DISCUSSION

Experimental findings presented in this work have helped in understanding the physiological as well as metabolic status during the life cycle of Cumin plant. Further, in the light of experimental observations, a clear picture of the effect of pretreatments of seeds with temperature, ascorbic acid and H_{2}O_{2} singly as well as in combination upon enzymic and metabolic activities, growth, development and yield has been obtained. The discussion will pertain to the above aspects.

Germination:

In Cumin germination of seeds starts with the imbibition of water and as a result of which moisture content, fresh weight and seedling length increases while dry weight decreases with increase in germination period (Plate 2). This uptake of water by seeds activates the entire metabolism within the seed (Ferry and Ward, 1959; Woodstock and Feeley, 1965; Woodstock and Grabe, 1967).

Ascorbic acid turnover (AA): AA content decreases upto 9 days after which it increases at 12 days (Plate 3.1). Decline in AA content is presumably due to a considerable increase in its
utilization between 6-9 days (Plate 3.2), thus indicating active participation of AA in the germination process. This increased utilization of AA during juvenile differentiation is in conformity with previous findings in case of wheat (Abraham, 1969; Pandya, 1969; Saxena, 1969), groundnut (Saxena, 1969); gram (Saxena, 1969) and sesamum (Shah, 1972; Ghesani, 1972). ASG content shows a fluctuating trend while NAB (AA-MH-complex) content shows an increasing trend during germination (Plates 3.3, 3.4) which may be helpful in release of AA when needed during active cell division and its more and more complexing with macromolecules when not required by the system (Chinoy et al., 1971). This is further supported by the decline in total sugar content during germination and is in flower bud stage (Plate 6.2, 15.3) as AA can be synthesised from the carbonhydrates as shown earlier by Chinoy et al. (1958, 1961). The above mentioned findings suggests that mobilization of food materials is governed by energy provided by the utilization of AA during germination and flower bud stage.

Increase in AA-FR-peroxidase activity (Plate 7.2) which catalyzes the production of the free radical of AA (MDHA) and more production of it with progress in germination will stimulate the growth of embryo axis (Piette et al., 1961; Yamazaki, 1962; Gurevich, 1963, 1968; Chinoy, 1967, 1969; Chinoy et al., 1969, 1969a; Ghesani, 1972). The increase in
AA content and AA utilization in the seedlings of pretreated seeds than those of control is more pronounced in 70°D + AA + H2O2 pretreated seeds (Plate 3.1, 3.2). These observations suggest that the pretreatments of the seeds stimulates AA biosynthesis as well as its utilization. During vegetative shoot apex - flower bud stage, AA content, AA utilization and AA-MM-complex increase (Plates 10.1, 10.3, 11.3) while ASG remains at the same level.

Similar trend is found in the corresponding leaf except ASG content where a sharp decline of ASG content in vegetative shoot apex- flower bud stage is noted (Plate 11.2). This points to the active formation as well as translocation of AA and its utilization to the flower bud stage, which suggests an active role of AA during reproductive differentiation. Thus, by increased utilization, the reductive atmosphere which is necessary for synthetic processes in plants increase and hence the active cell division and cell differentiation take place. This finding is in conformity with previous findings reported by Chinoy (1967), Chinoy et al. (1969, 1970a, 1971), Tsi'bulko (1968), Pandya (1969) and Saxena, (1969).

The AA biosynthesis increases at seed-I stage, also then it declines sharply in seed-II stage (senescence phase). All pretreatments increase AA metabolism but the marked effect was seen in 70°D + AA + H2O2 treatment. In this way there is a faster mobilization of ascorbic acid during ripening period.
as indicated by the data of AA turnover. Presoaking drying treatment of seeds has its effect on post-inductive stages of plant growth by changing the course of AA metabolism. An upsurge in AA and ASG content during the period of reproductive differentiation suggests that AA turnover provides electron energy for physiological processes leading to flowering.

Michlewicz (1961) found that AA content increased during the course of ontogenic development and reached its maximum just before flowering.

