the young and the old leaves the sugar content was decreased from the vegetative to the flowering stage, increased slightly at early pod filling stage and then sharply increased at the late pod filling stage. At the vegetative stage (8 WAS), the young and the old leaves had almost similar sugar content. However, the reduction in sugar content was greater at the flowering stage in the old leaves as compared to the young leaves. At the late pod filling stage, the sugar content was the highest than at all other stages, both in the old and the young leaves. Treatments and stages of plant growth interacted at highly significant level ($P< 0.001$) in affecting the sugar content in chickpea leaves, whereas the effect of leaf age was non significant (see appendix II, Table 43).

Kinetin treatments significantly increased the sugar content of chickpea leaves at all growth stages. Kinetin was more effective at 10^{-5}M in increasing the sugar content (19%) over the control as compared to 10^{-6}M kinetin, that increased the sugar content by 8%. However, the effect of both concentrations of kinetin were non significantly different from each other. The effect of kinetin was more profound in the young leaves as compared to the old leaves. The decrease in sugar content noted in kinetin treatments at flowering stage was greater in the old leaves as compared to the young leaves, and also the increase in sugar content at later stages was greater in the old leaves as compared to the young leaves. Kinetin (10^{-5}M) increased sugar content significantly at the late pod filling stage.

The application of ABA reduced the sugar content in the treated plant leaves non-significantly as compared to the control at all growth stages. Although there was no significant difference between the ABA treatments, 10^{-5}M ABA was more effective in decreasing the sugar content of the leaves (21%). At the late pod filling

stage, 10^{-5}M ABA was highly significant in decreasing the sugar content in the old leaves, whereas its effect on the young leaves was non significant (see appendix I. Table 20).

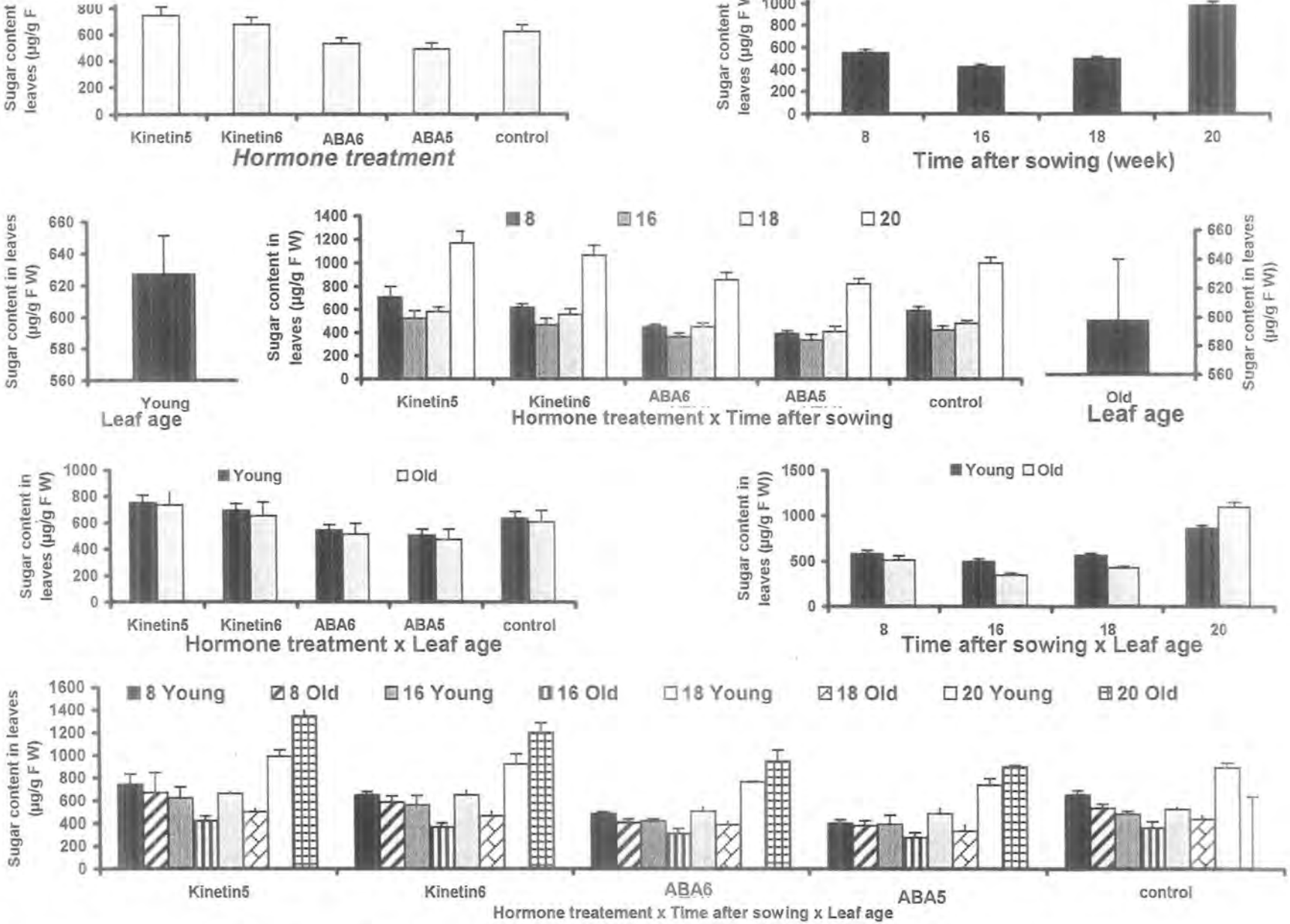


Figure 8: The effect of interactions between treatments, plant growth stages and leaf age on sugar content (µg/g F W) in young and old leaves of *Cicer arietinum* L. cv. CM88 (year 1999).

3.7 The Effect of Interactions Between Treatments, Plant Growth Stages and Leaf Age on Chlorophyll, Protein, Proline and Sugar Content:

The possible interactions presented in Figure 5 show that kinetin (irrespective to growth stages) increased the chlorophyll content while ABA decreased it as compared to the control. Regarding growth stages, the vegetative stage (8WAS) had the highest chlorophyll content but showed a decrease at the flowering stage and the late pod filling stage. The interaction between hormone treatments and leaf age showed higher chlorophyll content in the young as compared to the old leaves in all the treatments being higher with kinetin treatment.

The possible interactions presented in Figure 6 showed that kinetin (irrespective to growth stages) was stimulatory to protein content while ABA decreased it as compared to the control. Regarding growth stages, the flowering stage (16WAS) had the maximum protein content followed by a decrease at the early pod filling stage. The interaction between hormone treatments and leaf age showed higher protein content in the young leaf as compared to the old leaf in all treatments, whereas it was highest with kinetin treatment. ABA decreased the protein content in the young and the old leaves as compared to the control. Protein content in young and old leaves was almost similar with ABA treatment. The interaction between time after sowing and leaf age showed that there was no significant difference in the protein content in the young and the old leaves at all stages of plant growth, except the late pod filling stage, where the young leaves showed significant higher protein content as compared to the old leaves.

The interactions presented in Figure 7 show that applied hormones affected proline content irrespective of growth stages. Kinetin decreased the proline content,

while ABA significantly increased it as compared to the control. This effect was concentration-dependent. Regarding the growth stages, a linear increase in the proline content was noted from the vegetative stage to the early pod filling stage followed by a sharp decrease at the late pod filling stage. The interaction between hormone treatments and leaf age showed higher proline content in the old leaves as compared to the young leaves in all treatments; being higher in ABA treatment.

The interactions presented in Figure 8 show that kinetin (irrespective of growth stages) increased the sugar content, while ABA decreased it as compared to the control. Regarding the growth stages, a linear increase in the sugar content was noted from the flowering to the maturity stage (20WAS). The interaction between the hormone treatments and leaf age showed that the applied hormones affected the young and the old leaves to the same extent. The interaction between time after sowing and leaf age showed that the sugar content significantly increased only at the late pod filling stage, where the old leaves had higher sugar content as compared to the young leaves.

Chlorophyll content was higher at the vegetative stage in the young leaves, and maximum protein content at the flowering stage reflects the higher activity of the plant at these stages. The proline content decreased in the old leaves at the late pod filling stage when their was no further need for osmoprotectant. The high sugar content in the old leaves at the late pod filling stage was perhaps, not associated with senescence.

3.8 Endogenous level of ABA:

Only two stages were selected to detect the endogenous level of ABA in plant leaves, namely the vegetative (8WAS) and the late pod filling stages (20WAS) in the year 2000.

The results (Table 1) show that a significant increase in ABA in the control treatment was noted at the late pod filling stage (20 WAS) as compared to the vegetative stage (8WAS). The interaction between treatments and plant growth stages was highly significant in affecting the endogenous level of ABA (see appendix II, Table 44).

Kinetin at 10^{-5}M concentration decreased the endogenous level of ABA (16%) as compared to the control at both growth stages of plant, where it was more effective at the vegetative as compared to the late pod filling stage. Kinetin at 10^{-6}M did not significantly decrease the endogenous level of ABA at the vegetative stage. However ABA concentration in plant leaves at the late pod filling stage was higher as compared to the control. Exogenous application of ABA at 10^{-5}M increased the endogenous level of ABA by 89% at the vegetative stage as compared to 81% at the late stage, whereas the application of ABA (10^{-6}M) was less effective in increasing the endogenous level of ABA.

Table 1: DMRT of means showing the endogenous level of ABA (μg g^{-1} freeze dried leaves) in *Cicer arietinum* L.cv. CM88 at different plant growth stages and the effect of plant growth regulators (year 2000).

Treatments	–Weeks After Sowing–		
	8	20	Mean
Control	92.0 g	236.7 cd	164.4 D
Kinetin (10^{-5} M)	50.7 h	225.2 d	138.0 E
Kinetin (10^{-6} M)	89.3 g	354.4 b	221.9 B
ABA (10^{-5} M)	174.3 e	429.1 a	301.7 A
ABA (10^{-6} M)	127.2 f	242.7 c	184.9 C
Mean	106.7 B	297.6 A	

All such means which share a common English letter are statistically similar otherwise are different at $\alpha = 0.05$.

3.9 IAA Content:

The results in figure 9 indicate a slight decrease in IAA content at the flowering stage (16 WAS) followed by a sharp increase at the early pod filling stage (18 WAS) and subsequent sharp decrease at the late pod filling stage (20 WAS). All the treatments showed a non significant difference in the IAA content at the vegetative stage, the flowering and late pod filling stages.

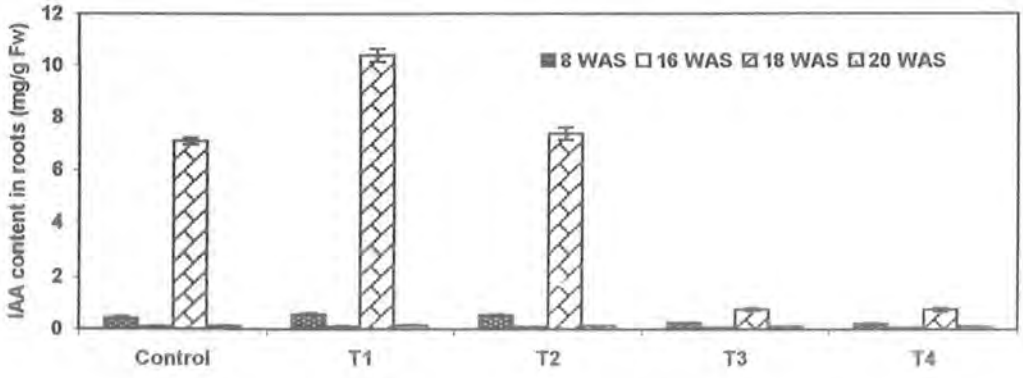
The results indicate the significant effect of treatments and growth stages on the IAA content (see appendix II, Tables 45-46).

The application of kinetin increased the IAA content in plant roots as compared to the control at the early pod filling stage. Kinetin increased accumulation of the IAA content during plant growth. During 1999, 10^{-5}M kinetin was the most effective in increasing the IAA content as compared to the control (44%), whereas 10^{-6}M kinetin was more effective in increasing the IAA content (having 37% increase over control) during the year 2000.

At the early pod filling stage (18WAS), IAA content increased significantly in kinetin treatments as compared to the control. The higher kinetin (10^{-5}M) concentration had significantly higher IAA content than noted with the lower kinetin (10^{-6}M) concentration.

ABA decreased (non significantly) the accumulation of IAA during different stages as compared to the control. Only at the early pod filling stage, ABA treatments significantly decreased the IAA content as compared to the control. The effect of ABA at both concentrations was non significantly different from each other in decreasing the IAA content. 10^{-5}M concentration ABA decreased the IAA content as compared to the control by 83% and 60%, respectively, in the two consecutive years.

whereas 10^{-6}M ABA decreased the IAA content by 84% and 52%, respectively. The IAA content was many fold higher at the early pod filling stage than at all other stages in both years. Also, the changes due to ABA application were significant at this stage. Both years showed same trend of changes regarding the application of hormones at different concentrations (see appendix I, Tables 21-22).



gure 9: IAA content in roots (mg/g FW) of *Cicer arietinum* L. cv. CM88 at different growth stages and the effect of plant growth regulators (year 1999). Vertical bars showed the standard error.

3.10 Fresh Weight, Dry Weight of Nodules:

The results presented in figure 10 and 11 show an increase in fresh and dry weight of nodules during plant growth until the early pod filling stage. This increase was more clear and significant at the flowering stage (16 WAS) and the early pod filling stage (18WAS), which was followed by decrease in fresh weight of the nodules at the late pod filling stage (20WAS). Dry weight of the nodules was the highest at the early pod filling stage and decreased at the late pod filling stage (20WAS). This dry weight was maximum at the early pod filling stage, followed by the flowering stage in all treatments.

The interaction between the treatments and the growth stages was non significant. However, the effect of growth stages was significant on the fresh weight of the nodules ($P< 0.001$), while it was not significant on the dry weight of the nodules ($P>0.05$, see appendix II, Tables 47-48).

Kinetin (10^{-5}M) increased the fresh weight and dry weight of the nodules. On the average, the increase over the control was 11%. However, 10^{-6}M kinetin did not affect the nodules weight. Kinetin at 10^{-5}M concentration increased the fresh weight of the nodules at the flowering stage and the early pod filling stage by 50%, whereas its effect on the dry weight of the nodules was highest at the early pod filling stage.

