DISCUSSION

Experimental findings presented in this work on the metabolic changes in the germinating seedling as affected by water stress as well as the effect of periodic wilting and continuous water stress on growth, yield, and metabolism of barley varieties chiefly pertain to the following two aspects of the subject:

I. Effect of low moisture level on metabolism of barley in the juvenile phase.

II. Study of varietal resistance to different levels of water stress and to wilting in relation to growth, yield and metabolism.

(a) Effect of continuous water stress and short term wilting on growth and yield of barley varieties.

(b) Metabolic changes taking place during and after drought treatments.

I. The external water supply affects the water content of both embryo axis and endosperm and a direct relationship between tissue water content and moisture supplied upto a certain limit can be established. During initial growth (upto 96 hours) the low moisture
level reduces the water content of the embryo axis and of the endosperm when compared to the water content of the embryo axis and the endosperm of seedlings germinated under adequate moisture level (Plate 2.1). With imbibition the moisture level of the seed rapidly increases during the first 24 hours. Imbibition of water by embryo axis and endosperm continues even after 48 hours, but at a slower rate. Sufficient evidence has accumulated to suggest that the elongation of the embryonal axis is mainly brought about by the imbibition of water and not by cell division or due to the addition of dry matter in the initial stages of germination (Toole, et al. 1956; Brown, 1965; Patel, 1967). Delayed germination under low moisture level treatment can be clearly seen from reduction in the dry matter content of embryo axis under reduced water supply. However, the endosperm contains higher amount of dry matter in comparison to that of adequately watered one (Plate 2.2) suggesting that under low moisture level the full mobilization of food material to the growing embryo axis does not take place. Further, the rate of germination is also delayed. Plate 1 which shows the seedling growth during experimental period
under adequate and low moisture level clearly brings out the fact that the rate of germination is delayed under water stress. Similar results were also reported by McGinnies (1960) for range grass, Springfield (1966) for saltbush, Mehrotra et al., (1967) for wheat and Indian mustard, John (1969) for barley, and Kaufmann and Ross, (1970) for lettuce and wheat. West (1962) reported a reduction in fresh weight and dry weight of corn seedlings germinated under water stress; John (1969) also found a decrease in dry weight of barley seedling under water stress condition. The net increase in water content adds to the internal turgor pressure but more importantly the increased water content is primarily responsible for the reaction of the various controlling system in the cell which in turn step up all physiological processes in the seed (Ferry and Ward, 1959; Woodstock and Feeley, 1965; Woodstock and Grable, 1967). Due to very low water content (10-20%) in air dry seeds metabolic activity is practically at a stand still (Street, 1966). Presumably there should also be a time lag between the initiation of imbibition and mobilization of nutrients from the endosperm or cotyledon by the activation of the enzymatic processes. The results leave no doubt
whatsoever that major centre of the enzymic activity is embryo and not the endosperm (Brown, 1965; Chinoy, 1969a; Chinoy et al., 1969, 1970) Patel, (1967) separated the embryonal axis from the cotyledon without moistening the seed. There could not have been any transfer of enzymes from the latter to the former. With an increase in water content of the seed the radical begins to elongate and at the same time metabolic activity is also accelerated (Street, 1966). Adequate moisture is most essential for active growth. During germination food reserves are mobilized and synthesis of necessary cell constituents takes place culminating in growth. The carbohydrate and soluble nitrogen are generally the main forms of transports from the storage tissue (Endosperm or cotyledon). The substances like phosphorus compound, vitamin and growth substances are also supplied by the same tissue. The mechanism and the factors which direct the flow of above mentioned substances from endosperm or cotyledon to the embryo axis (growth centre) are not yet understood properly and fully.

In the embryo axis the chain reaction of synthesis (metabolic processes) continually proceeds as a result of water absorption. Due to the catabolic processes the food materials are degraded and the
energy released. This energy is utilized to carry out the processes of metabolism. Considering the ascorbic acid metabolism of the seedlings, the AA content of embryo axis shows a progressive increase with the advance in seedling growth as Chinoy, 1969a; Chinoy et al., 1969, 1970, Acharya 1968; Jani 1969, have shown. AA concentration is reduced under the influence of low moisture level treatment. The bound form of AA i.e. ascorbigen (ASG) is also low in the embryo axis of seedlings germinated in low moisture level, however, it remains at a higher level during the 48 hours of germination; subsequently it declines in the endosperm of the same seedlings (Plate 3.1 and 3.2). Regarding the utilization of ascorbic acid (AAU), embryo axis shows enhanced utilization under low moisture level treatment and this is true for endosperm also. The AAU in case of embryo axis remains more or less at the same level during the first 96 hours of germination; on the other hand AAU in the case of endosperm increases with the progress of germination under both the series (Plate 4.1). It proves that mobilization of food materials can be governed by energy provided by the utilization of AA in endosperm during seedling growth.

The data regarding the AA, ASG and AAU confirm
the earlier findings of Chinoy et al., (1970a) who also found a low amount of AA, ASG and high utilization of AA and low magnitude of free radicals of AA in desiccated seedlings of gram. As AA and ASG contents are low and the AAU being high, the redox balance which is maintained by the AA turnover shifts slightly towards the oxidative side since the free as well as the bound form of AA decrease during germination under low moisture level. This further strengthens the belief that low moisture level causes the increase in oxidative processes as the catalase is also found at a very high level (Plate 9.1). This finding corroborates the observations of Stocker (1960) and Chinoy et al., (1970a). Evenari (1962) also reported that under wilting the oxidation processes are more than the reduction processes in the plants. Since AA is utilized as a protecting agent there is not enough of it for the formation of charge transfer complexes (CTC) with macromolecules and hence all the biosynthetic processes as well as all enzymatic processes are at a lower level under inadequate water. Even cell division and extension growth is low and germination rate is delayed. In this connection, it is interesting to recall here the work of Saxena et al., 1969, who have ascribed
the role of an enzyme mobilizing hormone to ascorbic acid.

