CHAPTER V

DISCUSSION OF FINDINGS ON WHEAT SEEDLINGS
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Experimental findings reported here throw some light on the metabolic alterations caused in the germinating seeds of two diverse food reserves, viz. Starch represented by Wheat and Proteins by Blackgram due to water stress imposed by Osmotica and Desiccation and stress caused by Low temperature. The experimental work was conducted to investigate:

I. Effect of Different Osmotica on the Metabolism of Wheat Seedlings during Germination.

II. Effect of Desiccation Treatments on the Metabolism of the Germinated Wheat Seedlings.

III. Effect of Low Temperature Treatments on the Metabolism of the Germinated Wheat Seedlings.

Experiment I

Effect of Different Osmotica on the Metabolism of Wheat Seedlings during Germination.

Germination:
Various external conditions control the germination. They are temperature, light, oxygen and the external water supply. Water in the environment of seeds is most important
for not only the initiation of seed germination as it is required for the imbibition processes, but also the continuation of germination (Carl Leopold and Kriedemann). Any extension growth is accompanied by increasing turgor (Cleland, 1958). Extension growth of root and shoot also depends upon the availability of water. Due to inadequate supply of water in the medium, seedling growth is arrested and germination is reduced. Availability of water in the germination medium influences the moisture uptake of both embryo axis and endosperm of wheat seedlings (Figs. 1 & 3) of (Plate 1 & 2). As is evident, from the % moisture content of embryo axis (Plate 1 - Fig. 1) is lowered by all osmotica, the greatest effect being of 5 osmotica. The effect of individual osmotica i.e., NaCl, Sucrose, etc., is not well marked, i.e., all osmotica in their respective potentials caused more or less equal effect. Low uptake of water by embryo axis in the osmotic media indicates that a water deficit was caused. Further, moisture content increased from 48 to 120 hours of germination. This indicates an increased uptake of water as germination advances. Lowered uptake of water by osmotically stressed seedlings of Wheat Embryo
Axis and Endosperm is in agreement with the observations of previous workers (Parmar and Moore, 1968; Prisco and O'Leary, 1970 and Pandya et al., 1972). As the seed imbibes water from the medium, the germination initiates and the seed increases in its weight and volume. During the early stages of germination, elongation of embryonal axis is mainly brought about by the imbibition of water and not by cell division or increase of dry matter (Toole et al., 1956 and Brown, 1965).

All osmotica had caused a water stress as evident by moisture uptake, this low moisture available to seedlings had a marked effect on elongation of root and shoot (Fig. 4 & 5 - Plate 1). Measurement after 120 hours of germination, showed a suppression in extension growth of root and shoot under all 5 osmotica, the maximum being caused under 5 PEG. 1 NaCl and 1 Mannitol increased the root and shoot growth in comparison to seedlings germinated in D.W. Enhancement by 1 NaCl and 1 Mannitol was also found in the case of Oat and Guar seedlings by Patel (1979). It is well known that NaCl acts favourably due to its ionic value and causes succulence (Hieomon, 1962). Mannitol as a sugar may
have a nutrient effect as reported by Jackson (1965) who observed stimulated root growth of *Aegopodium* by Mannitol. 5 PEG caused maximum reduction of root and shoot growth which was also reported for Corn seedlings by Parmar and Moore (1968).

Other parameters of growth of seedlings are the fresh weight and dry weight. Considering, therefore, the fresh weight of embryonic axis, it is seen from (Fig. 2 - Plate 1) that the fresh weight of embryonic axis increased from 48 hours to 120 hours of germination. All 7 osmotica slightly increased the fresh weight at 48 hours of germination, while 5 osmotica lowered it, which was maximum under 5 PEG. All osmotica including 7, behaved as nutritive and hence slightly increased the fresh weight. However, at 120 hours of germination, 7 NaCl and 7 Mannitol showed increased fresh weight. As discussed earlier, fresh weight depends upon moisture uptake, and since it is lowered by osmotica, fresh weight was also lowered. Thus, there is a direct correlation of fresh weight of embryonic axis and its moisture uptake. Fresh weight of endosperm (Fig. 1 - Plate 2) were also adversely affected by all osmotica; unlike the fresh weights
of embryo axis, they, however, declined with the progress of germination, although moisture uptake had increased. This may directly be correlated with declining dry weights of endosperm. None of the osmotica allowed full or proper depletion of food reserves from endosperm indicating water shortage. Consequently the dry weights of endosperm were more under osmotica than in D.W. There was, however, utilization of food reserves, since dry weights of endosperm declined with time (Fig. 2 - Plate 2). Here also 5 osmotica, chiefly 5 PEG seedlings, had maximum dry weights. Dry weights of embryo axis were raised by 1 osmotica, all of which may be acting as nutrients and therefore did not cause any adverse effect (Fig. 3 - Plate 1). Increase in dry weight of embryo axis with advancing germination is bound to occur, since embryo axis is a growing organ and increase in dry weight is considered as a growth parameter. Reduction in dry weights by 5 osmotica in the case of embryo axis is in agreement with the findings of Parmar and Moore (1969) in Corn seedlings and Pandya et al (1972) in Brassica seedlings.
Germination is described as the resumption of metabolic activity and is initiated by the uptake of water. It is the uptake of water by seedlings which is primarily responsible for the reaction of the various controlling systems in the cell, which in turn, set up all physiological processes in the seed (Ferry and Ward, 1959; Woodstock and Feeley, 1965 and Woodstock and Grabe, 1967). Since in the air dry seed, there is a very low water content (10-20%), the metabolic activity is practically at standstill (Street, 1966). Further, it is well established that the main centre of the enzymic activity is embryo axis and not the endosperm or cotyledon (Brown, 1965). By the uptake of water and subsequent increase in water content of the seed, the radicle begins to elongate and at the same time, the metabolic activity is also accelerated (Street, 1966). As germination advances, food reserves in the endosperm or cotyledon are hydrolysed and mobilized to the growing embryo axis; simultaneously there occurs a synthesis of new and essential cell constituents, ultimately culminating in growth. The food, thus made available to embryo axis provides it with energy which is utilized to continue the process of germination.
Carbohydrate Metabolism