ABA applied as foliar spray decreased the fresh weight of the nodules as compared to the control during the growing season by 8% and the dry weight of the nodules by 22%. It is noteworthy that the fresh weight and dry weight of the nodules was higher with 10^{-5}M ABA as compared to 10^{-6}M ABA at all growth stages (see appendix I, Tables 23-24).

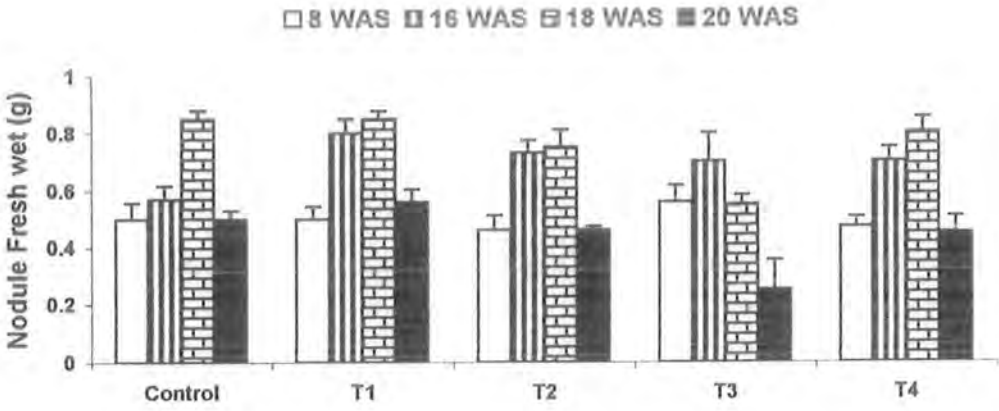


Figure 10: Weight of fresh nodules (g/palnt) in *Cicer arietinum* L. cv. CM88 at different growth stages and the effect of growth regulators (year 2000). Vertical bars error showed the standard error.

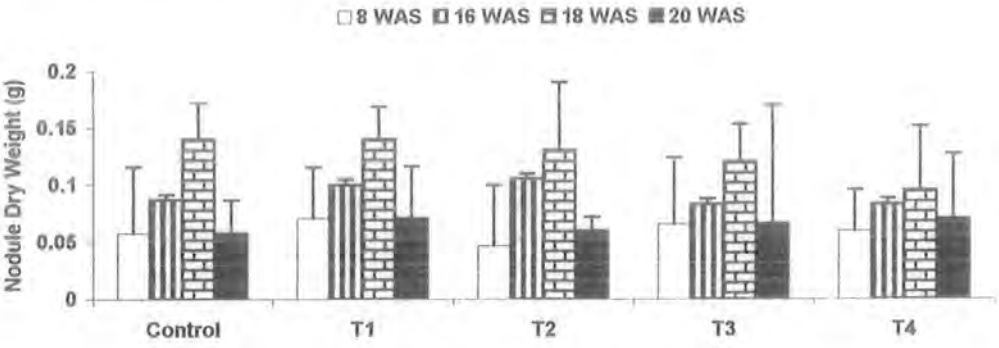


Figure 11: Weight of dry nodules (g/palnt) in *Cicer arietinum* L. cv. CM88 at different growth stages and the effect of growth regulators (year 2000). Vertical bars showed the standard error.

3.11 Diameter of Pink Bacteroids Tissue:

The results in figure 12 indicate an increase in the diameter of the pink bacteroid tissue during the different growth stages, with its maximum at the early pod filling stage (18 WAS) followed by significant decrease at the late pod filling stage. The increase in the diameter of the pink bacteroid tissue was greater at the flowering stage as compared to the increase noted at the early pod filling stage. The effect of treatments and plant growth stages and their interaction were highly significant (see appendix II, Tables 49-51).

Kinetin significantly increased the diameter of the bacteroids tissue as compared to the control at different stages of plant growth except at the vegetative stage. At all stages of plant growth, kinetin treatments were non significantly different from each other. The effect of kinetin was significantly the highest at the flowering stage and at the early pod filling stage. Kinetin-treated plants showed the minimum decrease (as compared to control) in the diameter of the pink bacteroid tissue at the late pod filling stage. Kinetin (10^{-5}M) treatment was more effective in increasing the diameter of bacteroid tissue in the three consecutive years (10%, 30% and 18%, respectively) as compared to the lower concentration in the three consecutive years (6%, 21% and 7%, respectively).

Application of ABA non-significantly (as compared to control) decreased the diameter of the bacteroids tissue of the nodules of treated plants in all stages of growth. ABA was more effective at 10^{-6}M in decreasing the diameter of the bacteroid tissue in three consecutive years (18%16% and 19%, respectively) as compared to

10^{-5}M concentration (6%, 8%,12%). The effect of ABA (10^{-6}M) treatment was non-significantly greater than that of 10^{-5}M ABA in decreasing the diameter of the bacteroid tissue. ABA treatments showed higher decrease in the bacteroid tissue as compared to the control at the late pod filling stage.

The data of the three years were slightly different from each other but trend of changes was almost similar due to application of the hormones in the three years. The diameter of the pink bacteroid tissue was larger in 1999 than in the other two years (see appendix I, Tables 25-27).

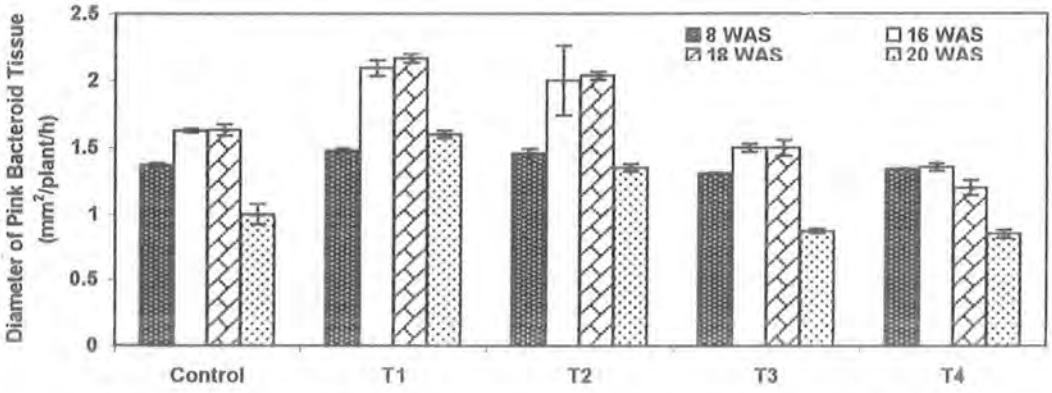


Figure 12: Diameter of pink bacteroid tissue (mm/plant/h) at different growth stages in *Cicer arietinum* L. Cv. CM88 and the effect of plant growth regulators (year 1999). Vertical bars showed the standard error.

3.12 Nitrogenase Activity:

The results in figure 13 indicate that the changes in nitrogenase activity due to the hormones were similar to those of the pink bacteroid tissue. The nitrogenase activity increased during plant growth. A significant increase in nitrogenase activity was noted at the flowering stage (16WAS), the increase being highest at the early pod filling stage (18 WAS) followed by a significant decrease at the late pod filling stage (20 WAS). At the vegetative stage and the late pod filling stage, all the treatments were non significantly different from the control, wherein at the late pod filling stage were lower than the initial readings at the vegetative stage. The nitrogenase activity in kinetin treatment was the highest as compared to the control (see appendix I, Tables 28-30).

Nitrogenase activity was significantly affected by the treatments at $P< 0.001$ (see appendix II, Tables 52-54). Among the different treatments, kinetin application showed a significant increase in nitrogenase activity as compared to the control. The highest effect of kinetin at both concentrations was noted at the flowering and the early pod filling stages, whereas at the late pod filling stage the nitrogenase activity was non significantly different from the control. Kinetin treatments showed increase by 100% at the early pod filling stage. Kinetin treatment at 10^{-5}M, increased the nitrogenase activity by 196%, 84% and 80%, respectively, in the three consecutive years, whereas 10^{-6}M kinetin showed lesser increase (82%, 30% and 38%, increase over the control). The effect of 10^{-5}M kinetin was slightly non-significantly different from the effect of 10^{-6}M kinetin except at the flowering and the early pod filling stage.

ABA decreased the nitrogenase activity significantly as compared to the control at all growth stages except at the late pod filling stage, where the effect of

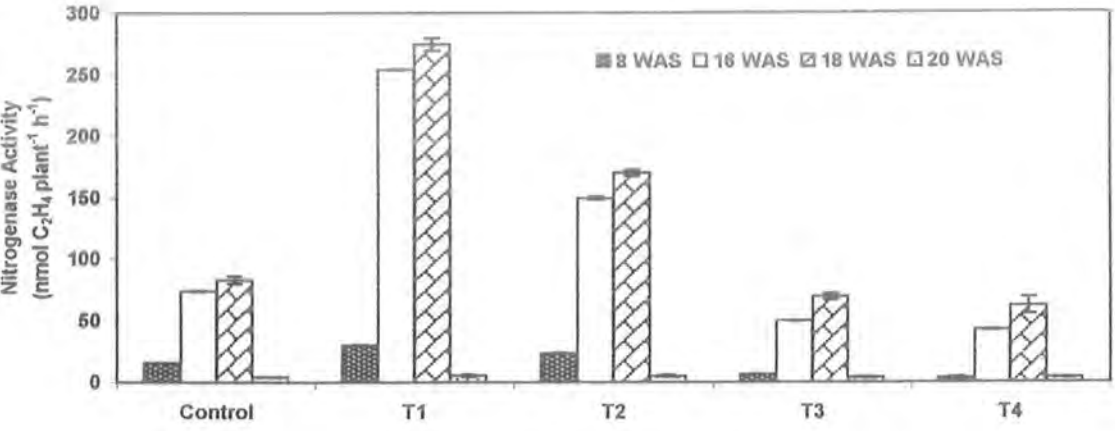
ABA was non significantly different from the control. ABA treatments had greater effect at the early pod filling stage, which was significantly lower than the control. The two concentrations of ABA used were not significantly different from each other in decreasing the nitrogenase activity. However, 10^{-5}M ABA was slightly less effective in decreasing the nitrogenase activity as compared to 10^{-6}M ABA. The ABA 10^{-5}M decreased nitrogenase activity over the control by 32%, 46% and 21%, respectively, in the three consecutive years, whereas 10^{-6}M ABA decreased it over the control by 41%, 43% and 31%, respectively. The same trend of changes was noted following the treatments during the three years. However, in 1999 all treatments showed higher level of nitrogenase activity as compared to 1998 and 2000.

Figure 14 show that the nitrogenase activity per mm pink bacteroid tissue increased during plant growth. A significant increase in nitrogenase activity per mm pink bacteroid tissue was noted at the flowering stage (16WAS) and at the early pod filling stage (18 WAS) followed by a significant decrease at the late pod filling stage (20 WAS). At the late pod filling stage, all the treatments were non significant different.

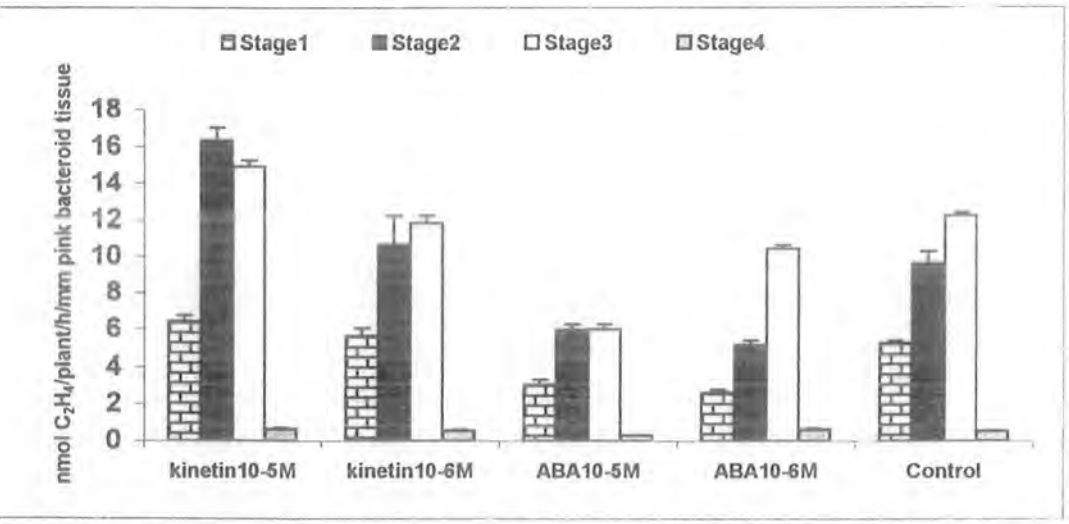
Among the different treatments, 10^{-5}M kinetin application showed a highest significant increase in nitrogenase activity per mm pink bacteroid tissue as compared to the control. The highest effect of kinetin at both concentrations was noted at the flowering and the early pod filling stages, whereas at the late pod filling stage the nitrogenase activity per mm pink bacteroid was non significantly different from the control.

ABA decreased the nitrogenase activity per mm pink bacteroid tissue significantly as compared to the control at all growth stages except at the late pod filling stage, where the effect of ABA was non significantly different from the

control. ABA treatments had greater effect at the flowering and at the early pod filling stages, which was significantly lower than the control. The two concentrations of ABA used were not significantly different from each other in decreasing the nitrogenase activity per mm pink bacteroid tissue except at the early pod filling stage where 10^{-6}M ABA was significant higher than 10^{-5}M ABA.



igure 13. Nitrogenase activity of nodules (nmol C_2H_4/plant/h) in *Cicer arietinum* L.cv. CM88 at different growth stages and the effect of plant growth regulators (year 1999). Vertical bars showed the standard error.



igure 14: Nitrogenase activity/ mm of pink bacteroid tissue in Nodules of Cicer arietinum L. cv.CM88 at different growth stages and the effect of plant growth regulators (year 1999). Vertical bars showed the standard error.

3.13 Available Nitrogen Content (NO_3-N) in Soil:

The results show that treatments interaction revealed significant effects on N in the soil (see appendix II, Table 55). Kinetin application increased the available N as compared to the control in the three consecutive years (Table 3). Kinetin treatment at a concentration of 10^{-5}M showed the highest soil NO_3-N among the treatments (790% and 249%, respectively), which was significantly higher than the effect of 10^{-6}M kinetin during years 1998 and 2000 (458% and 39%, respectively).

ABA treatment also increased the available soil N content as compared to the control. The 10^{-5}M ABA increased the N content by 178%, 28% and 75%, respectively, in the three consecutive years. The 10^{-6}M ABA on the other hand did not affect the available soil N content during the year 1998 but (non significantly) decreased the soil N content slightly during the following two years.

