CHAPTER XIII

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Dwarf varieties of several crops outyield their tall counterparts. It is well recognized that over-vegetativeness, such as tillering in cereals, may act to the detriment of grain production unless the environmental conditions are conducive for the utilization of all the growth for grain production. On the other hand, if the developmental process of a plant is accelerated beyond a certain limit, growth may be reduced to such an extent that the yield may suffer. The plant height is controlled by a set of dwarfing genes \( d \) recessive to normal \( D \). The manifestation of dwarf genes in comparison with their normal counterparts, is expressed clearly at organ level. In wheat, the dwarfness is governed by three recessive Norin genes, and accordingly there are, single-, double-, and triple-gene dwarf varieties. In maize, there are about a dozen of dwarf mutants. In certain other crops, growth is controlled by one or two inhibitory factors. In still others, complementary factors appear to control plant height. Besides, even within the dwarf varieties there are differences in their degree of dwarfness. Therefore, it is important to know the effects and interactions of these genes on physiological and developmental mechanism(s). Moreover, any alternation in plant frame changes the physiology, photosynthetic efficiency, nutrient uptake and finally the harvest index. Although dwarf cultivars are being extensively used yet the
present status of research in this area is not adequate for explaining the mechanism(s) of dwarfism in a satisfactory manner. However, 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. Hence, in present investigation, a concerted attempt was made to delineate the processes responsible for growth during, early Juvenile phase as well as early flowering stage (i.e., when plant tolls its maximum height) and their relationship with dwarf growth, so as to obtain an integrated picture of dwarfism in wheat (Triticum aestivum L.) and maize (Zea mays L.).

Three varieties 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 their plant height at maturity, were selected for study. Various biochemical studies were confined to: root, shoot and endosperm in case of seedling were carried out at 24 hourly, intervals upto 96 hours after germination (Laboratory Experiments), as well as internodes in case of fully grown field plants - at earing in wheat and tasselling in maize (Field Experiments).

Following experiments were undertaken:

1. GROWTH PATTERNS OF DWARF AND TALL CULTIVARS IN RELATION TO DWARFISM (CHAPTER-III)

2. IAA OXIDISING SYSTEM IN RELATION TO DWARFISH (CHAPTER-IV)
3. PHENOL COMPLEX IN RELATION TO DWARFISM (CHAPTER-V)
4. KINETICS OF NITRATE UPTAKE AND NITRATE REDUCTASE IN RELATION TO DWARFISM (CHAPTER-VI)
5. AMYLASE ACTIVITY IN RELATION TO DWARFISM (CHAPTER-VII)
6. EFFECT OF GIBBERELLIC ACID (GA₃) ON SEEDLING GROWTH AND ENDOGENOUS LEVELS OF GROWTH HORMONES IN RELATION TO DWARFISM (CHAPTER-VIII)
7. ELECTRON SPIN RESONANCE (ESR) STUDIES ON MANGANESE (Mn²⁺) CONTENT IN DRY SEED, EMBRYO AXIS AND ENDOSPERM IN RELATION TO DWARFISM (CHAPTER-IX)
8. SCANNING ELECTRON MICROSCOPY (SEM) OF MATURE EMBRYO AND SEED COAT IN RELATION TO DWARFISM (CHAPTER-X)
9. EFFECT OF PRETREATMENTS AND FOLIAR SPRAYS ON GROWTH, DEVELOPMENT AND YIELD IN RELATION TO DWARFISM (CHAPTER-XI)

The results of these experiments are already discussed (in details) separately in various chapters (vide ante: III to XI). The differential growth rates of internodes of the same plant and of different cultivars precluded mainly any correlation between the above mentioned parameters and dwarfism in plant(s). Therefore, to nullify the effects of these variables and intricacies in explanations, the results of another set of experiments (Laboratory Experiments) - where young seedlings were used and some of these difficulties were not encountered and thus a number of correlations became evident, are mainly stressed. These findings are summarised and general conclusions are drawn as follows:
GROWTH PATTERN STUDIES (CHAPTER-III)

a) Laboratory Experiments

Growth in terms of seedling length, fresh and dry weights of root, shoot and endosperm of 25 uniform seedlings grown in Hoagland media, was measured at 24, 48, 72 and 96 hours after germination.

