Chapter XII

GENERAL DISCUSSION
Growth may be regarded as an irreversible increase in size, mass or volume on account of conversion of relatively simple inorganic substances (water, carbon dioxide and minerals) into increasing quantities of proteins, carbohydrates and to lesser extent fats. The fundamental components constitute multiplication of cells by mitosis and enlargement of individual cell by vacuolation.

Dwarfness and tallness are the two contradictory aspects of quantitative growth. The basic machinery of growth lies in the cell (the fundamental unit of structure and function). The cell grows either by division or by elongation and this fact ultimately affects the height of the plant. The subapical meristematic region (the primary elongating meristem) is the major site of cell multiplication and cell extension, contributing to stem elongation and thus ultimately to plant height. But as great number of factors affecting the activity of this region, precludes a general and simple explanation for control of stem elongation. Dwarfism is however, a natural phenomenon occurring among plants and it is generally under the genetic control. A complex metabolism is governed by specific genes and which control the cell activity. Moreover, growth being a multiplicative system, is the net expression of several interacting biological systems and may be correlated with the
rates of different metabolic reactions triggered by hormones and/or substrates.

Upon educing genetic dwarfs to behave as normal plants by application of gibberellic acid (GA), Brian and Hemming (1958) propounded that dwarfism in plant is hormonally regulated. Phinney, (1961) expanded this idea by proposing that dwarfism in Corn (Zea mays) is controlled by non-allelic genes, each of which governs a different step in gibberellin biosynthesis. On contrary to this hypothesis, dwarf forms that respond less than the normal plants have been reported in wheat (Radley, 1970), peach (Wylie and Ryugo, 1971), rice (Harada and Vergara, 1972) and other plants (see Pelton, 1964). Further, it has been insinuated that expression of dwarfism may not be explained on the basis of the differences in the levels of either endogenous GA (Jones and Lang, 1968) and/or endogenous inhibitors (Goto, 1978; Wylie and Ryugo, 1971). However, the spectra of overlap in the physiological activities of both GA and IAA, influences mitotic activity and cell extension and this led many workers to allude that there is an interaction between these two phytohormones or that GA may affect the metabolism of IAA. The interaction of GA and IAA has been studied in many excised (Brian and Hemming, 1961; Paleg, 1965; Ockerse and Galston, 1967; Kazama and Katsumi, 1974) and intact plants (Grunwald and Lockard, 1973), and a marked synergistic response has been observed (Brain, 1966; Zeroni and Hall, 1980). Therefore, it has been advocated that GA action must precede auxin action.
Although the mechanism of this synergism has not been elucidated fully, yet the available evidences hitherto indicate that GA may: (1) directly influence the biosynthesis of IAA, (ii) inhibit peroxidase system and thereby increase auxin, (iii) lead to the production of an inhibitor(s) which retards the auxin destroying system and thereby permits sparing of IAA.

The present study is an attempt to evaluate: the validity of all the above cited hypotheses viz., IAA oxidising system (IAA oxidase and peroxidase), IAA protecting system (phenol complex) and endogenous levels of GA and IAA vis-a-vis saturation kinetics of nitrate uptake, nitrate reductase, amylase, ESR spectra of seed for Mn^{2+} content (free radicals), SEM of mature embryo and seed coat, and effects of pretreatments and foliar sprays on growth, development and yield in relation to dwarfism in wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.). Three varieties each of wheat (viz., cvs., WL-1562, dwarf; PNC-1, medium dwarf mutant and C-306, tall) and maize (viz., cvs., J-202, dwarf mutant; Vijay, medium tall and African, tall) varying in plant height at maturity were selected for study. The studies were confined to, the seedling and were carried out at 24 hourly intervals upto 96 hours after germination as well as internodes of fully field grown plants (i.e. at earing and tasselling stages in wheat and maize respectively). The results of these experiments have been already discussed separately in preceding chapters.
and here in this chapter, general discussion and conclusions are included.

Data on growth analysis (vide, Chapter III) displayed that the final plant height in wheat and maize, is a product of the number of internodes and the length of each internode. The dwarf cultivars had shorter (wheat and maize) and fewer (maize) internodes as compared to the tall ones. The growth behaviour of young seedlings also showed clear differences between tall and dwarf cultivars, the coleoptile length being correlative with final plant height at maturity, in both wheat and maize cultivars. Similar correlations in wheat, sorghum and bajra have been reported by Allan and Vogel (1964), Vaishnav (1978) and Krishnan (1982) respectively.

