CHAPTER-I
INTRODUCTION

The effect of gravity on plant physiological processes, as a result of which upward growth of shoot and downward growth of root takes place is known as gravitropism. Though the idea of gravitropism previously known as geotropism is an old one, the attention of plant physiologists of different countries of the world have of late been attracted greatly by gravitropism or in other words movement of plant organs with particular reference to gravitational pull towards the centre of gravity only at the end of the twentieth century. Studies have been made throughout the world about various physiological changes which take place at the molecular level underlying gravitropism. The 'Gravitational plant physiology' has of late emerged as a new and interesting branch of plant physiology for studying the plant processes under the influence of gravity (1g) in general and the influence of microgravity (<1g) or macrogravity (>1g) in particular on plant physiological processes with reference to germination, root and shoot growth. The very idea of the influence of micro and macrogravity on growth processes is very recent and thus has been able to draw the attention of gravitational plant physiologists.

Gravitropism has an important impact on plant, because of which a plant is in its position, the shoot resume upward growth after prostration by any external influence, the root grows downward to ensure water and mineral uptake from the soil and holding the plant in an upright position to facilitate the primary physiological processes like photosynthesis, respiration, absorption, conduction etc.
The tropic movement of radially symmetrical organs of plans such as root, stem, coleoptile etc. are the examples of gravitropism. The movement may be towards (positive) or away (negative) from the earth's gravitational pull.

The axis of the tip of the root or the shoot if reoriented by 90° with respect to the direction of the gravitational pull, again alter direction of growth, curving until it is again vertical in response to the gravitational force. The upper side of the horizontally placed coleoptile, hypocotyle or stem ceases growing almost immediately, while the lower side may continue to grow at about the same rate or may grow somewhat faster resulting upward bending in response to gravity showing negative gravitropism. However, reverse is the reaction in case of roots (positive geotropism).

The response to gravitational stimulation by geosensitive plant organs is dependent on both the magnitude and direction components of the gravity force (Dedolf et al, 1965). When a body is at rest on the earth, the force of inertia of motion is absent and it is in the normal state of gravity i.e. 1g. Again, the efficiency of any gravitropic stimulation is determined both by the intensity and the duration of the applied stimulus. Gravitropic response often has been assumed to be related to the product of intensity (acceleration, g-level) and duration (t) e.g. the response should be a linear function (g \times t). The (g \times t) product is usually referred to as "stimulus quantity" (Volkmann and Sievers, 1979).

The attention of gravitational plant physiologists of the world has been concentrated specially upon the effect of gravity less than 1g on plant physiological processes. With the development of modern technology human civilization has been extended upto space and attempts has therefore been made
to set up permanent establishment of space colony, for which, supply of food would be one of the most important requirements which is entirely dependent either directly or indirectly on plant till today, since there is no alternative source within the limit of present human knowledge.

At what level a plant perceives the gravity signal is the most important aspect. The lowest limit of gravity stimulus which is perceptible to a plant is referred to as the threshold effect. The threshold values vary from 30 to 250g s (Johnsson and Pickard, 1979). Recent measurement indicates threshold values generally less than 100g s; some even less than 30g s. But *Avena* coleoptile grown in space show threshold for a gravitropic response less than 0.1g (Brown *et al*, 1995) and 0.2 -12g s (Johnsson, 1965).

In earth-bound laboratories the slowly rotating clinostat has been widely used as a tool to alter the normal gravity condition to which the plant is exposed (Moore, 1990; Brown and Piastuch, 1994; Hilaire *et al*, 1995). The underlying assumption is that clinostat 'nullify' the action of the gravitational stimuli. With the help of a three dimensional clinostat to stimulate microgravity condition for germinating spores of *Adiantum capilus-veneris* Kasahara *et al* (1994) have reported that germination of spores under this condition is not affected, but the protonemata are shorter than that of the controls. A very slow rotating clinostat has also been developed for present research work.

