CHAPTER I
INTRODUCTION

As a result of intensive studies extending over many years, it is now known that plant growth regulators play a vital role in the control of growth, not only within the plant as a whole but also apparently within individual organs.

We may reasonably assume that the distribution and concentration of auxins and gibberellins, together with other types of regulators such as abscisic acid and ethylene, are vital factors in the overall control of growth and differentiation in the whole plant. Nevertheless, with regard to the space, time and concentration, the pattern of distribution of growth regulators in the plant is itself controlled by interactions between the environment and the genetic make-up of the plant. That is, growth regulators serve merely as agents, albeit very influential agents, in the overall integration and co-ordination of growth and differentiation.

The influence of growth regulators extends to all phases of development of the whole plant, including germination, dormancy, flowering, senescence and growth movement.

Gibberellic acid: Growth regulators like Gibberellic acid have been used to augment the germination of seeds of various species. Avery and Johnson (1947) and Pearse (1948) have reviewed this subject, Rao, et al.,(1963) observed that Morus indica seeds treated with CA. increased the percentage of germination and enlargement of seedling growth. Similar results were obtained by Caldwell and Sciuchetti (1963) with Datura Stramonium seeds,
but the fresh weight of the seedlings were less than that of the control.

Seth (1962) soaked coffee seeds for 48 hours in 1000 ppm $\text{GA}_3$ solution and noticed a 80% reduction in germination and treatment with 10 ppm caused a 10% inhibition. As a consequence of the $\text{GA}_3$ treatment a sixfold increase in the height of the dwarf pea seedlings, compared to the control seedlings, was observed by Moore (1958). Paleg (1960, 1962) found an increase in germination of barley seeds as a result of $\text{GA}_3$ treatment. Similar reports were filed by Shrivastava, et al. (1963). Hashimoto, et al. (1961) reported that $\text{GA}_3$ stimulated the germination of tobacco seeds.

Ever since the isolation of gibberellins by Yabuta and Sumik (1938), the physiological role of gibberellic acid has been demonstrated on many plants, as especially observed in stem elongation.

It has been shown that genetically dwarf species contain less endogenous $\text{GA}_3$ like substances than do their normal counterparts, (Proano and Greens, 1968; Gotah, 1970), indicating that the effect of a single gene dwarf species is often mediated through a block in the endogenous synthesis of gibberellic acid.

$\text{GA}_3$ has been noted to act within the tissue during the process of cell expansion, but this point still remains controversial due to lack of direct evidence (McComb, 1964). Alvim (1960) found an enhancement of both net assimilation rate and relative growth rate of kidney beans grown in a culture solution, as a result of spraying the plants with gibberellic acid. The increase in growth was also observed as a consequence of applying $\text{GA}_3$ to roots (Gleria and Bartfay, 1963) or to seeds (Gray, 1957). Selman and Bora (1968) observed an increased growth in tomato plants due to the foliar application of appropriate concentration of $\text{GA}_3$. 
Sachs, et al. (1959) concluded that growth after GA$_3$ application was solely a result of cell division, since no increase in cell size occurred during that period. GA$_3$ application was also shown to increase cell number in petioles, leaves and stem of caulescent plants. These observations have led to the conclusion that GA$_3$ stimulates growth via its effect on cell division (Sachs, 1965).

Many reports have been concerned with gross changes in nucleic acid or protein metabolism, but the mechanism of the action of GA$_3$ in the production of these compounds has not been completely understood. In 1965, Nitsan and Lang demonstrated the dependence of GA$_3$ stimulated growth on the synthesis of DNA. Holmes and Key (1967) and Broughton (1968, 1969) could not find a correlation between DNA synthesis and cell elongation after GA$_3$ application in soybean and pea, respectively.

Chen Yun-Mi (1962) performed an experiment with two-year old seedlings of *Populus balsamifera* and one-year old seedlings of *Populus suaveolens* by spraying them with a 400 ppm GA$_3$ solution. Control samples were simultaneously sprayed with water. In both the one and two-year old seedlings treated with GA$_3$, the height increased by 276%, while the fresh and dry weight increased by 250% and 350%, respectively. However, the length, diameter and weight of the root decreased: the fresh weight and dry weight of root decreased by 46% and 63%, respectively.

