EXPERIMENTAL FINDINGS

Experiment - I : Effect of different osmotica on biochemical changes during germination of Oat and Guar.

Graded and dehusked seeds of Oat (Var. N.P. Hyb-1) and Guar (Cv. Nau Bahar) were germinated in sterilized petridishes (9.0 cm² diameter) lined with sterilized filter paper (Whatman No. 1) in glass distilled water (D.W.) and various osmotica -1 and -5 of NaCl, mannitol and polyethylene glycol (PEG) upto 96 hours at room temperature (28 ± 2°C) in normal day light. The following metabolites and enzymic activities were estimated from embryo axis and endosperm (Oat) or cotyledon (Guar). The data have been compared with D.W. as control. The following observations refer to effects of osmotica on embryo axis and endosperm of Oat seedlings.

Root length (Plate 1.1) : Elongation growth of root increased progressively with germination. The extension growth of root was reduced by all osmotica but mannitol -1 and -5 showed less effect although at 24 hours of germination -1 NaCl and mannitol showed slight promotion of growth as compared to that in D.W. The higher concentration i.e.

* First figure indicates plate number and the figure following it indicates the number on the plate. This method of presentation of data has been used throughout in this thesis.
OAT VAR. N.P. HYBRID-1

GERMINATION - HOURS

1. D.W.
2. NaCl
3. NaCl
4. MANNITOL
5. MANNITOL
6. PEG
7. PEG

Germination times: 24, 48, 72, 96 hours
osmotica at -5 levels showed marked reduction in root length. -5 NaCl caused maximum reduction throughout the germination.

**Shoot length** (Plate 1.2) : Extension growth of shoot increased with the advancement of germination. It was observed that -1 NaCl showed best growth of all other osmotica. Throughout the germination, except -1 NaCl all osmotica arrested shoot length. -5 osmotics caused more arrest as compared of -1 ones. -5 PEG caused maximum reduction of shoot length. The least retarding effect was that of -1 NaCl, followed by -1 mannitol and -1 PEG.

**Fresh weights** of embryo axis (Plate 2.1) : increased continuously with an increase in the germination period. They were reduced by all osmotica throughout the germination except at 24 hours of germination where -1 NaCl and -1 mannitol showed a small increase in fresh weight. In all the cases, all -5 osmotica showed marked reduction in fresh weight. -5 NaCl and -5 PEG were equally harmful.

A decreasing trend for fresh weights of endosperm (Plate 2.2) observed throughout the germination. All osmotica reduced fresh weights of endosperm. However, their individual effects as well as the effects of osmotic levels were variable. At 24 hours -1 mannitol and -1 PEG caused least reduction. Both -1 and -5 NaCl caused more reduction
throughout germination. At 48 hours also, -1 NaCl caused maximum reduction; while -1 mannitol had no effect while -1 PEG reduced fresh weight. -5 osmotica caused less reduction than -1 osmotica. At 72 hours -1 mannitol showed no change, -1 NaCl caused maximum reduction and -1 PEG more than -5 PEG. At 96 hours -1 NaCl, -1 and -5 PEG caused maximum reduction followed by -5 mannitol. -5 NaCl and -1 mannitol slightly lowered fresh weight.

**Dry weights of embryo axis (Plate 1.3):** Dry weights increased with the march of germination; a reduction in dry weight except at 24 hours of germination, was noted in all the osmotica. At 24 hours, -5 NaCl reduced dry weight. At 48 hours, no effect of osmotica was discerned by -1 NaCl and PEG; other osmotica lowered it. All -5 osmotica caused more reduction; -5 mannitol lesser than NaCl and PEG. At 72 hours, all -1 osmotica had effect on reduction. -5 osmotica caused more dry weight reductions and maximum -1 NaCl. At 96 hours also -5 osmotica caused more reduction than -1 osmotica and -1 PEG caused least reduction.

**Dry weights of endosperm (Plate 1.4):** A decreasing trend for dry weight was observed throughout the germination, the decline in dry weight was less in osmotica as compared to D.W. Dry weights on the whole, were higher in endosperm in -5 osmotica. Effect of mannitol concentrations was discerned
Effects of Osmotica on
1. Root length
2. Shoot length
3. Dry weights of embryo axis and
4. Endosperm

during germination.
Effects of Osmotica on

1. Fresh weights of embryo axis
2. Endosperm
3. % moisture content of embryo axis and endosperm
   during germination.

PLATE - 2
at 72 hours and 96 hours when dry weights were lower in -1 mannitol than -5 mannitol. Effect of PEG was not consistent. At 24 hours and 96 hours, -1 PEG had more dry weight than -5. At 48 hours and 72 hours, it was reverse. NaCl seedlings showed less dry weight as compared to D.W. Dry weights were lesser in -1 NaCl as -5 NaCl.

Percent moisture content of embryo axis (Plate 2.3): upto 72 hours of germination increasing trend was observed for percent moisture content which was subsequently maintained. No significant effects of osmotica were seen after 24 hours of germination in any case, marked increase in moisture content was noted in -5 NaCl as compared to D.W. and other osmotica, the other osmotica showed decrease in moisture content in comparison to D.W. at 24 hours of germination.

Percent moisture content of endosperm (Plate 2.4): increased as germination advanced. Initially, osmotica caused a slight reduction of percent moisture content. As germination advanced, the reduction was more pronounced especially in the case of -5 osmotica. There was lesser reduction by -1 osmotica than -5 osmotica. Both -1 and -5 PEG caused maximum reduction of percent moisture content, while -1 mannitol caused lesser reduction.
Starch content: Throughout the germination no starch content was observed in embryo axis.

Starch content of endosperm (Plate 3.3): was depleted with the march of germination. Starch content of endosperm under all osmotica was lower in comparison to D.W. Though through the germination, -1 PEG showed highest reduction of starch content, while in -5 PEG the reduction was less. There was no effect of osmotic levels of NaCl except at 96 hours. In mannitol between -1 and -5 bars, -5 bar caused more reduction of starch content.

Amylase activity of embryo axis showed a rising trend (Plate 3.1). It was stimulated by -1 NaCl throughout the germination; -1 mannitol depressed it upto 48 hours and stimulated it thereafter. -1 PEG stimulated it only at 48 hours, otherwise it depressed the enzymic activity.

Considering the effects of -5 osmotica, it is seen that -5 NaCl and -5 mannitol stimulated the amylase activity upto 48 hours, which subsequently, depressed it. While -5 PEG stimulating it at 48 hours, depressed it at other times.

Amylase activity of endosperm (Plate 3.2): increased with the advancement of germination. All osmotica depressed the enzymic activity except -1 mannitol at 72 hours. However, the extent of depression was variable. Thus between -1 and -5 NaCl and -1 and -5 mannitol, -5 levels caused more depression,
Effects of Osmotica on

1. Amylase activity of embryo axis
2. Endospera and
3. Starch content of endosperm during germination.
but in the case of PEG, a greater depression was caused by -1 PEG than by -5 PEG.

