INTRODUCTION

The phenomenon of growth remains among the most mysterious and fascinating of all biological phenomena. The growth of a plant is a dynamic and complex, yet strictly controlled process (Wareing and Phillips 1970). Growth may refer to the changes that occur in the protoplasm, in the cell wall, in the cell considered as a unit, in plant parts or organs, or in the entire plant considered as a whole. It involves metabolic chain reactions that are triggered off by some internal factors. The ultimate result is manifested in irreversible increase in size and volume, coupled with increase in dry matter that contributes mostly to the bulk increase of protoplasm and nucleus. This situation calls for a careful approach towards unravelling the secrets of growth and differentiation. The intact plant cells growing within the framework of organized plant tissues do not permit a critical study of the factors responsible for continued growth and differentiation. Because, such studies need to be extended not only to the observable changes in form and structure but also to the internal process and factors controlling the development of organs and tissues so that these processes must ultimately be explainable in terms of physics and chemistry. A frequently employed technique consists of removal from the plant either an entire organ or part and segment thereof, isolated cells or very small cell aggregates and isolated mature or immature embryos. Such a technique has been defined as tissue culture, suspension culture, or embryo culture (Street 1973) respectively. Plant tissue culture methods have advanced considerably during
recent years and are now firmly established in the repertoire of biological techniques. It is becoming increasingly clear that such cultures, grown under precisely controlled conditions and in the absence of contaminant micro-organism, provide excellent experimental materials for studying many intriguing physiological problems.

The growth of tissue explants and cells involves many distinctive steps or processes, any one of which may be rendered limiting and therefore make regulation possible. This regulation could be mediated by the exogenous supply of a missing factor or by the endogenous presence or disappearance of an inhibitor.

German Botanist Gottlieb Haberlandt (1902) was the pioneer to set forth the purposes and potentialities of cell culture in plants. He made several attempts but without any success due primarily to his wrong selection of cells and partly to the inadequate knowledge of those days about the nutritional requirements of plants. Although Haberlandt did not have any success himself but his disciple Kotte (1922) and subsequently Robbins (1922) in USA succeeded in culturing tissues in an artificial medium.

The first culture unlike that of the organ cultures of Robbins and Kotte was aimed at the establishment of their survival. During this phase of research in this line, Gautheret (1939), Nobécourt (1939) and White (1943, 1946) concentrated their attention on methodology and defining suitable culture medium. These successes were followed by a period of development when culture media and technical methods were improved, permitting the culture of wide range of organs and callus
from many different species. Since 1960, methods have progressively become more advanced and have culminated in highly specialized techniques for culturing single cells, cell suspension and naked protoplast.

Leaves probably start to expand when stimulated by growth substances from cotyledons or from older leaves that have attained maximum expansion. In turn, this initiation of expansion in younger leaves may stop the older leaves expanding further and influence their senescence by changing their supply of growth substances. Leaf growth is found to occur only to a slight extent in darkness but is greatly accelerated by small intensities and duration of small illumination (Went 1928).

In the present investigation attempt has been made to raise the seedlings in dark to induce etiolation. Etiolated leaves lack the power of synthesizing the food materials necessary for further growth and development. Rather, they develop a hunger for various food materials. Special interest lies in excising the leaves from the mother plant and subjected the disks cut from them to grow in an artificial medium supplemented with various growth promoters and inhibitors.

The carbohydrates are the vital factors in all cell extension. Sugar besides providing cell wall materials like cellulose, hemicellulose and pectic materials provides the source of energy derived in the process of photophosphorylation, part of respiration. Therefore, sugar proves to be inevitable for plant growth. Cultures of plant tissues in vitro are usually supplied with an exogenous source
of carbon and energy. Even tissues rich in chloroplasts will not survive for long period in culture in light in the absence of an external supply of carbohydrates (Hanson and Edelman 1972, Hildebrandt et al. 1963). In dark grown cultures the requirement for an exogenous carbon source is obligatory for growth and differentiation (Gautheret 1955). Over many years glucose (Gautheret 1942) or sucrose (White 1943) had been widely used in culture media. Dale (1967) observed that leaf-tissues grown in the light also require a supply of exogenous carbohydrates. He envisaged that in terms of potentiality sugars viz. glucose, fructose and sucrose at a concentration of 2 % are almost equal. Miller (1951, 1952) applied 3 % D-glucose in cultures of etiolated bean leaf disks and such basal medium was later on employed by Scott and Liverman (1956) and Powell and Griffith (1960). Miller (1956) also favoured the idea of supplementing the basal medium with $5.8 \times 10^{-2}$ M sucrose. Sarma (1971) screened the efficiency of several sugars on the culture of etiolated bean leaf disks and confirmed the superiority of sucrose over other sugars. Such a situation calls for a comprehensive investigation with at least 3 sugars viz. glucose, fructose and sucrose.