Considering the nucleic acid metabolism the DNA content is higher in the embryo axis than in the endosperm, but both show generally an increasing trend with march of germination. Statistically significant reduction of DNA content is observed in embryo axis of seedlings of both the varieties germinated under low moisture supply (Plate 4.2). This may be the reason for reduced CTC formation in seedlings supplied with inadequate moisture which further affects the growth of seedlings as discussed earlier. RNase activity is increased in both organs with increasing seedling growth. The RNase activity is found at a slower rate in both embryo axis and endosperm under low moisture level treatment (Plate 5.1). Generally the RNA content increases with advancement in germination and the level of RNA content remains at a lower level in seedlings germinated at low moisture levels in cv. N.P. 20 (Plate 5.2). The decrease in nucleic acid content supports the findings of Kessler (1959), Todd and Basher (1965), Dove (1967), Maranville (1967) and Stutte (1968). Decreased RNase activity under the water stress condition is contradictory to the observations of Dove (1967) and Maranville (1967) who
reported enhanced activity of the enzyme under scarcity of water. It must be pointed out here that the organs in which increased RNase activity reported by Dove (1967) and Maranville (1967) was adult leaf; whereas in the present study the organs are different, i.e. seedling. Chinoy (1947a, 1960a, 1961, 1961a, 1962, 1962a), Chinoy et al., (1971); and Evenari (1962) have suggested that different organs at different periods of growth may behave differently under the influence of water stress. As reported by a number of workers embryonic stages are more drought resistant than other stages of growth and development. Moreover, drought hardiness of the seedlings decreased with the progress of growth and development (Milthorpe, 1950; Levitt, 1956; Chinoy, 1962, 1962a; Chen et al., 1968). As RNase activity is measured in juvenile phase i.e. the drought hardened phase it becomes a synthesizing enzyme rather than a hydrolyzing one. Its low activity under water stress confirms the low RNA synthesis and its enhanced activity results into more RNA being synthesized.

Saxena et al., (1969) have pointed out that ascorbic acid works as an enzyme mobilizing hormone (EMH). They found that when isolated endosperm of barley, cv. N.P. 20 was incubated in different
concentrations of ascorbic acid and gibberellic acid (GA) the former proved to be superior to GA at all concentrations tried in accelerating the release of sugars. Chinoy and Saxena (1971) reported that exogenous application of AA induced biosynthesis of RNA. Similar type of results were also reported by Price (1966), Schopfer (1967). Chinoy and Saxena (1971) also showed that RNase activity is stimulated in AA fed embryo axis. In the present work under low moisture level, AA is utilized at a faster rate for the protection against peroxidative damage. As AA is low in the embryo axis of seedlings under low moisture level the enzyme protein is also low. Moreover, the RNase activity is also low and thus the synthesis of RNA is impaired and eventually there is a decreased synthesis of nucleic acids under low moisture level which may be responsible for reduced growth under the supply of low moisture.

The activity of enzyme like protease is greatly enhanced in the embryo axis under low moisture level and it goes on increasing with advancement in germination. On the other hand endosperm shows low activity under deficit of water (Plate 6.1). The protein content is reduced in both the organs under low moisture level treatment
compared to that in adequately watered one (Plate 6.2). Quantitative reduction in protein content was observed in the water stressed corn seedlings (West, 1962). Positive correlation between water content of the tissue and protein level and inverse correlation between protein level and water loss rate were also found by Chen et al., (1964) in citrus seedlings. Genkel et al., (1967) reported that protein synthesis was disrupted in wilted plants in the vegetative phase. A number of workers have reported that protein is hydrolyzed in stressed condition (Petrie and Wood, 1938; Chibnall, 1954; Mothes, 1956; Stocker, 1960; Todd and Basher, 1965; Stutte and Todd, 1967, 1969; Savitskaya, 1968). The proteins are hydrolyzed by a number of proteolytic enzymes yielding a mixture of amino acids, amides and simple peptides (Street, 1966). The amino acid formation due to protein hydrolysis in the leaves of wheat, tobacco and rice plants under water stress has been reported by Vozhenko and Shkolnik, (1963). Chen et al., (1964) also reported the increase in amino acids in corn seedlings under water stress. A marked increase in proline and alanine has been reported by Chen et al., (1964), Barnett and Naylor (1969), Palfi and Juhasz (1968) and Protsenko et al., (1968). Protsenko et al., (1968) found that drought resistant variety of wheat showed more proline content.
under scarcity of water. They suggested that proline and asparagine favour the increase in relative hydration of protoplasm and in this way play a protective role during unfavourable condition. In the present investigation it is found that protease activity increased and protein content decreased in embryo axis under low moisture supply, conclusively proving that protein hydrolysis is going on and there may be the formation of amino acids amongst which proline may be one of them. The formation of proline thus plays a protective part as Protsenko et al., (1968) suggested.

One of the interesting observations is that the protein content in the endosperm goes on increasing with advancement in germination under both the moisture levels indicating that during early stages of germination of seeds, active synthesis of new protein and hence certain enzyme synthesis takes place as earlier reported by Mayer and Poljakoff-Mayber (1963), Cherry (1963), Bonner and Varner (1965), and Saxena (1969).

