DISCUSSION

Experimental findings presented in this work have helped in understanding the physiological and metabolic activities in the germinating seedlings as well as the effect of wilting and continuous water stress on growth and yield of plants raised from untreated and pretreated seeds. The discussion will pertain to the following two aspects of the subject:

I. Effect of different periods of desiccation treatments on enzymatic activities like catalase, lipase and amylase as well as carbohydrate, -SH and ascorbic acid metabolism during the juvenile phase.

II. Study of resistance to wilting and continuous water stress of adult plants raised from pretreated and untreated seeds in relation to growth, development and yield:

a) Effect of wilting and continuous water stress on growth and yield.

b) Effect of pretreatment.
I. The enhanced catalase activity in the germinating embryo axis with advance of growth is worthy of note especially in those revived after undergoing desiccation treatments (Plate 2). Endosperm shows a lower value of catalase activity as compared to that of the embryo axis, thus indicating that the major centre of enzyme activity is the embryo axis and not the endosperm. It also indicates its important role during the regain of turgor during revival.

This trend is followed by the lipase activity (Plate 3) which supports the above conclusion. The higher lipase activity in desiccated embryo axis suggests its role of affording a protection to seedling under desiccated condition by playing a vital role in the changed colloidal systems in the tissues of the seedling.

The enhanced hydrolytic activity of amylase in the desiccated, undesiccated and revived embryo axes leaves no room for doubt that hydrolysis of starch is going on at a much faster rate during the growth of the seedling. It means that there is a quicker
mobilization of the reserve carbohydrates in the embryo axis from the endosperm (Plates 4, 5, 6). This would naturally imply a faster utilization of those mobilized reserves for growth. Further, the higher amylase activity in desiccated embryo axis suggests that due to the presence of larger quantities of soluble carbohydrates, the loss of free water from the tissue is controlled which is helpful to the seedling for revival and further growth.

Moreover, due to the enhanced hydrolytic activity of amylase the starch of embryo axis gets complete conversion to reducing sugar and as a result it is having no starch at all the stages of germination, during undesiccated, desiccated or revived conditions (Plate 7). This is further evidenced by the fact that reducing sugar increases in the embryo axis. Larger quantities of reducing sugar in desiccated embryo axis also supports the conclusion that it possesses enhanced hydrolytic activity of amylase in comparison with the undesiccated or revived embryo axes (Plate 8). Acharya (1968) also showed the enhanced amylase activity in desiccated tissues of
wheat, ragi and sesamum. Sakai (1962, 1966) observed a close relationship between carbohydrate concentration and frost resistance. He, further, showed that sugars serve as the protective solutes during frost conditions and their permeation into a cell tends to protect it against frost injury.

Similarly, sulfhydryl content of the embryo axis also shows a rising trend with advance in germination, especially in the embryo axes revived after undergoing desiccation treatments (Plate 9). A good correlation between -SH content and frost hardiness has also been observed by Levitt and his co-workers (1961) and also by Schmutz et al. (1961).

Ascorbic acid content shows a progressive increase with the advance in stages of seedling growth in the embryo axis (Plate 10). AA concentration increases during desiccation periods as well as after revival. It is mentioned in the earlier part of this discussion that desiccated embryo axis possesses greater sugar content. This increase in the sugar content helps in the synthesis of increased

Ascorbigen content is also higher in the embryo axis revived after undergoing desiccation treatments (Plate 11). It indicates that ascorbic acid is released from its bound state existing during the desiccated condition and then it is further utilized for the metabolism of the seedling during growth.

Ascorbic acid utilization shows a progressive increase with advance in stages of seedling growth in the embryo axis (Plates 12, 13, 14). This shows that ascorbic acid plays a vital role in the growth processes of the plants. Moreover, decrease in the AA utilization in desiccated embryo axis further supports the view that free ascorbic acid gets bounded (in the state of ascorbigen) which results into its lower utilization. When the embryo axis is revived, the bound ascorbic acid (i.e. ascorbigen) gets released and its higher utilization is essential for the further growth of the seedling. These are very
significant results which clearly show that ascorbic acid plays a vital role in providing redox energy to the embryo axis in its onward march towards the adult phase.