From an examination of the starch content of endosperm (Fig. 4 - Plate 4), it is evident that from 48 hours to 120 hours of germination, the starch content was depleted, indicating its breakdown and utilization for the growing embryo axis. Initially, at 48 hours, more starch was depleted by osmotica and on the whole NaCl osmotica caused more depletion of starch as compared to PEG. At 120 hours, sucrose caused the least depletion and PEG caused the maximum depletion of starch content. It is well known that under water stress, starch content depletes due to hydrolytic activity of enzymes, amylase to provide sugars (Vasdie et al., 1961). From 48 hours to 120 hours the amylase activity rose, indicating lesser starch content of endosperms at 120 hours. At 48 hours, however, the amylase activity of endosperms (Fig. 3 - Plate 4) was more or less equally depressed by all osmotica, greater depression being by all PEG osmotica; and yet the starch content was lower in osmotic seedlings. Even at 120 hours of germination except NaCl, PEG and Mannitol all other osmotica had suppressed amylase activity; NaCl and Mannitol stimulated it. Although stimulation of hydrolyzing enzymes takes place under water stress, in the
present study, depressed activity was noted. This is contradictory to the general observations of an increased amylase activity due to water stress (Eaton and Ergle, 1948; Vieira-de-Silva, 1970 and Wilson, 1971).

The behaviour of enzyme activity depends on the nature of stress imposed. Most of the methods simulating water deficits involve withholding of water, restricted watering, desiccation, salinity, osmotica, etc. However, in this experiment, osmotica was used; hence the differential response of amylase activity. Depressed amylase activity in water stressed endosperm of barley seedlings was reported by Vyas (1971) who, however, found enhanced enzymic activity in the embryo axis. NaCl caused enhanced amylase activity of maize seedlings while mannitol and PEG depressed amylase activity of these seedlings as reported by Vora et al (1976). A mild stress stimulated, whereas a severe stress depressed enzymic activities (Todd, 1972). Depressed amylase activity by 5 osmotica may be explained as a result of severe stress. Stimulated amylase activity of endosperm by 1 NaCl, 1 PEG and 5 mannitol confirm the general observations that stress causes stimulation of enzymic activities. Amylase activity
was lower in embryo axis than in endosperm - this may be due to the fact that the major site of enzymic activity is with the substrate, i.e., in this case endosperm containing starch.

Carbohydrates, especially the sugars are the chief sources of energy. In embryo axis, at 48 hours of germination, 1 and 5 Sucrose, and 1 Mannitol enhanced the total sugar content, while at 120 hours only 1 NaCl enhanced this sugar content; all the other osmotica lowered this total sugar concentrations (Fig. 4 - Plate 5). Further, sugars increased with germination time. Different osmotic potentials (1 and 3) did not produce any change in sugar content indicating that all osmotic potentials caused an equal effect. In the case of endosperm (Fig. 4 - Plate 6), all 3 osmotica lowered this sugars more than 1 osmotica during both the germination hours. From 48 to 120 hours the endospermic sugars also increased. Lower sugar concentration by osmotica suggests that they were being utilized and oxidized, providing energy to embryo axis as well as to endosperm. Increased sugars by 1 osmotica, Sucrose and Mannitol at 48 hours and 1 NaCl at 120 hours indicate a transient accumulation of sugar for future utilization.
Reducing Sugar Content of embryo axis (Fig. 2 - Plate 5) enhanced by all osmotica except 5 PEG at 48 hours of germination. However, at 120 hours of germination this sugar content was increased substantially by 5 Sucrose. There were no prominent effects of other osmotica. Enhancement of sugars or their accumulation under water stress is well documented. Reducing sugars serve as a respiratory source and it is known that respiration increases under water deficit (Gates, 1964; Ilgin, 1957; Rajai and Loomis, 1954). At 48 hours of germination reducing sugar content of endosperm (Fig. 2 - Plate 6) was enhanced by all 1 osmotica but lowered by 5 osmotica. But at 120 hours of germination, they were lowered by all osmotica, the lowering being maximum by 5 osmotica. Under prolonged stress, sugars were either being utilized in metabolism or being oxidized.

Several workers have reported high amount of reducing sugars under water stress (Julander, 1945; Amer and Williams, 1958; Naidu, 1967 and Hodges and Lorio, 1969).

Non-reducing sugar content of embryo axis (Fig. 3 - Plate 5) was lowered by all osmotica except 1 and 5 Sucrose, which in fact slightly enhanced at 48 hours of germination.
and at 120 hours, \( \overline{1} \) NaCl enhanced this sugar content. On the whole, \( \overline{5} \) osmotica caused more reduction than \( \overline{1} \). Non-reducing sugar content of endosperm (Fig. 3 - Plate 6) increased with the advancement of germination. All osmotica lowered this sugar content, the maximum lowering being by \( \overline{5} \) osmotica. Non-reducing sugars increased with time. This is probably because of degradation of starch, which depleted. Lowered non-reducing sugar content of both organs indicate their conversion to reducing sugars through invertase activity or their utilization by oxidation (Woodane and Kozlowski, 1954) or their conversion to \( \alpha \)-Keto glutamate and NADP (H) for proline synthesis (Stewart et al., 1966). Proline is known to increase relative hydration of protoplasm under water stress.

Invertase Activity of embryo axis (Fig. 1 - Plate 5) and of endosperm (Fig. 1 - Plate 6) was suppressed by all osmotica except \( \overline{1} \) NaCl and \( \overline{1} \) Mannitol, which in fact stimulated this activity of embryo axis at 48 hours of germination. \( \overline{5} \) osmotica caused greater suppression than \( \overline{1} \). PEG (both \( \overline{1} \) and \( \overline{5} \)) caused the maximum suppression, than all other osmotica which had more or less the same effect. Maranville and Paulsen (1970) had observed that under water stress
invertase activity was suppressed causing an accumulation of Sucrose. Thus, the depressed invertase activity by osmotica could be considered as an adaptation under stress to allow accumulation of sugars by not hydrolysing it. But the non-reducing sugars do not accumulate under osmotica, they are probably converted to $\alpha$-Keto glutamate and NADP(H) or utilized directly for cellular respiration.