Amylase activity of both adequate moisture and low moisture series increases upto 72 hours of germination followed by a decline. The activity in endosperm at both the moisture levels also show
a gradual rising trend up to 72 hours of germination after which the activity sharply rises (Plate 7.1).

An interesting point is that the embryo axis shows higher amylase activity in the initial stages of growth as compared to the endosperm inspite of the absence of starch. Thus, this finding supports the findings of Brown (1965), Chinoy et al., (1970a), Saxena et al., (1969), Chinoy et al., (1969), Chinoy and Saxena (1971) all of whom report that embryo is the major seat of enzyme activity. The low moisture level enhances the amylase activity in the embryo axis while the reverse is true for the endosperm (Plate 7.1). Thus due to the lower moisture level the starch is not mobilized or it is less mobilized by preventing the proper hydration of the hydrolytic enzyme-amylase as endosperm contains high starch under low moisture level (Plate 7.2).

Due to the enhanced hydrolytic activity of amylase the starch of embryo axis is completely converted to reducing sugar and as a result the embryo axis is having no starch at all during the initial period of germination. The enhanced amylase activity in the embryo axis as influenced by low moisture level brings about an increase in the concentration of non-reducing sugar in the initial stages of embryo axis. This may be due to the higher amylase
activity and less activity of enzyme invertase as shown in Plate 8.1. It may be noted that generally the enzyme amylase is more prevalent in barley seeds as compared with other cereals and this accounts for the accumulation of non-reducing sugar (Plate 7.3). The endosperm shows a lower amount of non-reducing sugar as the starch is not hydrolyzed (Plate 7.3). As the amylase activity increases with progress in germination the non-reducing sugar content is also increased. The invertase activity is considerably reduced in the embryo axis under low moisture level while the reverse is true for endosperm (Plate 8.1). The reducing sugar concentration is low in both organs under low moisture level treatment when compared with that of adequately watered one (Plate 8.2). The low activity of invertase during the initial stages of germination under low moisture level is suggestive of the fact that this enzyme remains inactive under deficit of water as the high activity is found in the embryo axis of adequately watered seedlings. The lesser amount of non-reducing sugar and higher invertase activity in endosperm (Plates 7.3 and 8.1) suggest that there may be the formation of reducing sugar but from Plate 8.2 it is clearly seen that reducing sugar is low. Saunier et al., (1968) also found a decline in glucose and sucrose in droughted Larrea divaricata leaves and
this may be due to the more rapid respiration in the droughted plants (Vaadia et al., 1961). In the later period of seedling growth, the invertase activity is slightly increased, reducing sugar is also increased in the embryo axis under low moisture level suggesting that the protection is afforded at later stages of germination by this sugar under unfavourable conditions as discussed by Parker (1968). The decrease in sugar content may be due to their conversion into amino acids (Stewart et al., 1966). According to these authors the oxidation of sugar furnishes \( \alpha \)-ketoglutarate and NAD(P)H which are responsible for proline synthesis under water stress which favour the hydration of protoplasm.

Catalase activity is considerably enhanced in the embryo axis under reduced water level (Plate 9.1) confirming that respiration and oxidation processes are at higher levels under deficit of water (Chinoy, 1962, 1962a, 1969; Chinoy et al., 1966, 1970a). Higher utilization of AA is correlated with higher rate of catalase in that the faster production of \( \text{H}_2\text{O}_2 \) during enhanced utilization of AA is used as substrate for increased catalase activity thereby affording protection to plants against peroxidative damage. The increased oxidative processes under water
stress may increase \( \text{H}_2\text{O}_2 \) production; but the increased activity of catalase will oxidize the \( \text{H}_2\text{O}_2 \) thus preventing any peroxidative damage. Higher catalase activity under water stress has been found by Polimbetova et al., (1964), Lukicheva (1968) and Chinoy et al., (1970a). Under water stressed condition all oxidation reduction processes occurred at faster rates. This was apparently not due to an increase in metabolism but utilization of energy in vital processes (Chinoy, 1969b; Chinoy et al., 1970a).

The rate of catalase activity is increased in endosperm with progress in germination under both the moisture levels suggesting that oxidative processes are at a higher level in endosperm during germination processes.

Sulfhydryl (SH) content generally increases with progress of germination showing that enzymic activities are at a higher level and are very actively being utilized. Under low moisture level SH content goes down in the embryo axis showing that the water deficiency results in decreased SH which possibly may be oxidized to SS groups as Levitt et al., (1962) and Gaff (1966) have suggested. The decrease in SH content of the desiccated seedlings of barley has been observed by Jani (1969), in wheat, ragi and
sesamum seedlings under desiccation by Acharya (1968), and in desiccated cicer seedlings by Chinoy et al., (1970a). This strongly points to the fact that barley seedlings under low amount of water maintain oxidative processes at a high level. In the present work under moisture stress there is noted a decrease in protein level in seedlings compared to the seedlings supplied with adequate moisture (Plate 6.2). This when viewed with decreased -SH level (Plate 9.2) under moisture stress is in full agreement with the views expressed by Levitt et al., (1962) and Levitt (1967).

Peroxidase and AA-FR peroxidase activities are also reduced in embryo axis under low moisture levels; endosperm also shows the same trend at later stages of growth (Plates 10.1 and 10.2). Todd and Yoo (1964), Lukicheva (1968) and Chinoy et al., (1970a), have also reported a decrease in peroxidase activity under water stress condition. Galston and Dalberg (1954), Siegel and Galston (1966) ascribed the significant role of peroxidase in the regulation of cell growth and differentiation. Ramazanova (1968) reported that peroxidase activity decreased with increase or decrease in optimum ratio of free to bound water in the rye seedlings. The
reduced rate of AA-FR peroxidase in embryo axis of the seedlings of low moisture level suggests that free radical of AA is produced at a slower rate. The formation of monodehydroascorbic acid (MDHA) thus is less, and therefore, there are less possibilities for the formation of CTC thus reducing the electron energy available to the growing system. This leads to the arrest of anabolic activity, catabolic activity being enhanced. The biosynthesis of nucleic acids, protein and carbohydrates thus gets reduced under continuous water stress condition as suggested by Chinoy, (1969), and Chinoy et al., (1970a).