From the point of view of a better understanding of the significance of enhanced ascorbic acid content under the influence of water stress, it would be well at this stage to discuss some of the work undertaken in this laboratory on the role of ascorbic acid in metabolism, growth and development.

Chinoy, Nanda and Garg (1957) suggested that the transformation of the shoot apex from vegetative to reproductive stage is brought about by a change in the redox system of the plant as well as in other properties of the cytoplasmic and other nuclear colloids paving the way ultimately to meiotic division. Since then the dynamics of ascorbic acid production and utilization as well as the metabolic drifts of nucleic acids, proteins and the cell constituents have been studied in the shoot apex, differentiating floral organs and leaves of a number of species under different photoperiodic and vernalization treatments.
(Chinoy, Singh and Sirohi, 1957a; Chinoy and Nanda, 1959; Garg, 1960; Chinoy, 1962a, 1964a, 1967, 1968; Mansuri, 1965; Chinoy and Mansuri, 1966; Patel, 1967). Under appropriate temperature and light conditions, production and utilization of ascorbic acid take place at enhanced rates, thus, producing a highly activated metabolic state in the shoot apex at the time of floral initiation as well as subsequently in the developing spike. Increased electron flow during the period of reproductive differentiation augments the formation of ATP. The greater availability of ATP stimulates the synthesis of DNA and RNA and as a consequence of that proteins, enzyme-proteins and other cell constituents are also synthesized at faster rates. Further, it has been shown that over and above the increase in $\sim P$ bond energy during the period of reproductive differentiation, the flow of electron energy from two other sources also increases. These sources are, (i) the free radical of ascorbic acid; (ii) the formation of a charge transfer complex (CTC) between DNA and ascorbic acid (Chinoy, 1967, 1967a, 1968). A number of workers have demonstrated the formation
of free radical of ascorbic acid called monodehydro-
ascorbic acid (MDHA) during its peroxidative
oxidation (Krasnovskii and Brin, 1946; Piette et al.,
Yamazaki and Piette (1961) have come to the
conclusion that a free radical mechanism is the main
pathway involved in the ascorbic acid oxidase and
peroxidase reaction. Gurevich (1963) has demonstrated
the presence of a special peroxidase in wheat and
corn seedlings which monovalently oxidizes ascorbic
acid in the presence of oxygen on hydrogen peroxidase
to its free radical MDHA. Work in this laboratory on
a number of crop plants has conclusively shown that
the activity of this AA - free radical producing
peroxidase increases considerably, suggesting that the
formation of MDHA is appreciably enhanced during the
period of reproductive differentiation (Chinoy, 1967,

The faster production of free radicals has also been
confirmed by studies of electron spin resonance (ESR)
of vegetative shoot apex as well as of various
reproductive organs, such as primordial spike, anther,
carpel and developing embryo (Chinoy et al., 1968). The ESR signals were decidedly more intense and prolonged in the case of the primordial spike, anther and carpel compared to that obtained from the vegetative shoot apex.

It was also shown that the complexing ability of ascorbic acid with macromolecules like DNA is considerably augmented during the period of reproductive differentiation. On the basis of the foregoing work Chinoy (1967, 1967a) postulated that floral initiation and differentiation were brought about by the increased supply of energy of the free radical as well as of the charge transfer over and above that of augmented ATP pool which leads to the formation of structural proteins, enzymes and other organic constituents of the nucleus, the cytoplasm and the cell wall at enhanced rates. All this intense metabolic activity results in accelerated cell division and more rapid formation of new growth centres. In the initial stages of floral differentiation the rates of cell division and formation of new growth centres far outstrip the rate of cell maturation resulting in the laying down of the primordia of different organs of the spike and the
flower in quick succession (Chinoy, 1967, 1967a, 1968; Chinoy and Mansuri, 1966). Some of the above mentioned processes are so greatly accelerated in some cells in comparison to others that two cellular divisions take place in quick succession, whereas the chromosome complement is duplicated only once. This brings about a change in the norm of mitotic division and results into meiosis.