Table 2: DMRT of means showing the available N content (mg/ kg) of soil planted with *Cicer arietinum* L. cv. CM88 and the effect of plant growth regulators in three consecutive years.

Treatments	NO_3-N		
	1998	1999	2000
Control	0.60 D	1.53	1.07 D
Kinetin (10^{-5} M)	5.34 A	2.07	3.73 A
Kinetin (10^{-6} M)	3.35 B	2.12	2.66 B
ABA (10^{-5} M)	1.67 C	1.96	1.87 C
ABA (10^{-6} M)	0.63 D	1.29	0.96 D

All such means which share a common English letter are statistically similar otherwise are different at $\alpha = 0.05$.

3.14 Yield:

The results show that the treatments had highly significant effect on chickpea yield in respect to grain weight, number of pods per plant and weight of 100 grains per treatment in the three consecutive years (see appendix II, Table 58-66). In three consecutive years (Table 4), kinetin application increased the yield significantly as compared to the control. Kinetin applied as seed soaking (10^{-5}M) increased the grain weight by 45%, 42% and 68%, weight of 100 grains by 17%, 33% and 15% and number of pods per plant by 50%, 12% and 77%, respectively, in the three consecutive years. Kinetin at a concentration of 10^{-5}M had a significantly greater effect in increasing yield as compared to 10^{-6}M kinetin that was applied as foliar spray.

While ABA significantly decreased the yield as compared to the control, but when applied as seed-soaking at 10^{-5}M concentration it was more effective in decreasing the yield as compared to 10^{-6}M ABA applied as foliar spray. The higher concentration of ABA (10^{-5}M) decreased the grain weight by 58%, 34% and 25%, weight of 100 grain by 18%, 16% and19%, and number of pods per plant by 17%, 50%, and 17%, respectively, in the three consecutive years. Generally, there was no significant difference at the two concentrations of ABA.

In 1999, the grain weight and number of pods per plant increased 2-fold, whereas the weight of 100 grains increased by 40% as compared to the year 1998 and 2000. In 1998 and 2000 the yield was almost similar.

Table 3: DMRT of means showing yield of *Cicer arietinum* L.cv. CM88 harvested at edible pod stage (22 weeks after sowing) and the effect of plant growth regulators in three consecutive years.

Treatments	Grain Weight (g)			Weight of 100grains (g)			No. of Pods plant $^{-1}$		
	1998	1999	2000	1998	1999	2000	1998	1999	2000
Control	3.1 B	6.7 C	3.1 B	76.2 B	96.0 B	77.2 B	3.0 C	7.4 B	3.0 BC
Kinetin (10^{-5} M)	4.5 A	9.5 A	5.2 A	88.9 A	128.0 A	88.9 A	4.5 A	8.3 A	5.3 A
Kinetin (10^{-6} M)	3.3 B	8.0 B	4.3 B	79.2 B	117.0 B	80.2 B	3.8 B	8.3 A	4.3 B
ABA (10^{-5} M)	1.3 D	4.4 D	2.3 D	62.4 C	81.0 C	61.8 C	2.5 D	4.0 D	3.5 D
ABA (10^{-6} M)	2.3 C	4.7 D	3.5 C	60.0 C	81.0 C	64.2 C	2.7 D	4.7 D	2.6 E

All such means which share a common English letter are statistically similar otherwise are different at $\alpha = 0.05$.

DISCUSSION

4.1 Dry Weight of Root and Shoot:

During vegetative stage, shoot and root dry weight showed a linear increase until the flowering stage and reached its peak at the early pod filling stage. At the late pod filling stage, dry weight of the shoot and the root decreased. This decrease, perhaps, was due to abscission of old leaves resulting in a decrease in net storage of the assimilates in the tops and translocation of assimilates to the pods, which may have led to decrease in dry weight of the root. In Pigeon pea, leaf dry matter reached it's maximum at early pod development and then decreased rapidly, perhaps, due to leaf abscission and carbon translocation to other plant parts (Sanetra *et al.*, 1998) or due to accumulation of CK at this stage as reported by Emery *et al.* (2000). Kinetin treated plants showed maximum increase in dry weight of shoot and root at the early pod filling stage. Application of kinetin led to increased dry weight of the shoot, perhaps, by increasing shoot length and the number of leaves per plant. Cytokinins are known to stimulate cell division and regulate cell differentiation (Jacqumand *et al.*, 1994, Morris, 1997 and D'Agostino and Kieber, 1999). However, ABA was found to decrease dry weight of the shoot and the root at the vegetative stage and at the late pod filling stage. The increase in ABA correlated closely with inhibition of shoot growth as reported by Hansen and Grossmann (2000). Cramer *et al.* (1998) have reported that ABA reduces root elongation and inhibits shoot growth (Creelman *et al.*, 1990). ABA reduces the plant growth and enhances plant senescence (He *et al.*, 2001). It has been found that ABA reduces dry weight of the shoot in soybean (Cho

and Harper, 1993), whereas Bano *et al.* (1983) have reported that ABA reduces dry weight of the root and the shoot in Faba bean.

4.2 Chlorophyll Content:

Woolhouse, (1984) and Grove *et al.*, (1986) have reported that reduction of chlorophyll is one of the initial symptoms of leaf senescence. In the present study, the decrease in chlorophyll content may have started after the flowering stage (16 WAS) and became more evident at the early pod filling stage (18 WAS). Lindoo and Nooden (1977), Wittenbach *et al.* (1980), Grover *et al.* (1985) and Congming and Jianhuazhang, (1998) have reported that senescence of leaves initiates and progresses after flowering. The age of the plants and senescence could be the basic factors responsible for decrease in chlorophyll content.

As reported presently, chlorophyll content in all treatments was higher in the young leaves as compared to the old leaves, whereas the process of leaf senescence was initiated in the old leaves. This sequential senescence may explain the decrease in the chlorophyll content in the old leaves at the late pod filling stage. Imre *et al.* (1981) and Sanetra *et al.* (1998) have shown that the senescence signal is transported basipetaly and accordingly the span of the leaf layer in the canopy tends to be longer toward the top of the plant at harvest. In soybean, the leaves near the bottom of the canopy have lower chlorophyll content as compared to the upper leaves (Congming and Jianhuanzhang, 1998).

Nooden *et al.* (1990) have reported that the decrease in cytokinins generally is required for monocarpic senescence. Hajouj *et al.* (2000) have reported that CK delays the initiation of leaf senescence. So, it is expected that exogenous application

of kinetin overcomes the decrease in CK level, which takes place in the mature tissue and at later stages of growth.

Kinetin-treated plants showed increase in chlorophyll content in the young and the old leaves at all stages of plant growth. Kinetin possibly alleviates and/or inhibits some or all of the abscission inducing factors (Carlson *et al.*, 1987, Chaloupkova and Smart, 1994 and Hare *et al.*, 1997). The effect of kinetin as seed soaking had profound effect on the old leaves. Perhaps, it delays leaf senescence by inducing re-greening in the leaves as has been shown by Marek and Stewart (1992).

At the early and the late pod filling stages, when the old leaves abscised, cytokinin produced by the roots might have been transported only to the young leaves that played role in increasing the chlorophyll content in the young leaves as compared to the old leaves. It has been reported that application of cytokinins promotes photosynthetic activity mainly by means of increase in chlorophyll content (Caers and Vendring, 1986). Van Staden and Carmi (1982) have suggested that the effect of partial defoliation on the remaining leaves results in accumulation of more cytokinin.

Cytokinins are known to inhibit the activity of IAA oxidase and other peroxidases (Garg *et al.*, 1995). Kinetin applied at 10^{-5}M as seed soaking was more effective. The higher concentration of kinetin is possibly required to antagonize the inhibiting effect of plant growth retardants on the chlorophyll content and enhances the effect of other growth promoters such as IAA.

Application of ABA caused reduction in the chlorophyll content in both the young and the old leaves at the all stages of plant growth. The effect of ABA was more evident at the early pod filling stage in the old leaves. Application of ABA and enhanced leaf senescence. It has been reported that ABA enhances leaf senescence in bean (Imre *et al.*, 1981) and in oat (Gepstein and Thimann, 1980). ABA application

greatly enhances senescence in the old leaves as has been reported by Lindoo and Nooden (1977), Samet and Sinclair (1980) and Imre *et al.* (1981) whereas the young leaves were affected less by ABA. ABA is synthesized in the young tissue to a lesser degree than in mature tissues (Chanson and Pilet, 1982 and Zeevart and Boyer, 1984). Exogenous application of ABA in the present study, enhanced the endogenous level of ABA, which further augmented degradation of chlorophyll in the treated plant leaves. Greg *et al.* (1991) and ChaloupKova and Smart, (1994) have demonstrated that the application of ABA significantly decreases the chlorophyll content.

4.3 Protein Content:

The present results showed that protein content increased at the flowering stage in the young and the old leaves in all treatments. This increase reflects that the plants store more protein in their leaves throughout the vegetative to flowering stages. The demand for protein for fruit setting and seed formation is enhanced at the early pod filling stage and this may explain the decrease in protein content in chickpea leaves at this stage. Woolhouse (1982) has also reported that, the demand for protein is high at the flowering stage. He has shown that loss of protein and chlorophyll begins before leaf yellowing, indicating the onset of senescence process. The significant decrease of protein content noted at late pod-filling stages in the old leaves was concomitant with increase in protein content in the young leaves. It is possible that during senescence transfer of stored protein occurs from the old leaves to the young leaves. Wollaston (1997) has suggested that the protein content in the leaves can be rapidly degraded according to the need by other plant tissues.

Grover *et al.* (1985) and Staswick (1989) have reported that protein synthesis decreases in the old leaves. Sanetra *et al.* (1998) has shown that N demand for seed

production in several legumes cannot be met by N uptake alone. Therefore, the any remaining deficiency has to be made up by the vegetative tissue. During the remobilization phase, leaves start to behave as source organs, translocating carbon and organic molecules to ensure the formation of new developing tissues and/or storage such as seeds tissues involved in plant survival (Masclaux *et al.*, 2000). Remobilization of nitrogen has been shown to be dependent on leaf longevity (Rajan and Tollenaar, 1999a, 1999b). The nutrients are recycled from senescing cells to such other parts of the plants as meristem, young leaves and flowers (Nooden *et al.*, 1997). The mature protein (slowly matured to make soluble protease) accumulates in association with leaf senescence (Yamada *et al.*, 2001). Increase in protein content was noted presently in kinetin treatments at all growth stages. Kinetin might have enhanced the activity of the young leaves for protein synthesis as compared to the old leaves. It has been proposed that kinetin may be involved directly in the process of regulating protein transcription and translation (Crowell and Amasino, 1994 and Brinegar, 1994), and it may alleviate the promoters of senescence-induced protease (Gan and Amasino, 1995). The increase in protein content in the old leaves at maturity (20WAS) due to kinetin treatment may be due to the fact that exogenous application of kinetin enhanced the endogenous level of CKs in the old leaves at maturity (20 WAS) and increased their protein content. Beck (1992) has reported that a higher nitrogen status of the plants is always correlated with higher endogenous level of cytokinin. Exogenous application of benzyl adenine (BA) increases the availability of cytokinin in wheat (Rabie, 1996). It is possible that kinetin has the same effect. Kinetin may recover the decline of protein during senescence (Mancera *et al.*, 1999). The nitrogen metabolism of crops has been considered to improve the yield leaf protein (Imsande, 1988). Kinetin increased the nodule activity, which may

explain the increase in protein in the plant leaves. Dayal and Bharti (1991) and Garg *et al.* (1995) have reported that kinetin induces increase in N_2-ase activity.

It is shown here that ABA decreased the protein content in the young and the old leaves at all growth stages; the decrease was greater in the old leaves at the late pod filling stage. Perhaps ABA affects protein content by decreasing its synthesis and/or enhancing its degradation (Rock and Quatrano, 1996). Thus ABA-induced protease production in plant leaves (Mortin and Thimann, 1972, Fam *et al.* 1973 and Rock and Quatrano, 1996). Exogenous application of ABA increased the endogenous level of ABA at the vegetative stage as well as at the late pod filling stage. This increase may cause more degradation of protein content in the young leaves and the old leaves at both stages. In addition, leaf senescence as a factor combined with ABA-treated plants to cause leaf abscission more rapidly in the old leaves as compared to the young leaves. ABA has the potential to antagonize other growth hormones such as IAA (Greg *et al.*, 1991) and CKs (ChaloupKova and Smart, 1994). The above mentioned workers have suggested that ABA rapidly induces elevated level of mRNA transcript encoding basic peroxidase.

4.4 Proline Content:

Proline synthesis has a role in regulating several physiological responses, including developmental transitions as reported by Hare *et al.* (1997). Proline acts as osmoprotectant (Yoshiba *et al.*, 1997) and as a storage compound for nitrogen and carbon when starch and protein synthesis are inhibited (Flowers *et al.*, 1977, Chauhan *et al.*, 1980 and Naidue *et al.*, 1990). The accumulation of proline is accompanied by increase in soluble sugar content (Chen *et al.*, 1998 and Clifford *et al.*, 1998). In *Phseolus aureus*, Huq and Larher (1983) has reported that the production of organic solutes such

as asparagines, proline, glycine, betaine and free amino acids was increased in salt stress. Huq claimed that these organic solutes are a characteristic of stress conditions.

The present results show that at the initial vegetative stage, the old leaves had higher proline content as compared to the young leaves. An increase in proline content was noted in the old leaves from flowering till maturation. Proline accumulation may be related to leaf senescence because senescence of the plant and old leaves require more osmoregulation. Zhang *et al.* (1995) have reported that high level of sugar in leaves keeps proline high. At the late pod filling stage (20WAS) proline content decreased and switch off and start to decline in both the young and the old leaves because senescence was almost complete and no more osmoprotectant was needed.

ABA is well known to retard plant growth, accelerate leaf senescence and increase proline content. Exogenous application of ABA has been shown to induce accumulation of proline in plant leaves (Stewart and Voetberg, 1985, Hare *et al.*,1997 and Hare *et al.*, 1999). The increase in proline content due to exogenous application of ABA may reflect the causal link between ABA and proline accumulation (Xin and Li, 1993 and Savoure *et al.*, 1997). ABA might have enhanced proline synthesis, or might have been involved in the redistribution of proline as has been reported by Dallmier and Stewart (1992). The increase in proline content in ABA treatment may act as a mechanism to provide tolerance to the plants possibly due to reduction in nutrients and assimilates. Chauhan *et al.* (1980) and Naidu *et al.* (1990) have reported that proline accumulation enhances the tolerance of plants to stress.