Nucleic acid metabolism:

DNA content is maximum at 3 days after which it decreases gradually; reaching a very low level at 12 days (Plate 4.3) which may be due to its breakdown into simpler nucleotides required for the growth of embryo axis. RNA content also decreases with the germination period (Plate 4.2), with an increasing activity at 12 days of germination. Similar degradation of RNA has also been observed during germination by Beevers and Guernsey (1966). During germination RNA content declines sharply, presumably degraded by endogenous enzymes with the component nucleotides being transferred to the developing embryo axis (Cherry et al., 1962). This degradation of RNA is caused by increased activity of the enzyme RNase during germination and this
increased RNAse activity occurs during germination (Ledoux et al., 1962; Grellet et al., 1968).

Gradual rise in RNA content and steep rise in DNA content at the time of the onset of flowering (Plates 12.3, 13.1) is a significant event. Similar trend found in the corresponding leaf points to the active formation as well as translocation of nucleic acids to the apical region.

Hess (1961b) believes that successful floral induction involves a synthesis of a new reproductive ribonucleic acid and of new proteins in the leaf. Results confirm that a marked increase in DNA is associated with active cell division and increased RNA content is associated with cell enlargement (Silberger, 1953; Silberger and Skoog, 1953; Porter, 1953; Naylor et al., 1954; Jablonski and Skoog, 1954). Evans (1959) obtained some promotion of flower formation in a late variety of *Vicia faba* by a mixture of nucleotides.

The pretreatments of the seeds increases biosynthesis of RNA and DNA throughout the whole life cycle and the 700D + AA + H2O2 pretreatment of the seeds shows a highly significant enhancement in biosynthesis of RNA and DNA. Suge and Yamada (1965) have suggested that nucleic acid metabolism plays an important role in thermal induction of flowering. This increase of nucleic acids during growth and differentiation is also correlated positively with protein synthesis (Murakami, 1962).
Proteins: Proteins also play an important role during germination. There is a gradual decrease of proteins and histones with increase in germination period (Plate 5.2, 5.3) synchronizing with increased protease activity except at 12 days (Plate 5.1). Increased protease activity with advance seedling growth during the course of germination is in agreement with the findings of Wiley and Ashton in *Cucurbita maxima* (1967), Josef et al. (1966) in *Phaseolus vulgaris* and Penner and Ashton (1967) is squash cotyledons. Continuous breakdown of proteins is the result of increased protease activity resulting in free amine acids and amides which are then translocated and utilized for the growth of embryo axis. Mulliken et al. (1970) also found in *Abutilon theophrasti* a rapid loss of reserve proteins accompanied by the rapid growth of seedling during germination. As histone content is maximum at 6 days of germination decreasing afterwards during further period, it points to its participation in germination process. Histones take part in the trigger mechanism of germination and in switching over to the genome programme (Nezgovorova and Borisova, 1970). According to Bonner's (1967) concept histones play an important role in the regulation of protein biosynthesis by acting as repressors of DNA synthesis. $^{70}\text{D} + \text{AA} + \text{H}_2\text{O}_2$ treatment brings about an enhancement in the synthesis of proteins.
From vegetative to reproductive differentiation, protein and histone contents increase (Plates 14.3, 16.3). Corresponding leaf follows the same trend (Plates 14.4, 16.4). Here also 70°D + AA + H₂O₂ shows a marked enhancement in the synthesis of proteins. The present findings are similar to the above observations. There is also an increased histone content in reproductive organs and their corresponding leaves of pretreated plants (Saxena, 1969; Shah, 1973).

Sulfhydryl (SH): Sulfhydryl content generally increases with progress of germination (Spragg et al., 1962). During 6 days of germination, -SH content remains at the same level, then it declines at 9-12 days of germination, it remains more or less at the same level (Plate 6.4). It shows a fluctuating trend throughout the juvenile differentiation indicating the relationship between SH compounds and growth. This findings support the earlier observation of Milkowska (1964), who has reported that the acceleration of germination caused an increase in the ratio between SH and SS groups.

Spragg and Yemm (1959) have shown that reduced glutathione is essential for the activation of SH containing enzymes. There are abundant reports showing a relationship between SH compounds and growth (Hammett, 1933, 1933a; Hammett and Hammett, 1933).

In Cumin during reproductive differentiation, SH content increases (Plate 18.3) showing a similar trend with
oxidative enzymes like catalase, peroxidase, AA-PR-peroxidase (Plates 18.1, 17.1, 17.3). Hopkins and Elliott (1931) noticed that glutathione did not function as a hydrogen carrier for the bulk of tissue oxidations but that its function in oxidation-reduction was more specialized.