The finding thus proves that germination under low moisture level causes shifts in the redox balance of the tissue to oxidative side which adversely affects the synthetic activity in the embryo axis thus retarding the growth of the seedlings.

IIa. The survival value of plants after wilting treatment is found to be less in those cases where wilting treatment is given at later stages of growth (Table 20). This may be due to the fact that the oxidative as well as hydrolyzing enzymes are having a higher rate of activity. On the other hand survival value of the plants of continuous water
stress series wilted at shooting stage and anthesis stage is lesser than that of plants wilted at shooting stage in adequate water series (Table 20). This is due to the fact that during recovery period the available water is at a low level which arrests the physiological processes and thus full recovery may not be possible and hence less number of plants are revived.

Growth characters like height, tiller and leaf number are reduced in plants of continuous water stress series in comparison to those of plants grown under adequate moisture level (Plate 12, 13, and 14). A number of workers have observed a reduction in growth characters when plants were raised under continuous water stress (Khudairi et al., 1961; Etherington, 1967; Vaclavik, 1968; Fulton, 1968; Chinoy, 1969; Jani, 1969; John, 1969; Maurer et al., 1969). The plants under continuous water stress (7 to 10%) also show retardation in yield character like length of main spike, spikelet number, total grain number and grain weight per plant and 1000 kernel weight when compared to those of plants grown under adequate moisture level, i.e., 15 to 20% soil moisture (Plates 15 and 16). Reduction in yield under low moisture level has been reported by Abd el Rahman and Batanouny (1967) for
barley, Pringle and Chary (1968) for wheat, barley and peas, Fulton (1968) in oat plants, Maurer et al., (1969) in bean plants, Campbell et al., (1969) in wheat plants, Chinoy (1969); Jani (1969) and John (1969) in case of barley. The retardation in growth and yield is quite evident from the fact that throughout life cycle water has become a limiting factor in their growth. The leaf area exposed to the radiation is small and hence the carbohydrate available to the plants also becomes a limiting factor as it is evident from Table 27 that NAR of the continuous water stressed plant is lower in comparison to that of adequately watered one. Etherington (1967) also showed a decrease in dry matter production, photosynthesis rate and translocation in the plants under low water potential. Vaclavik (1968) reported a change in photosynthesis, reduction in leaf area and dry matter production in maize plants grown under low moisture level. As a consequence of reduced photosynthesis, the NAR is reduced which is responsible for the decrease in RGR as is seen in this experiment (Table 26). Moreover, the year emergence is delayed in the early varieties because of water deficiency and the grain formation is delayed and thus grain filling processes are shifted to a higher range of temperature (because
of the approaching summer) and thereby grain filling processes are retarded or completely reduced (Plate 16). Thus under scarcity of water, physiological processes like photosynthesis are affected adversely. Translocation was also inhibited and ultimately mobilization and elaboration of food materials decreased thus reflecting in retarded growth and yield as Chinoy (1969) suggested. Asana (1965), Peterson (1965), Campbell and Read (1968), and Chinoy (1947a, 1962, 1964, 1969) have also concluded that delayed ear emergence adversely affects the grain filling processes because of the increasing day temperature (early summer temperature).

There was retardation in growth and yield characters especially in the late variety when the wilting treatment was given at the shooting stage under both the moisture levels. On the other hand wilting at anthesis stage has resulted in a noticeable decrease in yield characters in the plants of adequate moisture series whereas in the plants maintained at continuous water stress there is a retardation in yield as well as tiller and leaf number. Moreover, the reduction in yield is greater in a late variety. Even normally watered plants give a poor yield under adequate moisture level and those plants of Munsing-6009 (a late variety) raised
at continuous water stress failed in yield (Plates 12, 13, 14, 15 and 16). In the late variety Munsing-6009 the yield, inspite of the normal water supply, is reduced because the grain filling processes start with the onset of summer. When these plants were raised under continuous water stress the ear emergence is very much delayed (in the month of April) and the temperature prevailing during this period in this region is sufficiently high to completely prevent the formation of grain as is evident from Plate 16.

Thus, it proves that as the vegetative period of the plant lengthens under restricted water supply higher temperature becomes responsible for the drying up of tillers and leaves in all the varieties under continuous water stress. The plants under adequate water supply when wilted at anthesis stage do not show reduction in vegetative characters but show poor yield. A number of workers have reported that drought hardiness of the seedlings decreased with the progress of growth and development (Milthorpe, 1958; Levitt, 1956; Chinoy, 1947a, 1962, 1969b; Chen et al., 1968). Distinct phase of growth differs in its sensitivity to water stress (Burstorm, 1956; Iljin, 1957; Chinoy 1947a, 1960a, 1961a, 1962, 1962a, 1964, 1968). Some phases are critical than
TABLE 31
Analysis of Variance of Data of Grain Number