There has been some controversy, however, about the usefulness of clinorotation as a stimulator of microgravity (Brown and Chapman, 1985; Moore, 1990). Lorenzi and Perbal (1990) have shown that the clinostat does not stimulate microgravity correctly. That clinorotation is not an useful method for stimulating
microgravity at the cellular level and that clinostatic experiments performed with rotation at 2rpm and 4rpm show some disturbance in cellular construction have been reported (Hilaire et al., 1995). Shen-Miller (1970) has used centrifuges and slowly rotating clinostats for exploring gravitropic thresholds of the Oat coleoptile. However, studies under clinorotation have shown changes in the cell cycle (Legué et al., 1992), the production of ethylene (Leather et al., 1972; Salisbury and Wheeler, 1981), the nastic response of maize coleoptile (Nick and Schäfer, 1989), root curvature (Hestnes and Iversen, 1978), soluble protein profile and expression (Piastuch and Brown, 1995; Schulz et al., 1992), calcium distribution (Hilair et al., 1995), plant morphology (Brown et al., 1976; Hilair et al., 1995), some enzymatic activities (Brown and Piastuch, 1994) and starch metabolism (Brown and Piastuch, 1994; Obenland and Brown, 1994).

The development of new and modern space technology has made possible to carry out different experiments to study the plant growth and development under the influence of real microgravity. Conditions of microgravity may be obtained for a period upto 15 minutes in launching rockets, while on board space shuttle provides microgravity periods for several days and months. But these two conditions are accompanied by technical problems like mechanical stress due to high level of vibration during take-off and landing. In the "International Space Station" which is under construction and to be completed by 2006 is expected to be a permanent orbital space station near the earth, would provide microgravity condition continuously for years without technical problems.

During their 185 - day space journey, Salyut-6 (launched in 1977) cosmonauts took to gravity free gardening. Cell division in higher plants and the
growth of *Chlorella* were also studied (Rajan, 1995).

Plant cell cultures were used for the first time in space biology studies by Krikorian and Steward (1978). In June, 1991 a shuttle carried a spacelab dedicated entirely to life science. It was the space mission since the Skylab (1973), designed for the most detailed and interrelated physiological measurements.

The growth and development of protoplasts of rapeseed (*Brassica napus* L. cv. Line) and carrot (*Daucus carota* L. cv. Navona) were studied on board the space shuttle "Discovery", during the first International Microgravity Laboratory (IML-1). Protoplasts exposed to microgravity conditions showed a delay in cell wall synthesis. The absolute threshold for a gravitropic response in *Avena* coleoptile obtained in the mission (IML-1) was less than 0.1g (Brown et al, 1995).

A second 'International Microgravity Laboratory' (IML-2) was placed into the orbit in July, 1994. The study on microgravity on cells, tissues and plants has thus been continued.

In physical terms, the force of gravity can deform or displace objects of specific mass. Hence, in biology regarding orientation of plant organs, there are some sensitive substance known as 'susceptor' which perceives the physical information generated by the gravity force. Although gravitropism has been widely studied, relatively little is known about the molecules (i.e. susceptor) involved in sensing the gravitational signal and transducing it. In higher plants the gravity susceptors are believed to be statoliths and they are further believed to be starch containing plastids known as amyloplasts in dense proportion. These statoliths are sedimented in specialized cells known as statocytes (Haberlandt, 1900; Nemec,
The amyloplast containing cells are located at endodermis, the starch parenchyma cells that surround vascular tissues in shoot, and in root they are located in the columella of the cap (Sack, 1991). The statoliths sediment with the direction of the g-force vector direction, that may sediment within tens of seconds or a few minutes after reorientation of an organ tip. The statocytes are highly polarized cells that contain a peripheral Endoplasmic Reticulum (ER) membrane located along the lateral cell wall, a nucleus positioned in the middle or at the top, and dense amyloplasts sediment at the bottom. If the statocyte reorient along with the organ the amyloplast sediments to the new physical bottom.

Detailed comparative studies of gravireactivity in a mutant and a wild type of Arabidopsis thaliana, have shown that starch grains are necessary for full sensitivity (Kiss et al., 1989; Saether and Iversen, 1991). But starchless mutants are still responsive to gravity, but the gravitropic curvature delayed, suggesting that the amyloplasts change the rate of gravitropic response, but may not be essential in signal perceptions and resulting transduction cascade (Caspar and Pickard, 1989; Kiss et al., 1989). The transduction mechanism by which the positional information is translated by the plant into biological (biochemical?) changes so that a differential growth response causes an adjustment of the growth direction is not fully understood.