**Total sugar content of embryo axis (Plate 4.2):** In all media, sugar concentrations increased with advancing germination. All osmotica lowered sugar concentrations up to 72 hours of germination. At 96 hours -1 and -5 mannitol and -1 NaCl enhanced the total sugar content, other osmotica lowered them. It may be noted that up to 48 hours of germination, all -1 osmotica caused more reduction of sugars than -5 osmotica. At 72 hours, -5 osmotica and -1 PEG caused a greater reduction of sugar than the rest of osmotica.

**Total sugar content of endosperm (Plate 5.2):** Like embryo axis, endospermic sugars also exhibited a rising trend. Effects of osmotica were variable with respect to their O.P. levels and nature. Thus, at 24 hours all -1 osmotica enhanced sugar; -5 PEG also enhanced sugars, while -5 NaCl and -5 mannitol lowered them. At 48 hours of germination, all osmotica except -5 PEG lowered sugar concentrations; -5 PEG enhanced them. At 72 hours, while -1 NaCl and -1 mannitol considerably enhanced sugars, others caused reduction of sugar content. All osmotica lowered sugar concentrations at 96 hours.

**Reducing sugar content of embryo axis (Plate 4.3):** increased as germination advanced. The effects of osmotica were
OAT VAR. N.P. HYBRID-1

1. Invertase activity of embryo axis
2. Total sugar content
3. Reducing sugar content during germination.

GERMINATION- HOURS
PLATE-4
Effects of Osmotica on
1. Invertase activity of endosperm
2. Total sugar content and
3. Reducing sugar content during germination.
inconsistent at 24 hours and 48 hours, -1 NaCl lowered and -5 NaCl enhanced; mannitol and PEG had no conspicuous effect. At 72 and 96 hours, all osmotica except -1 NaCl lowered reducing sugars, while -1 NaCl enhanced them. The effects of mannitol and PEG were not conspicuous up to 48 hours, -1 NaCl lowered Reducing Sugar (RS) concentration as compared to -5 osmotica. At 72 hours all -1 osmotica showed more reducing sugars than -5 osmotica. At 96 hours, -1 NaCl and -1 mannitol increased reducing sugars as compared to -5 NaCl and mannitol, while -1 PEG caused a decrease in Reducing Sugar.

Reducing Sugar content of endosperm (Plate 5.3): increased with the advance of germination period. Upto 72 hours germination, all -1 osmotica enhanced reducing sugar concentration at 96 hours, when they lowered them all -5 osmotica lowered reducing sugars, PEG showing maximum effect.

Non Reducing Sugar (NRS): content of embryo axis (Table followed an increasing trend and at 96 hours, they increased tremendously. All osmotica lowered NRS concentrations being more by -5 osmotica. Hence, -1 and -5 mannitol increased NRS at 96 hours.

NRS content of endosperm (Table ): followed a rising trend for all seedlings except -1 mannitol and -1 PEG; in the
Table - 1

Effects of osmotica on Non-Reducing Sugar Content mg/gr. dry wt. of embryo axis and endosperm of Oat during germination.

<table>
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<tr>
<th>Germination hours</th>
<th>D.W.</th>
<th>-1 NaCl</th>
<th>-5 NaCl</th>
<th>-1 Mannitol</th>
<th>-5 Mannitol</th>
<th>-1 PEG</th>
<th>-5 PEG</th>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>24</td>
<td>32.85</td>
<td>8.40</td>
<td>16.60</td>
<td>6.20</td>
<td>6.00</td>
<td>2.17</td>
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<tr>
<td>48</td>
<td>96.01</td>
<td>41.08</td>
<td>17.40</td>
<td>18.39</td>
<td>22.94</td>
<td>10.49</td>
<td>19.26</td>
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<tr>
<td>72</td>
<td>120.59</td>
<td>22.7</td>
<td>28.34</td>
<td>105.20</td>
<td>64.95</td>
<td>40.97</td>
<td>35.43</td>
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<td>96</td>
<td>239.62</td>
<td>245.7</td>
<td>82.48</td>
<td>598.99</td>
<td>529.87</td>
<td>310.78</td>
<td>180.34</td>
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<tr>
<td><strong>Endosperm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>3.62</td>
<td>7.44</td>
<td>7.11</td>
<td>5.41</td>
<td>6.22</td>
<td>5.14</td>
<td>14.76</td>
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<td>36.7</td>
<td>6.57</td>
<td>66.22</td>
<td>23.78</td>
<td>8.47</td>
<td>17.49</td>
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<tr>
<td>96</td>
<td>47.21</td>
<td>50.57</td>
<td>24.21</td>
<td>41.25</td>
<td>30.59</td>
<td>24.79</td>
<td>51.82</td>
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</table>
they increased up to 72 hours and decline later on; in the later, they declined at 72 hours and increased afterwards. Response of seedlings to osmotica were inconsistent. They were enhanced by -1 NaCl, lowered by -1 PEG. -5 NaCl and -5 mannitol; -5 PEG enhanced them.

**Invertase activity of embryo axis (Plate 4.1):** was depressed by osmotica except -5 NaCl at 24 hours. -1 NaCl and -1 mannitol at 72 and 96 hours, -5 NaCl depressed enzymic activity. As compared to -1 NaCl, mannitol and PEG had no marked effects up to 48 hours but at 72 hours and 96 hours -5 mannitol depressed the enzymic activity while -5 PEG depressed at 96 hours only.

**Invertase activity of endosperm (Plate 5.1):** was variable with time. Throughout germination seedlings in -1 osmotica showed greater enzymic activity than -5 osmotica. At 24 hours, all osmotica depressed it, the depression being greater by NaCl. At 48 hours, all osmotica depressed. -1 NaCl slightly enhanced it. At 72 hours, all -5 osmotica depressed activity while -1 mannitol enhanced it. At 96 hours, all osmotica depressed the activity except -1 mannitol which enhanced it. At 24 and 48 hours, all -1 osmotica showed enhanced activity.

**Protein content of embryo axis (Plate 6.3):** A fluctuating trend is discerned with respect to time. However, -1 NaCl
Effects of Osmotica on

1. Protease activity of embryo axis
2. Endosperm
3. Protein content of embryo axis and
4. Endosperm
during germination.
seedlings showed a rising trend for proteins. On the whole, the protein content was lowered by osmotica except -5 NaCl at 24 hours. There was a greater reduction of protein content by -5 osmotica than -1 osmotica, although -1 NaCl at 24 hours and -1 mannitol at 48 hours caused a more reduction than -5 osmotica. The osmotic reduction of protein content was more apparent during 72 and 96 hours of germination, especially, in the case of -5 osmotica.

Protein content of endosperm (Plate 6.4): went on declining upto 72 hours of germination after which it rose. Protein content was enhanced by osmotica. Further, seedlings in -5 osmotica had more protein content than -1 osmotica especially during first 48 hours; later on, however, no appreciable difference was discerned. Seedlings in -1 mannitol had more endospermic protein content than -1 PEG and -1 NaCl.