Seedling length, fresh and dry weights of root and shoot showed positive correlations with plant height at maturity. However, these correlations became more pronounced at latter stages of growth (viz., at 72 and 96 hours). Endosperm exhibited a decrease in dry weight with the advancement of growth and its inverse correlation with plant height at maturity in both wheat and maize, points out to the fact that the mobilization of reserve food materials is at faster rate in tall cultivars than dwarf ones, since endosperm is the sole source of food materials during initial stages of growth.

b) Field Experiments

The heights of ten plants for each cultivar of wheat and maize, grown in the field following standard agronomic practices, were measured at 20 days intervals till maturity. Mature plants were harvested and length of each internode and number of internodes for each cultivar were recorded.

Under field conditions, tall cultivars have greater internodal length (wheat and maize) and higher number of internodes (maize), indicating thereby, that the final height
of the plant is the product of these two parameters. Furthermore, the difference in final plant height became more apparent at flowering stage.

IAA OXIDISING SYSTEM (CHAPTER IV)

a) Laboratory Experiments

Cytoplasmic as well as ionically wallbound IAA oxidase and peroxidase activities in shoot recorded significant inverse correlation with plant height at maturity thus, indicating that oxidation of IAA proceeds at faster rate in shoots of dwarf cultivars than the tall ones both in wheat and maize. Cytoplasmic isoenzymes of IAA oxidase and peroxidase showed marked changes with ontogeny and tissue specificity. In general, isoperoxidases in dwarf cultivars appeared at early stages of germination than tall ones. However, no significant relationship between isoenzyme patterns (IAA oxidase and peroxidase) and dwarfism was noticed. Isoenzymes of cytoplasmic IAA oxidase and peroxidase appeared at similar Rf values probably indicating that both the activities are associated with a single enzyme protein.

b) Field Experiments

Cytoplasmic IAA oxidase activity in general exhibited decrease with the increase in internodal length. However, wall bound IAA oxidase activity versus internode number showed maximum in the apical. The activity exhibited a direct relationship with internodal length. Cytoplasmic
 Peroxidase activity in different internodes of wheat and maize was lowest in the apex and it increased basipetally. The activity did not show any general correlation which may be due to differences in developmental and ageing rates of different cultivars.

The distribution of ionically wall bound peroxidase showed an acropetal pattern in all the cultivars under study i.e. the activity was minimum in the apex and maximum towards the base. The activity in general revealed an increasing trend with an increase in the internodal length. Cytoplasmic isoenzymes of IAA oxidase were more in number in the basal internodes. Taller cultivars normally have lesser degree of polymorphism in IAA oxidase as compared to dwarf ones. Isoenzymes of peroxidase with higher mobility exhibited significant differences. However, no relationship between isoenzymes of peroxidase and dwarfism was evident.

PHENOL COMPLEX (CHAPTER V)

a) Laboratory Experiments

Total phenolic content in shoot recorded an inverse relationship with plant height at maturity. Further monophenols (which are usually co-factor of IAA oxidase), showed an inverse relationship while o-diphenols (generally acting as auxin protectors) in shoot recorded a direct correlation with plant height at maturity, pointing out the fact that tall cultivars possess a strong auxin protecting system(s). O-Diphenol oxidase activity (cytoplasmic and wall bound) in shoot exhibited an inverse relationship with plant
height at maturity. Thus high levels of wall bound o-diphenol oxidase and peroxidase in shoots of dwarfs possibly may be playing causative role in cessation of elongation of growth. Isoenzyme of o-diphenol oxidase did not discern any relationship with dwarfism. However, isoenzymes did show changes with ontogeny and tissue specificity.

b) **Field Experiments**

The distribution pattern showed that total-, mono-, di-phenols were lowest in the basal internode and increased towards apex in all the cultivars of wheat and maize. Phenolic content (Total, mono- and o-diphenols) versus internodal length showed highest content in young internode and with the elongation of internode, a sharp decrease was observed. **Cytoplasmic o-diphenol oxidase** did not reveal any definite trend in terms of distribution pattern and internodal length. However, **wall bound o-diphenol oxidase** was maximum in basal internode and a progressive decrease in its activity was recorded towards apex. The activity versus internodal length exhibited an increasing trend with the elongation of internode. Highest activity was recorded in fully elongated mature internode. It was observed that dwarf cultivars in general, have slightly more isoenzymes of o-diphenol oxidase than tall ones.