All data shows the superior effect of $70^\circ$D + AA + H$_2$O$_2$ treatment than any other pretreatment. And the data presented here suggest that in Cumin during transformation from vegetative-flower bud stage, the various enzymic activities increase. Thus, an increase in all enzymic activities during flowering suggests an increase in protein synthesis which can only be brought about by enhanced biosynthesis of nucleic acids. Increased biosynthesis of macromolecules during flowering require a reductive atmosphere which is provided by increased turnover of AA during reproductive differentiation (Chinoy, 1962, 1967, 1969). There is also an increase in SH content during reproductive differentiation. Even the SH content is found more in the reproductive apex as well as different stages of development of plants of $70^\circ$D + AA + H$_2$O$_2$ pretreated seeds compared with that in the other pretreatments and control ones. Levitt (1967) suggested that the increase in SH during hardening is more apparent than real. But the apparent increase in SH on hardening is just as important as, if it is real.

According to the hypothesis of Levitt (1956) for SH-SS, we can explain both SH$\rightarrow$SS conversion during thermal
stress. High SH may be partly due to glutathione (GSH) which can act as the primer for such chain reactions between proteins. Another noteworthy point is that it increased during hardening. Spragg et al. (1962) decreased the SH:SS ratio in proteins of pea seeds and found that germination decreased. Szent Gyorgyi (1928) was the first to suggest that glutathione (GSH) donated hydrogen to dehydroascorbic acid (DHA). Retardation in AA oxidation keeps glutathione in a reduced form thereby accelerating growth. According to Purr (1933), ascorbic acid assumes a protective role against the oxidation of SH compounds in organism.

Carbohydrate metabolism:
Sugar content decreases (total and reducing) with germination period (Plate 6.2, 6.3). The trend was observed in vegetative-flower bud stage and in seed-I, seed-II stage, it increases (Plates 15.3, 16.1). This decline in sugar content shows that AA can be synthesized from the carbohydrates as shown earlier by Chinoy et al. (1958, 1961). Sugar requirement is very high during the period of reproductive differentiation which serves as the basic material for chain reactions leading to transamination, peptidisation as well as linking of these peptides for the formation of macromolecules (Gurumurti et al., 1968). The leaf also shows a parallel trend during various stages in the development of the apical
organs (Plates 15.4, 16.2), indicating an active synthesis as well as translocation of active metabolites as shown earlier. All pretreatments show beneficial promoting effect.

Oxidative enzymes:

Peroxidase: Peroxidase activity increases with advancement in germination period (Plate 7.3). Similar increase in peroxidase activity during germination of sorghum and mung has been found by Gopalachari (1964). Vegetative shoot apex, seed-I stage shows an increasing trend in peroxidase activity. After that it declines. Similar trend was observed in corresponding leaves.

Plants grown from $70^\circ$D + AA + H$_2$O$_2$ pretreated seeds of Cumin show a higher rate of peroxidase activity during the formation of flower bud as well as differentiation of reproductive organs as compared with that in the other pretreatments and control. In the seed-II stage it does not show any increase. This finding supports the previous findings of Srinivason and Rao (1971). They have observed a higher peroxidase activity in the reproductive shoots than in the vegetative shoots of grapevine. Shkol'nik et al. (1961) have shown an increase in peroxidase activity at the time of flowering.

Krishnanmurty (1972) has indicated a 100 fold stimulation of peroxidase activity after 48 hours of germination compared with that of dormant seed. Yang (1969)
suggested the function of peroxidase as the electron oxidizing agent.

Chappet et al. (1970) has correlated peroxidase activity with cell elongation in wheat coleoptile. Galston and Dalberg, (1954); Siegel and Galston (1966) ascribed a significant role to peroxidase in the regulation of cell growth and differentiation. Siegel (1955) has demonstrated a broad hydrogen donor specificity of plant peroxidases. Peroxidase activity has been shown to have a direct correlation with the process of cell differentiation (Lalorsya, 1973). Similar observations have been obtained in case of sesamum by Chinoy et al. (1973).