Protein Metabolism

Protein content of embryo axis (Fig. 2 - Plate 7) of all seedlings fell from 48 hours to 120 hours of germination except in the controlled (D.W.) seedlings where this content increased. At 120 hours of germination, the protein content of embryo axis of controlled (D.W.) seedlings was raised many fold. $\text{NaCl}$ slightly enhanced it and all other osmotica lowered it. But that of endosperm (Fig. 2 - Plate 8), protein content increased with time. All osmotica lowered protein content of endosperm except at 48 hours of germination, $\text{I Sucrose}$ and $\text{I Mannitol}$ enhanced protein content and at 120 hours only $\text{I Sucrose}$ increased it. $\text{I osmotica}$ caused more reduction of protein content than $\text{I osmotica}$. Further greater reduction was observed at 120 hours.
than at 48 hours. The extent of reduction of protein content in endosperm was more or less same by all osmotica. Several workers have reported decreased protein content under water stress. Ben-Zioni et al (1967) observed severe inhibition of protein synthesis under water stress in tobacco leaves. Greenway et al (1972) also reported inhibition of protein synthesis at higher osmotic potentials. Reduction in protein or its synthesis in Corn under water stress has been noted by West (1962), Maranville and Paulsen (1972) in Corn seedlings and Chen et al (1964) in Citrus seedlings found decreased protein content under water stress. Degradation of protein fractions into amino acids under water stress has been recorded by Petrie and Wood (1933), Barnett and Naylor (1966), Todd et al (1970) and Mir et al (1970). Mannitol caused lower protein synthesis in Maize root tip than PEG (Greenway et al, 1972). In the present investigation both 1 and 5 PEG caused maximum reduction of protein synthesis. In general, all 5 osmotica caused more reduction of protein. Recently, Dhindsa and Cleland (1975) also noted decreased protein synthesis by Mannitol and PEG (4,000 Mol. Wt). Prisco and O'Leary (1970) reported lowered protein synthesizing capacity in Phaseolus.
seedlings in osmotic media. On the contrary, Pandya et al. (1972) observed no change on crude protein by PEG, but they did report lowering of ammoniacal nitrogenous substances. Thus lowered protein content by osmotica is in confirmation with the findings of above workers. It cannot be emphatically stated here, whether the lowered protein content was due to its lowered synthesis or its degradation into amino acids.

The findings on the protease activity indicates that degradation of protein into amino acids is unlikely. Protease activity of embryo axis (Fig. 1 - Plate 7) and of endosperm (Fig. 1 - Plate 8) was suppressed by all osmotica except \( \overline{1} \) NaCl, which enhanced this activity at 120 hours of germination in embryo axis as well as in endosperm. Here also, \( \overline{5} \) osmotica caused more suppression than \( \overline{1} \). As discussed earlier, \( \overline{5} \) osmotica caused a greater water stress than \( \overline{1} \), hence the activity was suppressed. Vyas (1971) reported enhanced protease activity in water stressed barley seedlings. Patel (1979) also reported that protease activity of Oat and Guer seedlings was stimulated by \( \overline{1} \) osmotica but was suppressed by \( \overline{5} \) osmotica. In the present study, however, all osmotica caused suppressed protease activity. Therefore,
there may not be any possibility of breakdown of proteins.

Bhavsar (1977) also observed suppressed protease activity of Cajanus cajan seedlings of osmotics. It is known that water deficit generally causes an overall decrease in enzyme level (Todd, 1972) and the synthesis of Macro-molecule is reduced (Kozlowski, 1964). The data on lowered protein content with suppressed protease activity may be due to inhibited protein synthesis.

Histone and Nucleic Acid Metabolism:

During germination from 48 hours to 120 hours, the histone content of embryo axis (Fig. 3 - Plate 7) and endosperm (Fig. 3 - Plate 8) rose, indicating that synthesis of histone had occurred. All osmotics lowered it, as expectable, since the proteins are also low. Also DNA content increased with germination and was lowered by osmotics. Thus a direct relation between histone and DNA can be established. Since both histone and DNA contents are lowered by osmotics, lowered protein synthesis and protein content are expected. This is supported by the observation in the preceding section.
RNA content of embryo axis (Fig. 2 - Plate 9) decreased with the advancement of germination but that of endosperm (Fig. 2 - Plate 10) increased with the march of germination. All osmotica lowered the RNA content but the osmotic effect was not conspicuous. RNAse activity of embryo axis (Fig. 1 - Plate 9) and of endosperm (Fig. 1 - Plate 10) was suppressed by all osmotica. At 48 hours of germination, the activity was suppressed more or less equally by all osmotica in embryo axis. The endospermic suppression of RNAse activity was greater by all 5 osmotica than 1, i.e. when the stress was very severe.

The above findings on nucleic acid metabolism are in agreement with those of the previous workers. (Kessler, 1961; Shah and Loomis, 1965; Todd and Bashir, 1965; Dove, 1967; Marenville, 1967; Stutte, 1968; Tvorus, 1970 and Vyas, 1971).

However, West (1962) observed an increase in RNA content of water stressed corn seedlings. Further Ghazaleh and Madershoot (1967) noted higher RNA and DNA content of leaves of droughted sweet orange plants. More recently, Dhindsa and Bewley (1976) reported that polysomes levels substantially declined in desiccated Tortula Moss, although there was no stimulation of RNAse activity. They also
reported that ribosomes in the desiccated moss failed to
unite with m-RNA fragments. In the present study, no
stimulation of RNase activity was observed on wheat
seedlings; on the contrary, there was a suppression of the
RNase activity. Thus under osmotic stress, decreased RNA
levels in wheat seedlings, both in embryo axis and endosperm,
might be due to reduced RNA synthesis probably due to the
failure of ribosome-mRNA complex. Shah and Loomis (1965)
reported a decline in RNA and DNA contents under water
deficit. A decline in both RNA and DNA contents as well as
in free nucleotides was also observed by Maranville (1967).

Suppressed RNase activity under the osmotic stress is
in conflict with the findings of Dove (1967) and Maranville
and Paulsen (1972). Decrease in RNA content during water
stress has been attributed to increased RNase activity by
Kessler and Tishel (1962). But in the present investigation
both RNA content and RNase activity are lowered by osmotica.
While most of the workers have worked on wilted leaves or
entire seedlings; in the present work separate embryo axis
and endosperm from wheat seedlings are taken up for altera-
tion in metabolites. Hence the results may be varied. Further,
water stress by low or inadequate water supply may generate response in tissues different from the water deficit simulated by osmotica. Hence the findings differed here on RNase activity. Ivanova (1965) and Tvorus (1970) found decrease in nucleic acids in the water stressed wheat plants and an accumulation of intermediate products of nucleic acids.