Fresh and dry weight measurements in different organs of the seedling viz., root shoot and endosperm, revealed higher water uptake in tall cultivars. The decrease in dry weight of endosperm and its inverse correlation with plant height at maturity coupled with direct correlation of amylase activity (Chapter VII) points out the fact the mobilization of food material (a function of GA) is at a faster rate in tall cultivars of both wheat and maize than in dwarf ones. In starchy seeds (like of maize and wheat) reducing sugars are important substrates for respiration, osmoregulation and cell wall synthesis. Thus low rate of starch hydrolysis in the dwarf cultivars may become a limiting factor for all these processes and hence for growth itself in wheat and maize.
IAA oxidising system viz., IAA oxidase and peroxidase (both cytoplasmic and ionically wall bound) in shoot, (Laboratory Experiments - Chapter IV) recorded a significant inverse correlation with plant height at maturity. Besides this, monophenols (Chapter V) which are usually co-factors of IAA oxidase also showed an inverse correlation whereas o-diphenols (Chapter V), generally acting as auxin protectors, recorded a direct relationship with plant height at maturity, indicating that oxidation of IAA proceeds at faster rate in the dwarf cultivars than in the tall ones. In field grown plants of both wheat and maize, the distribution pattern of IAA oxidising system and phenol complex did not explicit any clear cut correlation with dwarfism. This is probably because of the fact that in the internodes of different cultivars there are no well marked regions of cell division, elongation and maturation. Often, the regions in which cell elongation is most rapid were those in which cell division was also maximal, and cell division and elongation proceeded in the midst of fully differentiated cells. Furthermore the different internodes in the same plant and in different cultivars attained different developmental stages at different times which brought about a significant alternation in different enzymes and metabolites, thus making the comparison more intricate. However, in laboratory experiments where young seedlings were employed, some of these difficulties were not encountered and hence a number of correlations became evident.
In present investigation, levels of ionically wall bound IAA oxidase, peroxidase and o-diphenol oxidase, activities recorded an inverse relationship with plant height at maturity. Gardiner and Cleland (1974) propounded that wall peroxidases may play a causative role in cessation of elongation growth. However, these workers have further emphasized that cell elongation may not be under the control of a single factor but may be influenced by a series of factors including peroxidase induced auxin destruction, lignification and 'extension' induced wall stiffening.

Phenols are reported to be potentially important in determining the physical properties of cell walls and their occurrence in primary (growing) walls of monocotyledons and dicotyledons. At the very outset lignin is a well known phenolic component of secondary (non growing) cell walls, where it replaces the water and prevents further growth (Northcote 1972). However, one of the major phenols identified in both primary and secondary cell walls of grasses is ferulic acid (Harris and Hartley, 1976) which is probably ester linked to wall polymers (Whitmore, 1974). Peroxidase catalyzed oxidative coupling reactions result in binding of phenol-bearing polysaccharides together within the cell wall, perhaps thereby restricting the growth. Fry (1979, 1980, 1982) proposed and gave evidences to show that phenolic units within the primary cell wall were subjected to oxidation by extracellular peroxidase to yield hydrophobic and cross-linked derivatives that would lower the wall
extensibility. In present study, high levels of wall peroxidase and o-diphenol oxidase in the shoots of dwarf cultivars of wheat and maize, support this hypothesis and possibly reflect a causal relationship. The activity of wall IAA oxidase was also more in shoots of dwarf cultivars probably indicating that IAA and peroxidase activities are associated with a single enzyme protein.

A perusal of data on saturation kinetics of nitrate uptake in different cultivars reveals the differences in carrier system; a different amount of carrier appearing as different V_{max} and a changed affinity for the substrate(NO\textsubscript{3}\textsuperscript{-}) as K_{m} value (Doddema and Telkamp, 1979). In present investigation, V_{max} for nitrate uptake showed an inverse correlation in wheat, indicating clearly that the rate of nitrate uptake is significantly more in dwarf cultivars than tall one(s). However, in maize cultivars employed herein, V_{max} for nitrate uptake was vice versa to that of wheat cultivars further the activity of in vivo nitrate reductase (NR) - a substrate inducible enzyme, displayed an inverse relationship in both wheat and maize. It is now well recognized that compartmentalization exists in most plant tissues (Ferrari et al., 1973; Aslam and Oaks, 1975; Jackson, 1978, Oak and Gadal 1980) and that the storage pool (presumably in vacuoles) cannot readily supply nitrate to metabolic pool (presumably in cytoplasm) either for a sustained deposition of nitrate in the xylem (Jackson, 1978). However, there is hardly any information available which
explains how nitrate movement is regulated from the storage locations (source) to metabolic pools (sink). Jackson (1978) emphasized that for an efficient usage of fertilizer nitrogen, it is imperative to understand how the transport into and out of the storage pool is regulated. The low NR activity in tall cultivars of wheat and maize (even despite a high rate of NO$_3^-$ uptake in maize) recorded in present investigation, indicates that there may be an accumulation of nitrate in these plants, while, dwarf cultivars with higher levels of NR activity reduce nitrate more efficiently and hence probably have a smaller storage pool.