Exogenous application of kinetin appeared to reduce proline content as compared to the control in the young and the old leaves at all growth stages. Kinetin at 10^{-5}M concentration was more effective in delaying leaf senescence and it showed

less proline content in the plant leaves as compared to kinetin used at a 10 fold lower concentration. In Faba bean, kinetin application reduces proline accumulation (Bano, 1986).

4.5 Sugar Content:

Sugar is the principal form and the major export and storage form of photoassimilate in leaves (Sheem, 1994, Koch 1996 and Patrick and Offler, 1996). A decrease in sugar content was noted in the young and the old leaves at the flowering stage (16 WAS). This may be attributed to mobilization of C and N to reproductive parts at this critical stage as reported by Grover *et al.* (1985). Sugar is possibly transported from the fully expanded mature leaves to the young developing leaves (Brandner *et al.*, 1984b). The increase in sugar content in the young and the old leaves was linear from flowering to maturation. McCabe *et al.* (2001) have reported that an increase in sucrose after the onset of flowering in lettuce. They have explained this possibly, results from starch breakdown or reduction in sucrose consumption. In the present study sugar increased in the young leaves as well as in the old leaves when the seed demand for nutrient was maximum at the late pod filling stage (18 WAS). Delhon *et al.* (1996) have found that sugar is effective in stimulating N product from chickpea roots after flowering. Senescence of whole soybean plant during mid pod set suggests that the supply of photoassimilate exceeds the accompanying demand by the process of growth and maintenance (Brandner *et al.*, 1984b and Gwathmey *et al.*, 1992c). Photosynthetic assimilates determine the availability of carbon in leaves and accordingly determines the production and accumulation of sugar (Stitt, 1986, Prioul, 1996 and Foyer and Galtier, 1996).

Application of kinetin increased sugar content in the young leaves and the old leaves at all growth stages. The increase was greater in the young leaves, perhaps, due to increased photosynthetic assimilates in the leaves. It is well known that CKs increase the internal production of reducing sugars as reported by McCabe *et al.* (2001). Patrik and Offler (1996) have reported that kinetin enhances photosynthetic assimilates which are transported to the recipient cells in the form of sucrose. Gwathmey *et al.* (1992a) have found that starch and sucrose accumulate in stems of delay leaf senescence (DLS) genotype of *Vigna radiata* as compared to the leaf senescence genotype. Kinetin showed effect in delay leaf senescence and the accumulation of sugar may be attributed to the same reason. Erwin (1996) has reported that cytokinin balance of plants results in disproportionate distribution of the assimilate in favor of the cytokinin-enriched shoot.

Application of ABA reduced sugar content in the young and the old leaves at all growth stages but was more evident at the flowering stage. The decrease is greater in the old leaves because of increased ABA synthesis in the old leaves (Samet and Sinclair, 1980). Brandner *et al.* (1984a) have reported that sugar is depleted from the stems of cowpea variety that shows leaf senescence.

4.6 Nodule weight, Diameter of Pink Bacteroid Tissue, Nitrogenase Activity and Nitrogenase Activity per Pink Bacteroid Tissue:

The period from the flowering stage to the early pod filling stage (16-18 WAS) was the critical stage during which nodule activity per mm pink bacteoid tissue, including nodule weight and diameter of pink bacteroid tissue, was the highest. The ARA per plant increases with time as the nodules grow (Pawlowski, 1997). Wollaston (1997) has also found similar results. Bacteroid gives information about

physiological conditions of nitrogen fixation, it reflects the effects of combined nitrogen and the appearance of nodule senescence (Rigaud, 1984). The decrease in these parameters noted presently at the late pod filling stage (20 WAS) may be due to degradation and decrease in the diameter of the pink bacteroid tissue, the active site of N_2-fixation. Similar findings have been reported by Lawn and Burn (1974) and Grover *et al.* (1985). Chlorophyll and ARA are the limitation of senescence that coincides with the approximate time when chlorophyll concentrations are higher in the upper canopy than in the lower canopy of leaves (Brandner *et al.*, 1984a). Roots die after the shoot has undergone other senescence processes, such as leaf drop and pod dry-down (Fisher *et al.*, 2002).

Kinetin treatment increased the fresh weight of the nodules and the diameter of the pink bacteroid tissue and significantly enhanced the nodule activity per mm pink bacteoid tissue. Foliar spray of kinetin is known to bring about considerable increase in nodulation parameters (Singh, 1993). Kinetin increased the N_2-fixation process and increased protein content in plant leaves during flowering and early pod filling stages. Roa *et al.* (1984) has reported that kinetin increases the efficiency of nitrogen fixation. Dayal and Bharti (1991) and Garg *et al.* (1995) have also reported that kinetin induces increase in dry weight of the nodules as well as N_2-ase activity. It has been demonstrated that mRNA, protein concentration and CK content of nodules increases early in nodules ontogeny and decreases as the nodule grow and total nodules activity develops (Cho and Harper, 1993). Singh (1993) has shown that kinetin causes increase in leghaemoglobin content and nodule bacteroidal region. Kinetin applied as seed soaking in the present study was more effective in increasing nitrogen fixation as compared to kinetin applied as foliar spray. Zarrin and Bano

(1998) provided similar results and explained that this is perhaps, due to poor absorption and reduced mobility of kinetin applied to the leaves.

Abscisic acid reduced nodule weight and diameter of bacteroid tissue and accordingly nitrogenase activity per mm pink bacteoid tissue was also reduced. Bano and Hillman (1986) have reported that ABA treatment results in delay in nodule initiation and inhibits nodule growth and development as well as the number of nodules per plant with reduction in functional bacteroids per nodule. Cho and Harper (1993) have reported that exogenous application of ABA results in decrease in nodule weight.

Application of exogenous ABA decreases nodulation, perhaps, by inhibiting the cortical cell divisions required for root nodule development and induces senescence (Moro *et al.*, 1992). ABA decreases weight of nodules with reduction in the volume of pink bacteroid tissue and specific nitrogenase activity (B*ano et al.*, 1983). Sood (1996) has observed that the ABA-treated plants suppress nitrate reductase activity. ABA applied as seed soaking (10^{-5}M) was less effective in the present study in decreasing nodule weight and nodule activity as compared to the effect of 10^{-6}M ABA. The early abscission of the old leaves treated with ABA led to reduction in nodule activity because the old leaves might supply the nodules with products of photosynthesis.

4.7 IAA Content of Roots:

The present results show that IAA content in the roots increased during the vegetative stage, which reached its peak at the early pod filling stage, followed by decrease at the late pod filling stage. At maturity (20WAS), the IAA content decreased, perhaps, due to senescence. The decrease in IAA may be either due to

decrease in its synthesis or by enhancement of its degradation. Greg *et al.* (1991) and ChaloupKova and Smart (1994) have reported that peroxidase activity and esterification of IAA increase during senescence at a later stage when the nodules become soft and fragile. The IAA oxidase activity increases when senescence is almost complete.

Kinetin increased IAA content at all growth stages. The increase was high at the early pod filling stage and low at the late pod filling stage. Kinetin seemed to be effective in increasing the content of IAA perhaps by an increase in IAA synthesis or by inhibition of IAA oxidase activity. Garg (1992, 1995) have reported that cytokinins inhibit the activity of IAA oxidase.

Presently, exogenous application of ABA decreased IAA content in the treated plants at all growth stages. Such application of ABA causes increase in endogenous level of ABA, which augments the effect of ABA on inactivation of IAA. Dunlop and Rodbacker (1990) have reported that ABA enhances esterification of IAA.

4.8 Soil analysis:

Nitrogen is one of the nutrients that most limits plant productivity maximally (Valverde *et al.*, 2002). An advantage of economic value of symbiotic assimilation with N_2 fixing rhizobia is that it provides the crop with N and precludes the need for costly addition of fertilizer (McNeil *et al.*, 1996). The bacteria present in the nodules fix atmospheric N and provide more than 80 kg N ha^{-1} (Sindhu *et al.*, 1992). Aslam *et al.* (2000) have reported that chickpea crop enhances N fertility level of the soil.

Water status of plants and soil has also been found to be affected by phytohormones. Westgate *et al.* (1996) have found that water uptake and water potential are directly affected by ABA, cytokinin and auxin. ABA may be the

triggering agent of cellular degradation process initiated by water stress (Aloni and Pressman, 1981).

Kinetin treatment showed increase in nodule activity over the control and increased the available amount of nitrogen in soil as compared to the control. It is possible that N accumulation is related to biomass accumulation and seed growth. Zarrin *et al.* (1998) have demonstrated that soil nitrate is higher when kinetin is applied as seed soaking or foliar spray.

ABA applied as seed soaking increased the nitrate content in the soil as compared to the control. Perhaps, ABA reduced the uptake and the relative contribution of both nitrogen (nitrate-nitrogen and total nitrogen) sources, mainly influenced by the amount and availability of nitrogen in the soil as suggested by Zarrin (2001). The absolute amount of nitrogen fixed by chickpea is normally decreased with increased availability of mineral nitrogen (Kage, 1995).

4.9 Yield:

Plant hormones are considered as key regulators of seed development (Brenner and Cheikh, 1995). Application of kinetin increased the yield of chickpea by increasing plant growth and plant assimilates through its effect in decreasing retardants like ABA, and by increasing the number of pods per plant and weight of seeds per treatment. Hirel *et al.* (2001) have reported that the increase in maize productivity is due to efficient remobilization of stored nitrogen during grain filling. Since kinetin increases the nitrogen fixation, it is possible that kinetin increases the yield for the same reason. Grover *et al.* (1985) have reported that the rate of pod

growth exceeds the growth rate of the total tops, thus indicating mobilization and translocation of previously stored assimilates from the other plant parts into the pods. In soybean, cytokinins affect seed growth positively by attracting nutrients to the developing fruit or indirectly by protein synthesis and are involved in regulation of seed growth by triggering cell division (Hein *et al.*, 1986). BA increases the number of pods plant $^{-1}$ and seed yield in Pigeonpea (Karan and Kakarlaya, 1997) and in soybean (Reinbott and Blevins, 1998).

Hansen and Grossmann (2000) have reported that ABA are also involved in regulating grain development. In the present study, ABA decreased the yield by increasing the endogenous level of ABA leading to decrease the plant growth and accumulation of dry matter. It also decreased the number of pods and partitioning of the assimilates to the pods. ABA has been found to be most associated with decrease in grain yield of maize (Sanguineti *et al.*, 1999).

Table 5: Physiological ranking of the treatments effect in young and old leaves of *Cicer arietinum L.*cv CM88 as compared to control at four stages of plant growth.

Parameters	Young leaves ——Weeks after sowing——				Old leaves ——Weeks after sowing——			
	8	16	18	20	8	16	18	20
kinetin at 10^{-5}M								
Chlorophyll	higher increase	higher	higher	higher	higher	higher	higher	higher
Protein	higher	higher increase	higher increase	higher increase	higher	higher	higher	higher
Proline	lower	lower	lower	lower	lower	lower	lower	lower
Sugar	higher	higher	higher	higher	higher	higher	higher	higher increase
kinetin at 10^{-6}M								
Chlorophyll	higher	higher	higher	higher	higher	higher	higher	higher
Protein	higher	higher	higher	higher	higher	higher	higher	higher
Proline	lower	lower	lower	lower	lower	lower	lower	lower
Sugar	No difference	higher	higher	higher	higher	higher	higher	higher
ABA at 10^{-5}M								
Chlorophyll	lower	lower	lower	lower	lower	lower	lower	lower
Protein	lower	lower	lower	lower	lower	lower	lower	lower
Proline	higher	higher	higher increase	higher	higher	higher	higher increase	higher
Sugar	lower	lower	lower	lower	lower	lower	lower	higher decrease
ABA at 10^{-6}M								
Chlorophyll	lower	lower	lower	lower	lower	lower	lower	lower
Protein	lower	lower	lower	higher decrease	lower	lower	lower	lower
Proline	higher	higher	higher	higher	higher	higher	higher increase	higher
Sugar	lower	lower	lower	lower	lower	lower	lower	lower

Table 6: Physiological ranking showing the effect of kinetin treatments on *Cicer Arietinum L.*cv. CM88 as compared to control at four stages of plant growth.

Parameters	kinetin at 10^{-5}M ——Weeks after sowing——				kinetin at 10^{-6}M ——Weeks after sowing——			
	8	16	18	20	8	16	18	20
Shoot fresh weight	higher increase	higher increase	higher increase	higher increase	higher increase	higher increase	higher increase	higher increase
Shoot dry weight	higher	higher increase	higher increase	higher increase	higher	higher increase	No difference	higher
Root fresh weight	higher	higher increase	higher increase	higher	higher	higher	higher	higher
Root dry weight	higher increase	higher increase	higher increase	higher increase	higher	higher	higher	higher
Nodule fresh weight	No difference	higher	No difference	higher	No difference	higher	lower	lower
Nodule dry weight	higher	higher	No difference	higher	lower	higher	No difference	higher
Diameter of pink bacteroid tissue	higher	higher increase	higher increase	higher increase	higher	higher increase	higher increase	higher increase
Nitrogenase activity	higher	higher increase	higher increase	higher increase	higher	higher increase	higher increase	higher
IAA content	higher increase	No difference	higher	higher	higher	No difference	higher increase	higher
ABA content in leaves	higher decrease			lower	lower			higher

Table 7: Physiological ranking showing the effect of ABA treatments on *Cicer Arietinum L.*cv. CM88 as compared to control at four stages of plant growth.

Parameters	ABA at 10^{-5}M ——Weeks after sowing——				ABA at 10^{-6}M ——Weeks after sowing——			
	8	16	18	20	8	16	18	20
Shoot fresh weight	lower	lower	higher decrease	lower	lower	lower	higher decrease	higher decrease
Shoot dry weight	No difference	lower	higher decrease	higher decrease	No difference	lower	higher decrease	higher decrease
Root fresh weight	lower	No difference	lower	lower	lower	lower	lower	lower
Root dry weight	lower	higher decrease	higher decrease	higher decrease	higher decrease	higher decrease	higher decrease	higher decrease
Nodule fresh weight	lower	higher	lower	lower	higher	higher	lower	lower
Nodule dry weight	lower	lower	lower	higher	No difference	lower	lower	No difference
Diameter of pink bacteroid tissue	lower	lower	lower	lower	lower	higher decrease	higher decrease	lower
Nitrogenase activity	higher decrease	higher decrease	higher decrease	lower	higher decrease	higher decrease	higher decrease	lower
IAA content	lower	lower	higher decrease	lower	lower	lower	higher decrease	lower
ABA content in leaves	higher increase			higher increase	higher increase			higher

Conclusion

Proline as an osmoprotectant, increased with the age of the plants as well as with ABA treatment, while kinetin decreased it.