**KINETICS OF NITRATE UPTAKE AND NITRATE REDUCTASE (CHAPTER VI)**

a) **Laboratory Experiments**

The kinetics of nitrate uptake during early seedling
stage indicated that $V_{\text{max}}$ (a quantitative parameter of nitrate uptake) was highest in dwarf cultivar (viz., WL-1562) which decreased with increasing tallness in wheat. However, this difference was not substantial in maize cultivars under study. Further $K_m$ value did not reveal relationship with plant height at maturity. The activity of \textit{in vivo} nitrate reductase (NR) in root as well as shoot exhibited an inverse relationship with plant height at maturity.

b) \textbf{Field Experiments}

Changes in \textit{in vivo} NR-activity of different internodes revealed that lower internodes have low activity of NR and it increased towards apex. Maximum activity was recorded in young internodes and during elongation marked decrease in NR activity was discernible. However, a direct positive relationship was noticed between the integrated values of NR activity and soluble proteins.

\textbf{AMYLASE ACTIVITY (CHAPTER VII)}

a) \textbf{Laboratory Experiments}

Amylase activity in endosperm (both cytoplasmic and wall bound) exhibited a direct significant relationship with plant height at maturity, thereby indicating that mobilization of the reserve materials from endosperm to growing organs is faster in the tall cultivars than dwarf ones. Furthermore, in starchy seeds (like of maize and wheat), where reducing sugars are important substrates for respiration, osmoregulation and cell wall synthesis, low rate of starch hydrolysis in endosperm(s) of dwarf cultivars may
become a limiting factor for all these processes and hence for growth. Endosperm had the most intense bands of isoenzymes of amylase, indicating clearly that the activity was maximum in this organ. Further ontogenic changes and organ specificity were evident. However, no qualitative differences in the isoenzyme patterns of amylase (and even of IAA oxidase, peroxidase and o-diphenol oxidase) were detected in tall and dwarf cultivars thus suggesting thereby similarity in genetic makeup of tall and dwarf cultivars and the difference among these cultivars is of quantitative nature.

b) Field Experiments

The distribution of amylase activity showed an increase in activity towards apex. The activity plotted against internode length recorded an increasing trend with increase in length of the internode. Highest activity was recorded in apical internode. In all, more amylase activity was observed in the internodes of tall cultivars of wheat and maize than dwarf ones.

EFFECT OF GA₃ ON SEEDLING GROWTH AND ENDOGENOUS HORMONES (CHAPTER VIII)

GA₃(10⁻⁵M) prepared in Hoagland medium (half concentration) was applied to uniformly germinated seedlings of both wheat and maize. Growth in terms of length, of root and shoot was measured at 48 hours after germination. Percent increase in length of root and shoot over control was
calculated. As regards endogenous hormones, GA- and IAA-like substances were estimated using laboratory grown 96 hours old seedlings (after germination), of both wheat and maize cultivars.

Effect of GA₃ on seedling growth was inhibitory in dwarf wheat cv.WL-1562 which in turn exhibited high levels of endogenous GA-like substances. All other cultivars of wheat and maize showed promotion in root and shoot growth over controls. Mutant dwarfs of both wheat (cv.PNC-1) and maize (cv.J-202) responded more to GA₃ than their normal counterparts (viz., cvs. C-306 and Vijay respectively). The depressed root and shoot growth in cv.WL-1562 due to exogenous application of GA₃ revealed a block at GA-synthesis as well as its utilization, as length of the control seedlings was more than the treated ones. In wheat, endogenous levels of GA-like substance were more in cv.WL-1562 than that of cvs.C-306 and PNC-1, while in maize, more levels of GA-like substances were observed in cv.Vijay than its mutant dwarf cv.J-202. However, endogenous levels of IAA-like substances were in general, more in tall cultivars (of both wheat and maize) than dwarf ones.

ESR-STUDIES ON MANGANESE (Mn²⁺) CONTENT IN DRY SEED, EMBRYO AXIS AND ENDOSPERM (CHAPTER IX)

Perchloric acid (PCA) extracts of dry seeds; embryo axis and endosperms of 48 hours old seedlings (laboratory grown) were used for recording ESR spectrum on ESR spectrometer (Varian Associate, Model E-4).
Mn$^{2+}$ content in the dry seeds of tall cultivars was more as compared to dwarf ones. However, its inverse correlation in embryo axis (of 48 hours old seedling) indicates that its mobilization to growing embryo axis from endosperm was more in dwarf cultivars than tall ones, thus suggesting thereby, the possibility of high IAA oxidase activity (for which it acts as a co-factor) in dwarf shoot(s). This is however, in conformity with the present results (vide ante: IAA oxidising system), where IAA oxidase and peroxidase activities in shoot exhibited inverse correlations with plant height at maturity.