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Factor</th>
<th>Degree of freedom</th>
<th>Variance</th>
<th>F Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Variety (V)</td>
<td>2</td>
<td>6362.08</td>
<td>43.80 S</td>
</tr>
<tr>
<td>2.</td>
<td>Soil moisture regime (SMR)</td>
<td>1</td>
<td>141512.28</td>
<td>974.46 S</td>
</tr>
<tr>
<td>3.</td>
<td>Wilting treatment (WT)</td>
<td>2</td>
<td>569.04</td>
<td>3.91 S*</td>
</tr>
<tr>
<td>4.</td>
<td>Replicate (R)</td>
<td>9</td>
<td>57.35</td>
<td>0.39</td>
</tr>
<tr>
<td>5.</td>
<td>V x SMR</td>
<td>2</td>
<td>1722.67</td>
<td>11.86 S</td>
</tr>
<tr>
<td>6.</td>
<td>V x WT</td>
<td>4</td>
<td>93.47</td>
<td>0.64</td>
</tr>
<tr>
<td>7.</td>
<td>SMR x WT</td>
<td>2</td>
<td>506.60</td>
<td>3.48 S*</td>
</tr>
<tr>
<td>8.</td>
<td>Error</td>
<td>157</td>
<td>145.22</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>Total</td>
<td>179</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
the other hand Stocker (1961) suggested that flower formation is a critical phase in the cereal. In a number of cereals, the stage between shooting and coming into ear i.e. initiation and formation of reproductive organs is proved to be a critical phase. Hence the damage is more when the plants are subjected to water stress at such a critical stage (Sabonski and Lipinska, 1960; Lehane and Staple, 1962; Robins and Domingo, 1962; Lerman, 1963; Aspinall et al., 1964; Aspinall, 1965; Belyakov, 1964; Chinoy, 1962, 1962a, 1964, 1966, 1969a; Lypshina, 1967; Acharya, 1968; Milaca and Juncu, 1968; Kuzniar, 1968; Wilson, 1968; Day and Intalap, 1970). The height is reduced when wilting treatment was given at shooting stage under both the soil moisture series, on the other hand it is unaffected when wilting treatment was given at anthesis stage under both the soil moisture regimes (Plate 12). This finding is in agreement with that of Day and Intalap (1970). Decrease in plant height was found by Dimitrijevic and Saric (1965) in wheat plants when water stress was given at heading stage. Joffe and Small (1964), Kudrev and Tyankov (1965), Chinoy (1947a, 1961, 1961a, 1962, 1964, 1969), Acharya (1968), Jani (1969), John (1969), reported a decrease in dry matter and yield when plants were severely wilted at shooting stage. In the present
study the wilting treatment is not so severe, and the reduction in yield is found only in wilted plants of cv. Munsing-6009. Even normally watered plants of that variety also show poor yield in comparison with wilted plants of cv. N.P. 20, cv. Peatland and cv. Presto. It proves that temperature is the main factor for reduction in yield (Asana and Saini, 1962; Asana and Joseph, 1964; Asana and Williams, 1965; Chinoy, 1947a, 1949, 1962, 1963, 1964, 1969a; Chinoy and Sharma, 1951; Nanda and Chinoy, 1957, 1958), as the late variety further becomes a late by drought treatment. Under this condition as respiration is enhanced and catabolic processes are enhanced, food reserves are depleted and as a consequence poor grain filling is observed.

Considering the effect of wilting at the anthesis stage the yield characters especially the grain weight are reduced. Quite a large number of workers have reported a decrease in yield when plants were stressed at flowering stage (Robins and Domingo, 1962; Chinoy, 1947a, 1962, 1964, 1966, 1969; Chinoy et al., 1970a; Aspinall et al., 1964; Belyakov, 1964; Kudrev and Tyankov, 1965; Wilson, 1968; Acharya, 1968; Mulalic, 1968; Kuzniar, 1968; Jani, 1969; Day and Intalap, 1970).
Late variety Munsing-6009 shows greater reduction in grain weight supporting the earlier findings of Chinoy (1947, 1962, 1964, 1969), and Jani (1969). The reduction in yield is due to higher temperature as discussed earlier. Moreover, the leaves become senescent when drought is given at anthesis stage. Asana (1965) observed that after ear emergence the leaves become rapidly yellow and effective photosynthetic surface consists only of the ear and a part of the stem. The grains were filled by carbohydrates derived from photosynthesis of the green ear and to some extent from the sugar accumulated in the stem. Asana (1965) also observed that intermittent drought from anthesis onwards and higher temperature brought about a more rapid yellowing of the ear and a decrease in the level of stem sugar. Wardlaw (1967) noticed that from the onset of wilting there was a progressive reduction in the rate of photosynthesis but grain growth remained unaffected by several days of leaf wilting during grain development in wheat. Thus he suggests that in this instant water stress acts directly on the leaf rather than indirectly through effect on growth or on sugar movement within the conducting tissue. Aspinall (1965) pointed out that there was a reduction in chlorophyll content of spikelet when wilting treatment was given at the stage of grain
development. Chinoy (1962) showed that higher temperature increased the rate of moisture loss during the wilting period thus enhancing the intensity of drought. Low number of grain may be responsible for the reduction in yield. Low water potential in the plant at the time of differentiation of reproductive tissue limits the number of grains capable of development. Wilson (1968) showed that later drought disrupted the normal processes leading to accumulation of dry matter in the grain and part of this limitation was probably due to the early leaf senescence.

The increased drought intensity concomitant with reduced availability of water and higher temperature causes reduction in yield by preventing the translocation of photosynthates and thereby causing sterility especially of those plants, grown under low moisture regime, and further subjected to water stress at anthesis period. Similar observations were also reported by Peterson (1965). The data presented here are in full agreement with earlier findings of Chinoy (1947, 1947a, 1960, 1961a, 1962, 1962a, 1962b, 1963, 1964, 1965, 1968, 1968a, 1969a; Chinoy and Sharma, 1957, 1958; Chinoy et al., 1966) that there is very little difference in the drought resistance of different varieties of wheat at any
growth stage if the differences due to developmental process are eliminated.