On the basis of $K_m$ values, the cultivars under investigation of wheat and maize, are grouped into three categories i.e. cultivars with low, medium and high $K_m$ values. Working on a number of annual range grasses, Lancaster (1976) showed that those grass species which have more affinity for nitrate (i.e. low $K_m$) were more efficient in absorbing nitrate from low concentrations in natural conditions and were found in less fertile soils (low nitrate levels), while species with high $K_m$ for nitrate uptake were less efficient in acquiring nitrate in natural environment and generally required a relatively higher fertility to compete with a mix of other grasses. Herein the present study, no significant correlation between $K_m$ values and dwarfism could be established. However, these studies suggest that such a short-term kinetic analysis of nitrate uptake have validity and may be useful in describing plant responses to nitrate
under natural conditions, hence may prove to be of immense practical value in breeding programmes.

No qualitative differences in the isoenzyme patterns of IAA oxidase, peroxidase, o-diphenol oxidase and amylase were detected in tall or dwarf cultivars suggesting thereby similarity in genetic make up of tall and dwarf cultivars and the difference among these cultivars is only of quantitative nature. However, isoenzymes did display differences with ontogeny and tissue specificity.

Effect of GA$_3$ on seedling growth in terms of percent over control revealed that all the varieties of wheat (except that of triple dwarf cv.WL-1562) and maize exhibited promotion in root and shoot growth. The cultivar WL-1562 did not respond at all to applied GA$_3$ rather it displayed an inhibition in its root and shoot growth. Mutant dwarfs of both wheat (cv.PNC-1) and maize (cv.J-202) responded more to applied GA$_3$ than their normal counterparts (viz. cvs.C-306 and Vijay respectively). GA-insensitivity likewise, was also reported by Gale and Marshall (1973b) while working with Norin-10 wheat. Endogenous levels of GA-like substances were more in wheat cultivar WL-1562 (dwarf) than that of C-306 (Tall) and PNC-1 (medium dwarf mutant). However, in maize more levels of endogenous GA-like substances were observed in cv. Vijay (medium tall) than cv.J-202 (dwarf mutant). Endogenous IAA-like substances were overall more in tall cultivars than dwarf ones in both wheat and maize.
The levels of endogenous GA content in plants play a determining role for responding plant height after GA3 application. The reason that triple dwarf strain WL-1562 which normally do not respond to exogenous GA3 is attributed to the presence of a high level of endogenous GA content (Radley 1970; Singh et al., 1978) This tendency of the dwarf strains to exogenous GA3 suggests that these strains were impaired either at normal GA production or at its proper utilization. It was therefore, argued that semidwarf and dwarf strains responding to GA3 application probably had a block in GA production, while the non-responsive ones had a block in GA utilization (Gale and law, 1973). The depressed root and shoot growth in cv.WL-1562 due to exogenous GA3 application in present study revealed a block at GA synthesis as well as its utilization as length of the control seedlings was more than the treated ones.