SEM-STUDIES ON MATURE EMBRYO AND SEED COAT (CHAPTER X)

Dehydrated materials (embryo and seed coat) coated with 200 Å thick Au-Pd film were scanned and photographed with Cambridge Sterio-Scanner (Model S4-10). The sizes of the various morphological characteristics according to their greater dimensions were taken at different magnifications for better understanding, but comparative evaluations were made at similar magnifications.

SEM-study of mature embryo revealed that dwarf cultivars of wheat and maize under study, possess embryos small in size than tall ones. However, differences in shapes of the embryos are less pronounced and basic patterns are also similar. Scanning microphotographs of seed coats, of both wheat and maize cultivars, displayed cellular differences in finer seed coat structures between tall and
dwarf cultivars. However, the difference between seed surface structures of dwarf mutants and their tall versions are less pronounced and although the basic patterns are similar, yet they differ in size of their cells. It is a matter of interest herein that dwarf genes are operative during development of seed and thus owing to their genetic control, the difference in size of the embryo and patterns of seed coat was observed.

**EFFECT OF PRETREATMENTS AND FOLIAR SPRAYS ON GROWTH DEVELOPMENT AND YIELD UNDER FIELD CONDITIONS (CHAPTER XI)**

Uniform seeds of wheat and maize cultivars were pretreated with suitable concentrations of growth regulators (viz., GA₃⁻, KIN⁻, IAA⁻₁₀⁻⁵ M and CCC⁻₁₀⁻⁴ M) and were sown in the field along with untreated and DW-treated controls following standard agronomic practices (as cited in Chapter II) for watering, manuring and thinning. Various growth parameters like plant height, leaf number, leaf length and breadth, dry weights of root, stem, leaf and reproductive parts (spikes/cobs) were recorded at 20 days intervals. Tiller number in wheat, was also recorded. Untreated plants were sprayed at anthesis stage (earing in wheat and tasselling in maize) with aforementioned growth regulators (viz; GA₃⁻, KIN⁻, IAA⁻₁₀⁻⁵ M and CCC⁻₁₀⁻⁴ M) and harvest data recorded for both pretreatments and foliar sprays, were compared.

Vegetative growth represented by plant height, dry weights of root, shoot and reproductive parts (spike/cob),
leaf area, RGR, NAR, LWR (in both wheat and maize) and tiller number (in wheat only) were higher in plants raised from pretreated seeds under field conditions. In wheat, tiller number increased with GA$_3$ and CCC pretreatments in cvs: C-306; PNC-1 and to some extent WL-1562. Pretreatment with GA$_3$(10$^{-5}$M) utterly failed to increase plant height in cv. WL-1562 while CCC (10$^{-4}$M) could reduce plant height in all the cultivars of wheat, though more substantially only in cv. C-306 (tall). It is interesting to note that although tall wheat cv. C-306 produced more tiller than dwarf cv. WL-1562 yet effective tillers (ear bearing) were more in cv. WL-1562. Since, cv. WL-1562 had relatively more GA-like substances than cv. C-306 (as evidenced in Chapter VIII), it would seem that tiller survival and endogenous GA (in shoot) are probably linked in some manner.

Foliar sprays with growth regulators (viz., GA$_3$, KIN, IAA and CCC) at anthesis stage did not show any marked effect on plant height, dry matter production of root, shoot and leaf (in both wheat and maize) and tiller number (in wheat only). However, foliar sprays were 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 while in maize, cob length, cob weight, grain weight/plant and 100-grain weight were also increased.

Pretreatments with GA$_3$, KIN, IAA and CCC, were able to
enhance reproductive growth resulting in higher grain yield. Growth and yield attributes like, plant height (except pretreatment of; CCC and GA₃ only in cv.WL-1562), dry weights of root, stem, leaf and plant in both wheat and maize, whereas 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. Pretreatments were however, superior to foliar sprays. Dwarf wheat (cv. WL 1562) produced heavier grains than tall (viz., cv.C-306). Beneficial effects of pretreatments therefore, do not cease upto vegetative growth only, rather they are maintained throughout the life cycle of plant.

Finally studies presented in this thesis help in understanding the complex problem of dwarfism in wheat and maize. Dwarf cultivars - having higher nitrate uptake coupled with higher NR-activity and reduced height to prevent lodging, may prove to be promising in genetic manipulation.
Following important points emerge from the results presented in this thesis:

1. Seedling length, fresh and dry weights of root and shoot showed positive correlations with plant height at maturity. However these correlations became more pronounced at latter stages of seedling growth. Endosperm exhibited a decrease in dry weight with advancement of growth and recorded an inverse correlation with plant height at maturity. The final height of plant under field conditions, is the product of internodal length and internode number. Tall cultivars have greater internodal length (wheat and maize) and higher number of internodes (maize) than dwarf ones. However, the difference in plant height became more apparent at flowering stage.