AA-FR-peroxidase:

AA-FR-peroxidase activity increases continuously with an increase in germination period (Plate 7.2). As free radical of AA is formed due to the activity of this enzyme, and it is a more powerful reducing agent than AA itself, its production with progress in germination enhances the growth of embryo-axis (Piette et al., 1961; Yamazaki, 1962; Gurevich, 1963; Chinoy, 1967, 1969; Chinoy et al., 1969, 1969a). Yamazaki and Piette (1961) have also concluded that a free-radical mechanism is the main pathway involved in AA-oxidase and peroxidase reactions. Increased free radical formation using electron paramagnetic resonance spectrometer has also been reported by Chinoy et al. (1969, 1970a). Thus,
AA-FR-peroxidase activity is actively associated with systems actively growing in a number of crop plants.

The AA-FR-peroxidase activity also increases during floral differentiation as well as in corresponding leaves (Plate 17.3, 17.4). Similar increase has also been reported by Pandya (1969), Saxena (1969), Jaikaria (1971), Shah (1972) and Ghesari (1972). Increase in free radical signal during transformation from vegetative to reproductive stage, when intense cellular division also occurs, has been reported by Chinoy et al. (1969, b, 1970, 1971).

The AA-FR-peroxidase activity is also more during various stages of reproductive differentiation in plants raised from pretreated seeds especially from $70^\circ$D + AA + H$_2$O$_2$ treated seeds have shown extensive effect. Presumably increased AA-FR-peroxidase activity results in increased production of free radicals. Chinoy et al. (1969b, 1971) report that the enhanced concentration of free radicals of AA during the period of reproductive differentiation, supply, enhanced electronic energy for accelerated cell division.

Increased production of free radicals, may release more energy essential for early flowering in the plants of $70^\circ$D + AA + H$_2$O$_2$ followed by all other pretreatments and a higher AA-FR-peroxidase activity clearly suggests that free radicals play an important role in growth.

Catalase: Catalase activity increases and reaches its maximum at 9 days after which it declines (Plate 7.3). An increase
in catalase activity with increase in germination period has also been found by Michniewicz and Stanislawski (1962) and Chinoy et al. (1969). Altman et al. (1966) established a positive correlation between activities of peroxidase and catalase during respiration. This increased catalase and peroxidase activity causes the breakdown of complex food reserves into simple and soluble substances which are then utilized for the growth of embryo-axis.

The catalase activity increases from vegetative shoot apex and fertilized carpel stage then it shows a continuous decline up to senescence (Plate 18.1). In case of corresponding leaves it increases up to seed-I stage then there was a sharp fall in the activity (Plate 18.2). Besides this pretreatments of the seeds enhance significantly catalase activity thereby suggesting that oxidative processes are increased and hence the respiration also increases.

Hydrolytic enzymes:
RNase: RNase activity decreases up to 9 days then it increases in 12 days of germination (Plate 4.1). These findings support the earlier findings of Matsuhita (1959, 1959a) who has found that there is a several fold increase in RNase of the wheat endosperm during germination. Besides this, same result was obtained in barley endosperm by Ledoux et al. (1962) during germination. Srivastava (1964) has also observed that
RNase activity increases during germination, in barley. Generally, the activity of hydrolytic enzymes increases under the influence of drought and high temperature (Sisakyan, 1940).

RNase activity also increases during the flower bud and flower stage (Plate 12.1), otherwise it is very low at the end of the life cycle. The leaf subtending reproductive organ shows the same trend (Plate 12.2) but the most important point is that the enzyme activity is enhanced in the plants of all pretreated seeds, during whole life cycle. Holden and Pirie (1955) have shown that RNase of tobbaco leaf is more thermostable than any enzyme. RNase is fairly resistant to high temperatures. Udvardya et al. (1967, 1969) have pointed out a possibility that RNase can exist in plant tissues in a complexed state, from which it is liberated during conditions unfavourable to the plants. This appears to be a very important speculation, which also confirms present data for RNase activity.

Protease: Protease activity increases up to 9 days of germination then it declines (Plate 5.1). These findings are in conformity with that of Wiley and Ashton (1967) and Donald and Ashton (1967). They found that during the course of germination of **Cucurbita maxima** and squash seeds, the level of proteolytic enzyme activity increased in the cotyledons through the second day and third day respectively.
and then decreased. Rameshwar et al. (1971) have observed that the rapid loss of protease activity coincided with the decreased loss of protein in cotyledon.