Protease activity of embryo axis (Plate 6.1): increased with time. The effects of osmotica were not seen at 24 hours, when the activity was enhanced by -5 NaCl. At 48 hours, the activity was depressed by all osmotica, the depression being greater by -1 NaCl and -5 mannitol and -5 PEG; however, during 72 and 96 hours, the activity was enhanced by -1 NaCl and -1 mannitol but was depressed by all -5 osmotica as well as by -1 PEG.
Protease activity of endosperm (Plate 6.2): A rising trend for the protease activity was observed throughout the germination. Initially up to 48 hours of germination, no clear difference was observed for different osmotica, subsequently, the activity was suppressed by all osmotica. Both -1 and -5 mannitol caused lesser effect on the enzymic activity.

DNA content of embryo axis (Plate 7.2): Increasing trend was observed up to 72 hours of germination which declined later on. Effects of various osmotica as well as their concentrations were variable. During 24 hours, NaCl, mannitol raised DNA content while PEG lowered it. All -5 osmotica increased DNA content as compared to -1 osmotica. At 48 hours, all osmotica lowered the amount of DNA there being greater lowering by -5 NaCl and -5 PEG. At 72 hours -1 NaCl enhanced DNA content, -1 mannitol had no effect on DNA content, while -5 osmotica lowered it, being maximum by PEG and least by NaCl. At 96 hours, -1 NaCl and -5 mannitol enhanced DNA content -1 mannitol and -5 PEG no effect, -5 NaCl and -1 PEG lowered it.

DNA content of endosperm (Plate 8.2): remained steady, although there was a slight fell or rise. Osmotic effects were also not conspicuous. At 24 hours, there was a slight enhancement of DNA content by osmotica. At 48 hours, only
-1 and -5 mannitol enhanced DNA, -5 NaCl and -5 mannitol lowered it, while -1 NaCl and -1 PEG did not show any effect. At 72 hours of germination, only -1 mannitol increased DNA content, other osmotica lowered it, and PEG caused maximum lowering. All osmotica lowered DNA content at 96 hours when -5 NaCl lowered to a maximum, -1 mannitol having no effect.

**RNA content** of embryo axis (Plate 7.1): throughout the germination, showed an increasing trend. Considering the osmotic effects, it was seen that they were inconsistent. Thus, at 24 hours -1 NaCl and PEG slight raised it, while mannitol -1 and -5 had no conspicuous effect. -5 NaCl enhanced it, while -5 PEG lowered it. At 48 hours all osmotica except -5 NaCl and -1 mannitol reduced RNA content almost to the same extent. At 72 hours, -1 NaCl and -1 mannitol considerably enhanced RNA content, other osmotica lowered it. -5- PEG caused maximum reduction. At 96 hours also, -1 NaCl and -1 mannitol lowered RNA, other osmotica caused reduction, Both -1 and -5 PEG had the same effect.

**RNA content** of endosperm (Plate 8.1): of seedlings in D.W. and -1 NaCl increased initially, then decreased and again increased. In the case of -1 mannitol it increased upto 72 hours, and declined later on. In the case of -1 PEG there was a continuous decline of RNA content. A declining trend is
Effects of Osmotica on
1. RNA content of embryo axis
2. DNA content and
3. RNase activity

during germination.
OAT VAR. N.P. HYBRID-1

Effects of Osmotic Agents on
1. RNA content of endosperm
2. DNA content and
3. RNase activity
during germination.
observed for RNA content in all -5 osmotics seedlings osmotic effects are also inconsistent. At 24 hours, a slight enhancement was caused by -1 and -5 NaCl, all other osmotics lowered content. At 48 hours enhancement was seen in the case of NaCl endosperm, otherwise, there was a reduction caused by other osmotics. At 72 hours, all osmotics lowered RNA content. At 96 hours, all osmotics considerably reduced RNA content. PEG both -1 and -5 caused maximum reduction of RNA content.

**RNase activity** of embryo axis (Plate 7.3): increased with time. Osmotic effects varied with time. At 24 hours all osmotica except -5 NaCl depressed the enzymic activity. At 48 hours -1 PEG stimulated the activity; other osmotica depressed it. Stimulation of the enzymic activity was seen by -1 NaCl and -1 mannitol during 72 and 96 hours of germination, all osmotica depressed it. -5 PEG caused a greater depression.

**RNase activity** of endosperm (Plate 8.3): like embryo axis showed a rising trend. Effects of osmotica were less conspicuous. There was either a slight stimulation or a slight depression caused by osmotics upto 72 hours of germination. However, at 96 hours, all osmotics depressed the enzymic activity which was greater by -1 PEG and -5 NaCl.
Catalase activity of embryo axis (Plate 9.1) showed increasing trend up to 72 hours of germination after which it remained steady. All osmotica depressed the activity except \(-1\) NaCl and \(-1\) mannitol which stimulated it at 72 hours of germination and \(-5\) NaCl slightly raised it at 24 hours. Depressing effect of osmotica was almost the same in mannitol, PEG and NaCl. Generally, all \(-5\) osmotica caused greater depression.

Catalase activity of endosperm (Plate 9.2): showed an increasing trend throughout the germination. All osmotica depressed it except \(-1\) NaCl and \(-1\) mannitol, which stimulated it at 24 and 72 hours and \(-1\) mannitol at 96 hours. Upto 72 hours both \(-1\) and \(-5\) PEG caused maximum depression of the enzymic activity, \(-5\) being greater than \(-1\). At 96 hours the maximum depression was caused by \(-5\) NaCl.

Peroxidase activity of embryo axis (Plate 9.3): increased with the germination period. The effects of the osmotica were not consistent; thus at 24 hours no conspicuous effect was seen. At 48 hours all osmotica except \(-1\) NaCl stimulated it, \(-1\) mannitol caused maximum stimulation. At 72 hours of germination also stimulation by osmotica was seen. \(-1\) mannitol caused maximum stimulation followed by \(-1\) NaCl, however, 96 hours all osmotica depressed it, \(-5\) NaCl caused the maximum depression.
Effects of osmotica on
1. Catalase activity of embryo axis
2. Endosperm
3. Peroxidase activity of embryo axis and endosperm during germination.
Peroxidase activity of endosperm (Plate 9.4): increased with time. All osmotica depressed the activity where maximum depression was caused by -5 PEG, of the all osmotica, NaCl caused minimum depression and at 72 hours both -1 mannitol and -1 and -5 NaCl slightly stimulated the activity.

Statistical analysis of data : (Table 7):

The results presented in this experiment have been analyzed by the method of analysis of variance for starch content, total sugars, reducing sugar, invertase, protein, protease, DNA, RNA, RNase, catalase and peroxidase activity for both embryo axis as well as endosperm of Oat seedling. The summarized data for degree of freedom, variance and F. value are presented for these estimations. The significance of data has been tested at 1% and 5% level.

In case of embryo axis, all data on the whole, were significant at 1% level i.e. germination period and osmotica except PEG in peroxidase and mannitol for DNA was not significant. Interaction of all estimations between germination period and osmotica were significant at 1% level (Table 7).