IIb. Ascorbic acid content is reduced under the wilting treatment (Plate 17) which supports the earlier findings of Tombesi (1951), Ivanova and Karakash (1964), Chinoy et al., (1970a). However cv. Peatland shows higher AA content when wilted under continuous water stress (Plate 17). This may be due to the varietal differences as cv. Peatland is a late variety in comparison to cv. N.P. 20. A number of workers observed an increase in AA content in plants under wilting (Solomon, 1955; Novitskaya, 1958; Stocker, 1960; Shcherbakova, 1963; Acharya, 1968). AA is known to reduce bleaching of chlorophyll solutions (Rudolph and Bukatsch, 1967). They fed the leaves of Rumex alpinus with AA and found reduced photooxidation of chlorophyll. They indicated that an increased content of AA reduces the pressure of oxygen in the cells. Furthermore, there could be an increased electron transfer to pigments which would also reduce the extent of chlorophyll photooxidation. Thus it appears that low content of AA during wilting treatments at later stages of growth may accelerate the photooxidation of chlorophyll and thus enhance the process of senescence. The revived plants of
drought series reach more or less the same level or even surpass that of normal water series under adequate moisture level as shown by Acharya (1968) and Chinoy et al., (1970a, 1971), but it remains low in the revived plants of continuous water stress subjected to wilting treatment at anthesis stage. This is one of the reasons why these plants show poor yield (Plate 16), as leaf senescence sets in earlier. Increased concentration of AA after the wilting treatment (during revival) suggests that redox balance is maintained on reductive side and thus metabolic activity is increased and biosynthetic processes are going on at a normal level. The bound form of AA (i.e. ASG) is higher under continuous water stress in comparison with that of adequately watered plants (Plate 18). It is reduced under wilting treatment in the plants raised at adequate moisture level but the reverse trend is seen for the plants of continuous water stress regime. Moreover, revived plants show higher amount of ASG than wilted plants (Plate 18). This data partially confirm the result of Acharya (1968) and Chinoy et al., (1970a, 1971). It is possible that AA may be released from its bound state during moisture stress and utilised to protect the organs concerned from peroxidative damage. Generally the wilting treatment lowers the utilization of AA (AAU) under both the soil moisture
regimes. It increases during revival (Plate 19) suggesting that plants overcome the adverse effect of drought by higher AAU i.e. higher energy production during the recovery period. The AAU is higher during shooting stage than at anthesis stage (Plate 19), on the other hand plants suffer less when drought was given at shooting stage than at anthesis stage as it is evident from the yield characters (Plates 15 and 16).

Overall picture of AA turnover can be summarised thus: AA, ASG and AAU are low under wilting treatment. On revival the concentration of AA increases and AAU also goes on increasing thus playing a vital role in maintaining the redox balance towards the reductive side which is so vital for synthetic activity in the plants for maintaining growth.

RNase activity is appreciably reduced under water stress and after the termination of wilting and on addition of water the activity goes on increasing and even surpasses sometimes that of normally watered plants (Plate 20). This is clearly seen in the revived plants which were subjected to wilting at anthesis stage under both the soil moisture regimes. These results are different from the observations of Kessler (1959), Maranville (1967), and Dove (1967).
They noticed higher RNase activity in stressed leaves and suggested that RNase being free and it hydrolyses the RNA and thus RNA content is also reduced under water stress condition. In the present investigation it is found that RNA content is also decreased under wilting treatment, conclusively proving that RNase being not free, and it remains as a synthesizing enzyme. Its synthetic activity is decreased under stress condition because of the low AA present at that time, as Chioyn and Saxena (1971) have suggested that AA may be responsible for RNA biosynthesis by enhancing the RNase activity. The enzyme may be free and may behave as a hydrolysing enzyme when plants suffer from atmospheric drought and soil drought. It is seen from Plate 20 that RNase activity is high and RNA content is reduced in cv. Peatland, comparatively being a late variety than cv. N.P.20, when wilted at anthesis stage under continuous stress condition. After rewatering the RNA content goes on increasing in the plants which were subjected to wilting treatment at shooting stage but it remains at a low level in the revived plants which were subjected to wilting treatment at anthesis stage. The results are in agreement with that of Simonova (1968) who reported decreased level of RNA in pollen grain of barley under wilting treatment given at the phase of stamen
primordium to the appearance of pollen mother cells. Dewatering leads to an increase of RNA, but when drought was given from the time of formation of mother cells to fertilization, the changes were irreversible. These changes can be related with growth and yield. Adequately watered plants contain higher RNA and show better growth and yield when compared to that of continuously water-stressed plants which show retardation in growth and yield. The DNA content follows the same trend as that of RNA during wilting and revival (Plates 21, 22). However, the continuously water-stressed plants which were wilted at anthesis stage and then subsequently revived show lesser DNA content compared to that of normally watered plants under same regime (Plate 21). It shows that DNA biosynthesis is impaired irreversibly when there is atmospheric drought and severe soil drought. The decreased biosynthesis of nucleic acids under wilting treatment has been observed by Kessler (1959, 1961), Shah and Loomis (1965), Dove (1967), Maranville (1967), Stutte (1968), Simonova (1968), and it is one of the reasons for poor growth of the plants subjected to wilting treatment.