Application of kinetin (10^{-5}M) as seed soaking can be implicated to delay senescence (in both leaves and nodules) and consequently result in increase in the productivity of the plants. However, the cost benefit ratio does not permit kinetin to be used in commercial scale, the alternative is to imply the plant growth promoter Rhizobateria (PGPR) that produce cytokinin (Atzo *et al.*, 1988; Basten *et al.*, 1998) as well as other growth promoting hormones in association with rates of plants to form biofertilizer production.

The research insight should also be made to detect the quantity and quality of protein and fatty acids in seeds. Study should be extended to monitor the translocation of assimilates from leaves to the developing pods and seeds as well as from the leaves to nodules.

The future work should include the soil physical conditions including soil moisture, pH, temperature, electron conductivity values *etc.* on the leaf and nodule senescence with particular emphasis on the changing level of endogenous phytohormones particularly ABA and cytokinin.

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APPENDICES

APPENDIX I

List of DMRT Tables, containing data of parameters investigated at four growth stages in *Cicer arietinum* L. cv. CM88 treated with plant growth regulators and grown under natural condition. Each figure represent a mean of three replicates.

Table 8: DMRT of means showing the effect of plant growth regulators on fresh shoot weight (g) of *Cicer arietinum* L. cv. CM88 measured at different plant growth stages (year 2000).

Treatments	Weeks After Sowing				
	8	16	18	20	Mean
Control	2.09 l	9.33 e	14.70 c	6.83 h	8.24 C
Kinetin (10^{-5} M)	4.32 j	13.77 c	20.93 a	9.17 ef	12.05 A
Kinetin (10^{-6} M)	3.33 k	10.70 d	15.67 b	8.27 fg	9.49 B
ABA (10^{-5} M)	2.00 l	8.90 ef	9.40 e	5.50 i	6.45 D
ABA (10^{-6} M)	1.72 l	7.60 gh	8.97 ef	4.60 ij	5.72 E
Mean	2.69 D	10.06 B	13.93 A	6.87 C	

All such means which share a common English letter are statistically similar otherwise are different at $\alpha = 0.05$.

Table 9: DMRT of means showing the effect of plant growth regulators on dry shoot weight (g) of *Cicer arietinum* L.cv. CM88 measured at different plant growth stages (year 2000).

Treatments	Weeks After Sowing				
	8	16	18	20	Mean
Control	0.30 i	1.90 e-g	3.57 b	2.03 d-f	1.95 B
Kinetin (10^{-5} M)	0.43 i	2.83 c	5.52 a	2.77 c	2.89 A
Kinetin (10^{-6} M)	0.42 i	2.43 cd	3.30 b	2.33 d	2.12 B
ABA (10^{-5} M)	0.30 i	1.67 f-h	2.43 cd	1.57 gh	1.49 C
ABA (10^{-6} M)	0.31 i	1.63 f-h	2.23 de	1.43 h	1.40 C
Mean	0.35 C	2.09 B	3.41 A	2.03 B	

All such means which share a common English letter are statistically similar otherwise are different at $\alpha = 0.05$.

Table 10: DMRT of means showing effect of plant growth regulators on root fresh weight (g) of *Cicer arietinum* L.cv. CM 88 measured at different plant growth stages (year 2000).

Treatments	Weeks After Sowing				
	8	16	18	20	Mean
Control	1.21d-g	1.67 bc	1.47 b-d	1.40 c-e	1.44 BC
Kinetin (10^{-5} M)	1.53 b-d	2.47 a	2.87 a	1.90 b	2.19 A
Kinetin (10^{-6} M)	1.29 c-f	1.73 bc	1.57 b-d	1.63 b-d	1.56 B
ABA (10^{-5} M)	0.85 fg	1.65 bc	1.40 c-e	0.97 e-g	1.22 CD
ABA (10^{-6} M)	0.82 g	1.48 b-d	1.42 cd	0.73 g	1.11 D
Mean	1.14 B	1.80 A	1.74 A	1.33 B	

All such means which share a common English letter are statistically similar otherwise are different at $\alpha = 0.05$.

Table 11: DMRT of means showing the effect of plant growth regulators on dry root weight (g) of *Cicer arietinum* L.cv. CM 88 measured at different plant growth stages (year 2000).

Treatments	Weeks After Sowing				
	8	16	18	20	Mean
Control	0.140 lm	0.264 ef	0.292 d	0.279 de	0.244 C
Kinetin (10^{-5} M)	0.208 gh	0.334 c	0.413 a	0.353 b	0.327 A
Kinetin (10^{-6} M)	0.161 jk	0.280 de	0.326 c	0.330 c	0.274 B
ABA (10^{-5} M)	0.135 m	0.220 g	0.266 ef	0.178 ij	0.200 D
ABA (10^{-6} M)	0.106 n	0.194 hi	0.256 f	0.157 kl	0.178 E
Mean	0.150 C	0.258 B	0.310 A	0.259 B	

All such means which share a common English letter are statistically similar otherwise are different at $\alpha = 0.05$.

Table 12: DMRT of means showing total chlorophyll content (mgl^{-1}) in young and old leaves of *Cicer arietinum L.*cv. CM88 at different plant growth stages and the effect of plant growth regulators (year 1998).

Treatments	Young Leaves					Old Leaves					Grand Mean
	Weeks After Sowing					Weeks After Sowing					
	8	16	18	20	Mean	8	16	18	20	Mean	
Control	249.6 ab	233.1 a-c	68.0 fg	49.7 fg	150.1 AB	204.5 b-e	168.9 e	41.6 g	28.0 g	110.7 CD	130.4 A-C
Kinetn (10^{-5} M)	268.0 a	249.5 ab	99.0 f	60.0 fg	169.1 A	210.6 b-e	178.9 de	49.8 fg	39.3 g	119.6 CD	144.4 A
Kinetin (10^{-6} M)	268.4 a	243.5 ab	69.8 fg	53.9 fg	158.9 A	207.1 b-e	172.9 e	41.7 g	37.2 g	114.7 CD	136.8 AB
ABA (10^{-5} M)	238.1 ab	212.3 b-e	48.8 fg	38.0 g	134.3 BC	182.1 c-e	162.0 e	32.1 g	20.7 g	99.2 D	116.8 C
ABA (10^{-6} M)	247.1 ab	225.2 a-d	61.5 fg	47.5 fg	145.3 AB	201.6 b-e	160.1 e	33.6 g	26.9 g	105.6 D	125.5 BC
Mean	254.2 A	232.7 B	69.4 E	49.8 EF		201.2 C	168.6 D	39.8 F	30.4 F		

All such means which share a common English letter are statistically similar otherwise are different at $\alpha = 0.05$.

Table 13: DMRT of means showing total chlorophyll content (mgl^{-1}) in young and old leaves of *Cicer arietinum L.*cv. CM88 at different plant growth stages and the effect of plant growth regulators (year 1999).

Treatments	Young Leaves					Old Leaves					Grand Mean
	Weeks After Sowing					Weeks After Sowing					
	8	16	18	20	Mean	8	16	18	20	Mean	
Control	206.4 a-c	167.2 c-e	221.4 ab	78.7 h-l	168.4 AB	107.3 g-j	44.3 lm	43.8 lm	33.2 lm	57.2 D	112.8 BC
Kinetin (10^{-5} M)	232.5 ab	197.0 a-c	239.4 a	107.3 g-j	194.0 A	117.2 e-h	62.3 i-m	82.9 g-l	46.9 lm	77.3 CD	135.7 A
Kinetin (10^{-6} M)	232.7 ab	185.2 b-d	237.4 ab	107.1 g-j	190.6 A	112.4 f-i	57.2 j-m	59.7 i-m	44.5 lm	68.4 D	129.5 AB
ABA (10^{-5} M)	240.4 a	135.3 d-g	192.2 a-c	50.0 k-m	154.5 B	102.4 g-k	33.3 lm	24.4 m	20.1 m	45.04 D	99.8 C
ABA (10^{-6} M)	192.3 ab	160.8 c-f	197.5 a-c	51.8 k-m	150.6 B	105.4 g-j	38.7 lm	33.8 lm	32.6 lm	52.64 D	101.6 C
Mean	220.9 A	169.1 B	217.6 A	79.0 CD		109.0 C	47.2 E	48.9 DE	35.5 E		

All such means which share a common English letter are statistically similar otherwise are different at $\alpha = 0.05$.

Table 14: DMRT of means showing total chlorophyll content on total chlorophyll content (mgl^{-1}) in young and old leaves of *Cicer arietinum* L.cv. CM88 and the effect of plant growth regulators at different plant growth stages (year 2000).

Treatments	Young Leaves					Old Leaves					Grand Mean
	Weeks After Sowing					Weeks After Sowing					
	8	16	18	20	Mean	8	16	18	20	Mean	
Control	302.3 a	299.5 a	231.3 b	44.2 fg	219.3 A	301.6 a	294.2 a	110.0 e	23.4 fg	154.8 CD	182.3 C
Kinetin (10^{-5} M)	304.3 a	301.9 a	236.3 b	54.0 f	224.1 A	303.7 a	301.8 a	118.7 de	41.4 fg	162.7 C	191.4 BC
Kinetin (10^{-6} M)	303.4 a	301.5 a	233.3 b	45.3 fg	220.9 A	301.8 a	295.4 a	112.1 e	30.6 fg	157.1 CD	185.0 C
ABA (10^{-5} M)	258.8 b	289.1 a	145.7 d	27.9 fg	180.4 C	300.8 a	291.0 a	90.3 e	21.2 g	147.2 D	175.8 C
ABA (10^{-6} M)	301.9 a	293.4 a	195.0 c	34.2 fg	206.1 AB	301.3 a	298.5 a	90.7 e	21.8 g	150.7 D	178.1 C
Mean	294.1 A	297.1 A	208.3 B	41.1 D		301.8 A	296.2 A	104.3 C	27.7 D		

All such means which share a common English letter are statistically similar otherwise are different at $\alpha = 0.05$.

Table 15: **DMRT of means showing protein content (µg g^{-1} FW) in young and old leaves of *Cicer arietinum* L.cv. CM88 at different plant growth stages and the effect of plant growth regulators (year 1998).**

Treatments	Young Leaves					Old Leaves					Grand Mean
	Weeks After Sowing					Weeks After Sowing					
	8	16	18	20	Mean	8	16	18	20	Mean	
Control	1220 h-m	7250 a-c	1458 h-k	3200 fg	3282 BC	1257 h-m	7367 a-c	1525 h-j	703 j-m	2713 D	2997 BC
Kinetin (10^{-5} M)	1355 h-m	7983 a	1791 h	3550 f	3670 A	1427 h-k	7800 ab	1616 h-i	1001 h-m	2961 CD	3316 A
Kinetin (10^{-6} M)	1280 h-m	7533 ab	1601 h-i	3483 f	3475 AB	1358 h-m	7617 ab	1650 h-i	903 i-m	2882 D	3178 AB
ABA (10^{-5} M)	971 h-m	6417 d	612 k-m	2583 g	2646 D	1100 h-m	5550 e	1292 h-m	514 m	2114 E	2380 D
ABA (10^{-6} M)	1141 h-m	6700 cd	1268 h-m	2617 g	2931 CD	1240 h-m	7167 bc	1383 h-l	552 lm	2586D	2758 C
Mean	1193 C	7177 A	1346 C	3087 B		1276 C	7100 A	1493 C	735 D		

All such means which share a common English letter are statistically similar otherwise are different at $\alpha = 0.05$.

Table 16: DMRT of means showing protein content (μg g^{-1} FW) in young and old leaves of *Cicer arietinum L.*cv. CM88 at different plant growth stages and the effect of plant growth regulators (year 1999).

Treatments	Young Leaves					Old Leaves					Grand Mean
	Weeks After Sowing					Weeks After Sowing					
	8	16	18	20	Mean	8	16	18	20	Mean	
Control	1267 h-m	1650 c-k	1100 i-o	3017 b	1758 BC	1317 g-m	1767 c-i	967 l-o	1341 f-m	1348 DE	1553 BC
Kinetin (10^{-5} M)	1517 d-m	3200 ab	2000 c-f	3783 a	2625 A	1400 f-m	2133 cd	1200 h-n	1508 d-m	1560 CD	2093 A
Kinetin (10^{-6} M)	1400 f-m	2100 c-e	1750 c-i	3217 ab	2117 B	1383 f-m	1950 c-g	1050 k-o	1452 e-m	1459 C-E	1788 B
ABA (10^{-5} M)	1150 h-o	1107 i-o	867 m-o	1817 c-h	1235 DE	1133 i-o	1633 c-l	500 o	1108 i-o	1094 E	1164 D
ABA (10^{-6} M)	1217 h-n	1350 f-m	1067 j-o	2250 c	1471 C-E	1233 h-n	1733 c-j	583 no	1202 h-n	1188 DE	1329 CD
Mean	1310 C	1881 B	1357 C	2817 A		1293 C	1843 B	860 D	1322 C		

All such means which share a common English letter are statistically similar otherwise are different at $\alpha = 0.05$.

Table 17: **DMRT of means showing protein content (μg g^{-1} FW) in young and old leaves of *Cicer arietinum* L.cv. CM88 at different plant growth stages and the effect of plant growth regulators (year 2000).**

Treatments	Young Leaves					Old Leaves					Grand Mean
	Weeks After Sowing					Weeks After Sowing					
	8	16	18	20	Mean	8	16	18	20	Mean	
Control	1150 c-j	800 i-l	1267 b-h	1500 a-d	1179 A-D	1333 a-g	1100 e-k	1083 f-k	1350 a-g	1217 A-D	1198 BC
Kinetin (10^{-5} M)	1183 c-i	1033 g-k	1483 a-e	1717 a	1354 AB	1433 a-f	1400 a-g	1100 e-k	1600 ab	1383 A	1369 A
Kinetin (10^{-6} M)	1167 c-j	817 i-l	1450 a-f	1533 a-c	1242 A-D	1350 a-g	1250 b-h	1100 e-k	1517 a-c	1304 A-C	1273 AB
ABA (10^{-5} M)	783 j-l	633 l	1117 d-k	1267 b-h	950 E	1083 f-k	800 i-l	1017 g-l	1283 b-h	1046 DE	998 D
ABA (10^{-6} M)	1067 f-k	750 kl	1250 b-h	1283 b-h	1088 C-E	1267 b-h	933 h-l	1033 g-k	1300 b-h	1133 B-E	1110 CD
Mean	1070 C	807 D	1313 AB	1460 A		1293 A-C	1097 BC	1067 C	1410 A		

All such means which share a common English letter are statistically similar otherwise are different at $\alpha = 0.05$.