The protease activity increases in flower bud stage then it declined in flower stage. It increases gradually throughout the life cycle. Same trend was followed by corresponding leaf. All pretreatments accelerate the activity compared with that in the control.

Invertase: Invertase activity decreases slightly with the advancement of germination. It then remains more or less at the same level (Plate 6.1). Similar results have been obtained by Saxena (1969) in wheat as well as in gram and Gurumurti et al. (1969) in Arachis hypogea. This increase in invertase activity may result in release of reducing sugars that can be used up in further growth of the embryo axis.

Invertase activity also increases during floral differentiation in reproductive organs as well as in the leaf of Cumin (Plate 15.1, 15.2). A marked stimulating effect was seen in $70^\circ D + AA + H_2O_2$ pretreated seeds. This increased activity augments the supply of total and reducing sugars to the cells of plants.

Effect of pretreatments on growth development and yield:

In the present investigation, growth, characters like dry weight of root, stem, leaves and whole plant are
directly correlated with the pretreatments (Plate 23.1, 23.2, 23.3, 23.4) causing an increase in the dry weight of the root, stem leaves and whole plant. Generally, growth is accompanied by changes in the form and physiological activities. Chinoy (1966, 1968) showed that major growth and yield characters of plants, such as rates of photosynthesis and respiration, height, number of leaves fresh and dry weight of stem, leaves and root, number of ears, spikelets and grain, 1000 kernel weight and others are correlated with the length of growth period. The results presented here point to the fact that the growth characters are generally enhanced by different pretreatments. Most marked enhancement was seen in $70^\circ\mathrm{D} + \mathrm{AA} + \mathrm{H}_2\mathrm{O}_2$ followed by $\mathrm{AA} + \mathrm{H}_2\mathrm{O}_2$ pretreated seeds. In Cumin, the maximum value is found in the plants of $70^\circ\mathrm{D} + \mathrm{AA} + \mathrm{H}_2\mathrm{O}_2$. Other growth characters, such as height, total leaf number, number of inflorescence, dry weight of root, stem, leaves and whole plant are generally more in the plants of $70^\circ\mathrm{D} + \mathrm{AA} + \mathrm{H}_2\mathrm{O}_2$ followed by other pretreatments and the control.

Most interesting feature is the earlier initiation of flowering by pretreatments. It synchronizes with an upsurge in metabolic activities. A reductive atmosphere is created earlier by the early enhancement in SH (Plate 13.3) and AA (Plate 10.1) during the reproductive stages of plants.
raised from pretreated seeds compared with that in the control. Various enzymic activities such as peroxidase (Plate 17.1), AA-FR-peroxidase (Plate 17.3), RNase (Plate 12.2), invertase (Plate 15.1) and AAU (Plate 10.3) are also at higher level in plants raised from \(70^\circ\text{D} + \text{AA} + \text{H}_2\text{O}_2\) followed by other pretreatments and the control.

During the period of reproductive differentiation there is a sharp breakdown of reserve carbohydrates into reducing sugars (Plates 15.3, 16.1) by increasing invertase activity (Plate 15.1). This results into enhanced biosynthesis of ascorbic acid by the plants. Peroxidase and AA-FR peroxidase are the enzymes which catalyze the oxidation of AA in plant tissues (Bonner, 1957 and Mapson, 1958). From the data reported by Chinoy et al. (1957, 1958) and Chinoy (1962, 1969), it is clear that AA is the most important constituent of the redox system which influences the process of development. The redox system regulated by glutathione and other constituents may also be involved in growth of plants.

In the present work the vegetative growth in terms of dry weight of root, stem and leaves as well as reproductive growth in terms of flower bud, flower, fertilized carpel and seed are enhanced by pretreatments. Thus it seems that the overall energy level is being
affected by pretreatments of the seeds by which plants enter the reproductive phase from vegetative phase very early.

The time of flowering of plant greatly influences the height, leaf number and yield (Chinoy, 1966, 1968; Mansuri, 1965; Pandya, 1969). They have correlated the stem elongation with the time of flowering. This is supported by the present data on relative growth rate (RGR), where the mean RGR for root, stem and leaf (Table 8) is highest under the 70°D + AA + H₂O₂, pretreatment followed by other pretreatments compared with that in the control. This higher RGR value indicates the rapid growth in plants raised from pretreated seeds. Flowering in turn can be hastened by accelerating their rate of metabolic activity. Thus, growth and development are controlled by the metabolic activity.