In endosperm all osmotica were significant at 1% level with germination period; NaCl and mannitol were not significant while PEG was significant at 5% level with DNA.
<table>
<thead>
<tr>
<th>Factors</th>
<th>D.F.</th>
<th>Variance</th>
<th>F Value</th>
<th>Variance</th>
<th>F Value</th>
<th>Variance</th>
<th>F Value</th>
<th>Variance</th>
<th>F Value</th>
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<td>2</td>
<td>1666.6</td>
<td>5.24</td>
<td>15.0</td>
<td>2.72</td>
<td>159.7</td>
<td>3.20</td>
<td>177.6</td>
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<td>26.8</td>
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<tr>
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<td>4.4</td>
<td>0.80</td>
<td>4.4</td>
<td>0.80</td>
<td>4.4</td>
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<td>GBOTLCA (O)</td>
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<td>4.5</td>
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<td>4.8</td>
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<td>4.8</td>
<td>1.00</td>
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* Significant at 5% level
All osmotica were on the whole significant at 1\% level although starch with NaCl is at 5\% level and protein with NaCl was not significant; NaCl and PEG at 5\% level for DNA, while mannitol with DNA was not significant. All interactions were significant at 1\% level, while interaction between germination period and osmotica was not significant for starch in NaCl and PEG. With protein PEG was significant at 5\% while in protease activity NaCl and mannitol was significant at 5\% level and PEG at 1\% level.

Interaction in DNA, NaCl was at 5\% level and mannitol and PEG was not significant. Thus the findings reported here are highly significant for the period of germination and effect of osmotica as well as its interaction.

The following metabolites and enzymic activities were estimated from embryo axis and cotyledon for Guar seedling.

Root length (Plate 10.1) : The extension growth of root increased as germination advanced. Throughout the germination, root growth was enhanced by -1 NaCl. During 72 and 96 hours of germination, it was also enhanced by -1 PEG. There was reduction of root length by other osmotica and the maximum reduction was caused by -5 PEG, throughout the germination. -1 osmotica was less suppressive than -5 osmotica.

Shoot length (Plate 10.2) : There was a continuous growth
GUAR CV. NAU BAHAR

GERMINATION-HOURS

1. D.W.
2. \( \bar{\text{NaCl}} \)
3. \( \bar{5} \text{ NaCl} \)
4. \( \bar{\text{Mannitol}} \)
5. \( \bar{5} \text{ Mannitol} \)
6. \( \bar{\text{PEG}} \)
7. \( \bar{5} \text{ PEG} \)
of shoot. All osmotica except -1 NaCl supressed the shoot growth. -1 NaCl caused slight enhancement. Although at 48 hours, it was supressed. Maximum supression of shoot growth throughout germination was caused by -1 PEG at both 0.P.

**Fresh weights of embryo axis (Plate 11.1):** increased as germination advanced. -1 NaCl enhanced the fresh weights of embryo axis. At 96 hours even -1 mannitol also enhanced, other osmotica decreased. The decrease in fresh weight of embryo axis was greater in the case of PEG.

**Fresh weights of cotyledon (Plate 11.2):** of seedlings increased with time. -1 mannitol enhanced the fresh weights of cotyledons, while -1 NaCl enhanced it upto 48 hours; otherwise, there was a retardation of fresh weights by osmotica and PEG caused maximum retardation.

**Dry weights of embryo axis (Plate 10.3):** An increasing trend for the dry weights of embryo axis was seen. -1 mannitol increased the dry weight at 48 and 72 hours of germination. -1 and -5 NaCl and PEG and -5 mannitol lowered the dry weights. PEG caused greater reduction of dry weight.

**Dry weights of cotyledon (Plate 10.4):** went on decreasing as germination proceeded. Cotyledons of seedlings in osmotica had more dry weights than in D.W. of various osmotica, -1 and -5 NaCl caused enhancement of dry weights to a smaller extent.
Effects of Osmotica on
1. Root length
2. Shoot length
3. Dry weights of embryo axis and
4. Cotyledon
during germination.
Effects of Osmotica on
1. Fresh weights of embryo axis
2. Cotyledon
3. % moisture content of embryo axis and
during germination.
4. Cotyledon
Percent moisture content of embryo axis (Plate 11.3): rose to a considerable extent from 24 to 48 hours of germination after which the increase in moisture content was less. -1 and -5 NaCl slightly increased percent moisture in comparison to D.W. Other osmotica lowered percent moisture content of the embryo axis and -5 PEG caused maximum reduction of percent moisture up to 72 hours. At 96 hours, however, all osmotica showed either less adverse effect or none at all.

Percent moisture content of cotyledon (Plate 11.4): showed an increasing trend. All osmotica lowered the percent moisture content, although -1 NaCl at 72 hours, caused a slight enhancement. -5 PEG considerably lowered percent moisture.

Protein content of embryo axis (Plate 12.3): of seedlings under different media increased at 48 hours of germination and declined. -1 and -5 NaCl increased protein, while -1 and -5 mannitol and PEG reduced the protein content, the reduction being greater by -5 than -1 osmotica. The maximum reduction was caused by -5 PEG.

Protein content of cotyledon (Plate 12.4): of seedling exhibited the trend of rise and fell during germination in all media. The protein content declined from 24 to 48 hours and increased at 72 hours of germination and declined
Effects of Osmotica on
1. Protease activity of embryo axis
2. Cotyledon
3. Protein content of embryo axis and
4. Cotyledon
during germination.
again at 96 hours of germination. All osmotica lowered the protein content except -5 NaCl which at 96 hours raised it. PEG lowered protein content to the maximum.

Protease activity of embryo axis (Plate 12.1) : of the seedlings increased with time. Throughout the germination period, -1 and -5 NaCl stimulated the protease activity. -1 mannitol and PEG also stimulated the activity, the activity, however, was suppressed by -5 mannitol and -5 PEG.

Protease activity of cotyledon (Plate 12.2) : of the seedlings exhibited a rise and fall trend. It rose from 24 to 48 hours, declined during 72 hours and again rose at 96 hours. The activity was stimulated by -1 NaCl at 24 hours, by -1 mannitol at 96 hours and -1 PEG at 72 hours; otherwise it was suppressed by osmotica and the maximum suppression was seen in -5 PEG seedlings.

Total sugars content of embryo axis (Plate 13.2) : showed varied responses with time and media. Thus in D.W. and -1 NaCl they exhibited a rise and fall trend; in -1 and -5 mannitol and PEG, they exhibited a rising trend, while in -5 NaCl and mannitol, sugar increased up to 72 hours and then declined. -1 and -5 NaCl increased sugar concentrations. -1 Mannitol also increased sugar concentration and -1 PEG increased sugar content at 72 and 96 hours; -5 PEG raised
sugar concentrations only at 96 hours. All seedlings at 96 hours showed a much concentration of sugars.