Loss of chlorophyll content was found in the leaves of the GA$_3$-treated plants. The shape of the leaves of these treated plants was lanceolate. However, stalk growth, cell elongation and rapid cell division were stimulated. Bostrack and Struckmeyer (1964) also found that GA$_3$-treated leaves were rather thin and pale green. Whether this can be
interpreted as a symptom of nitrogen deficiency is open to question. 
Artananov (1967) further observed that when gibberellin (20mg/l) 
was sprayed on sugar beet leaves, inhibition of chlorophyll synthesis 
occurred. This adverse effect of \( \text{GA}_3 \) was shown to be reversed by applying 
Vitamin \( \text{B}_2 \) (50mg/l). The decrease in the chlorophyll content paralleled 
a decrease in the protein content.

While much is known about the action of \( \text{GA}_3 \) on the growth of stems 
and coleoptiles, its influence on the root growth is still not completely 
understood. Some Japanese workers reported inhibition of root growth by 
fungal extracts (Imamura, et al., 1960) as well as crystalline gibberellin 
(Hayashi, et al., 1953). The root growth of rice plant was decreased 
when \( \text{GA}_3 \) was applied to the aerial parts of the plants (Erygin, et al., 1962). 
Root crops like potatoes and carrots showed a reduction in yield when 
treated with \( \text{GA}_3 \), while the aerial parts showed the usual elongation 
(Morgan and Mess, 1956).

**Indole-3-acetic acid:**

Went (1928) showed that the growth of the sub-apical tissues of oat 
coleoptile was dependent on a substance that diffused from the tips. Went 
proved that these substances, called auxins, could move into the adjacent 
tissues and promote cell elongation. This substance was later identified 
as Indole-3-acetic acid.

The physiological activity of IAA was later demonstrated in the 
Kogl's laboratory. This discovery prompted chemists and biochemists to
examine compounds of analogous structure. The results obtained from these research work have led to important developments in plant physiology and in agriculture; these results have proved to be especially fruitful in the studies of the relationship between the chemical structure and biological activity. They have shown that the chemical regulation of plant growth and interaction of various types of physiologically active compound is a highly complicated process within living cells, these growth factors operate in a delicately balanced way.

The possibility that IAA has an effect on germination has been the subject of inquiry (Audus, 1959), but the experimental evidence is extremely scant. It seems possible that the divergent results obtained with IAA may be related to the stage of dormancy. This compound may stimulate germination only in very dormant seeds, and even then the effect is very small (Poljakoff-Mayber, 1959; Söding and Wagner, 1955). Sinha (1969) conducted two years of trial experiments by pre-treating seeds with aqueous solutions of NAA and IAA at concentrations of 50, 75 and 100 ppm for 24 hours. He observed an increase in the dry weight of the plants. Dexter (1940) reported that treatment of sugar beet seeds with IAA, IBA, NAA, PAA did not hasten germination or growth. However, contrary to the above report, Darra, et al. (1971) observed beneficial effects of pre-treating seeds with IAA, IBA, NAA, GA and 2,4-D on germination. Inhibition of germination was also reported by Daletskya (1964); Nikolaeva (1967) and Barton (1960). Nikolaeva, et al. (1974) further reported differences in sensitivity to IAA of different species of seeds. A threshold of sensitivity was observed in Indian mustard (Brassica juncea, L) at a concentration of $10^{-5}$ IAA. Concentration of $10^{-4}$% and above depressed the germination of the silver maple seeds.
It has been hypothesized that the effect of IAA depends on the IAA content in the embryos, the degree of their differentiation, the site of localization and the interaction of IAA with other hormones. The effectiveness of the action of IAA is determined by the balance of the native hormones and by the biological characteristic of the seeds.

Auxins have been shown to cause cell division in some tissues, but not in all. The most apparent involvement of auxin controlling cell division is in cambium. The action of synthetic auxin on tissues varies; some promote only cell enlargement, such as in tobacco pith, and others evoke cell division (Steward and Shantz, 1965).

The method by which auxin induces cell elongation is still not clearly understood. In the first phase of elongation, the cell wall may actually get thinner (Brown and Sutcliffe, 1950) implying a stretching of the cell wall without an accompanying cell wall synthesis. However, at the end of a period of cell elongation, the wall in many cases gets thicker (Audus, 1959), suggesting that new cell wall material may be synthesized after the initial stages of extension.