Thus the overall effect of osmotica was that of decline in the levels of all Macro-molecules like nucleic acids and proteins. Nucleic acids and histones were lowered and this reflected adversely on the nucleic acid directed protein synthesis which was also lowered. This in turn lowered proteins including enzyme protein.

Oxidizing Enzymes:

Catalase activity was quite low during the early period of germination, which however sharply rose during the longer periods of germination. The enzymatic activity of embryo axis (Fig. 1 - Plate 3) and endosperm (Fig. 1 - Plate 4) of the seedlings of 48 hours of germination was not affected by osmotica but there was osmotic effect in the seedlings of 120 hours of germination. In embryo axis, NaCl stimulated
the catalase activity and all other osmotica suppressed it, the greater suppression being by 1 and 5 Sucrose and 1 PEG. The endospermic catalase activity was suppressed more by 5 osmotica than 1, the maximum suppression being by 5 NaCl.

Thus, on the whole, there was an osmotic suppression of catalase activity. Suppressed catalase activity was reported in desiccated wheat seedlings by Parkas and Rajathay (1955), Todd and Yoo (1964), Lukicheva (1968), Takeoki (1966), Chintoy et al (1969) and Acharya (1974). With restricted moisture level, catalase activity of embryo axis and endosperm of Sorghum seedling was low (Vora et al, 1974). Patel (1979) also observed depressed catalase activity in embryo axis and endosperm or cotyledon by osmotica in oat and Guar seedlings. However, George (1975) reported increased catalase activity of Maize seedlings by osmotica. Nanda (1950), Chikasue (1953) and Delkov and Makedonoka (1969) correlated catalase activity with rate of germination and growth. Catalase activity has been correlated with germination vigour. The rise in catalase activity from 48 hours to 120 hours of germination indicates germination vigour. Suppressed catalase activity in seedlings by
osmotica may thus be correlated with arrested seedling
growth. Galston (1951) and Halevy (1964) have however
shown an inverse relationship between catalase activity and
growth.

Activity of another oxidizing enzyme – the peroxidase –
of embryo axis (Fig. 2 – Plate 3) and of endosperm
(Fig. 2 – Plate 4) increased with the germination period.
Peroxidase enzyme has been associated with differentiation
and cell elongation (Siegel and Galston, 1967). Rise in
the peroxidase activity of embryo axis thus can be associated
with its differentiation and growth. All osmotica suppressed
peroxidase activity of embryo axis. At 48 hours of germina-
tion, there was more or less equal suppression by different
osmotica as well as their osmotic potentials. But at 120
hours of germination, there was more suppression by \( \text{X} \)
osmotica than 5. In the case of endosperm, at 48 hours of
germination, 5 Sucrose stimulated this enzymic activity and
\( \text{X} \) Mannitol caused the maximum suppression. At 120 hours of
germination, all osmotica suppressed peroxidase activity
except \( \text{X} \) PEG, which stimulated this enzymic activity. \( \text{X} \)
Mannitol caused the maximum suppression. The stimulation
may be considered as the effect of a particular osmotic
effect. In general, however, there was a suppressed peroxidase activity. A positive correlation between respiration rate, catalase and peroxidase activities in growing roots of citrus was reported by Altman et al. (1965). All osmotica suppressed the catalase and peroxidase activity thus indicating a lowered respiration rate which affected the seedling growth (Fig. 4 & 5 - Plate 1). Further, as mentioned earlier, differentiation and growth are positively correlated with peroxidase activity by Siegal and Galston (1967). Suppressed peroxidase activity by osmotica resulted into poor differentiation and growth of seedlings. The depressed or inhibited catalase and peroxidase activities indicate that there is no availability of metabolically derived $H_2O_2$ which can be used as oxidant for reduced NADPH in the pentose pathway of glucose (Hendricks and Taylorson, 1975). There was thus an overall depressed state of seedlings. Carbohydrates, i.e., all sugars, depleted, probably through oxidation to provide energy to stressed seedlings and hence enough material was not available for cell wall formation. Thus, possibly, arrested the seedling growth (Lockhart, 1965). All macromolecules like proteins and nucleic acids were also low in amount. Therefore, the seedling growth was retarded.
Experiment II

Effect of Desiccation Treatments on the Metabolism of the Germinated Wheat Seedlings.

One of the methods to simulate water deficit is to subject plants, leaves, seedlings, etc. to desiccation treatment using suitable desiccants like \( \text{H}_2\text{SO}_4 \), etc. In Experiment I, wheat seeds were germinated in various osmotic media thereby restricting water availability to these seedlings due to osmotic stress. In Experiment II, germinated wheat seedlings were subjected to desiccation, thereby causing a gradual dehydration of tissues as water is being absorbed by the desiccants used. Thus, there is a difference between osmotic and desiccation, although both create water deficit.

Per cent (%) Moisture Loss/Desiccated Weight:

Considering firstly the changes in moisture content, it is seen that in embryo axis (Fig. 2 - Plate 19) and in endosperm (Fig. 2 - Plate 20) in the seedlings of 48 hours and 120 hours of germination, there was a gradual fall in moisture content as the treatment period was prolonged i.e. from 48 hours to 120 hours of treatment, the rate of moisture
loss increased. (Readings shown in Histogram (Fig. 2 - Plate 19 & 20) indicate per cent (%) moisture loss). In fact, there was a progressive moisture loss, which was less during the initial stage and by longer treatment periods, the moisture loss was more and more. This is reflected on desiccated weight of the embryo axis. Dry weight of embryo axis was minimum in undesiccated seedlings showing maximum water content (Fig. 1 - Plate 19).

Desiccated weight showed a direct relation with moisture loss, i.e. at 24 hours of desiccation, desiccated weight was maximum. More desiccated weight suggest the lesser utilisation of starch, at least in endosperm (Fig. 1 - Plate 20).