**Total sugars of cotyledon (Plate 14.2):** The cotyledonary sugars also showed a trend of rise and fall. They increased at 48 hours, declined at 72 hours and again increased at 96 hours. In -5 PEG, however, sugars continued to increase up to 72 hours and then declined. -1 NaCl increased sugar up to 72 hours, -5 NaCl increased up to 48 hours, sugar content was lowered by -1 and -5 mannitol. -1 PEG lowered sugar content at 24 and 96 hours, whereas -5 PEG increased sugar content at 48 and 72 hours of germination.

**Reducing sugar (RS) content of emrbryo axis (Plate 13.3):** Concentrations of Reducing Sugar (RS) did not remain steady. Reducing sugar of seedlings in D.W. and -1 and -5 NaCl increased up to 48 hours, declined at 72 hours and again rose. RS concentration of seedlings in -1 and -5 mannitol and PEG showed a rising trend. Effects of osmotica were also variable. Thus, -1 and -5 NaCl and -1 mannitol consistently enhanced. RS concentrations, -1 PEG caused a decrease up to 48 hours and then raised RS concentrations. -5 mannitol and -5 PEG lowered them at 24 hours but subsequently, they raised them. A point to note is that at 96 hours, all osmotica increased Reducing Sugar content.
Effects of Osmotica on
1. Invertase activity of embryo axis
2. Total sugar content and
3. Reducing sugar content
during germination.
Effects of Osmotica on
1. Invertase activity of cotyledon
2. Total sugar content and
3. Reducing sugar content during germination.
Reducing sugar of cotyledon (Plat 14.3) of seedlings in D.W. and -1 and -5 PEG showed a rising trend; in -1 and -5 NaCl. Reducing sugar went on decline upto 72 hours and then rose sharply. In -1 mannitol sugar decreased at 48 hours, increased at 72 and 96 hours and in -5 mannitol sugars almost remained steady and rose at 96 hours.

Non Reducing Sugar (NRS) content of embryo axis (Table 2) of seedlings in -1 and mannitol, -1 PEG and all -5 osmotica exhibited a rising trend. In D.W. and NaCl, they followed a rise and fall pattern. -1 NaCl, -1 mannitol, -5 NaCl caused enhancement of NRS. -1 and -5 PEG lowered NRS upto 72 hours afterwhich they increased NRS. -5 mannitol increased upto 72 hours and subsequently lowered it.

Non Reducing Sugar content of cotyledon (Table 2) of D.W. seedlings remained more or less at the same level upto 72 hours of germination, afterwhich they rose considerably. In all other seedlings, they showed a rise and fall pattern. They rose at 48 hours, fell at 72 hours and again rose at 96 hours, -1 NaCl increased NRS concentration upto 72 hours of germination, afterwhich it lowered them. -1 mannitol and -1 PEG lowered NRS at 24 hours of germination, increased at 48 hours, Subsequently lowered NRS. -5 mannitol and NaCl enhanced NRS upto 48 hours and then lowered them. -5 PEG increased upto 72 hours and then lowered them.
Invertase activity of embryo axis (Plate 13.1): A rising trend for the activity was discerned. The activity was stimulated by -1 and -5 NaCl and -1 mannitol but was depressed by -5 mannitol and PEG; -1 PEG depressed it at 24 hours but otherwise it was stimulated. Maximum stimulation was caused by -1 and -5 NaCl.

Invertase activity of cotyledon (Plate 14.1): was followed an increasing trend throughout germination, in comparison to embryonal activity. The cotyledonary activity was very low. The effects of osmotica varied with germination period. -1 NaCl stimulated it up to 72 hours. At 96 hours, however, it depressed the enzymic activity. -1 mannitol depressed it at 48 hours, otherwise it caused stimulation. -1 PEG depressed it up to 48 hours and then it stimulated; -5 NaCl stimulated it throughout the germination period, while -5 mannitol and PEG had a consistent depressing effect.

DNA content of embryo axis (Plate 15.2): of seedlings in D.W., -1 NaCl, -1 PEG, -5 mannitol and PEG increased initially and then declined; again at 96 hours of germination it increased. In the case of seedlings, -1 mannitol and -5 NaCl, however, there was a continuous rise. Considering the osmotic effects, it is seen that DNA content was lowered by all osmotica except -1 NaCl at 24
Table - 2

Effects of osmotic a on Non-Reducing Sugar Content mg/gr. dry wt. of embryo axis and cotyledon of Guar during germination.

<table>
<thead>
<tr>
<th>Germination hours</th>
<th>D.W.</th>
<th>-1 NaCl</th>
<th>-5 NaCl</th>
<th>-1 Mannitol</th>
<th>-5 Mannitol</th>
<th>-1 PEG</th>
<th>-5 PGE</th>
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<tr>
<td>Embryo axis</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>24</td>
<td>242.31</td>
<td>326.19</td>
<td>315.06</td>
<td>406.37</td>
<td>266.60</td>
<td>105.23</td>
<td>181.73</td>
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<td>48</td>
<td>500.17</td>
<td>602.20</td>
<td>806.15</td>
<td>865.62</td>
<td>490.97</td>
<td>222.66</td>
<td>157.18</td>
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<tr>
<td>72</td>
<td>450.15</td>
<td>309.00</td>
<td>968.91</td>
<td>1249.50</td>
<td>613.48</td>
<td>221.33</td>
<td>192.86</td>
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<tr>
<td>96</td>
<td>308.32</td>
<td>891.61</td>
<td>528.32</td>
<td>1185.12</td>
<td>247.37</td>
<td>970.55</td>
<td>490.90</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>24</td>
<td>133.74</td>
<td>179.53</td>
<td>197.21</td>
<td>81.34</td>
<td>138.01</td>
<td>111.20</td>
<td>170.83</td>
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<td>246.55</td>
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<td>151.64</td>
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<td>153.03</td>
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<td>129.28</td>
<td>199.46</td>
<td>111.73</td>
<td>83.44</td>
<td>72.68</td>
<td>114.93</td>
<td>176.50</td>
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<tr>
<td>96</td>
<td>355.82</td>
<td>275.22</td>
<td>290.97</td>
<td>306.83</td>
<td>98.27</td>
<td>200.12</td>
<td>47.37</td>
</tr>
</tbody>
</table>
and 48 hours of germination, which enhanced it. At 72 hours of germination, -1 and -5 NaCl raised DNA content; all other osmotica lowered it. At 96 hours, -1 and -5 NaCl and -1 mannitol increased and the rest of osmotica lowered it. Thus, -1 NaCl had a consistent increasing effect on DNA content.

DNA content of cotyledon (Plate 16.2): decreased from 24 to 72 hours but again at 96 hours, cotyledon of all seedlings registered a rise in DNA content. All osmotica reduced it and maximum reduction was caused by -5 PEG. DNA content of cotyledon was much less than that of embryo axis.