Since the discovery of the phytohormone IAA and isolation of this active substance by Kögl et al. (1934) it has found wide application on intact plants or isolated plant parts for clearer appraisal of its effect on control of growth of stems, internodes, coleoptiles and roots. But its mechanism on the expansion growth of leaves has not been thoroughly investigated. Gregory (1928) first proposed the hormonal control of leaf growth. Thimann (1935) applied IAA to intact
leaves and concluded that it stimulates the extension growth of veins and inhibits the mesophyll growth. Such a view was corroborated by Wheeler (1959) experimenting with Phaseolus vulgaris leaves. However, stimulation of growth of disks obtained from stipules and leaves of Pisum (Hashimoto 1959) and etiolated leaves of Phaseolus vulgaris (Dale 1966, Sarma 1971) have been reported. Controversies still prevail regarding the actual mode of action of auxin in the growth of leaves. Since in the growing young leaves auxin is known to be synthesized in appreciable quantity, how far externally added auxin is effective in this respect poses a problem for investigation.

The gibberellins are a large group of closely related diterpene acids that constitute an important class of natural plant growth hormones. Discovered much more recently than the auxins, gibberellins are known to affect plant growth and metabolism at fantastically low concentrations. At least 52 types of gibberellins (GA1 - GA52) have been identified so far, of which gibberellic acid (GA3), the most accessible form of gibberellins has since been applied on intact plants or isolated plant tissues to study a variety of responses affecting plant growth and development. The most typical and striking plant response to gibberellins is the stem elongation, actually internodal elongation, limited exclusively to younger tissues which are still growing. Gibberellins applied in one part of the plant or even to the soil can stimulate growth in all the growing parts of the plant especially of dwarf varieties. Gibberellic acid (GA3) accelerates cell elongation and at least in certain instances
it is reported to have induced cell division.

Humphries and Wheeler (1963) envisaged that growth of mesophylls as well as the veins is elicited by GA. It is well documented that GA$_3$ causes stimulation of extension growth of stem, coleoptile and hypocotyls (Greulach and Haesloop 1958, Sarma and Borah 1972-74, 1974; Kaufman and Jones 1974) and expansion growth of bean leaf disks (Humphries 1958, Sarma 1971). Interaction between GA$_3$ and IAA has also been reported by several workers on a variety of plants and tissues (Saeb-Stein 1960, Sarma 1971, 1979; Sarma and Borah 1974). Humphries and Wheeler (1960) reported that GA increased cell size and also cell number in bean leaf disks but the stimulation of cell division only occurred in the dark. Light enhanced the GA stimulation of cell expansion.

Gibberellic acid is also reported to promote the elongation of leafsheaths of rice plants (Hayashi et al. 1956), rice coleoptile sections (Soni and Kaufman 1972) and leaf sheaths of dwarf maize seedlings (Katsumi 1970). Katsumi (1970) observed that leaf sheaths on intact plants elongated but excised leaf sheaths did not respond to GA treatment.

1974) as well as IAA plus BA (Behki and Lesley 1976) could induce regeneration of plants from leaf explants. Therefore, it was decided to have a deeper insight into the effects of kinetin and IAA and interaction between kinetin and IAA and also between BA and GA\textsubscript{3} on etiolated leaf disks of bean.

**Coumarin** and a number of unsaturated lectones are known as naturally occurring growth inhibitors of plants. The views expressed by various workers on the possible mechanism of action of these inhibitors are rather conflicting (Torrey 1956). That coumarin inhibits root growth had been reported by Audus (1948) and Goodwin and Taves (1950). On the other hand, stimulation of growth by coumarin had been reported by several workers. Thimann and Bonner (1949) reported promotion of growth in *Avena* coleoptiles. Expansion growth of leaf disks of *Chenopodium album* (Miller and Mayer 1950-51) and etiolated bean (Sarma 1971) is reported to have been stimulated by coumarin. Thimann and Bonner (1949) and Gantzer (1960) observed synergistic effect of IAA and coumarin on the growth of oat coleoptile sections. Synergism between IAA and coumarin was also reported by Sarma and Deka (1977b) on the growth of bean hypocotyl segments. From reports available it appears that the effect of coumarin on growth is still a matter of conjecture.