The protease activity decreases under wilting.
treatment in both the soil moisture regimes but revived plants show higher protease activity under adequate moisture level, while the revived plants under continuous water stress show reduced protease activity (Plate 23). Protein content decreased in wilted plants when compared to that of normally watered plants. After revival the protein level goes on increasing and reaches more or less the same level as that of normally watered plants under adequate moisture supply but it remains at a low level in the revived plants of continuous water stress regime (Plate 24). Adverse effect of drought on protein content has been reported by Stocker (1960), Evenari (1962), Satarova and Tvorus (1965), Shah and Loomis (1965), Barnett and Naylor (1966), Thompson et al., (1966), Stutte and Todd (1967, 1969), Saunier et al., (1968), Chen et al., (1968), Miller (1968), Ramagopal and Hsiao (1970). Decreased protease activity has been reported by Stocker (1960). In the present study it can be said that synthesizing activity of protease decreases as Stocker (1960) suggested, which is confirmed by the decreased level of protein under water stress condition. Decreased protein synthesis under water stress condition has been reported by Barnett and Naylor (1966), Genkel et al., (1967),
Maranville (1967), Miller (1968), Protsenkov et al. (1968), although different workers have given different reasons for decrease in protein synthesis under water stress condition. As the protein synthesis is inhibited there may be the accumulation of amino acids as shown by Grinenko (1963), Barnett and Naylor (1966), Palfi (1968). Koretskaya and Zholkevich (1966) reported a slower rate of incorporation of amino acids into proteins in dehydrated leaves of Cucumis and Vicia. Reduction in protein synthesis in the present work is correlated with decreased RNA content as Genkel et al., (1967), Chen et al., (1968), and Ramgopal and Hsiao (1970) have already pointed out.

Rewatering leads to increase in protein content under adequate moisture regime but it remains at a lower rate under continuous water stress (Plate 24). This proves that macromolecule changes taking place under drought are reversible as Sedykh and Khokholora (1967) showed. On the other hand the changes are irreversible if the plants have suffered from inadequate water at the time of recovery period and that is why the continuously water-stressed plants cannot fully recover after drought treatment and show much more retardation in growth as well as in yield. Under wilting treatment the amino acids are accumulated like proline and alanine (Grinenko,
1963). Barnett and Naylor (1966) reported an increase in proline and asparagine in water stressed plants. Protsenko et al., (1968) also reported an accumulation of amino acids and suggested that proline and asparagine compounds are favoured for the rehydration of protoplasm and thus protect the plants against unfavourable conditions. In the present study the reduced protein content may result in the accumulation of amino acids and thus resist the drought conditions. Continuous-water-stress plants show reduced biosynthesis of protein and concomitant poor growth and yield.

Generally the SH content is reduced under water stress condition in both the regimes. In revived plants of adequate moisture regime the SH content again increases to more or less the same level or even surpasses that of adequately watered plants; but the plants of continuous water stress regime show less amount of SH content even after revival in comparison with that in normally watered plants (Plate 25). The decreased SH content under wilting has been reported by Gaff (1966), Jani (1969) and Chinoy et al., (1970). Levitt (1962) introduced the SH factor as an index for determination of cold hardiness and same perhaps applies to drought
hardiness also. They found that SH can be oxidized to SS either by cold or drought and the persistance of SS even after rewatering leads to the denaturation of protein. In the present study the plants of adequate moisture level show more drought hardiness than the plants of continuous water stress possibly because the former shows higher SS content in comparison with that of latter. Low SH content can be correlated with lower protein content (Plate 24 and 25), and thus make it drought susceptible one. Levitt et al., (1962) reported that increased SH content of plant tissue associated with hardiness is an increase in protein and not in non-protein fraction. SH content was directly related to cold hardiness. Significant reduction in SH content of total water soluble protein was found during hardening and frost death of cabbage leaves (Morton, 1969). When protoplasm changes from sol to gel there is a change from SS linkage to SH radicals. An unfolding of the protein and separation of the SS bonds could be related to greater elasticity of the ectoplasm during hardening (Parker, 1969). Greater ectoplasmic elasticity was found by Scarth and Levitt (1937) when certain plants were cold hardened. Genckel (1964)
emphasized that high protoplasmic elasticity was one of the consistent features of xerophyte.

Under wilting treatment the invertase activity behaves differently in both the cultivars. cv. N.P. 20 shows lower enzyme activity under wilting treatment. Generally the revived plants show similar or higher enzymic activity in comparison with that of normally watered plants (Plate 26), suggesting that sugar concentration increased during revival and that may be responsible for the higher AA content during revival. Levitt (1967) seems to feel that sugar can protect cellular proteins by helping the SH groups to remain far enough from each other to prevent SS formation.

Reducing sugar concentration is generally low in continuous-water-stressed plants than in adequately watered plants. Moreover, it is reduced during water stress under both the soil moisture regimes, which is in agreement with the result of Saunier et al., (1968). The revived plants show higher reducing sugar content than unwilted ones which is also in agreement with the findings of Amer and Williams (1957). The continuous-water-stressed plants show low amount of reducing sugar (Plate 27) synchronising with low invertase activity (Plate 26). Reduction in growth and yield of plants under continuous-water-stress may be due to the fact that it contains low amount of reducing sugar which acts
as one of the protecting agents (Parker, 1968). Moreover low protein content in these plants is due to the fact that sugar is less, which increases the possibility of SS formation which leads to the denaturation of protein as suggested by Levitt (1967). The lower protein content is one of the causes of retardation of growth of continuously water-stressed plants.