RNA content of embryo axis (Plate 15.1): fluctuated with time. It increased at 48 hours, decreased at 72 hours and again increased at 96 hours except in -5 mannitol and -5 NaCl. RNA content showed a rising trend. The osmotic effects were variable. Thus the RNA content was increased by -1 NaCl and -1 mannitol and -1 PEG, although mannitol at 48 hours and PEG at 24 hours caused reduction in it. -5 NaCl increased it while -5 mannitol and -5 PEG lowered it. On the whole, -5 PEG caused a greater reduction in RNA content.

RNA content of cotyledon (Plate 16.1): increased at 48 hours and then decreased at 72 hours; it again rose at 96
GUAR CV. NAU BAHAR

GERMINATION - HOURS
PLATE - 15

Effects of Osmotica on
1. RNA content of embryo axis
2. DNA content and
3. RNase activity
during germination.
Effects of Osmotica on
1. RNA content of cotyledon
2. DNA content and
3. RNase activity
during germination.
hours. The effects of osmotica were not conspicuous on RNA content; however, -1 NaCl caused a slight enhancement of RNA. -1 and -5 mannitol, -5 PEG reduced it up to 72 hours, after which there was no effect. -5 NaCl up to 48 hours had no marked effect, but subsequently it lowered RNA content, at 72 hours and subsequently it sharply increased. -5 mannitol and PEG had a lowering effect on RNA content, the effect being greater by -5 PEG.

**RNase activity** of embryo axis (Plate 15.3) of all seedlings increased with germination, osmotic effects varied. Thus, -1 NaCl stimulated it; -1 mannitol depressed it and -1 PEG stimulated it from 48 hours onward. -5 NaCl stimulated the RNase activity at 96 hours; otherwise it depressed the activity. Bot -5 mannitol and PEG depressed the RNase activity throughout the germination.

**RNase activity** of cotyledon (Plate 16.3): was much less as compared to that of embryo axis. It exhibited a rising pattern. Osmotic effects were less conspicuous up to 48 hours of germination, especially -1 osmotica -5 osmotica depressed the activity. During the later period of germination, the osmotica depressed the RNase activity, except -1 mannitol which stimulated it at 96 hours.

**Catalase activity** of embryo axis (Plate 17.1): exhibited a rising trend except in -1 NaCl and -5 mannitol. Osmotic
effects varied. -1 NaCl stimulated it up to 48 hours of germination but later it depressed the enzymic activity. Except a stimulation at 24 hours, -1 mannitol depressed the enzymic activity. Seedlings in -1 PEG showed depressed enzymic activity up to 48 hours of germination. Later on, however, the enzymic activity was stimulated by this osmoticum. All -5 osmotica depressed the catalase activity, the depression was minimum by -5 NaCl and maximum by -5 PEG.

Catalase activity of cotyledon (Plate 17.2): was lower than that of embryo axis up to 72 hours, but at 96 hours it was more than that of embryo axis. The activity showed an increasing trend. Enzymic activity was stimulated by -1 NaCl and mannitol stimulation being greater by -1 mannitol. -1 PEG stimulated it only at 24 hours otherwise, it depressed the activity. All -5 osmotica depressed the activity and depression was least in -1 NaCl. -5 PEG caused maximum depression.

Peroxidase activity of embryo axis (Plate 17.3): followed a pattern of rise and fall. Thus, it increased at 48 hours of germination and decreased at 72 hours; again it rose at 96 hours of germination. Effects of osmotic stress were not consistent -1 NaCl stimulated it; -1 mannitol stimulated it at 48 and 96 hours of germination; otherwise
Effects of Omectica on
1. Catalase activity of embryo axis
2. Cotyledon
3. Peroxidase activity of embryo axis and Cotyledon
during germination.
it depressed enzymic activity. -1 PEG initially depressed activity and later on stimulated it. -5 NaCl depressed the activity at 24 hours, but subsequently stimulated it. -5 mannitol consistently depressed the enzymic activity, a depressed activity was seen up to 48 hours and subsequently a stimulated one in the case of -5 PEG.

Peroxidase activity of cotyledon (Plate 17.4) : was very low as compared to embryo axis. The activity exhibited an increasing trend; except in D.W. -1 and -5 PEG it declined at 96 hours. The enzymic activity was stimulated by -1 NaCl depressed by -1 PEG and in mannitol. It was depressed at 72 hours but stimulated at 48 and 96 hours. -5 mannitol and PEG depressed the activity while in the case of NaCl, also, it was depressed except at 96 hours when it was stimulated.

Statistical analysis of the data (Table 8) :

The observations presented in this experiment have been analyzed so as to confirm this findings statistically for protein, protease, total sugars, reducing sugar, invertase, DNA, RNA, RNase, catalase and peroxidase activities in both embryo axis as well as cotyledon of Guar seedlings. Degree of freedom, variance and F.value for factors germination period, osmotica and interaction with germination period and osmotica are tabled in
summarized form. The validity of the statistically analyzed data has been tested for significance at 1% and 5% level.

In case of embryo axis, data on protein, protease, total sugars, reducing sugar, invertase, RNA, RNase and peroxidase activities were significant at 1% level for all osmotica, while in catalase there was no effect of NaCl. Germination period was also significant at 1% level for all the above. Interaction between germination period and osmotica was significant at 1% level for protein, protease, total sugars, invertase, RNA, catalase and peroxidase while at 5% level of reducing sugar in NaCl. Interaction in the case of DNA (PEG and NaCl) and RNase was not significant in all osmotica.

Data on cotyledon reveal that they were significant at 1% level for germination period for all osmotica, except RNA in mannitol which was significant at 5% level. All osmotica were significant at 1% level except NaCl. There was no significant effect of PEG for total sugars and mannitol for RNA. Briefly, NaCl did not show any significant effect. Interaction with germination period and osmotica was at 1% level significant, but with protein, total sugars and RNA in NaCl, peroxidase and DNA in mannitol and protein and DNA in PEG were significant at 5% level. While there were no significant effect with
### Table 1

Analysis of variance of data of biochemical analysis in soybean axis and cotyledon in Gen Cvs. Bas Bahar during germination in osmotic stress

protease, invertase and DNA in NaCl, RNA in PEG and reducing sugar and RNA in mannitol. RNase was not significant effect with interaction in all osmotica. Thus, the findings reported here are highly significant for the germination period and effect of osmotica as well as its interaction.
Experiment - II: Effect of desiccation on biochemical changes during germination of Guar and pretreated seeds of Oat.

Graded seeds of Oat Var. N.P. Hyb-1 were pretreated with the solution of GA-10 and 50 ppm, Kinetin-1 and 5 ppm and CCC-100 and 250 ppm and untreated seeds were kept as control. Control and pretreated seeds of Oat were germinated upto 96 hours at room temperature (28±2°C). Extension growth, fresh and dry weights and % moisture content of embryo axis and endosperm were recorded at 24 hourly interval upto 96 hours.

For the desiccation experiment, pretreatment of Oat seeds was restricted to GA-50 ppm, kinetin-1 ppm and CCC-100 ppm. Pretreated seeds of Oat were germinated upto 96 hours and to desiccation treatment for two periods - 24 and 96 hours then taken out from desiccator and estimated it.