As regards Mn\textsuperscript{2+} content (ESR-studies) in dry seeds of different cultivars of wheat and maize, it becomes clear that tall cultivars had more Mn\textsuperscript{2+} content in their seeds than dwarf ones. This difference was however more pronounced in wheat cultivars under study. Furthermore, it is interesting to note in present investigation that though tall cultivars possessed more Mn\textsuperscript{2+} in their seeds yet mobilization of Mn\textsuperscript{2+} content to embryo-axis was more in dwarfs than tall ones. The universal appearance of manganese - a trace element, in biological systems suggests its significance in living organisms. Various roles have been ascribed to manganese
viz., binding to sugar phosphate backbone of DNA (Eichhorn and Shin 1968); Mn$^{2+}$ dependant synthesis of RNA by chromatin bound RNA polymerase (Duda and Cherry, 1971); its involvement in the synthesis of chlorophyll and in the maintenance of its structural integrity (Khamara 1973); its participation in biosynthesis (Ogura et al., 1972) and oxidation (Graebe et al., 1974) of gibberellins; and above all its implication as a co-factor in IAA oxidising systems. (Morgan et al., 1966 and Taylor et al., 1968). High levels of manganese have also been shown to increase activity of IAA oxidase (Stonier et al., 1968 and Morgan et al., 1976). It is therefore, ascertained that the oxidation of IAA in plant is regulated at two levels: the enzymatic and manganese level. In tall cultivars, because of their ability to accumulate more of Mn$^{2+}$ in their seeds, it may not be a limiting factor while in dwarfs, it may be a limiting factor. As the element (Mn$^{2+}$) is involved in oxidation of IAA and the tall cultivars possess higher manganese content in present investigation, one may lead to believe the concept of Meudt (1967, 1972) that the oxidative transformation of IAA activates IAA for its physiological response. But this is not the case with the present results (Chapter IV) which showed less IAA oxidase activity in tall cultivars and in addition to this, a strong auxin protecting system in them due to the presence of more diphenols, further checks the oxidation of IAA. Moreover, inverse correlation of Mn$^{2+}$ content in embryo-axis with plant height at maturity indicates thereby the possibility of high IAA oxidase activity in dwarf shoots.
which further corroborates the findings in present investigation that oxidation of IAA proceeds at faster rate in shoots of dwarfs than tall ones. This is still further supported by the fact that tall cultivars possessed more endogenous levels of IAA-like substances as compared to dwarf ones (see Chapters IV, V and VI).

A close scrutiny of the scanning microphotographs of wheat and maize embryos revealed that dwarf cultivars under study, possess embryos small in size than tall ones. However, differences in shapes of embryos are less pronounced and basic patterns are also similar. In tall wheat cultivar (viz. C-306) coleoptilar slit has remain compact while it is broad and ensheathing in dwarf cultivar (WL-1562). It is interesting to note in the present investigation that dwarf genes are operative during development of the seed and thus owing to their genetic control, the differences in size of the embryos are observed. Cytological investigations at cellular levels, revealed that less cell division, reduced rate of cell elongation and change in differentiation - may be operative either singly or collectively, may account for cellular basis of dwarfism (Enright and Cumbie, 1973). However, it is a matter of interest that dwarf genes were not seen operative herein present study at initial stages (viz., 24 and 48 hours) of germination. Hence, more coleoptile growth was observed in dwarf cultivar (WL-1562) of wheat (see, Chapter I). Scanning microphotographs of seed coats of wheat and maize displayed cellular differences in finer seed
coat structures between tall and dwarf cultivars. In dwarf wheat (cv.WL-1562), cellular pattern of seed coat, is composed of long turgid cells with stout cell walls, indicating thereby the maximum storage of food material in its seed. This criterion is also in conformity with the heavier grains of this dwarf wheat cultivar (viz. WL-1562) than the tall cv.C-306 (chapter XI). The differences between seed surface structure of dwarf mutants and their tall versions are less pronounced and although basic patterns are similar, yet they differ in size of their cells. Banerjee et al. (1979) had also observed distinct interspecific differences in *Melilotus* and *Ipomea* spp., but the intraspecific variations were insignificant. These studies, however, confirm the genetic control on seed coat pattern in wheat and maize thereby suggesting that finer seed coat structures are stable and could be used as important clues for identification of seeds as well as for ascertaining the parentage of tall and dwarf cultivars.