The non-reducing sugar content is more in continuously water-stressed plants than that in adequately watered plants (Plate 28). Moreover, it is also more at anthesis stage than at the shooting stage. Thus the trend of non-reducing sugar is just reverse that of reducing sugar (Plate 27 and 28). Wilting treatment reduces the non-reducing sugar as Saunier et al., (1968) also reported for sucrose in wilted leaves of Larrea divaricata. After the removal of water stress the revived plants show more or less the same trend of sugar as compared to that in normally watered plants under adequate moisture level. The revived plants of continuous water stress series on the other hand show lesser amount of non-reducing sugars compared to that of normally watered plants at anthesis stage in that regime (Plate 28). The decrease in non-reducing sugar content synchronized
with a decrease in the growth rate of plants as Hodges and Lovio (1969) suggested. Hiller and Greenway (1968) showed that synthesis of polysaccharides is suppressed under low water potential. Tarchevski (1963) found more labelled carbon in raffinose, valine and malic acid under the influence of drought. The decrease in non-reducing sugars may be due to the decrease in photosynthesis and ultimately sucrose production goes down as suggested by Kozlowaski (1965).

The increase in non-reducing sugars at anthesis stage and decrease in reducing sugar when compared with that of shooting stage proves that drought at anthesis stage is more harmful than drought at shooting stage. Murti and Srinivasulu (1968) suggested a similar relationship between drought susceptible and drought resistance varieties of wheat.

Catalase activity is lowered in the wilted leaves than in the normally watered plants of both the series (Plate 29). Takakoi (1968) reported decreased activity with increase or decrease in the soil moisture level in leaves of Cowpea plants. The decreased catalase activity during wilting treatment is compatible with the lower utilization of AA under the same condition. The revived plants
show higher activity than wilted ones, and some time even surpasses that of normally watered plants (Plate 29). The enhanced activity during recovery period points to the fact that the enzyme plays a vital role in regaining the norms of metabolic activities of the plant during revival as suggested by Chinoy (1969) and Chinoy et al., (1970, 1970a).

Peroxidase activity is generally reduced in droughted plants when drought intensity was higher (Plate 30). Todd and Yoo (1964) and Lukicheva (1968) also reported decreased peroxidase activity in water stressed wheat leaves. After rewatering the peroxidase activity goes on increasing and reaches more or less the same level as that of unwilted plants (Plate 30). The role of peroxidase activity can be described as a vital one in regaining the norm of metabolic activity after the adverse effect of drought.

Generally the AA-FR peroxidase activity is lower in the wilted plants under both the soil moisture regimes than that in unwilted plants. Moreover after rewatering the activity increases and some times surpasses that of normally watered plants, with the exception of plants revived after drought at anthesis stage in which there is lower
peroxidase activity under continuous water stress (Plate 31). The reduced AA-FR peroxidase activity, low AA content and low DNA content under wilting treatment suggests that CTC formation may be retarded under scarcity of water at later stages of growth. On revival the enhanced AA-FR peroxidase activity and AA content suggests that the redox balance is maintained on reductive side for enhancing the flow of electron energy which may be utilized for biosynthesis of cell constituents for maintaining normal growth.

Water stress given to a plant at any stage of growth has a profound influence on the metabolic patterns of the cell constituents of the plant. In the present study a set of plants was given continuous water stress with wilting at shooting and anthesis stages. Another set of plants was maintained at adequate moisture level with temporary stress given at shooting and anthesis stages. In general the water stress causes a reduction in the AA, ASG as well as utilization of AA. Concomitant with this it is also observed that there is a decrease in AA-FR peroxidase activity which decreases the electron energy supply for the synthesis of cell metabolites. This process thus leads to deficiency in the vital cell metabolites like RNA, DNA as well as proteins as
seen in Plates 21, 22, 24 during water stress. Further the sugar level in wilted plants declines (Plate 27) suggesting a possible utilization of sugars as substrates for increased respiration which generally is induced by water stress.

Another important observation is that the plants grown under adequate moisture level, when subjected to water stress and then revived, the disturbance caused in the metabolism by the water stress is generally made up during revival, the metabolic revival being better in plants receiving water stress at the shooting stage. In the plants raised under inadequate moisture level the synthesis of cell metabolites are already impaired by the inadequate supply of water. When these plants are given further water stress the disturbance caused in the metabolism is enhanced as is evident from the decreased level of AA, DNA, RNA, protein and SH content (Plate 17, 21, 22, 24 and 25). When these plants are revived by supplying limited amount of water the recovery is incomplete. Thus the levels of nucleic acids, protein and SH during revival do not reach to the same level as in normal watered plants. This must be due to the fact that metabolic machinery synthesizing the cell metabolites are permanently injured by the inadequate
water supply, which was further aggravated by water stress given at shooting and anthesis stage. Of the two water stresses given at the anthesis stage in inadequate moisture regime plants suffer the maximum damage as is clearly evident from the decreased yield in these plants. The lateness of the variety and drought given at the certain periods as well as moisture level maintained profoundly affect the yield. When plants of N.P. 20, an early cultivar, were droughted at anthesis stage the yield was adversely affected as compared to that of plants wilted at shooting stage. The same is true for other varieties. In the plants maintained at continuous water stress the vegetative period is lengthened. When these plants were given additional stress at anthesis and shooting stages the vegetative period was prolonged further. This causes the fertilization of carpel as well as the development of the grain to take place under high temperature caused by the onset of summer, the result being that the grain filling is either improper or nil as observed in Plate 16. Similar effect of high temperature on grain filling of oat grains was also reported by Vora (1969). Thus the reduced water stress causes the shift in the metabolic drifts, prolongs the vegetative period and adversely affects the yield. Varietal
differences in drought resistance of plants are mainly due to differences in environmental conditions during the different growth and developmental stages of varieties belonging to different flowering classes, and not due to any genetic difference in the capacity of different varieties to endure wilting as is clearly borne out by this thesis.