The phytohormone auxin which has an important role in vascular development and root formation may be another important susceptor to perceive the gravity signal. Auxin is synthesized in young leaves of the shoot system and transported downward to the root tip (Goldsmith, 1977). Indole-3-acetic acid (IAA) is the predominant form of active auxin found in plants. According to
Cholodny-Went Theory (Cholodny, 1928; Went and Thimann, 1937), IAA may be the message transmitted from the cap towards the response zone of the root (Shaw and Wilkins, 1973; Rivier and Pilete, 1974; Young et al, 1990). It has been shown that the transport of auxin in any given tissue is polar, i.e., it moves in one direction (Scott and Wilkins, 1968; Morris et al, 1969; Goldsmith, 1977; Kerk and Feldman, 1994, 1995). The statocyte containing endodermal tissue in shoot transport auxin from its site of synthesis in the shoot apex to its site of action (Lomax et al, 1995; Gälweiler et al, 1998). Hence, amyloplast sedimentation upon gravistimulation activates auxin-efflux promoting lateral transport to adjacent cortical and epidermal tissue. Auxin accumulates at the bottom side promoting differential cell elongation between upper and lower flanks, lead to upward shoot curvature (Lomax, 1997).

The molecules transducing the gravity signals in statocytes have not yet been definitely characterized. The sedimentation of amyloplasts can stretch plasma and/or ER membrane resulting in mechanosensitive channels, can allow a local transient increase in cytoplasmic Ca\(^{2+}\) levels that will trigger a signal leading to the production of physiological signals (Sievers et al, 1991). Furthermore, high concentration of Ca\(^{2+}\) has been detected in statocyte amyloplasts (Chandra et al, 1982). Under clinorotation calcium distribution is changed (Hilair et al, 1995). Recent experiments with Ca\(^{2+}\) reporter system have failed to identify gravity-induced transient changes in cytosolic Ca\(^{2+}\) levels (Legué et al, 1997).

Recently, Kim et al (2000) have established involvement of brassinosteroids (BRs) in gravitropic activity in the primary root of maize. Brassinosteroids may be involved in auxin-mediated processes for the gravitropic
Root gravitropism is the result of differential growth of the lower and upper sides of root that are not vertically oriented relative to the gravitational field (Jackson and Barlow, 1981; Barlow and Hofer, 1982; Darbelley and Perbal, 1984). Two regions of the root are responsible for proper orientation with respect to gravity vector: the root cap which is the site of gravity perception (Audus, 1962) and the elongation zone, where differential rates of cell elongation result in corrective root curvature. When a primary root is subjected to a change of orientation in the gravitational field, its tip bends and reorient itself with respect to gravity. This may also result in changes in the overall rate of root elongation relative to vertical roots (Finn and Digby, 1980; Pilet and Ney, 1981), and growth asymmetry varies during the gravitropic response (Selker and Sievers, 1987; Ishikawa et al, 1991; Zieschang and Sievers, 1991). Three different phases can be distinguished in the gravitropic curvature (Larsen, 1957): (1) the latent time, during which no curvature is observed, (2) the phase of fast bending and (3) a period of slowing down of the rate of curvature. It has been established that lateral IAA transport causes unequal rates of cell elongation, leading to curvature. Concentration of increased IAA, ABA and an unidentified inhibitor in the bottom halves of maize roots which may be responsible for growth curvature. Since root cells are significantly more sensitive to auxin concentration than the cells of shoot (Thimann, 1937), it seems unlikely that high concentrations of auxin could be tolerated in a growing root tip.

The gravitropic response of root varies as a function of the angle \( \theta \) of inclination of their axis with respect to gravity (Larsen, 1962; Perbal and
The response to a stimulus is a function of \( \sin \theta \) for short periods of stimulation (Larsen, 1962). Adventitious rooting on stem cutting of *Coleus blumei* L. is affected by inclination of the cutting with \( \rho \text{g} \sin \theta \) and \( \rho \text{g} \cos \theta \) (Das, 1993; Boissya and Das, 1995).

Interaction between root gravitropism and root phototropism have been reported in some genera of *Arabidopsis* (Mohr, 1961; Schneider, 1964; Okada and Shimura, 1992). Phototropism has an important account in the design of root gravitropism (Vitha et al., 2000). The strength of phototropism and gravitropism are comparable in *Arabidopsis* root (Okada and Shimura, 1992, 1994). Though the interaction between these two tropisms is a complex phenomenon, gravitropism is stronger than phototropism (Vitha et al., 2000).

To understand the gravity dependent physical function like hypoxia, in the roots of agar grown *Arabidopsis* exposed in microgravity condition in spaceflight were affected that may be caused by change in gravity mediated fluid and/or gas behaviour (Porterfield et al., 1997).