2. Cytoplasmic as well as ionically wall bound IAA oxidase and peroxidase activities in shoot, displayed significant inverse correlations with plant height at maturity. Isoenzymes of cytoplasmic IAA oxidase and peroxidase appeared at similar Rf values probably indicating that both the activities are associated with a single enzyme protein.

3. Monophenols (which are usually co-factors of IAA oxidase) showed inverse correlation while diphenols (generally acting as auxin protectors) in shoot
recorded a direct relationship with plant height at maturity, pointing out the fact that tall cultivars possess a strong auxin protecting system(s).

4. Ionically wall bound o-diphenol oxidase recorded an inverse relationship in shoot with plant height at maturity. High levels of wall bound o-diphenol oxidase and peroxidase in shoots of dwarfs possibly may be playing causative role in cessation of elongation growth.

5. The kinetics of nitrate uptake during early seedling stage revealed that Vmax (a quantitative parameter of nitrate uptake) was highest in the dwarf cultivar WL-1562 which decreased with increasing tallness in wheat. However, such difference was not seen in maize cultivars. Further Km value did not display any relationship with final plant height. The activity of nitrate reductase (NR) in the internodes of plants (field grown) as well as in the shoots of seedlings (grown under laboratory conditions) of wheat and maize, showed an inverse relationship. However direct positive relationship was discernible between the integrated values of NR-activity and soluble proteins.

6. Amylase activity in endosperm (both cytoplasmic and wall bound) exhibited a direct relationship with plant height at maturity, thereby indicating that the mobilization of the reserve food materials from
endosperm to growing organs is faster in tall cultivars than dwarf ones.

7. Isoenzymes exhibited differences with ontogeny and tissue specificity. No qualitative differences in isoenzyme patterns of IAA oxidase, peroxidase, o-diphenol oxidase and amylase were detected in tall or dwarf cultivars, suggesting thereby a similarity in the genetic make-up of tall and dwarf cultivars and the difference being only of the quantitative nature.

8. Effect of GA₃ on seedling growth, was inhibitory in dwarf wheat (cv. WL-1562), which in turn displayed high levels of endogenous GA-like substances. All other cultivars (of wheat and maize), showed promotion in root and shoot growth over controls. Dwarf mutant of wheat (cv. PNC-1) and maize (cv. J-202) responded more to GA₃ than their tall counterparts (cvs. C-306 and Vijay respectively). Endogenous IAA-like substances in general, were more in tall cultivars of both wheat and maize than dwarf ones.

9. Mn²⁺ content (ESR-studies) in the dry seeds of tall cultivars was more as compared to dwarf ones. However, Mn²⁺ content in the embryo axis (48 hours old seedling) displayed an inverse correlation with plant height at maturity, thus indicating its faster mobilization from endosperm to growing embryo axis in dwarf cultivars and thereby suggesting the possibility of high IAA oxidase
activity (for which it acts as a co-factor) in dwarf shoots.

10. SEM study of mature embryo revealed that dwarf cultivars of wheat and maize, possess embryos smaller in size than tall ones. However, differences in shape of embryos are less pronounced and basic patterns are also similar. Seed coats of wheat and maize displayed cellular differences in finer seed coat structures between tall and dwarf cultivars. However, the differences between seed surface structures of dwarf mutants and their tall versions are less pronounced, and although the basic patterns are similar, yet they differ in size of the cells.

11. Vegetative growth represented by plant height, dry weights of root, shoot and reproductive parts (spike/cob), leaf area, RGR, NAR, LWR (in wheat and maize) and tiller number (in wheat only) were more in plants raised from pretreated seeds under field conditions. Pretreatments as well as foliar sprays (at anthesis stage) with growth regulators (viz. GA₃, KIN, IAA and CCC) increased the final yield. Pretreatments were however superior to foliar sprays. Pretreatment effects do not cease upto vegetative growth only, rather they also greatly influence reproductive phase which ultimately resulted in the increased yields.
As an adjunct to the findings presented above, providing evidences for the metabolic regulation of dwarfism, it can therefore be said in final conclusion that 'phenomenon of dwarfism is controlled by complex and interwoven metabolic mechanisms of cells possessing many interlocked nuclear and cytoplasmic gears. Besides, it can be kept in motion only by a co-ordinated system'. Thus dwarf cultivars which possess higher nitrate uptake coupled with high NR-activity together with reduced lodging, may prove to be promising in genetic manipulations in future.