Pretreatments of seeds with growth regulators are well known to stimulate the germination, enhance vegetative growth and finally result in increased yields. (Henckel, 1964; Saxena 1974, 1979, 1983). A perusal of growth data (Chapter XI) of wheat and maize under field conditions clearly reveals that plant height, dry weights of root, shoot, leaf and reproductive parts increased as the growth advanced and were more in plants raised from pretreated seeds with GA₃, KIN, IAA and CCC than DW- and untreated controls. GA₃ treatment
utterly failed to increase plant height in cv.WL-1562 while CCC (used in higher concentration) could reduce the plant height in all the cultivars of wheat, though more substantially only in cv.C-306 (tall). Enhanced stem elongation with GA₃ and KIN pretreatments can be attributed to the probable increase in the levels of endogenous growth substances and slower growth registered in control, can be ascribed to lower levels of endogenous growth substances. In wheat, tiller number increased with GA₃ and CCC pretreatments in cvs.C-306, PNC-1 and to some extent in cv.WL-1562. It was noted that GA₃ in wheat delayed tillering and improved tiller survival (especially in cv.C-306). Singh et al., (1972); Saini et al., (1975) and Gupta (1978) also reported similar results. It is interesting to note that although tall wheat cv.C-306 produced more tillers than dwarf cv.WL-1562, yet effective tillers (ear bearing) were more in cv.WL-1562. The ability to maintain better tiller survival therefore, seems to be related to the synthesis/presence of relatively larger quantities of GA-like substances in the apices during early stages of plant growth. Since cultivar WL-1562 had relatively more amount of GA-like substances than cv.C-306 (see Chapter VIII), it would seem that tiller survival and endogenous GA are probably linked in some manner. Further, it was noticed that pretreatment of seeds with GA₃ and KIN have increased RGR, NAR and LWR effectively in plants raised from them in both wheat and maize. Thus higher rates photosynthesis in plant raised from pretreated seeds resulted
in higher NAR and RGR. Krishnamoorthy (1973) propounded that though final yield in *Sorghum* is the result of complementary action of many physiological growth components, but main factors seem to be photosynthetic leaf area exposed and photosynthetic efficiency or NAR. Inferences drawn from growth data in present study are in close agreement with the results obtained by Prasad (1983); Ray *et al.* (1983); Pakeeraiah (1985) and Mehra (1990) suggesting the beneficial effects of pretreatments as indicated by increased dry weights of root, shoot and leaf, which ultimately represent higher NAR and RGR. Pretreatments therefore, appear to accelerate metabolic activities resulting in production of more plastic materials which are mobilized to apical region at faster rates.

Foliar spray with growth regulators (viz., GA3, KIN, IAA and CCC) at anthesis stage did not show any marked effect on plant height, tiller number (only in wheat), dry matter production of root, shoot and leaf. Influence of growth regulators sprayed at anthesis stage, may have its own limitation(s) while expressing their action - as major vegetative growth thitherto, is over. However, foliar sprays are seen potent in altering reproductive growth, to produce heavier grains than controls. Foliar sprays significantly increased main spike length, main- and extra-spike weights, grain number and grain weight of main spike, grain weight/plant and 1000-grain weight in wheat. However in maize, cob length, cob weight, grain weight/plant and 100-
grain weight, were also increased significantly. Belluci et al., (1982) also reported increased pod number and seed number per plant in *vicia faba* by spraying GA3. In her seem, foliar spray at anthesis stage with GA3 and Ascorbic acid in combination, proved better than pretreatment (Saxena, 1979). Narula (1981) showed that foliar spray at anthesis with KIN is quite beneficial with respect to yield attributes and 1000-grain weight in mung and wheat. Findings in present study corroborate the results obtained by Prasad (1983) in wheat with foliar spray of CCC and GA3 at anthesis stage.

The beneficial effects of pretreatments do not cease upto vegetative growth only, rather they are maintained throughout the life cycle of the plant as evidenced from the harvest data (Chapter XI). Growth and yield attributes viz., plant height, dry weights of root, stem, leaf and plant, in both wheat and maize, while tiller number, main spike length, spike number, main- and extra-spike weights, grain number and grain weight of main spike, grain weight/ plant and 1000-grain weight in case of wheat and cob length, cob weight, grain weight/plant and 100-grain weight in case of maize, increased significantly in plants raised from pretreated seeds as compared to DW and untreated controls. In present investigation, IAA pretreatment as well as foliar spray could hold no good at par with GA3 KIN and CCC. Pretreatments however, were superior over foliar sprays. Dwarf wheat (cv.WL-1562) produced heavier grains than tall (cv.C-306) though similar difference was not observed in
maize cultivars under study. Inferences drawn from the present investigation on pretreatments and foliar sprays, by and large, uphold the findings of the previous workers (Henkel 1964; Chinoy, 1970; Saxena 1979; Sharma and Shah, 1981; Prasad, 1983; Pakeeraiah, 1985; Saxena et al., 1985; Pandey, 1987; Malhotra, 1990 and Mehra, 1990), who after reporting positive indications of prospective uses of growth regulators in increasing plant productivity through pretreatments or foliar sprays, suggested thereof, that presowing treatments exhibit beneficial influences on different developmental and physiological processes, which in turn, lead to increased yields. Furthermore, enhanced source output during grain filling achieved by virtue of pretreatments or foliar sprays with appropriate growth regulators under optimal cultural conditions (viz., fertilization and irrigation etc.) only, determined the final increase in the grain yield.