The habit of use of stem cuttings for quick vegetative propagation is much popular among the people from a very primitive time without knowing the science behind. It is the most economic and easier process and can produce large number of offsprings in a very short period within a limited place from a single donor plant. This process fulfils the growing demand of human beings specially in regard to maximise production; moreover, in ornamental plants and also in other economic plants like tea, coffee, rubber etc. cuttings are widely used for producing new plants. The plants raised from cuttings get earlier maturity and show much higher productivity than those from seeds. Unless rooting takes place
properly the potentiality of a cutting never flushes into a future suitable plant. So efficient rooting is the most important factor for a cutting. Roots that form on a cutting, directly from the stem or from the callus tissue, are adventitious. Sometimes the adventitious roots appear to be initiated by division in the cambial zone (Smith, 1936). Again adventitious root formation is a complex phenomenon. Rooting which takes place on a cutting is greatly affected by various internal factors present in the cutting and also by various external factors. Moreover, age of the donor plant, position of the stem from which the cutting has taken and season of the year or in other words physiological conditions of the donor plant as well as those of the cuttings are also very important factors.

The effect of gravity components on adventitious root initiation and their subsequent growth in *Coleus blumei* L. stem cutting has been established (Das, 1993; Boissya and Das, 1995). According to them cuttings cultured at 45° (θ =45°) inclination to the earth's gravitational field show earlier rooting but fail to exhibit maximum amount of IBA accumulation at the base of the cuttings, but the maximum amount of IBA accumulation is exhibited at 22.5° inclination. Their results clearly suggest that the gravity components viz ρg Sinθ and ρg Cosθ play some positive role on the rate of flow of IBA from the apex to the base and subsequent root production and their growth where θ is the angle of orientation of the cultured cuttings.

Greater accumulation of auxin inhibits cell elongation of roots resulting positive gravitropism, while in shoot it promotes cell elongation by increasing auxin concentration on the lower side, consequently resulting upward curvature. Shoots of several rice lines show agravitropic growth or reduced
gravitropism, the phenomenon being termed 'lazy'. Yamazaki (1985) has observed that 83% lazy-kamenoo seedlings show normal gravitropic curvature 3 days after inhibition, whereas 93% of 5-days-old seedlings are agravitropic. When seedling grows after germination, the shoot gradually shifts its growing site from the mesocotyle/coleoptile to the leaf. The gravitropic response of cereal grass shoot, however, occurs in the leaf sheath pulvini (Kaufman et al, 1987). Seedling of maize grown under the conditions of 3-d clinostat exhibit curvature in different portions (Hoson et al, 1995) viz; (1) the basal transition zone connecting root and mesocotyle, (2) the coleoptile node located between mesocotyle and coleoptiles, (3) the elongating region of the coleoptile. Control coleoptiles, however, elongate almost linearly, whereas coleoptiles on the clinostat bend either away or towards the caryopsis depending on the time of rotation.

In addition to microgravity, conditions of macrogravity (Hypergravity) i.e. at more than +1g are also useful for examination of the role of gravity on plant growth. It has also been reported that hypergravity affects germination, cell differentiation and height of fruiting bodies in the cellular slime mold Dictyostelium discoideum (Kawasaki et al, 1990). Cells of Melilotus alba L root under fast clinorotation (55 rpm) show an accumulation of a disorganized ER network at the proximal and distal poles and a random distribution of the amyloplasts (Hilaire et al, 1995). Ottaki (1963) reported that the developmental axis of a fern, Pteris vittata, is modified by centrifugal force.

Much have been reported about the effect of gravity pull on the curvature of plant organs or on differential growth rates on either side of plant organs. But so far no report is available on the effect of gravitational pull on
initiation of adventitious roots on cultured stem cuttings and their (roots) subsequent growth. However, credit goes to Boissya and Das (1995) for their remarkable pioneering work on the particular aspect of the complex problem. They have not only decided the effect of gravitational pull on root initiation and their growth on stem cuttings but also have thrown new light on the mechanism by which the pull mediate the rooting process and root growth on cuttings. They have gone further in establishing the fact that the angles of inclination of the cultured *Coleus blumei* cuttings have a prominent role in mediating certain physiological processes with concomitant effect of root (adventitious) initiation and their growth.

Therefore, the objective of this research is for furthering their research in more detail by going a step further with exposure of the stem cuttings of *Coleus blumei* L. plants to clinorotations (micro and macro) at various planes of inclination viz., 90°, 45°, and 0° (or 180°) to the surface of the earth. It is expected that some new lights about the media or susceptor through which the gravitational pull is translated into physiological activities under the changed circumstances of rotation at various planes which, however, is very unusual under normal pull of gravitation will emerge.