The most significant finding in the present work is the enhancement in the concentration of ascorbic acid during the period of desiccation. Desiccation, no doubt, increases the concentration of the cell constituents, raises the osmotic value and this brings a number of molecules of different kinds in closer aligment with each other, thus, promoting more efficient metabolic activity.

A number of investigators have also noted a similar increase in ascorbic acid content during the period of water stress (Stocker, 1966, 1961; Shcherbakov, 1963; Acharya, 1968).
Looking at the evidence presented in this thesis and also taking into consideration the evidence obtained by a number of investigators regarding the increase in the concentration of ascorbic acid and its metabolic utilization during desiccation one is led to the conclusion that ascorbic acid plays a paramount role in providing protection to the plant during drought by creating a suitable redox environment. Further, desiccation reduces the free water content of protoplasmic colloids, thus affording a greater chance to the molecules of DNA and ascorbic acid for forming a complex by enhancing the overlapping their electron clouds for charge transfer as well as for the accelerated production of free radicals. This enhanced flow of electron energy, which is the direct resultant of increased ascorbic acid turnover during desiccation, leads to accelerated synthesis of cell constituents and ultimately enhanced growth.

Recently, Chinoy (1969) has shown that ESR signals of the desiccated and revived embryo axes of barley Presto are more intense compared to undesiccated one. The 'g' value of the signals are close to the free
electron value of 2.000 to 2.004 which testifies the organic nature of the observed free radical species. Desiccation and revival in the juvenile phase can therefore be considered beneficial from the point of view of electron flow thereby causing a better growth, earlier development and higher yield.

II. (a) Effect of wilting and continuous water stress on growth and yield:

Taking into consideration the growth data of the four varieties of barley (Plates 16, 17, 18, 19, 20, 21, 22, 23, 24) it appears that the drought resistance of a plant depends on the growth stage at which the plant was subjected to wilting. Thus drought treatment at tiller initiation stage appears to be beneficial for its growth. Quite a large number of workers (Brounov, 1899; Pullman, 1905; Haver, 1908, 1915; Azzi, 1922; Aamodt and Johnston, 1936; Stefanovskii and Guscin, 1937; Konovalov, 1938; Petinov, 1938; Skazkin, 1938; Zablouda, 1938, 1940; Singh and Singh, 1939; Sokolenko, 1939; Fuchs and Rosenteil, 1939, 1940;
Chinoy, 1947a, 1960, 1961, 1961a, 1962, 1962a; Acharya, 1968) have shown that the degree of susceptibility and resistance to drought in plants differs with the stage of the development of a plant. It, therefore, becomes necessary to take into consideration, the developmental stage of the plant not only for the wilting treatment but also for evaluating the reaction of the plant to wilting. Milthorpe (1960) investigating the changes in the drought resistance of wheat varieties from the dormant embryo stages to successive germination stages, could not detect any difference in the drought resistance of seedlings of different varieties known to possess differential capacity of resistance to drought. Chinoy (1947, 1947a, 1960, 1961, 1961a, 1962, 1962a, 1962b) has also shown that there is very little difference in the drought resistance of different varieties of wheat, even at the tiller initiation stage and also at the shooting and flowering stages if the differences due to developmental process are eliminated.
The progressive effect of drought is clearly brought out by the drought coefficients for growth characters like stem height, leaf number, tiller number and for all yield characters worked out by taking the ratio of maximum value of a growth or yield character of a plant under any particular drought treatment by maximum value of the corresponding growth or yield character of the plant under full watering and then multiplying the quotient by 100. The greater the ratio the lesser will be the effects of drought or greater the recovery from drought. The drought coefficients are presented as histograms in Plates 23, 27, 30, 33. From these data it is clear that wilting at tiller initiation stage is beneficial to the plant for its vegetative growth and yield.