Carbohydrate Metabolism

Considering therefore, the starch content of endosperm (Fig. 6 - Plate 20) it is seen that in seedlings of 48 hours germination, the maximum starch content was observed in the endosperm of undesiccated seedlings and minimum in seedlings desiccated for 24 hours. With decreasing moisture content, there was lesser depletion of starch. It indicates that stress causes hydrolysis of starch, through
the activity of the hydrolyzing enzyme, amylase, as discussed earlier. The amylase activity, however, was depressed by all desiccation treatments, the least amylase activity being observed in embryo axis of 48 hours germinated seedlings desiccated for 24 hours (Fig. 5 - Plate 19). In 48 hours germinated seedlings moisture loss can be correlated with amylase activity. The endospermic amylase activity (Fig. 5 - Plate 20) was also suppressed during desiccation. Despite the lowest amylase activity, there was minimum starch content. Thus there is no relation between starch content and amylase activity. Wilson (1971) reported that in crested wheat grass seedlings, amylase activity was not affected at low water potentials, but water potentials at 20 atmospheres inhibited \( \alpha \)-amylase synthesis. The low amylase activity observed here is in good agreement with Wilson’s findings. Several workers have observed that starch content of the leaves is lowered due to water deficit. A progressive decline in starch content was correlated with increase in amylase activity of the desiccated barley seedlings by Jani et al (1968). Inhibition of GA induced synthesis of \( \alpha \)-amylase in barley half seed at water potentials above 20 atmospheres was
reported by Janes (1966). Hodges and Lorio (1969) also observed a depletion of starch content of *Loblolly pine* under moisture stress. Vyas (1971) reported low amylase activity and higher starch content of endosperm of Barley seedlings in restricted water supply. But in the present study, low amylase activity and low starch content are observed. As observed earlier for Sorghum, Vora et al (1976) reported depressed amylase activity in the endosperm of Maize seedlings under $\frac{1}{3}$ PEG, $\frac{1}{3}$ NaCl and $\frac{1}{3}$ Mannitol; however the starch depletion also was maximum in these endosperms. It is likely that although the amylase activity was depressed, some other enzyme like Phosphorylase might have hydrolysed starch (Stumpf, 1952). It may be mentioned that phosphorylase is known to increase 100%, in *Cannra* under water stress (Takeoki, 1968). Thus here also, starch depletion might have been brought about by the activation of other enzymes, like phosphorylase. It is also known that stress leads to denovo formation of new enzymes (Kessler, 1961), and this is considered as a bio-mechanism for allowing seedlings to survive the stress conditions.
Depletion of starch through hydrolysis during desiccation should result into formation of sugars. Reducing sugar content and non-reducing sugar content of embryo axis (Fig. 2 & 3 - Plate 21) and the non-reducing sugar content of endosperm (Fig. 3 - Plate 22) in both the seedlings of 48 hours and 120 hours of germination increased with period of desiccation treatment. It is well documented that stress leads to increased concentration of reducing sugars. (Julander, 1945; Amer and Williams, 1958; Vaadia et al., 1961; Chinoy et al., 1969 and Hodges and Lorio, 1969). Further, due to water deficit, sugars increase and may be utilized through increased respiratory activity. Gates (1964) noted that starch depletes and soluble carbohydrates and total sugars increase in droughted plants. Hodges and Lorio (1969) found that in Loblolly pine under drought, the rise in sugars was proportional to the fall in starch content. Enhancement of sugars with progressive increase of desiccation treatment, probably, aid the tissues to survive desiccation. According to Parker (1958) sugars increase in drought and the liquid water sugar mixture acts as a solvent as the cell dehydrates, thereby offering
protection to tissues against dehydration. Thus the rise in reducing sugar content in embryo axis can be considered as a bio-mechanism to survive the stress conditions.

Increasing concentration of low molecular weight carbohydrates in desiccated seedlings probably helps in retaining turgidity (Maranville and Paulsen, 1970). Thus the findings of sugar content here is in good agreement with this. As the desiccation period increased, the levels of sugars also increased. Further, sugars also help tissues against damage by either stabilising proteins (Klotz, 1958) or by replacing water crystals in protein (Parker, 1968).

As desiccation treatment increased, the invertase activity also increased concomitant with available sugars. The invertase activity of embryo axis (Fig. 1 - Plate 21) at 48 hours of germination was not adversely affected by desiccation except by 24 hours desiccation, which suppressed it. This activity increased with desiccation period. In the embryo axis of the seedlings of 48 hours of germination, the periods beyond 24 hours has not affected this activity.
but in the seedlings of 120 hours of germination, the
96 hours and 120 hours of treatment increased this activity
more than that of the undesiccated seedlings. In the case
of endosperm (Fig. 1 - Plate 22) also, this activity
was suppressed when compared with the undesiccated seedlings,
but with increased desiccation, the activity also increased.
There appears to be a direct relationship between invertase
activity and sugars and this relationship seems to be the
same in embryo axis as well as in endosperm. Thus a
"sink-source" relationship is maintained. Reducing sugar
content of endosperm (Fig. 2 - Plate 22) fell with desiccation
period and also this sugar content was lower than
those of the undesiccated seedlings. This shows that these
sugars were being mobilised to embryo axis augmenting
there, the sugar concentration. Embryo axis is a "growing
organ" and endosperm is an organ "for storage" of reserve
food, this is further proved by the observations, that all
sugars were more in embryo axis than in endosperm.

Stimulated invertase activity of embryo axis by later
desiccation period, and also its rise with increased
desiccation period, provided more sugars to the organs.
Decrease in sugar content in wilted condition was also
reported by Henrici (1952), Woodhams and Kozlowski (1954)

**Protein Metabolism**

The protein content of embryo axis (Fig. 2 - Plate 23) was lowered more or less equally by desiccation periods from 24 hours to 72 hours and 96 hours and 120 hours caused greater lowering than the above three periods, in the seedlings of 48 hours of germination. In the 120 hours of seedlings the lowering was less with increasing periods of treatment. Thus, the overall effect of desiccation was that it caused reduction in protein content. Protein content of endosperm of seedlings of 48 hours of germination was lowered by 24 hours and 48 hours of desiccation treatment and was enhanced by 72 hours and 96 hours and then decreased by 120 hours of treatment. However, all desiccation treatments lowered protein content of endosperm of seedlings of 120 hours of germination. Several workers have noted a decrease in protein content during stress, indicating their breakdown into soluble nitrogenous compounds of low molecular weight such as amino acids, amides and soluble proteins (Axelrod and Jagendorf, 1951; Robert and
Wood, 1951; Roberts, 1952; Mothes and Engelbrecht, 1956).