Table 18: DMRT of means showing proline content (μg g^{-1} FW) in young and old leaves of *Cicer arietinum L.*cv. CM88 at different plant growth stages and the effect of plant growth regulators (year 1999).

Treatments	Young Leaves					Old Leaves					Grand Mean
	Weeks After Sowing					Weeks After Sowing					
	8	16	18	20	Mean	8	16	18	20	Mean	
Control	645 n	6983 f-k	8200 e-i	4850 i-m	5170 D-F	757 n	8100 e-i	13633 bc	4017 j-n	6627 B-D	5898 BC
Kinetin (10^{-5} M)	468 n	4917 i-m	5400 h-m	3703 k-m	3622 F	533 n	5483 h-m	10837 c-e	2867 l-m	4930 D-F	4276 D
Kinetin (10^{-6} M)	510 n	5933 h-m	7683 e-j	2567 mn	4173 EF	722 n	7100 f-k	12550 b-d	3950 k-n	6080 C-E	5127 CD
ABA (10^{-5} M)	808 n	10100 c-f	14500 b	6317 g-l	7931 A-C	845 n	10817 c-e	18917 a	5133 h-m	8928 A	8430 A
ABA (10^{-6} M)	762 n	8817 e-h	9683 d-g	5267 h-m	6132 C-E	792 n	10217 c-f	18017 a	4133 j-n	8290 AB	7211 AB
Mean	639 D	7350 B	9093 B	4541 C		730 D	8343 B	14791 A	4020 C		

All such means which share a common English letter are statistically similar otherwise are different at $\alpha = 0.05$.

Table 19: DMRT of means showing proline content (μg g^{-1} FW) in young and old leaves of *Cicer arietinum L.*cv. CM88 at different plant growth stages and the effect of plant growth regulators (year 2000).

Treatments	Young Leaves					Old Leaves					Grand Mean
	Weeks After Sowing					Weeks After Sowing					
	8	16	18	20	Mean	8	16	18	20	Mean	
Control	550 mn	5200 h-k	7200 e-h	4500 h-k	4363 DE	1050 l-m	9000 c-f	11634 bc	3633 i-l	6329 BC	5346 BC
Kinetin (10^{-5} M)	550 mn	4750 h-k	6400 f-i	3100 j-n	3700 E	650 mn	6767 e-h	8670 d-f	2300 k-n	4597 DE	4148 C
Kinetin (10^{-6} M)	250 n	4933 h-k	6683 e-h	2450 k-n	3579 E	867 l-n	8283 d-g	10550 cd	3300 j-m	5750 B-D	4665 BC
ABA (10^{-5} M)	650 mn	7350 e-h	13506 b	6333 f-i	6960 AB	1500 l-n	9500 c-e	16917 a	5100 h-k	8254 A	7607 A
ABA (10^{-6} M)	600 mn	5267 h-k	8682 d-f	5583 g-j	5033 C-E	1400 l-n	9167 c-f	11217 b-d	4785 h-k	6642 A-C	5838 B
Mean	520 D	5500 C	8494 B	4393 C		1093 D	8543 B	11797 A	3824 C		

All such means which share a common English letter are statistically similar otherwise are different at $\alpha = 0.05$.

Table 20: DMRT of means showing sugar content (μg g^{-1} FW) in young and old leaves of *Cicer arietinum L.*cv. CM88 at different plant growth stages and the effect of plant growth regulators (year 2000).

Treatments	Young Leaves					Old Leaves					Grand Mean
	Weeks After Sowing					Weeks After Sowing					
	8	16	18	20	Mean	8	16	18	20	Mean	
Control	650 f-h	480 g-m	523 g-l	890 d-e	636 C	530 g-l	363 k-n	437 i-n	1100 bc	608 CD	622 B
Kinetin (10^{-5} M)	740 ef	627 f-i	663 f-h	990 cd	755 A	673 fg	420 j-n	503 g-m	1347 a	736 AB	745 A
Kinetin (10^{-6} M)	650 f-h	563 f-k	650 f-h	923 c-e	697 A-C	587 f-j	370 k-n	463 h-n	1200 ab	655 BC	676 B
ABA (10^{-5} M)	403 j-n	397 j-n	487 g-m	737 ef	506 E	383 j-n	277 n	333 l-n	900 de	473 E	490 C
ABA (10^{-6} M)	487 g-m	420 j-n	507 g-m	760 ef	543 DE	410 j-n	310 mn	393 j-n	950 cd	516 E	530 C
Mean	586 C	497 DE	566 CD	860 B		517 CD	348 F	426 E	1099 A		

All such means which share a common English letter are statistically similar otherwise are different at $\alpha = 0.05$.

Table 21: DMRT of means showing IAA content (mg g^{-1} root FW) of *Cicer arietinum* L.cv. CM88 at different plant growth stages and the effect of plant growth regulators (year 1999).

Treatments	8	16	18	20	Mean
	Weeks After Sowing				
Control	3.77 c	0.7 c	62.28 b	1.14 c	16.99 A
Kinetin (10^{-5} M)	5.1 c	0.79 c	91.1 a	1.3 c	24.62 A
Kinetin (10^{-6} M)	4.82 c	0.79 c	64.65 b	1.14 c	17.874 A
ABA (10^{-5} M)	1.93 c	0.06 c	6.66 c	1.12 c	2.54 B
ABA (10^{-6} M)	1.93 c	0.7 c	6.75 c	1.12 c	2.72 B
Mean	3.59 B	0.7 B	46.25 A	1.14 B	

All such means which share a common English letter are statistically similar otherwise are different at $\alpha = 0.05$.

Table 22: DMRT of means showing IAA content (mg g^{-1} root FW) of *Cicer arietinum* L.cv. CM88 at different plant growth stages and the effect of plant growth regulators (year 2000).

Treatments	Weeks After Sowing				Mean
	8	16	18	20	
Control	4.64 f	1.66 h	22.16 c	0.96 h	7.36 C
Kinetin (10^{-5} M)	5.96 e	1.66 h	23.48b	1.31 h	8.15 B
Kinetin (10^{-6} M)	5.69 e	1.66h	31.97a	1.40 h	10.16 A
ABA (10^{-5} M)	2.80 g	1.49h	6.31e	1.05 h	2.89D
ABA (10^{-6} M)	3.42 g	1.58 h	7.8 d	1.23h	3.5 D
Mean	4.47 B	1.58 C	18.31 A	1.23 C	

All such means which share a common English letter are statistically similar otherwise are different at $\alpha = 0.05$

Table 23: DMRT of means showing fresh nodule weight per plant (g) in *Cicer arietinum* L.cv. CM88 at different plant growth stages and the effect of plant growth regulators (year 2000).

Treatmnts	Weeks After Sowing				
	8	16	18	20	Mean
Control	0.5	0.57	0.85	0.50	0.61
Kinetin (10^{-5}M)	0.5	0.8	0.85	0.56	0.68
Kinetin (10^{-6}M)	0.46	0.73	0.75	0.46	0.61
ABA (10^{-5}M)	0.47	0.70	0.80	0.47	0.61
ABA (10^{-6}M)	0.56	0.70	0.55	0.25	0.56
Mean	0.49 B	0.70 A	0.76 A	0.48 B	

All such means which share a common English letter are statistically similar otherwise are different at $\alpha = 0.05$.

Table 24: DMRT of means showing dry nodule weight per plant (g) in *Cicer arietinum* L.cv. CM88 at different plant growth stages and the effect of plant growth regulators (year 2000).

Treatments	Weeks After Sowing				
	8	16	18	20	Mean
Control	0.058	0.087	0.14	0.058	0.09
Kinetin (10^{-5}M)	0.071	0.1	0.14	0.071	0.1
Kinetin (10^{-6}M)	0.047	0.105	0.13	0.06	0.09
ABA (10^{-5}M)	0.066	0.083	0.12	0.066	0.07
ABA (10^{-6}M)	0.06	0.083	0.095	0.06	0.07
Mean	0.06	0.09	0.13	0.061	

All such means which share a common English letter are statistically similar otherwise are different at $\alpha = 0.05$.

Table 25: DMRT of means showing diameter of pink bacteroid tissue (mm plant^{-1} h^{-1}) in root nodules of *Cicer arietinum* L.cv. CM88 at different plant growth stages and the effect of plant growth regulators (year 1998).

Treatments	Weeks After Sowing				Mean
	8	16	18	20	
Control	1.36 b-g	1.56 a-d	1.74 ab	0.96 gh	1.41 AB
Kinetin (10^{-5} M)	1.48 b-e	1.70 ab	1.88 a	1.16 d-h	1.56 A
Kinetin (10^{-6} M)	1.41 b-f	1.63 a-c	1.88 a	1.03 f-h	1.49AB
ABA (10^{-5} M)	1.26 c-h	1.46 b-e	1.56 a-d	1.00 gh	1.32 BC
ABA (10^{-6} M)	1.14 e-h	1.36 b-g	1.22 d-h	0.88 h	1.15 C
Mean	1.33 B	1.54 A	1.66 A	1.01 C	

All such means which share a common English letter are statistically similar otherwise are different at $\alpha = 0.05$.

Table 26: DMRT of means showing diameter of pink bacteroid tissue (mm plant^{-1} h^{-1}) in root nodules of *Cicer arietinum* L.cv. CM88 at different plant growth stages and the effect of plant growth regulators (year 1999).

Treatments	Weeks After Sowing				
	8	16	18	20	Mean
Control	1.37 cd	1.63 b	1.63 b	1.00 e	1.41 C
Kinetin (10^{-5} M)	1.48 bc	2.09 a	2.17 a	1.60 b	1.84 A
Kinetin (10^{-6} M)	1.46 bc	2.00 a	2.04 a	1.35 cd	1.71 B
ABA (10^{-5} M)	1.31 cd	1.50 bc	1.50 bc	0.87 e	1.30 D
ABA (10^{-6} M)	1.34 cd	1.36 cd	1.20 d	0.85 e	1.19 E
Mean	1.39 B	1.72 A	1.71 A	1.13 C	

All such means which share a common English letter are statistically similar otherwise are different at $\alpha = 0.05$.

Table 27: DMRT of means showing diameter of pink bacteroid tissue (mm plant^{-1} h^{-1}) in root nodule of *Cicer arietinum* L.cv. CM88 at different plant growth stages and the effect of plant growth regulators (year 2000).

Treatments	Weeks After Sowing				
	8	16	18	20	Mean
Control	1.05 jk	1.77 c-e	1.88 b-d	1.25 hi	1.49 C
Kinetin (10^{-5} M)	1.25 hi	2.04 ab	2.17 a	1.60 ef	1.76 A
Kinetin (10^{-6} M)	1.11 ij	1.92 bc	2.00 ab	1.38 gh	1.60 B
ABA (10^{-5} M)	0.93 j-l	1.70 d-f	1.65 ef	0.95 j-l	1.31 D
ABA (10^{-6} M)	0.85 l	1.57 f	1.52 fg	0.89 kl	1.21 E
Mean	1.04 C	1.80 A	1.84 A	1.21 B	

All such means which share a common English letter are statistically similar otherwise are different at $\alpha = 0.05$.

Table 28: DMRT of means showing nitrogenase activity (nmol C_2H_4 plant^{-1} h^{-1}) in root nodules of *Cicer arietinum* L.cv. CM88 at different plant growth stages and the effect of plant growth regulators (year 1998).

Treatments	Weeks After Sowing				Mean
	8	16	18	20	
Control	16.0 jk	74.3 ef	94.9 e	5.1 k	47.7 C
Kinetin (10^{-5} M)	30.2 ij	254.0 b	274.3 a	5.5 k	141.0 A
Kinetin (10^{-6} M)	23.4 i-k	149.3 d	169.7 c	5.1 k	86.9 B
ABA (10^{-5} M)	6.7 k	49.8 gh	69.8 ef	4.1 k	32.6 D
ABA (10^{-6} M)	3.7 k	42.6 hi	62.3 fg	3.9 k	28.1 D
Mean	16.0 C	114.0 B	134.9 A	4.7 D	

All such means which share a common English letter are statistically similar otherwise are different at $\alpha = 0.05$.

Table 29: DMRT of means showing nitrogenase activity (nmol C_2H_4 $plant^{-1}$ h^{-1}) in root nodules of *Cicer arietinum* L.cv. CM88 at different plant growth stages and the effect of plant growth regulators (year 1999).

Treatments	Weeks After Sowing				Mean
	8	16	18	20	
Control	7.23 ef	14.73 d	18.90 c	0.56 ef	10.5 C
Kinetin (10^{-5} M)	9.47 e	34.17 a	32.40 a	0.92 d	19.2 A
Kinetin (10^{-6} M)	8.23 e	20.43 c	24.07 b	0.73 h	13.6 B
ABA (10^{-5} M)	3.99 g	8.97 e	8.98 e	0.26 fg	5.5 D
ABA (10^{-6} M)	3.48 g	6.98 ef	12.47 g	0.52 fg	5.9 D
Mean	6.48 C	17.06 B	19.6 A	0.63 C	

All such means which share a common English letter are statistically similar otherwise are different at $\alpha = 0.05$.

Table 30: DMRT of means showing nitrogenase activity (nmol C_2H_4 $plant^{-1}$ h^{-1}) in root nodules of *Cicer arietinum* L.cv. CM88 at different plant growth stages and the effect of plant growth regulators (year 2000).

Treatments	Weeks After Sowing				Mean
	8	16	18	20	
Control	2.53 jk	5.39 e-h	8.53 c	2.4 d-g	4.7 C
Kinetin (10^{-5} M)	4.31 g-i	8.63 c	17.70 a	3.2 d-f	8.5 A
Kinetin (10^{-6} M)	3.32 ij	6.63 de	13.26 b	2.7 d-g	6.5 B
ABA (10^{-5} M)	1.86 jk	4.06 hi	7.08 d	1.7 f-i	3.7 D
ABA (10^{-6} M)	1.57 k	3.14 i-k	6.28 d-f	1.6 f-i	3.19 D
Mean	2.68 C	5.57 B	10.6 A	2.30 B	

All such means which share a common English letter are statistically similar otherwise are different at $\alpha = 0.05$.