Acharya (1968) showed that desiccation treatment of a suitable period increases the ascorbic acid content as well as some of the enzymatic activities of germinating seedling and in adult plants, thus, helping the plant to successfully resist the unfavourable conditions of water stress.
Recently, Chinoy (1968) has postulated: 'a wilting period of short duration during the early stage of a plant's life stimulates the participation of ascorbic acid in synthetic processes as an electron donor during the period of recovery, most probably by the accelerated production of free radicals of ascorbic acid and also by stimulating the formation of a charge transfer complex between ascorbic acid and macromolecules. Enhanced growth and flowering obtained in the case of plants undergoing wilting during the tiller initiation stage are the resultants of the activation of metabolism as stated above'.


The data also leaves no doubt that wilting at the shooting stage causes retardation in stem elongation and dry matter production. This is also evident from drought coefficients for height, tiller number and leaf number (Plate 23). This is because the
higher temperatures prevailing during stem elongation have an adverse effect which appears to be due to the dissipation of all food reserves in enhanced respiration. Iljin (1957) considered that harmful effect of water stress at the shooting stage in plant results into a 'mechanical injury' to the mature cells of stem which are unable to regain their original turgor even after rewatering.

Continuous water stress to which the plants are subjected for their whole life period results into a considerable retardation in growth characters (Plates 16, 17, 18, 19, 20, 21, 22, 23, 24) because mobilization and elaboration of food materials in plant's organs decrease.

The adverse effect of wilting at the shooting stage on grain yield and 1000 kernel weight indicates that temperature prevailing during this wilting period determines the extent of damage to yield of the plant (Plates 25, 26, 27, 28, 29, 30, 31, 32, 33). Further it is interesting to note that the intensity of this adverse effect progressively increases with the lateness of the variety.
Chinoy (1947, 1960, 1961, 1961a, 1962, 1962a, 1964) in a series of papers have shown that wilting at shooting and anthesis stages in the life of the plant directly affects important physiological processes involved in stem elongation, growth of the spike, micro and megasporogenesis, fertilization of the flower and the subsequent development of the embryo. Any deficiency of water at these stages will therefore, adversely affect growth and yield of the plant. He, further, concluded that as the vegetative periods of late varieties under all wilting treatments are further prolonged due to drought, the adverse effect of high temperature during the process of grain ripening is magnified. Consequently, reduction in grain yield and 1000 kernel weight of wilted plants of late varieties is much greater compared to that of normally watered plants of the same varieties.

Drought resistance of a plant appears to depend upon sugar content, osmotic value of cell sap, differences in environmental factors like temperature, humidity, light etc. at the time of wilting treatment given to the plant as well as
during some of the important stages of plant's life mentioned above. Continuous water stress always brings about retardation in all yield characters in comparison with the fully watered plants.

The results presented in this work indicate that variation in drought resistance of varieties are due to differences in environmental conditions during different growth and developmental stages of the plants.

b) Effect of pretreatment:

Considering the data of stem growth, leaf number, grain yield per plant and 1000 kernel weight (Plates 16 to 24) it appears that presowing hardening treatment has influenced the growth and yield characters. The progressive effect of drought is clearly brought out by the drought coefficients of growth and yield characters (Plates 23, 27, 30, 33). From these data it is clear that pretreatments on the whole give a better performance under drought treatments. Under drought at the tiller initiation stage, pretreatments appear to have gained an
appreciable advantage over the plants raised from untreated seeds. Under drought at shooting stage also the pretreated series show better performance in growth and yield as compared to the untreated series.

Results obtained in the present investigation conclusively show that pretreatment with ascorbic acid has beneficially affected growth and developmental characters of various varieties. Moreover, they are in conformity with the results obtained by other workers (Korneev, 1963; Dawson, 1965; Chinoy, 1968; Acharya, 1968). Abraham (1969) and Dave (1969) have also reported the beneficial effect of AA and sucrose pretreatments on various growth and developmental characters as well as on yield in a number of wheat varieties.