Adverse effect on proteins under water deficit is also well documented by (Satarova and Tvorov, 1965; Shah and Loomis, 1965; Thompson et al, 1966; Stutte and Todd, 1967, 1969; Saunier et al, 1968). Thus, there was either increased proteolysis due to water stress, or there was inhibition or reduced protein synthesis (Petrie and Wood, 1938; Naylor, 1966; Ben-Zioni et al, 1967 and Marsenville and Paulsen, 1972). Further, there may also be an accumulation of amino acids under water stress (Chen et al, 1968).

Especially in the embryo axis at 120 hours of germination, a direct relation between moisture content and proteins is observable. With increased desiccation, the % moisture loss went up with concomitant increase in proteins. Moreover, the rise in proteins after increased desiccation or prolonged desiccation may indicate their synthesis from already formed amino acids during earlier periods of desiccation. Genkel, (1970), believes that a high degree of resistance to drought is largely due to plants' ability to renew proteins during the stress period. Endospermic proteins showed a decreasing trend on desiccation treatment. The decline in endospermic proteins (Fig. 2 - Plate 24) may be due to its degradation to amino acids which are translocated to embryo axis. These
amino acids then augment up the building of new proteins or in the synthesis of proteins as discussed above.

Protease activity in embryo axis (Fig. 1 - Plate 23) like amylase and invertase activities, followed the same fall and rise, i.e., this activity was lowered by the shorter treatment periods and enhanced by the longer treatment periods. The rise in protease activity was such that it was more than that in the embryo axis of undesiocated seedlings.

The gradual rise of protease activity can be correlated with the fall in moisture content as well as with the rise in protein. Thus, this enzyme may show double behaviour. It acts as a hydrolyzing enzyme initially and then as a synthetic one to allow resynthesis of already formed amino acids — hence protein level goes up later. Lowered protease activity of water stressed corn seedlings was reported by Marsanville and Paulsen (1972). It is known that water deficit generally causes an overall decreased enzyme level (Todd, 1972).

According to Stocker (1960) during the reaction phase, protein content declines and during the restitution phase
there is a rise in protein content. Thus in the seedlings of 120 hours of germination the early decline and later rise in protein may be accordingly explained. Further, it may be that the later rise in protease activity may synthesize new proteins as there are similar or parallel changes between protease activity and protein content at 120 hours of desiccation treatment.

Histones level in embryo axis (Fig. 3 - Plate 23) also fell during shorter stress period and was lower than that of undesiccated seedlings but with increase in treatment period, they rose and the level surpassed that of even undesiccated seedlings. But in endosperm (Fig. 3 - Plate 24) histone content was lowered by 24 hours of treatment in seedlings of 48 hours and 120 hours of germination and with the increasing treatment, the histone content also increased and thus 120 hours of treatment enhanced this content when compared with the undesiccated seedlings. The changes in histones of embryo axis show that there might have been changes in nucleic acid synthesis. Alternatively, their behaviour also reflects in the behaviour of proteins since they breakdown into amino acids.
Nucleic Acid Metabolism

Considering the changes in nucleic acid by desiccation it is seen that RNA content of embryo axis (Fig. 2 - Plate 25) was equally lowered by all periods of desiccation treatment in the seedlings of 48 hours of germination. In 120 hours germinated seedlings also, RNA content was lowered, being maximum by 24 hours of desiccation period and minimum by 96 hours. DNA content of embryo axis (Fig. 3 - Plate 25) was also lowered, which was maximum by 24 hours and minimum by 120 hours of desiccation period. RNAase activity of embryo axis (Fig. 1 - Plate 25) was depressed by all desiccation treatment which was severe by 24 hours of treatment. The suppression of RNAase activity was ameliorated by desiccation time. It also bears an indirect relationship with moisture content. As moisture content decreased, i.e. when the stress increased, suppression of RNAase activity and reduction of DNA and RNA contents were lessened. In endosperm (Figs. 1, 2 & 3, Plate - 26) of the seedlings of 48 hours and 120 hours of germination the same effect of initial lowering and enhancement by longer periods of desiccation treatment on nucleic acid metabolism as seen in embryo axis was observed. Gates and Bonner (1959) found a reduction of total RNA content in water stressed tomato
leaves. They also observed that leaves did possess the
ability to incorporate P-labelled-phosphate in RNA — and
concluded that stress conditions led to increase in the
rate of destruction of RNA. Kessler (1961) also found a
decrease in RNA concentration per unit dry weight of tomato
plants and again it was not certain whether this was due
to increased RNA hydrolysis or due to reduced RNA
synthesis. Similarly, in the present investigation also,
it cannot be stated whether reduction in RNA content was
due to its decreased synthesis or increased RNAase activity.
The decline in RNA content and RNAase activity were parallel
indicating that RNAase might not have acted on the synthetic
side. Initially, desiccation period showed least RNAase
activity; when the moisture loss was minimum as the moisture
was being lost, there was lesser suppression and lesser
reduction of RNA content. Again, it cannot be considered
here that RNAase might have acted on the hydrolytic side,
because both RNAase activity and RNA content show similar
changes. West (1962) however, observed increase in RNA
content of corn seedlings under water stress. As discussed
earlier, Ivanova (1963) also reported a decrease in nucleic
acid content in leaves of droughted wheat plants. Shah and
Loomis (1965) observed a significant decline in RNA and DNA content in leaves of sugar beet even under moderate stress. Here also, in wheat seedlings nucleic acids fell depending upon the moisture status of the seedlings. Decline in RNA and DNA content in corn under moisture stress was also reported by Maranville (1967). He, however, found stimulated RNAase activity unlike the findings reported here. When drought tolerant moss, Tortula ruralis was desiccated, polysome levels were substantially declined without stimulated RNAase activity (Dhindsa and Bewley, 1976). Thus, the desiccation treatment reduced nucleic acids and proteins which do have an indirect relationship with moisture loss, the smaller the moisture loss, i.e., during the shorter periods of treatment, the greater was fall in these contents.