APPENDIX II

List of ANOVA Tables, containing data of parameters investigated at four growth stages in *Cicer arietinum* L. cv. CM88 treated with plant growth regulators and grown under natural condition.

Where

P is Significant at P<0.05

P is High significant at P<0.01

P is Very high significant at P<0.001

Table 31: Analysis of variance (ANOVA) for fresh shoot weight with two factors (treatments and stages of plant growth) year 2000.

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	Factor A	4	305.965	76.491	269.4936	0.0000
4	Factor B	3	1022.907	340.969	1201.3004	0.0000
6	AB	12	111.02	9.252	32.5955	0.0000
-7	Error	40	11.353	0.284		
	Total	59	1451.246			

Coefficient of Variation: 6.35%

Table 32: Analysis of variance (ANOVA) for dry shoot weight with two factors (treatments and stages of plant growth) year 2000.

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	Factor A	4	17.006	4.252	87.9124	0.0000
4	Factor B	3	70.577	23.526	486.4551	0.0000
6	AB	12	10.469	0.872	18.0387	0.0000
-7	Error	40	1.934	0.048		
	Total	59	99.987			

Coefficient of Variation: 11.15%

Table 33: Analysis of variance (ANOVA) for fresh root weight with two factors (treatments and stages of plant growth) year 2000.

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	Factor A	4	8.362	2.091	40.0751	0.0000
4	Factor B	3	4.657	1.552	29.7551	0.0000
6	AB	12	1.748	0.146	2.7929	0.0073
-7	Error	40	2.087	0.052		
	Total	59	16.854			

Coefficient of Variation: 15.13%

Table 34: Analysis of variance (ANOVA) for dry root weight with two factors (treatments and stages of plant growth) year 2000.

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	Factor A	4	0.169	0.042	89.8898	0.0000
4	Factor B	3	0.205	0.068	145.1693	0.0000
6	AB	12	0.025	0.002	4.5089	0.0001
-7	Error	40	0.019	0.000		
	Total	59	0.418			

Coefficient of Variation: 8.86%

Table 35: Analysis of variance (ANOVA) of chlorophyll content in chickpea leaves with three factors (treatments, plant growth stages and leaf age) year 1998.

Sourse	D.F.	S.S.	M.S.	F.	P.
Replication	2	4874.89	2437.445	3.5424	0.0337
Treatment (T)	4	10724.51	2681.127	3.8965	0.0062
Stage (S)	3	849014.5	283004.8	411.298	0
T x S	12	881.577	73.465	0.1068	
Leaf Age (A)	1	51850.26	51850.26	75.3553	0
T x A	4	719.209	179.802	0.2613	
S x A	3	9567.317	3189.106	4.6348	0.0049
T x S x A	12	899.234	74.936	0.1089	
Error	78	53670.03	688.077		
Total	119	982201.5			

Table 36: Analysis of variance (ANOVA) of chlorophyll content in chickpea leaves with three factors (treatments, plant growth stages and leaf age) year 1999.

Source	D.F.	S.S.	M.S.	F	p
Replication	2	413.604	206.802	0.2818	
Treatment (T)	4	25228.8	6307.199	8.5934	0
Stage (S)	3	186157.6	62052.52	84.5449	0
T x S	12	5982.895	498.575	0.6793	
Leaf Age (A)	1	373056.7	373056.7	508.2801	0
T x A	4	1972.394	493.099	0.6718	
S x A	3	60027.92	20009.31	27.2622	0
T x S x A	12	3904.011	325.334	0.4433	
Error	78	57248.8	733.959		
Total	119	713992.7			

Table 37: Analysis of variance (ANOVA) of chlorophyll content in chickpea leaves with three factors (treatments, plant growth stages and leaf age) year 2000.

Source	D.F.	S.S.	M.S.	F	p
Replication	2	286.483	143.242	0.569	
Treatment (T)	4	13071.6	3267.899	12.9809	0
Stage (S)	3	1446647	482215.6	1915.484	0
T x S	12	7273.6	606.133	2.4077	0.0104
Leaf Age (A)	1	22924.62	22924.62	91.0625	0
T x A	4	4293.065	1073.266	4.2633	0.0036
S x A	3	59952.03	19984.01	79.3816	0
T x S x A	12	2919.334	243.278	0.9664	
Error	78	19636.19	251.746		
Total	119	1577004			

Table 38: Analysis of variance (ANOVA) of protein content in chickpea leaves with three factors (treatments, plant growth stages and leaf age) year 1998.

Source	D.F.	S.S.	M.S.	F	p
Replication	2	920396.6	460198.3	2.5887	0.0816
Treatment (T)	4	13124225	3281056	18.4566	0
Stage (S)	3	7.17E+08	2.39E+08	1344.629	0
T x S	12	5143618	428634.8	2.4112	0.0103
Leaf Age (A)	1	9060706	9060706	50.9683	0
T x A	4	416785.3	104196.3	0.5861	
S x A	3	32684712	10894904	61.286	0
T x S x A	12	2099902	174991.9	0.9844	
Error	78	13866172	177771.4		
Total	119	7.94E+08			

Table 39: Analysis of variance (ANOVA) of protein content in chickpea leaves with three factors (treatments, plant growth stages and leaf age) year 1999.

Source	D.F.	S.S.	M.S.	F	p
Replication	2	107708.2	53854.08	0.4731	
Treatment (T)	4	13011825	3252956	28.5796	0
Stage (S)	3	18573294	6191098	54.3934	0
T x S	12	2592650	216054.1	1.8982	0.0472
Leaf Age (A)	1	7846922	7846922	68.941	0
T x A	4	3161111	790277.7	6.9432	0.0001
S x A	3	10765310	3588437	31.5271	0
T x S x A	12	1970297	164191.4	1.4425	0.1651
Error	78	8878027	113820.9		
Total	119	66907144			

Table 40: Analysis of variance (ANOVA) of protein content in chickpea leaves with three factors (treatments, plant growth stages and leaf age) year 2000.

Source	D.F.	S.S.	M.S.	F	p
Replication	2	541.667	270.833	0.0069	
Treatment (T)	4	1970833	492708.3	12.541	0
Stage (S)	3	3506896	1168965	29.7538	0
T x S	12	256333.3	21361.11	0.5437	
Leaf Age (A)	1	88020.83	88020.83	2.2404	0.1385
T x A	4	16666.67	4166.667	0.1061	
S x A	3	1391896	463965.3	11.8094	0
T x S x A	12	198833.3	16569.44	0.4217	
Error	78	3064458	39287.93		
Total	119	10494479			

Table 44: Analysis of variance (ANOVA) for endogenous level of ABA in chickpea leaves with two factors (treatments and stages of plant growth) year 2000.

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	Factor A	4	96882.121	24220.530	674.1402	0.0000
4	Factor B	1	273340.161	273340.161	7607.9912	0.0000
6	AB	4	26518.436	6629.609	184.5247	0.0000
-7	Error	20	718.561	35.928		
	Total	29	397459.280			

Coefficient of Variation: 2.96%

Table 45: Analysis of variance (ANOVA) for IAA content in root with two factors (treatments and stages of plant growth) year 1999.

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	Factor A	4	60.850	15.213	4.9865	0.0023
4	Factor B	3	290.754	96.918	31.7687	0.0000
6	AB	12	163.767	13.647	4.4734	0.0002
-7	Error	40	122.030	3.051		
	Total	59	637.402			

Coefficient of Variation: 118.35%

Table 46: Analysis of variance (ANOVA) for IAA content in root with two factors (treatments and stages of plant growth) year 2000.

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	Factor A	4	5.018	1.254	364.7911	0.0000
4	Factor B	4	42.153	10.538	3064.6265	0.0000
6	AB	16	14.208	0.888	258.2477	0.0000
-7	Error	50	0.172	0.003		
	Total	74	61.551			

Coefficient of Variation: 9.45%

Table 47: Analysis of variance (ANOVA) for fresh nodule weight with two factors (treatments and stages of plant growth) year 2000.

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	Factor A	4	0.081	0.020	1.2195	0.3178
4	Factor B	3	0.928	0.309	18.6459	0.0000
6	AB	12	0.327	0.027	1.6422	0.1186
-7	Error	40	0.664	0.017		
	Total	59	2.000			

Coefficient of Variation: 24.57%

Table 48: Analysis of variance (ANOVA) for dry nodule weight with two factors (treatments and stages of plant growth) year 2000.

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	Factor A	4	0.058	0.014	1.2254	0.3154
4	Factor B	3	0.065	0.022	1.8392	0.1556
6	AB	12	0.155	0.013	1.0910	0.3934
-7	Error	40	0.473	0.012		
	Total	59	0.751			

Coefficient of Variation: 110.25%

Table 49: Analysis of variance (ANOVA) for diameter of pink bacteroid tissue with two factors (treatments and stages of plant growth) year 1998.

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	Factor A	4	1.189	0.297	6.7048	0.0003
4	Factor B	3	3.662	1.221	27.5319	0.0000
6	AB	12	0.286	0.024	0.5380	
-7	Error	40	1.773	0.044		
	Total	59	6.910			

Coefficient of Variation: 15.21%

Table 50: Analysis of variance (ANOVA) for diameter of pink bacteroid tissue with two factors (treatments and stages of plant growth) year 1999.

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	Factor A	4	3.654	0.914	64.9739	0.0000
4	Factor B	3	3.512	1.171	83.2683	0.0000
6	AB	12	0.798	0.067	4.7317	0.0001
-7	Error	40	0.562	0.014		
	Total	59	8.527			

Coefficient of Variation: 7.97%

Table 51: Analysis of variance (ANOVA) for diameter of pink bacteroid tissue with two factors (treatments and stages of plant growth) year 2000.

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	Factor A	4	2.406	0.601	56.6019	0.0000
4	Factor B	3	7.492	2.497	234.9993	0.0000
6	AB	12	0.181	0.015	1.4191	0.1976
-7	Error	40	0.425	0.011		
	Total	59	10.504			

Coefficient of Variation: 7.00%

Table 52: Analysis of variance (ANOVA) for nitrogenase activity with two factors (treatments and stages of plant growth) year 1998.

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	Factor A	4	108836.489	27209.122	219.7131	0.0000
4	Factor B	3	193660.632	64553.544	521.2685	0.0000
6	AB	12	85842.511	7153.543	57.7647	0.0000
-7	Error	40	4953.573	123.839		
	Total	59	393293.205			

Coefficient of Variation: 16.70%

Table 53: Analysis of variance (ANOVA) for nitrogenase activity with two factors (treatments and stages of plant growth) year 1999.

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	Factor A	4	186526.075	46631.519	237.2313	0.0000
4	Factor B	3	213156.971	71052.324	361.4686	0.0000
6	AB	12	90367.574	7530.631	38.3110	0.0000
-7	Error	40	7862.627	196.566		
	Total	59	497913.246			

Coefficient of Variation: 11.39%

Table 54: Analysis of variance (ANOVA) for nitrogenase activity with two factors (treatments and stages of plant growth) year 2000.

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	Factor A	4	56838.210	14209.553	184.1140	0.0000
4	Factor B	3	125384.959	41794.986	541.5401	0.0000
6	AB	12	70314.768	5859.564	75.9227	0.0000
-7	Error	40	3087.120	77.178		
	Total	59	255625.058			

Coefficient of Variation: 12.36%

Table 55: Analysis of variance (ANOVA) for nitrate- nitrogen content in soil with two factors (treatments and replicates) year 1998.

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
1	Factor A	4	49.310	12.328	46.9155	0.0000
-3	Error	10	2.628	0.263		
	Total	14	51.938			

Coefficient of Variation: 22.12%

Table 56: Analysis of variance (ANOVA) for nitrate- nitrogen content in soil with two factors (treatments and replicates) year 1999.

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
1	Factor A	4	1.584	0.396	2.0738	0.1594
-3	Error	10	1.910	0.191		
	Total	14	3.494			

Coefficient of Variation: 24.38%

Table 57: Analysis of variance (ANOVA) for nitrate- nitrogen content in soil with two factors (treatments and replicates) year 2000.

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
1	Factor A	4	16.107	4.027	32.7901	0.0000
-3	Error	10	1.228	0.123		
	Total	14	17.335			

Coefficient of Variation: 17.03%

Table 58: Analysis of variance (ANOVA) for grain weight with two factors (treatments and replicates) year 1998.

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
1	Replication	2	0.124	0.062	1.1923	0.3522
2	Factor A	4	17.040	4.260	81.9231	0.0000
-3	Error	8	0.416	0.052		
	Total	14	17.580			

Coefficient of Variation: 7.86%

Table 59: Analysis of variance (ANOVA) for grain weight with two factors (treatments and replicates) year 1999.

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
1	Replication	2	1.036	0.518	15.6970	0.0017
2	Factor A	4	56.436	14.109	427.5458	0.0000
-3	Error	8	0.264	0.033		
	Total	14	57.736			

Coefficient of Variation: 2.73%

Table 60: Analysis of variance (ANOVA) for grain weight with two factors (treatments and replicates) year 2000.

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
1	Replication	2	0.048	0.024	0.4068	
2	Factor A	4	13.824	3.456	58.5763	0.0000
-3	Error	8	0.472	0.059		
	Total	14	14.344			

Coefficient of Variation: 8.43%

Table 61: Analysis of variance (ANOVA) for weight of 100 grains with two factors (treatments and replicates) year 1998.

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
1	Replication	2	2.764	1.382	0.0924	
2	Factor A	4	1746.816	436.704	29.1973	0.0001
-3	Error	8	119.656	14.957		
	Total	14	1869.236			

Coefficient of Variation: 5.27%

Table 62: Analysis of variance (ANOVA) for weight of 100 grains with two factors (treatments and replicates) year 1999.

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
1	Replication	2	32.716	16.358	2.4597	0.1470
2	Factor A	4	1611.600	402.900	60.5819	0.0000
-3	Error	8	53.204	6.650		
	Total	14	1697.520			

Coefficient of Variation: 3.55%

Table 63: Analysis of variance (ANOVA) for weight of 100 grains with two factors (treatments and replicates) year 2000.

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
1	Replication	2	7.104	3.552	0.4560	
2	Factor A	4	1543.536	385.884	49.5390	0.0000
-3	Error	8	62.316	7.789		
	Total	14	1612.956			

Coefficient of Variation: 3.75%

Table 64: Analysis of variance (ANOVA) for number of pods per plant with two factors (treatments and replicates) year 1998.

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
1	Replication	2	0.004	0.002	0.0123	
2	Factor A	4	4.164	1.041	6.4259	0.0129
-3	Error	8	1.296	0.162		
	Total	14	5.464			

Coefficient of Variation: 5.24%

Table 65: Analysis of variance (ANOVA) for number of pods per plant with two factors (treatments and replicates) year 1999.