Sirosis and Claude (1966) observed that the elongation of Avena coleoptiles was accelerated by the application of auxin. Thimann (1936), Bonner and Coepfli (1936) found that the growth of the coleoptiles and stem was very much accelerated by IAA at 1 ppm, whereas the same concentration caused an inhibition of root growth. Burg and Burg (1965) observed a marked elongation of pea stem sections when treated with $10^{-6}$ M IAA, but in the case of fresh weight only the higher concentrations caused increases. Thimann, et al. (1951) reported that when 20 mm pea stems were incubated for 24 hours in water and 1 mg/l IAA, the control
sections incubated in water increased in length by 20% and in fresh weight by 22%, as against a 50% increase in length and a 60% increase in fresh weight in the auxin-treated section. Audas and Das (1955) reported 20-25% stimulation of the pea root sections by .01 mg/l IAA. IAA up to 25 ppm increased the relative growth of *Lemma minor*, both in dry weight and in the frond area basis (Blackman, *et al.*, 1954); L. Minor grew better in the lower concentrations of IAA, which might suggest that the tissues contain sub-optimal and limiting levels of auxins.

**Cycocel:**

Plant growth retardants, a new group of synthetic chemicals, retard stem extension and increase stem thickness, thus causing the development of stocky plants without any physiological aberration. The application of these chemicals neither suppresses the growth permanently nor causes any deleterious effect on the vigour of the treated plants. Ever since the discovery of growth regarding chemicals, there has been substantial evidence to demonstrate the potential to decrease the plant height by reducing the internodal length and by slowing down cell division or cell elongation.

A physiological regulation of the plant height with cycocel (CCC) was reported in many species (Thomas, 1964 and El-Fowly, *et al.*, 1968; Hook, *et al.*, 1973). It was observed that plants treated with CCC were reduced in height. Such observations led to the use of CCC in agricultural practices, such as dwarfing tall varieties and reducing lodging in grain crops.

Wittwer and Tolbert (1960) found that the germination of lettuce seeds was strongly inhibited by CCC and related compounds. CCC could
suppress germination induced by gibberellin and red light (Cathey and Stuart, 1961). Rutgar, et al., (1965) studied the effect of the application of CCC on barley seeds and marked inhibition of seedling growth was observed during the initial stage of growth phase. Ray and Schroder (1966) using sunflower seeds, and Bose and Hore (1967) with Bouganvilleas El-Fowly (1968) using cotton seeds noted a reduction of height using CCC in the pre-treatment of seeds. A reduction up to 30% of leaf area was also reported by El-Fowly (1968).

Mukula, et al., (1967) observed that when CCC was applied as a foliar spray on seedlings of winter and spring wheat at 0, 2.5, 5.0 and 10 kg/ha, stem growth was reduced and lodging was prevented at 10 kg/ha.

Tolbert (1960) observed the effects of CCC on Thatcher wheat at concentrations of 10^{-2}M to 10^{-6}M, when applied as a soil drench, as a seed treatment and as a foliar spray. The most characteristic effects of CCC treatment were shorter and thicker stems, broader and greener leaves, earlier and stronger tillering and more uniform growth. Mohsin and Smith (1972) with Beans found a decrease in fresh and dry weight as a result of the application of 10^{-4} M to 10^{-3} M CCC on 7 day old seedlings. However, the leaves were observed to be thicker and greener.

Larter, et al. (1965) reported that the application of CCC as a soil drench was more effective than as a foliar application. The soil application of CCC at 10^{-10} and 10^{-4} M significantly increased the tiller number and grain yield in plants grown under a moisture regime.

Cathey (1964) reported that some plants were not responsive to CCC and that most of the Graminacious plants were insensitive. Black hull barley (c.i. 666), however, was found to respond to CCC quite rapidly. This result is in contrast to reports by Cathey (1964) and Humphries (1969).
This apparent contradiction was probably due to difference in sensitivity of different cultivars (Tolbert, 1960).

Abscisic acid:

Abscisic acid was first isolated from cotton plants (Liu, et al., 1961) and subsequently from young cotton fruit by Okhuma, et al., (1963), and its chemical structure was established by Okhuma, et al., (1965).