**Oxidizing Enzymes**

During desiccation, the catalase activity of embryo axis (Fig. 3 - Plate 19) was stimulated in the seedlings of 48 hours of germination and the stimulation was increased with desiccation time. In the seedlings of 120 hours of germination, the activity was, however, suppressed by
desiccation treatment up to 96 hours and the minimum activity was seen during 24 hours desiccation; the desiccation treatment of 120 hours, however, stimulated the activity. The endospermic catalase activity (Fig. 3 - Plate 20) was also stimulated by desiccation treatment in the seedlings of 48 hours of germination. However, it was depressed in the case of endosperm in the seedlings of 120 hours of germination. It is well documented that water stress enhances catalase activity. Lukicheva (1968) and Takaoki (1969) also reported stimulated catalase activity under water stress as observed here, in the embryo axis and endosperm of the seedlings of 48 hours of germination. However, with time, i.e., with increased desiccation period, the catalase activity increased. This shows that there was enhanced respiratory activity and toxicity as the treatment period increased, although the moisture loss increased. It may be stated that as the desiccation treatment was prolonged, there was increased toxicity and more \( \text{H}_2\text{O}_2 \) was evolved and in order to remove it by oxidation, catalase activity was stimulated. The seedlings of 120 hours of germination, however, behaved differently. In them, the enzymic activity was suppressed and the suppression can be directly related
to moisture loss. Here also, 120 hours of desiccation treatment stimulated the activity, indicating again increased respiration and toxicity. Parkas and Rajathay (1955); Polimbetova et al (1964) and Lukicheva (1968) reported increased catalase activity in wheat plants under water stress.

The oxidizing enzyme, peroxidase, was also affected by desiccation treatment. In embryo axis, the peroxidase activity (Fig. 4 - Plate 19) was lowered by the shorter treatment periods and the longer treatment period of 120 hours stimulated this activity. The same lowering and stimulation of this activity was found in the case of endosperm (Fig. 4 - Plate 20). According to Stocker (1960), the oxidative processes dominate during the reaction phase of drought resistance. Increased catalase and peroxidase activities during desiccation may thus be explained as a drought resistance mechanism.

**Experiment III**

**Effect of Low Temperature Treatments on the Metabolism in the Germinated Wheat Seedlings.**

Alden and Hermann (1971) have cited voluminous literature
on various aspects of cold hardiness, and yet the intricate mechanism and physiological processes involved in cold hardiness of plants, remains obscure and much remains to be studied. As Mazur (1969) has pointed out, this mechanism must involve some physiological and chemical events. Hence an attempt was made to investigate the biochemical changes occurring during the low temperature treatment in germinated wheat seedlings.

**Carbohydrate Metabolism**

A protective role to carbohydrate has been ascribed during freezing or low temperature condition (Alden and Hermann, 1971). Considering, therefore, the changes in carbohydrate content of tissues subjected to low temperature, it is seen that endospermic starch (Fig. 4 - Plate 36) in the seedlings of 48 hours and 120 hours of germination went on depleting from 24 hours to 120 hours of treatment. It is well documented that there is reduction of carbohydrate reserves under cold stress (Levitt, 1959). He observed conversion of starch to sugar during cold hardiness. Therefore, the faster depletion of endospermic starch here is in agreement with Levitt's observations. Ogolevets (1964) also observed depletion of starch and hemicellulose in the bark of Oak,
Apple and Birch at temperatures below 0°C.

Amylase activity of embryo axis (Fig. 3 - Plate 35) and of endosperm (Fig. 3 - Plate 36) was suppressed by all periods of low temperature treatment. Patel (1979) observed suppressed amylase activity of oat seedlings subjected to low temperature treatment. Thus the findings here on amylase activity is in agreement with Patel's observations.

Considering the changes in sugars, it is seen that the low temperature treatment lowered the total sugar content of embryo axis (Fig. 4 - Plate 37) and of endosperm (Fig. 4 - Plate 38) initially in the seedlings of 48 hours and 120 hours of germination. As the duration of low temperature treatment increased the total sugar content increased and in the embryo axis seedling of 48 hours of germination, the low temperature treatment of 120 hours was more than that of the undesiccated seedlings. Same initial lowering by shorter treatment periods and enhancement by longer treatment periods is also discerned for non-reducing sugar content of embryo axis (Fig. 3 - Plate 37) and for endosperm (Fig. 3 - Plate 38), in the seedlings of 48 hours and 120 hours of germination. Reducing sugar content of embryo axis...
in the seedlings of 48 hours of germination, however, behaved differently. This sugar content decreased with duration of treatment and the maximum reduction was caused by 120 hours of treatment. In the embryo axis of 120 hours germinated seedlings, the reducing sugar content showed a maximum decrease initially, then slightly increasing and again decreasing with increase in treatment period. In the endosperm of 120 hours germinated seedlings, this sugar content was lowered by low temperature treatment but they remained almost constant with the duration of treatment. Such fluctuations in the reducing sugar content show their active turnover. The initial decline in sugars and later their rise indicate that as the low temperature treatment was prolonged sugar rose, especially, non-reducing sugars and total sugars.

Invertase activity was equally suppressed throughout the low temperature treatment in the embryo axis (Fig. 1 - Plate 37) of seedlings of 48 hours and 120 hours of germination. In endosperm, also this activity was lowered, but the maximum suppression of the enzymic activity was by the
shorter period of treatment (i.e., 24 hours and 48 hours) and later on there was lesser suppression as the treatment was prolonged and the effect of these longer periods of treatment were almost equal. Suppressed invertase may allow sugars especially non-reducing sugars to accumulate. Although all sugar contents were lowered by low temperature treatment initially, they rose during the later period of low temperature treatment. Enhancement of sugar under low temperature treatment has been reported by several workers (Parker, 1963). Teltscherova (1967) reported that Sucrose, Polyfructose, Raffinose in addition to monosaccharides appeared in shoot apices of winter plants under low temperature of which Sucrose and Monosaccharides increased. Accumulation and increase of sugars during low temperature treatment may allow and help the seedlings to survive freezing condition. Protective role of sugars has been ascribed under stress. Sucrose and like substances are known to retard the growth of ice crystals and alter their pattern without depressing the point of ice nucleation beyond the freezing point; thereby possibly protecting the proteins of membranes and enzymes from sudden loss of water (Parker, 1963, 1972). Sugars are also known to protect proteins during
frost by replacing some of the water of hydration more firmly by hydrogen bonds (Ullrich and Heber, 1961). Further, Parker (1963) believed that sugars may alter the state of hydration of enzymes and protein membranes. The sharp depletion of sugars in the initial cold stress may be on account of their utilization as an energy source in the development of cold hardiness as well as their protective nature (Pyiklik, 1963; Murray and Cooper, 1967; Alekseeva, 1969 and Khisamutdinova and Vasileva, 1970). The continuous decline of reducing sugars of embryo axis during increased period of low temperature treatment indicate their utilization as energy source. In fact, embryo axis is a sensitive "growing organ" and as such it requires a greater protection. The fall and rise in reducing sugar content of embryo axis of 120 hours of germinated seedlings with duration of low temperature treatment may be explained in a similar way.