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
1	Replication	2	0.364	0.182	15.1667	0.0019
2	Factor A	4	14.340	3.585	298.7500	0.0000
-3	Error	8	0.096	0.012		
	Total	14	14.800			

Coefficient of Variation: 3.53%

Table 66: Analysis of variance (ANOVA) for number of pods per plant with two factors (treatments and replicates) year 2000.

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
1	Replication	2	0.228	0.114	1.0459	0.3949
2	Factor A	4	6.996	1.749	16.0459	0.0007
-3	Error	8	0.872	0.109		
	Total	14	8.096			

Coefficient of Variation: 4.38%

APPENDIX III

Photographs representing the different effects of plant growth regulators on different parts of *Cicer arietinm* L. cv. CM88 at flowering stage and early pod filling stage.

Figure 15: The shoot biomass of *Cicer arietinum* L. cv. CM88 as affected by kinetin applied as seed soaking and foliar spray at early pod filling Stage.

Table 41: Analysis of variance (ANOVA) of proline content in chickpea leaves with three factors (treatments, plant growth stages and leaf age) year 1999.

Source	D.F.	S.S.	M.S.	F	p
Replicatio n	2	8692455	4346227	1.2967	0.2793
Treatment (T)	4	2.62E+08	65619847	19.5773	0
Stage (S)	3	2.09E+09	3 697911	1 208.2	1 0.0000
T x S	12	1.47E+08	12240699	3.6519	0.0002
Leaf Age (A)	1	73501792	73501792	21.9288	0
T x A	4	5212648	1303162	0.3888	
S x A	3	1.79E+08	59813462	17.845	0
T x S x A	12	16604734	1383728	0.4128	
Error	78	2.61E+08	3351831		
Total	119	3.05E+09	7		

Table 42: Analysis of variance (ANOVA) of proline content in chickpea leaves with three factors (treatments, plant growth stages and leaf age) year 2000.

Source	D.F.	S.S.	M.S.	F	p
Replicatio n	2	5600442	2800221	1.2854	0.2823
Treatment (T)	4	1.7E+08	42599715	19.5542	0
Stage (S)	3	1.44E+09	5 478614	2 219.6	1 0.0000
T x S	12	1.09E+08	9114420	4.1837	0
Leaf Age (A)	1	75606275	75606275	34.705	0
T x A	4	6286608	1571652	0.7214	
S x A	3	80583913	26861304	12.3299	0
T x S x A	12	8003731	666977.6	0.3062	
Error	78	1.7E+08	2178541		
Total	119	2.06E+09	5		

Table 43: Analysis of variance (ANOVA) of sugar content in chickpea leaves with three factors (treatments, plant growth stages and leaf age) year 2000.

Source	D.F.	S.S.	M.S.	F	p
Replicatio n	2	49806.67	24903.33	2.4243	0.0952
Treatment (T)	4	1049895	262473.8	25.5509	0
Stage (S)	3	5644849	1881616	183.1691	0
T x S	12	112171.7	9347.639	0.91	
Leaf Age (A)	1	26700.83	26700.83	2.5992	0.111
T x A	4	1611.667	402.917	0.0392	
S x A	3	753209.2	251069.7	24.4408	0
T x S x A	12	64895	5407.917	0.5264	
Error	78	801260	10272.56		
Total	119	8504399			

Figure16: The effect of ABA applied as seed soaking and foliar spray on shoot biomass of *Cicer arietinum* L. cv. CM88 at early pod filling stage where ABA (10^{-5} M) shows yellow leaves as compared to control, and the height of the plant was less at both concentrations.

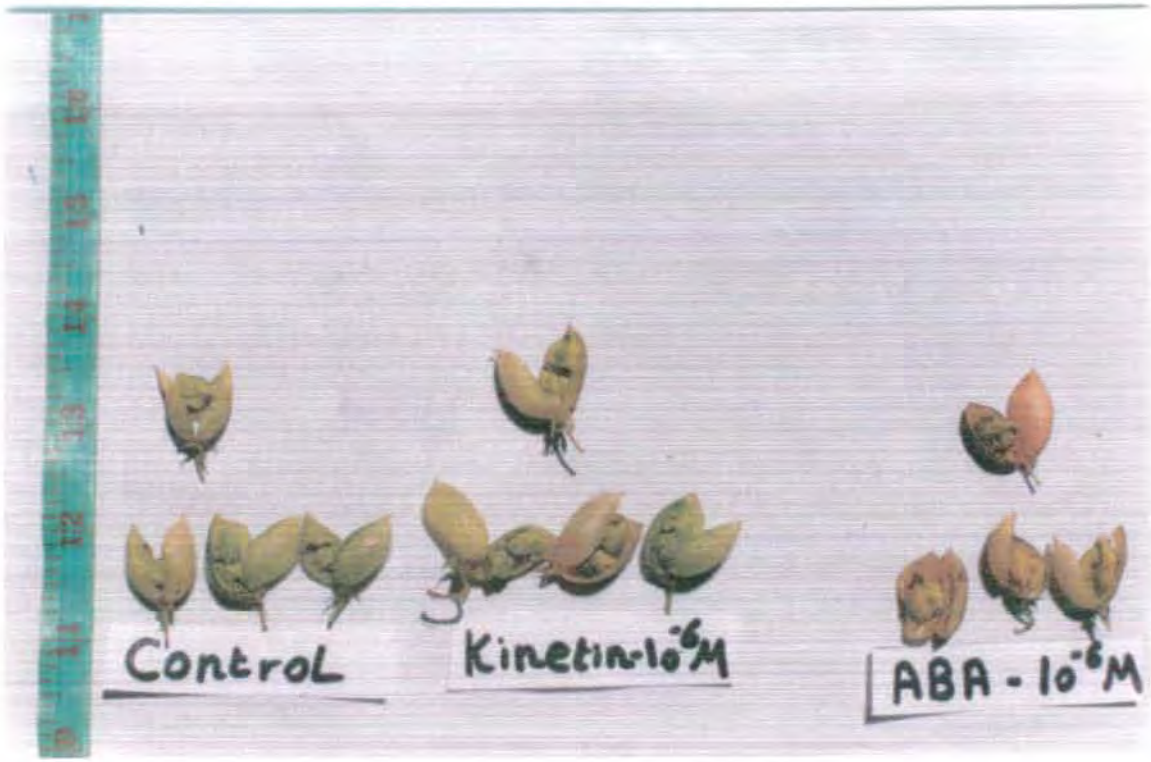

Figure 17: The effect of Kinetin and ABA on grains of *Cicer arietinum* L. cv. CM88 where kinetin treated plants showed larger pods and pods were still greenish, whereas ABA treated plants show early maturity of pods.

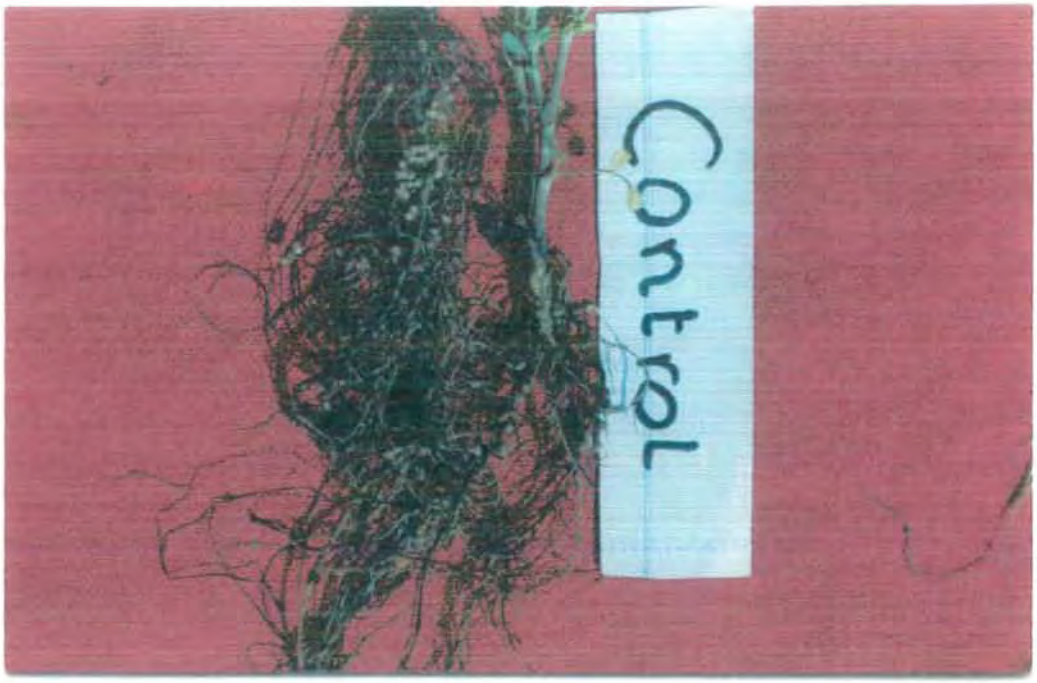

Figure 18: Nodules of *Cicer arietinum* L. cv. CM were grown basically on tap root. Irregular shapes of nodule tops that united at their bases make nodule counting difficult.

Figure 19: The effect of ABA applied as seed soaking and foliar spray on nodules of *Cicer arietinum* L.cv. CM88 at flowering stage. ABA decreased nodule number as compared to control. Nodules were restricted to the main root.

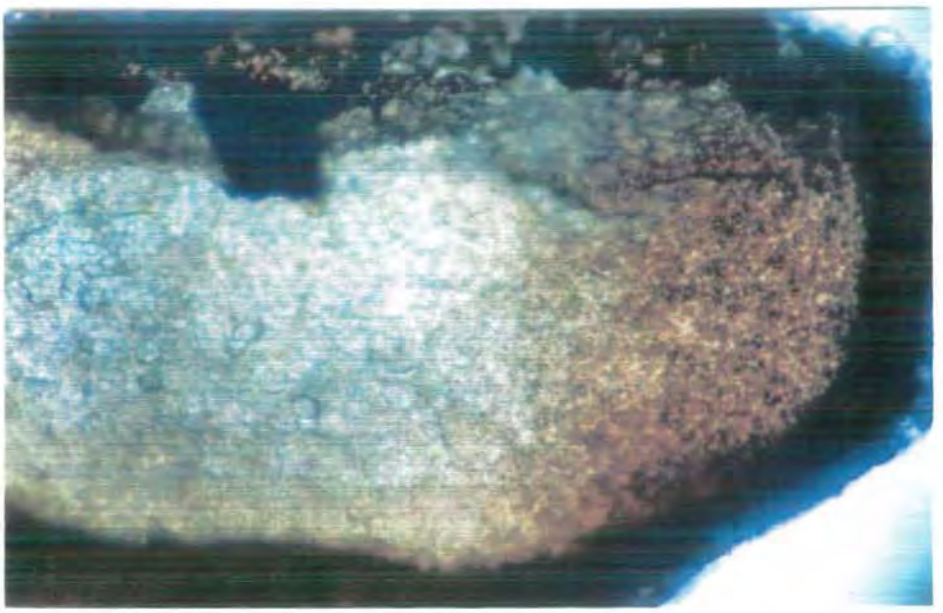

Figure 20: A dissection of untreated nodule at early pod filling stage of *Cicer arietinum* L. cv. CM where senescence of nodule started from the center of bacteroid tissue which became green while meristematic tips of nodule were still pink.

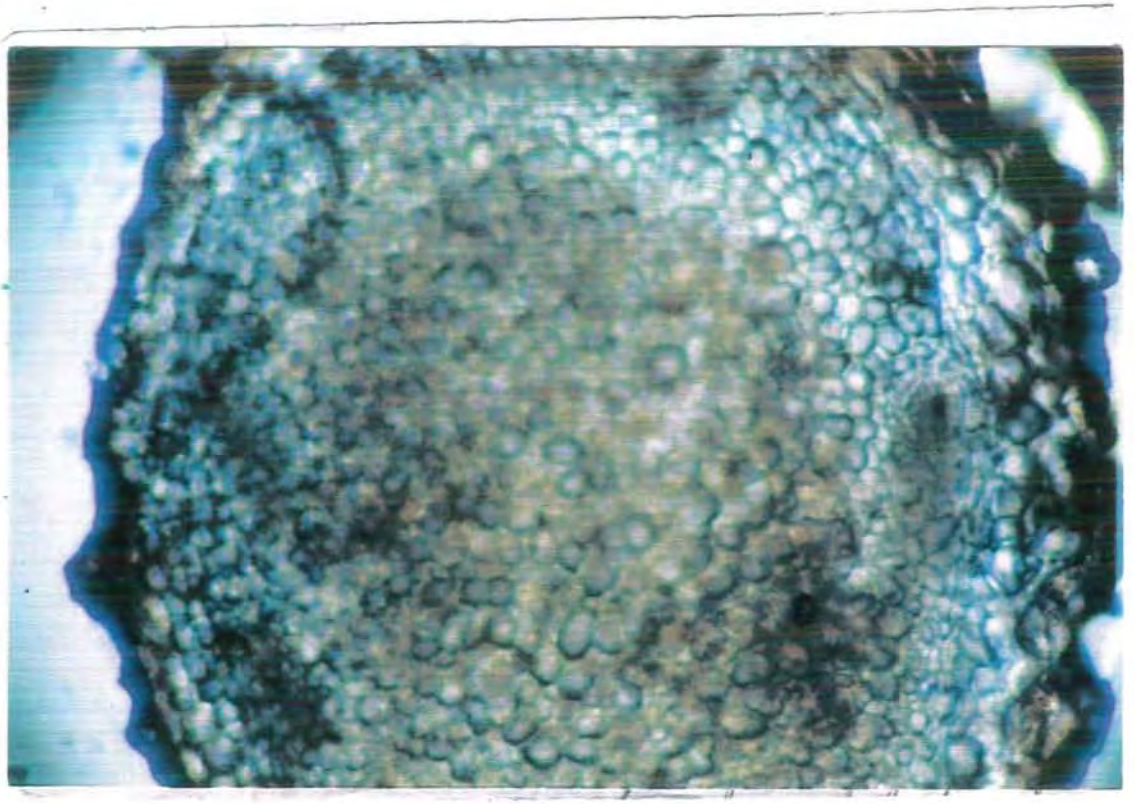

Figure 21: A dissection of nodule of *Cicer arietinum* L.cv. CM88 treated with ABA (10^{-5}M) at early pod filling stage where bacteroid tissue became greenish.