In case of Guar Cv. Nau Bahar, untreated seeds were germinated in D.W. upto 96 hours and to desiccation treatment for two periods - 24 and 96 hours.

For Oat, seedling growth was measured for root and shoots, while carbohydrate metabolism was estimated from embryo axis and endosperm separately.

Seedling growth: Root length (Plate 13.1): The extension growth of roots increased with progress of germination.
Throughout the germination, GA pretreated seeds had larger root length, which was maximum in 50 ppm GA. Kinetin 1 ppm treatment enhanced root growth only up to 48 hours of germination after which it retarded the root growth; 5 ppm kinetin enhanced root growth only at 24 hours of germination, after which it retarded, CCC pretreatment - 100 ppm also enhanced root growth except at 72 hours; CCC 250 ppm pretreatment except at 24 hours enhancement caused retardation of root growth.

Shoot length (Plate 18.2): Extension growth of shoot increased with time. All pretreatments caused enhancement of shoot length; GA treatment caused maximum enhancement followed by CCC 100 ppm and kinetin 1 ppm in kinetin shoot length. It may be noted that kinetin treated seedlings were succulent.

Fresh weight of embryo axis (Plate 18.5): increased as germination advanced. Except GA, all pretreatments lowered fresh weights of embryo axis, GA 50 ppm caused maximum fresh weight, while kinetin 5 ppm caused maximum reduction of fresh weight.

Fresh weight of endosperm (Plate 18.6): followed a declining trend. Endosperms of pretreated seedlings had lesser fresh weights compared to control. Endosperm of kinetin treated seedlings 5 ppm and CCC 250 ppm seedlings
Effects of pretreatments on
Lengths of (1) Root and (2) Shoot
Dry weights of (3) embryo axis and (4) endosperm
Fresh weights of (5) embryo axis and (6) endosperm
% moisture content of (7) embryo axis and (8) endosperm
had least fresh weights.

Dry weight of embryo axis (Plate 18.3): increased as the germination advanced. No conspicuous differences in dry weights due to pretreatments are discerned.

Dry weight of endosperm (Plate 18.4): went on declining with time. Endosperm of control seedlings showed minimum dry weight, followed by CCC treated seedlings, at 96 hours of germination. Endosperm of other pretreated seedlings had more dry weights.

Percent moisture content of embryo axis (Plate 18.7): followed a rising trend. Pretreatment did not show any marked effect on percent moisture content of embryo axis except that GA treated seedlings had lower moisture content initially;

Percent moisture content of endosperm (Plate 18.8): increased as germination progressed. All pretreatments lowered moisture content more or less equally.

Starch content of endosperm (Plate 19.3): of D.W. i.e., control seedlings and pretreated seedlings depleted as germination advanced. All pretreated seedlings had less starch content compared to control ones. On the whole, GA treated seedlings followed by CCC treated ones had lesser starch content.
24 hours desiccation treatment caused more starch depletion than 96 hours desiccation treatment in all seedlings. Starch depletion was faster at 24 hours desiccation treatment. In the case of CCC treated seedlings followed by GA and then kinetin and D.W. Depletion of starch, however, was faster in GA treated seedlings subjected to 96 hours desiccation, followed by kinetin and CCC. Thus, all pretreatments caused a greater depletion of starch as compared to D.W.

Amylase activity of embryo axis (Plate 19.1): of control seedlings increased up to 72 hours and then declined. In the GA treated seedlings, the activity fluctuated, a discerning trend is observed for amylase activity, in embryo axis of kinetin treated seedlings. Embryo axis of CCC treated seedlings showed a declining trend for the enzymic activity up to 72 hours after which it rose. Higher amylase activity was seen in the case of embryo axis of GA and CCC treated seedlings.

Desiccation treatment stimulated amylase activity of all seeds i.e. GA, CCC and kinetin treats. Control, desiccation treatment seedlings which was greater by 96 hours.

CCC treated seedlings showed maximum embryonal enzymic activity when desiccated to 96 hours followed by D.W., GA and kinetin.
Effects of pretreatment and desiccation on
1. Amylase activity of embryo axis
2. Endosperm and
3. Starch content of endosperm
during germination.
Amylase activity of endosperm (Plate 19.2): of control seedlings registered a continuous rise; the activity of endosperm of GA treated seedlings increased up to 72 hours of germination and fell thereafter. In the case of endosperm of kinetin treated seedlings, the amylase activity registered a continuous decline, while the amylase activity of CCC treated seedlings fluctuated between fall and rise.

Considering the effects of desiccation treatment, it is seen that both desiccation treatments stimulated amylase activity which was more by 96 hours desiccation treatment in all the seedlings. Maximum stimulation due to desiccation was seen in the case of endosperm of control seedlings, followed by CCC treated seedlings. The stimulation by desiccation was least in GA treated seedlings. In the case of non-desiccated seedlings, maximum enzymic activity was seen in control seedlings and minimum in the case of GA treated seedlings.

Total sugar content of embryo axis (Plate 20.2): of control seedlings and kinetin treated seedlings showed an increasing trend with time. In the case of GA treated seedlings, sugars after an initial decrease, went on increasing. In the CCC treated seedlings sugars went on increasing up to 72 hours of germination after which they decreased.
The effect of pretreatment on sugar concentrations were not consistent; on the whole, kinetin and CCC treatments enhanced sugars; GA treatment caused maximum enhancement at 24 and 96 hours of germination.

Both desiccation treatments enhanced sugar concentrations and the enhancement was more by 96 hours desiccation treatment, maximum sugars were found during 24 and 96 hours desiccation treatment in CCC treated seedlings; followed by D.W. or control seedlings and kinetin treated seedlings. GA treated seedlings showed large sugars also during desiccation.

Total sugars content of endosperm (Plate 21.2) : of control and CCC treated seedlings registered a continuous rise, the concentration of sugars of endosperm of GA treated seedlings increased up to 72 hours and thereafter declined. Sugar concentrations of kinetin treated seedlings after an initial slight fall, increased. Effects of pretreatment on sugars were in consistent. At 24 hours, all pretreatment enhanced sugars being maximum by kinetin. At 48 hours only GA caused enhancement. Kinetin and CCC lowered sugars. All treatment lowered sugar and 72 and 96 hours.

Considering the effect of desiccation treatment, it is seen that sugars were enhanced by that treatment. Desiccation treatment of 96 hours caused more enhancement.
Effects of pretreatment and desiccation on
1. Invertase activity of embryo axis
2. Total sugar content and
3. Reducing sugar content
during germination.
Effects of pretreatment and desiccation on
1. Invertase activity of endosperm
2. Total sugar content and
3. Reducing sugar content
during germination.

PLATE - 21
Both during 24 and 96 hours desiccation treatment GA caused maximum enhancement of sugars, followed by CCC and kinetin.

Reducing sugars (RS) of embryo axis (Plate 20.3): of control seedlings after an initial rise went on decreasing. Reducing sugar of embryo axis of GA treated seedlings increased up to 72 hours and decreased thereafter. Reducing sugars of embryo axis of both kinetin and CCC treated seedlings followed a declining trend. CCC and kinetin pretreatment caused enhancement of sugars while GA pretreatment lowered Reducing Sugar concentrations.