Reviewing the literature on pretreatment, Genkel (1964) concludes that presowing hardening is the result of extensive reorganization induced by the dehydration process. Protoplasmic colloids show increased hydrophillic viscosity and protoplasmic elasticity. These changes have been shown in case of plants like
tomoato, grasses, sunflower and others when exposed to presowing treatment. Osmotic changes were also noted. All these contribute to the water holding capacity of plants during water stress. Also hardened plants show an increased respiratory activity, maintain a high level of synthetic reactions during drought and finally a higher rate of photosynthetic activity. Dobrunov (cited by Genkel, 1964) found the process of hardening plus fertilization good for corn accompanied by a marked influence over net productivity of photosynthesis.

Genkel (1964) raised a pertinent question regarding the properties of pretreated plants. "Hardened plants have a higher metabolic rate even though their hydrophilic protoplasmic viscosity always increases". He, himself has sought the explanation to this contradiction by stating that it could be understood only if the energy level of the plant is considered, which is characteristic of compounds containing high energy bonds. The energy level is proportional to the concentration of such substances. Acharya (1968) carried out extensive work on
metabolic drifts in leaves of hardened plants subjected to wilting at various stages. She noticed a higher catalase and amylase activities in wheat and ragi. Dave (1969) also showed that AA pretreatment accelerates the metabolic activities resulting into production of more plastic materials which are mobilized from the leaf to the apical region at faster rates.

On the basis of a mass of data collected during the course of two decades in this laboratory regarding the role of ascorbic acid in metabolism, growth and development, the following postulation has been advanced (Chinoy, 1967a).

Germinative processes are accelerated in seeds pretreated with ascorbic acid, and the beneficial effects of this pretreatment persist upto the maturity of plant. Also, pretreatment with ascorbic acid enhances the biosynthesis of AA as well as ASG in the apical organs and the utilization remains at a higher level in comparison to that of the plants raised from the seeds pretreated with distilled water or untreated seeds. The beneficial effect of
the AA pretreatment is well manifested in the growth and development of the plants. Higher mean RGR values of different organs accompanied by a higher NAR clearly suggest a higher chlorophyll synthesis. This denotes a higher photosynthetic efficiency of the AA - pretreated plants. Also a higher turnover in case of AA - pretreated series enhanced the photosynthetic phosphorylation which increases the "P pool. It also indicates production of more free radicals and more active AA - macromolecule complexes. This helps the plant to escape from the injurious effect of subsequent higher temperature which is detrimental for the development of the primordial spike during the period of reproductive differentiation. On the other hand, delay in the ripening brought about as a result of AA pretreatment beneficially affects the grain filling processes, which is reflected in a higher 1000 kernel weight.

Brown (1940), Poljakoff-Mayber (1953), Waisel (1962) and Evanari (1966) failed to observe beneficial effect of presowing treatments. This is most probably due to the fact that their method of seed soaking was
defective. Submerging the seeds in water or solutions for more than 24 hours in their experiments possibly beyond imbibitional stage, forces the seeds to respire anaerobically, thus producing a deleterious effect on subsequent growth and yield. It may also be pointed out here that ascorbic acid which appears during early imbibitional period requires free oxygen for its turnover. In its absence germinative processes are affected deleteriously. This is also reflected in the post-germinative period in retarded growth and low yielding capacity. Other important differences in the method adopted by the above mentioned workers and that adopted in the present work are: (i) that in our work ascorbic acid, which is a universal constituent of the redox system in plants has been used as an agent of pretreatment; (ii) that the concentration of ascorbic acid was very low (i.e. 25 mg./litre) and (iii) that the pretreatment in the present work included three periods of drying for seeds undergoing pretreatment. All these three factors were absent from the methods used by above mentioned workers. All these factors are of paramount
importance for enhancing growth and yield in the adult phase of the plant. This is presumably done, as the mass of corroborative data obtained in the present investigation as well as by other workers in this laboratory, by the self triggering of the ascorbic acid turnover by the AA pretreatment. Desiccation further helps by still further augmenting the ascorbic acid turnover as shown recently in this laboratory (Chinoy, 1969).