Depressed invertase activity at low temperature might be due to the degradation of its proteins or its vitrification although much lower temperature (−10°C to −20°C) is reported for vitrification (Sakai, 1966). Ogolevets (1966) found a decline in amylase and invertase activities from
2°C to 20°C.

**Protein Metabolism:**

Protein content of embryo axis (Fig. 2 - Plate 39) was lowered by all periods of low temperature treatment. The fall in protein content was more in the seedlings of 120 hours of germination than in the seedlings of 48 hours. Protease activity of embryo axis (Fig. 1 - Plate 39) was depressed by low temperature treatment. Shorter periods of treatment caused maximum suppression and with increase in treatment the activity also increased. Histone content of embryo axis (Fig. 3 - Plate 39) was also lowered by low temperature treatment. In 48 hours germinated seedlings, this content increased with the duration of treatment. But in the seedlings of 120 hours, there was a sharp fall initially, then a sharp rise and then again a sharp fall in content was observed due to low temperature treatment.

Endospermic protein content (Fig. 2 - Plate 40) and protease activity (Fig. 1 - Plate 40) were suppressed by low temperature treatment in the seedlings of 48 hours and 120 hours of germination. Histones of endosperm (Fig. 3 - Plate 40) also were lowered by this temperature treatment.
Thus both in embryo axis and endosperm protein metabolism was adversely affected by low temperature treatment.

Fall in protein content indicates its degradation or breakdown during low temperature treatment resulting into the formation of amino acids. Changes in amino acid content were positively correlated with frost resistance by Li et al (1965). Parker (1963), however, was doubtful about the role of amino acids in frost hardness and considered them as poor indicators of protein levels. The fall observed in protein levels in the present study indicates their degradation and as such the formation of amino acids. These changes in protein content associated with the changes in nucleic acids are believed to affect the physical properties of cell protoplasm and enable the protoplasm to resist the stress from dehydration possibly caused by intercellular freezing (Kohn and Levitt, 1966).

Nucleic Acid Metabolism

Nucleic acid content of embryo axis was also adversely affected by low temperature. DNA content of embryo axis (Fig. 3 - Plate 41) and endosperm (Fig. 3 - Plate 42) was
reduced to the same extent by low temperature treatment in the seedlings of 48 hours and 120 hours of germination. More reduction in DNA content was seen in seedlings of 120 hours of germination than in 48 hours seedlings. RNA content of embryo axis (Fig. 2 - Plate 41) and of endosperm (Fig. 2 - Plate 42) was also lowered by low temperature treatment. The RNase activity of embryo axis (Fig. 1 - Plate 41) and of endosperm (Fig. 1 - Plate 42) was suppressed by all periods of low temperature treatment. Thus nucleic acid metabolism also was severely affected by low temperature treatment.

Decrease in RNase activity during cold hardiness was reported by Babenko et al. (1971) in Wheat and in Korean Boxwood by Gusta and Weiser (1972). Brown and Sasaki (1972) also noted a quantitative decline in m-RNA in Mimosa epicotyl during induction of cold hardiness; however in hypocotyl it remained constant. Brown and Bixby (1973) reported lowered RNase activity during induction of cold hardiness in epicotyl and hypocotyl tissues of Mimosa. Generally, an increase or constancy in nucleic acid content has been noted (Barskaya and Oknina, 1959; Siminovitch, 1963.
and Li and Weiser, 1968). In the present study, however, a decrease in nucleic acid content was observed. This is in agreement with the observations of Brown and Sasaki (1972), who believed that decrease in RNAase activity possibly indicated its role in RNA-protein synthesis. Results on DNA and RNA contents are not in agreement with the observations of others in the field. As mentioned earlier, protein content was also lowered by low temperature treatment. Thus the whole "Nucleic Acid-Protein Metabolism" were adversely affected by low temperature treatment.

Low temperature treatment slightly stimulated the catalase activity of embryo axis (Fig. 1 - Plate 35) of seedlings of 48 hours of germination but inhibited it very substantially in the embryo axis of seedlings of 120 hours of germination. Catalase activity of endosperm (Fig. 1 - Plate 36) was stimulated slightly with 48 hours germinated seedlings whereas it was highly suppressed in 120 hours germinated seedlings. Peroxidase activity of embryo axis (Fig. 2 - Plate 35) was inhibited by low temperature treatment. Peroxidase activity of endosperm (Fig. 2 - Plate 36) of the seedlings of 48 hours of germination was lowered by
shorter treatment periods and prolonged treatment enhance this activity more than that of the controlled (untreated) seedlings. In the 120 hours germinated seedlings, this activity was highly suppressed by low temperature treatment.

Lowered catalase activity of embryo axis and endosperm in the seedlings of 120 hours of germination indicates that the respiratory activity is at a lower state thus not evolving any extra \( \text{H}_2\text{O}_2 \) and which is perhaps not required during cold treatment. But increase in catalase activity of embryo axis and endosperm of 48 hours seedlings indicates a faster respiration and may offer cold resistance (Korovin and Barskaya, 1962) but they in fact observed no change in catalase activity under low temperature stress. Gerloff et al. (1967) reported an increase in the activity of catalase and peroxidase during cold hardiness of alfalfa roots. Changes in peroxidase enzyme have been considered to regulate permeability and protect membranes at low temperature and sub-freezing temperature (McCown et al., 1968). Depressed peroxidase activity of embryo axis in the seedlings of 120 hours of germination again points out a low respiratory activity, just to keep the seedlings only
to survive against frost. At low temperature, most of the biochemical activities perhaps remain at a standstill and, therefore, there may not be any intermediates accumulated including \( \text{H}_2\text{O}_2 \) and therefore catalase and peroxidase activities remain at a lower level. High catalase activity in the controlled (untreated) seedlings of 120 hours of germination indicates an active metabolism and vigour. Slightly stimulated catalase activity in embryo axis of seedlings of 48 hours germination may be an adaptation in helping the seedlings to harden.