Abscisic acid (ABA), another natural growth inhibitor is active mainly at concentrations 100-1000 times lower than the phenolic compounds (Kefeli and Kadyrov 1971). The inhibition of growth of whole plants, excised organs or seeds is the most easily measured response to natural(+) or racemic(+) ABA (Milborrow 1974).
It has been reported that ABA inhibits growth of all parts of plants and counteracts the stimulatory effects of the natural growth-promoting compounds when applied with them (Milborrow 1974). ABA is also reported to have counteracted the toxicity of supra-optimal concentrations of the growth-promoting compounds (Barlow et al. 1961, Milborrow 1966). On the other hand, ABA is reported to stimulate hypocotyl elongation of cucumber (Aspinall et al. 1967). That ABA brings about senescence in leaf disks or sections through gradual degradation of chlorophyll has also been reported (Aspinall et al. 1967, Back and Richmond 1971, De Leo and Sacher 1970).

Maleic hydrazide (1,2-dihydro-3,6-pyridazinedione) and 2,3,5-tri-iiodobenzoic acid (TIBA) are classified as anti-auxins from their inhibitory action (Sircar 1971). Both of them are considered to be of practical and theoretical interest as growth regulating chemicals that inhibit plant growth without causing obvious morphological abnormalities. The most evident morphological effect of MH and TIBA is a general stunting of the plants (Naylor and Davis 1950, Bush and Sims 1974, Khazhakyan and Chailakhyan 1977, Venkataratnam and Satyanarayanmurthy 1950). Sengupta and Chattopadhyay (1954) observed an inhibition of the rate of growth of the main stem and leaf formation and an increase in the rate of leaf fall in tomato plants treated with TIBA. Venkataratnam and Satyanarayanmurthy (1950) reported that young chilli seedlings sprayed with TIBA at 20 to 100 ppm, the apical portions and the lower leaves showed epinasty within 24 hr. TIPA (Venkataratnam and Satyanarayanmurthy 1950) and MH (Choudhury and Ramphal 1960) are reported to interrupt with the
Apical dominance. A distinct interaction between MH and IAA has also been reported on the extension growth of etiolated bean hypocotyl sections (Sarma and Borah 1973). They envisaged that MH imparted anti-auxin effects culminating in reduced extension growth. The effect of MH and TIBA on intact plants has been studied in some details, but their action on the growth of isolated tissues has rather been meagerly investigated.

It has been reported that a plant or isolated leaf tissue treated with growth retardant such as CCC exhibit reduction in growth which results directly from an inhibition of endogenous GA synthesis (Zeevaart and Osborne 1965, Bristow 1966, Cross et al. 1968, Dale and Felippe 1968, Felippe and Dale 1968, Ockerse 1970). On the other hand, some workers claimed to have increased the level of endogenous GA's treating with CCC (Reid and Crozier 1970, 1972; Halevy and Shilo 1970). Antagonism between CCC and GA₃ on the growth of bean hypocotyl segments has also been reported (Sarma and Deka 1977a).

That the growth retardants inhibit cell-division and cell-expansion has been reported (Sachs et al. 1960, Mahmoud and Steponkus 1970). Phosfon D has been characterized by Preston and Link (1958) as a compound inhibiting longitudinal growth. It has also been proposed that Phosfon D interacts directly with gibberellin (Cathey 1964).

Alar, B995 or SADH is also reported to cause growth inhibition through its action on GA biosynthesis (Ryugo and Sachs 1969). Application of GA₃ is known to reverse the growth retarding effect of SADH (Williams and Stahly 1970). Counteracting effects between growth
retardants (CCC and B995) and GA have also been reported by Dunberg and Eliasson (1972). The effects of the growth retardants on the growth of isolated plant tissues or parts have not yet been studied in details, although their effects on crop plants and intact plants have been investigated in greater details.

During the last decade there has been a rapid expansion in the application of plant tissues and cell culture techniques in almost all branches of pure and applied plant sciences. Plant tissue and cell cultures have emerged from mere curiosities and are proving to be powerful means of advancing plant science and solving long-standing problems in food science and agriculture. When Haberlandt attempted the first plant cell culture, his intentions were to develop a more versatile tool to explore morphogenesis and to demonstrate totipotentiality of plant cells. He probably did not visualise that the cell culture technique would prove a valuable aid in economically oriented activities.

The last decade has witnessed a very rapid rise in the number of plant scientists using the techniques of organ, tissue and cell culture in plant physiological researches. This is mainly due to important development and requirements in these techniques which now make possible an increasing range of reproducible and quantitative experiments involving plant cultures. It is also partly due to successful demonstration by a number of pioneer investigations that many problems in plant physiology otherwise inaccessible to study can be tackled by the imaginative exploitation of tissue culture techniques.
With the premise enunciated it was considered pertinent to have a further insight into the effects of individual compounds on the expansion growth of bean leaf disks. It was also decided to explore the possible interactions between the growth promoters and inhibitors or growth retardants on the isolated leaf tissues.