During early studies (Addicott, et al., 1964; Robinson and Wearing 1964) ABA was found to be a highly active growth inhibitor in various biological tests.

One of the most interesting features of abscisic acid is the wide range of physiological, biological and biochemical responses. Addicott and Lyon (1969) grouped these responses into two categories, promotive and inhibitory. Although the mechanism of the action of ABA is uncertain, biochemical studies have led to two main hypotheses: the first view is that ABA acts through inhibiting the production of m-RNA in plants (Villers, 1968), while the second is that ABA takes part in the inhibition of DNA synthesis (VanOverbeek, et al., 1967). The inhibition of RNA synthesis by ABA has also been observed by Van-Overbeek (1967) and Walton et al., (1970) and in both the cases inhibition seemed to be a general attribute in all cases of RNA synthesis.

The generalization of the response could be due to two possibilities: (a) that ABA acts on early common step in the synthesis of all types of RNA and DNA; or (b) that ABA inhibits cell division. However, Haber, et al., (1969) showed that ABA inhibited seed germination under
conditions in which DNA synthesis was not inhibited.

The dormancy of many seeds can be greatly prolonged by ABA. Some seeds will not germinate at all (Lipe, 1966; Pieniazek and Grochowska, 1967). It is worth noting that ABA in inhibiting the seed germination is relatively transient, and after rinsing away the inhibiting solution of ABA, germination occurs.

The senescence of detached leaves of many plants is retarded by cytokinin and GA₃ and is accelerated by ABA. High concentrations of cytokinin counteract the senescence accelerating effect of ABA (Back and Richmond, 1971).

ABA inhibits root elongation but not to the extent that IAA inhibits root growth. When ABA and IAA are used together, the reduction in elongation is less than that caused by IAA alone. Consequently ABA can be considered an antagonist of IAA. Khan and Downing (1968) showed that the growth inhibition in barley coleoptiles caused by abscisic acid could be arrested by benzyl adenine.

In conjugation with GA, ABA commonly counteracts or inhibits GA induced responses, especially responses like elongation, germination and senscence (Aspinall, et al., 1967). Kaufman and Jones (1973) showed that ABA as a potent inhibitor of gibberellic acid promoted growth in Avena stem segments at physiological concentrations of 10⁻⁵ to 10⁻⁶ M. This result compares favorably with results obtained in other systems where gibberellin-abscisic acid interactions have been studied (Addicott and Lyon, 1969; Aspinall, et al., 1967). In most other systems, when kinetic analysis was done, the interaction between ABA and Gibberellin was a non-competitive type (Addicott and Lyon, 1969).
Borman, et al., (1967) also reported that ABA accelerated the loss of chlorophyll and the loss of turgor in abscising petiole plants of explants. Color changes in leaves were a common response to the foliage application of ABA (Rudinicki et al., 1968). In isolated leaf disks ABA also accelerated the loss of chlorophyll in a number of species examined (Aspinall et al., 1967; Shankhla and Shankhla, 1968). The chlorophyll synthesis of seedlings of Fraxinus was also inhibited by ABA (Sondheimer and Galson, 1966).
EXPERIMENTAL OBJECTIVES

Many plant growth regulators have been studied extensively with respect to plant species, mode of application, concentrations of the chemicals, soil interactions and growing environment. It has been observed that concentration, time of treatment and duration of treatment are particularly important with regard to producing the desired affects in plants.

Manipulation of crop production processes with plant growth regulating chemicals may be one of the most important advances to be achieved in agriculture. Thus plant growth regulators have emerged as a new, rapidly growing segment of agricultural production. Further, a great deal is yet to be learned in the field of growth regulators, before one can make the most productive and purposeful use of these chemicals.

With the above in mind the following research objectives were formulated:

1. The effect of different concentrations of GA$_3$, IAA, CCC and ABA on germination and subsequent growth of vegetable crops.

2. The effects of methods of application (seed treatment and apical application) on growth and development of vegetable crops.

3. To study species variability with respect to growth and development as a result of method of application and concentration of the above growth regulators.

4. To study the effects of all the above growth regulators on chlorophyll content of pea under the above experimental conditions.

5. To study interactions of GA$_3$ and ABA; GA$_3$ and CCC with respect to seed treatment and apical application in pea on germination and subsequent growth.