Due to enhanced metabolic activity as well as enzymic activities, active translocation of various metabolites takes place in the plants raised from pretreated seeds and this is supported by the data of net assimilation rate (NAR) and it is more under the plants raised from 70°D + AA + H₂O₂ followed by other pretreatments and the control. Higher NAR value shows an increase in photosynthesis and a more rapid translocation of photosynthetates from the leaves to the stem (Alvim, 1960). Mean value of NAR is higher in the plants of 70°D + AA + H₂O₂ and other pretreatments of the seeds (Table 8). The plants raised from 70°D + AA + H₂O₂ pretreated
seeds have also higher number of leaves (Plate 22.2). This is further supported by the higher value of leaf-weight ratio (LWR) of the plants raised from pretreated seeds (Table 8).

The noteworthy point is that the pretreatments increase, all the above mentioned characters. Compared to all other pretreatments, 70°C + AA + H₂O₂ treated seeds shows a marked increase in all enzymic and metabolic activities, growth, development and yield characters.

There is an increase in 1000 kernel weight of pretreated plants and the yield increases by 30-50% especially in 70°C + AA + H₂O₂ pretreated plants. Pretreated plants are also free from Cumin blight.

Thus, from the foregoing discussion, it becomes clear that pretreatments of seeds have a profound influence on the metabolic patterns of the cell constituents of the plant. In general, by the pretreatments, AA, ASG, AA-MM-complexing are enhanced. Concomitant with this, it is also observed that there is an increase in AA-FR-peroxidase, peroxidase, catalase and other hydrolyzing enzymes such as protease, RNase, and invertase. The AA-FR-peroxidase, RNase, protease and AA oxidase (AAO) are the specific enzymes which take an active part during the reproductive differentiation of a plant. An increase in AA-FR-peroxidase activity accelerates the formation of free radical of AA under the pretreatments which may provide energy for the synthesis of
important cell metabolites. This process leads to an increase in the vital cell metabolites like RNA, DNA as well as proteins. Further, the sugar level in the plants of pretreatments declines suggesting a possible utilization of sugars as substrates for increased respiration as well as for the biosynthesis of AA. Price (1966), Schopfer (1967) as well as Chinoy and Saxena (1971) have shown increased m-RNA synthesis as a result of AA application which resulted in faster biosynthesis of metabolites and increase in rate of enzymic reaction. Chinoy et al. (1971) suggested that differentiation is controlled at the molecular level by the production of different types of structural as well as enzymic proteins.

Metabolic drifts of AA, nucleic acids, proteins and other cell constituents have been studied in the shoot apex and differentiating floral organs under high and low temperature (see; Chinoy, 1962, 1969, 1970; Chinoy et al., 1957; Chinoy and Nanda, 1959; Chinoy and Mansuri, 1966; Patel, 1967; Pandya, 1969; Vora, 1969). On the basis of this, ascorbic acid - nucleic acid - protein metabolism concept of growth and development was advanced by Chinoy 1962, 1969. This concept points out that the increased ascorbic acid, nucleic acid protein metabolism at the time of floral induction and during flower differentiation is due to the
production of a charge transfer complex between macromolecules and AA both contributing to the enhanced energy flow during the period of reproductive differentiation.

Thus the data presented in this thesis gives new information on various metabolic processes and enzymic activities as influenced by pretreatments during juvenile and adult phase in Cumin and supports earlier findings of this laboratory on ascorbic acid - nucleic acid - protein metabolism concept of growth and development.

The present work also demonstrates the validity of the ascorbic acid - nucleic acid - protein metabolism concept of growth and development by developing a method of pretreating the seeds with ascorbic acid + H₂O₂ after heating the seeds at 70°C for a week. The pretreated seeds have been sown in farmer's fields in a number of villages during 1973-74 and 1974-75 and an enhancement of 30-50 per cent in the yield of pretreated seed over that of the control has been obtained.

In the current Cumin season 1100 kgs. of seeds were pretreated (70°C + AA + H₂O₂) and given to 115 farmers of Gujarat State for large scale trials in their fields. Pretreated seeds are already showing better germination and stand of the crop compared with those of the control.