Sugar concentrations were increased by desiccation treatments in embryo axis of all seedlings, the increase was more by 96 hours desiccation. CCC pretreatment caused enhancement of Reducing Sugar also during desiccation treatments, followed by kinetin and GA.

Reducing Sugars (RS) of endosperm (Plate 21.3): of all seedlings except GA treated followed an increasing trend. Reducing sugars of endosperm of GA treated seedlings also increased up to 72 hours after which they declined. Reducing sugar concentrations were enhanced by desiccation treatments, however, responses were varied according to time and treatment. Thus, there was more enhancement of Reducing Sugar of control seedling by 24 hours desiccation than 96 hours.
In the case of GA, kinetin and CCC pretreatment seedlings, reducing sugar concentrations were enhanced in endosperm by 96 hours and maximum reducing sugars were, on the whole, more in desiccated GA seedlings. However, in the case of CCC and kinetin treated seedlings germinated at 96 hours, 24 hours desiccation treatment caused more enhancement of sugars compared to 96 hours desiccation.

Non reducing sugar (NRS) of embryo axis (Table 3) of seedlings in D.W., kinetin treated increased as germination advanced. NRS of GA treated seedlings fell sharply, remained steady and again rose to a high level; those of CCC treated seedlings increased up to 72 hours and declined subsequently. Pretreatments increased NRS. Desiccation treatments of control seedlings enhanced NRS concentration. In GA treated seedlings, NRS of embryo axis were lowered by 24 hours desiccation but enhanced by 96 hours desiccation. Kinetin treated seedlings during desiccation, generally, had more NRS. Sugars were enhanced in embryo axis of CCC treated seedlings, CCC treated seedlings had maximum concentration of sugars when desiccated at 96 hours.
Non-Reducing Sugar content of endosperm (Table 4) of control seedling increased with time; of GA treated seedlings after an initial increase, declined; NRS of endosperm of kinetin treated seedlings fell at 48 hours; then substantially increased and remained steady, while in CCC treated seedlings fell at 48 hours and thereafter increased. Effects of pretreatment on NRS concentrations were inconsistent. Desiccation treatment of 24 hours, on the whole, lowered NRS concentration of control seedlings except at 24 hours germination, when it enhanced. 96 hours desiccation treatment enhanced NRS of endosperm. Enhancing effects on NRS concentrations were discerned by desiccation treatments to the endosperm of GA treated seedlings. The enhancement being more by 96 hours desiccation. 24 hours desiccation treatment lowered NRS content of endosperm of kinetin treated seedlings. On the contrary, 96 hours desiccation treatment enhanced them. NRS of CCC treated endosperm, responded differently with desiccation treatment 24 hours desiccation treatment, on the whole lowered NRS, while 96 hours desiccation treatment enhanced them.
Table 3

Effects of pretreatment and desiccation on Non-Reducing Sugar Content mg/gr. dry wt. of embryo axis oat during germination

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<tr>
<th>Germination hours</th>
<th>Untreated D.W.</th>
<th>Treated GA 50 ppm</th>
<th>Treated K 1 ppm</th>
<th>Treated CCC 100 ppm</th>
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<td>Control</td>
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<td>140.91</td>
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<td>72</td>
<td>25.07</td>
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<td>57.91</td>
<td>6.45</td>
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<td>18.26</td>
<td>41.49</td>
<td>34.88</td>
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<td>3.45</td>
<td>23.47</td>
<td>38.83</td>
</tr>
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<td>96</td>
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<td>160.04</td>
<td>53.52</td>
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<td>27.87</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17.06</td>
<td>171.74</td>
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</tbody>
</table>
Effects of pretreatment and desiccation on Non-Reducing Sugar Content mg/gr.dry wt. of endosperm of * during germination

### Table 4

Effects of pretreatment and desiccation on Non-Reducing Sugar Content mg/gr. dry wt. of endosperm of * during germination

<table>
<thead>
<tr>
<th>Germination hours</th>
<th>Untreated D.W.</th>
<th>Treated GA 50 ppm</th>
<th>Treated K 1 ppm</th>
<th>Treated CCC 100 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>24 hrs. desi.</td>
<td>96 hrs. desi.</td>
<td>24 hrs. desi.</td>
</tr>
<tr>
<td>24</td>
<td>1.44</td>
<td>15.89 36.67</td>
<td>10.24</td>
<td>24.68 42.69</td>
</tr>
<tr>
<td>48</td>
<td>15.12</td>
<td>9.84 57.91</td>
<td>22.92</td>
<td>28.27 34.68</td>
</tr>
<tr>
<td>72</td>
<td>21.41</td>
<td>10.65 27.44</td>
<td>20.47</td>
<td>13.84 85.99</td>
</tr>
<tr>
<td>96</td>
<td>38.30</td>
<td>25.47 75.13</td>
<td>15.88</td>
<td>20.27 102.36</td>
</tr>
</tbody>
</table>
Invertase activity of embryo axis (Plate 20.1) of control and kinetin treated seedlings followed a declining pattern. In the embryo axis of GA treated seedlings, enzymic activity declined up to 72 hours; after which it rose. The enzymic activity of CCC treated seedlings initially rose after which, the activity declined. The enzymic activity was lowered by all the pretreatments, being lowest by GA.

Desiccation treatments depressed the enzymic activity which was greater by 96 hours of treatment; however, in kinetin treated seedlings, the enzymic activity was stimulated by desiccation treatment, which was more by 24 hours.

Invertase activity of endosperm (Plate 21.1) of control seedlings fluctuated with rise and fall, that of endosperm of kinetin and CCC treated seedlings followed a decline and rise pattern; while in the case of GA treated seedlings, it consistently declined.

24 hours desiccation treatments stimulated the invertase activity only at 24 hours germination of control seedlings, 96 hours desiccation did not cause any pronounced effect. Desiccation treatment of both 24 and 96 hours stimulated invertase activity of endosperm of GA treated seedlings, while in CCC treated seedlings, only 24 hours desiccation stimulated the enzymic activity. The response of kinetin treated seedlings also varied. There
was a slight stimulation of invertase activity in seedlings germinated at 24 and 48 hours subjected to desiccation; during later period of germination, there was a depression effect of desiccation.

For Guar, the following metabolites and enzymic activities were estimated from embryo axis and cotyledon separately.

Total sugars content of embryo axis (Plate 22.3): rose at 48 hours; declined at 72 hours and again increased at 96 hours of germination. Desiccation treatments lowered sugar concentrations, 96 hours desiccation treatment caused more reduction of sugar concentration than 24 hours.

Total sugars content of cotyledons (Plate 22.4): also increased at 48 hours; decreased at 72 hours and rose again at 96 hours of germination. Responses of sugars to desiccation treatments, however, varied. While, 24 hours desiccation treatment caused an enhancement of sugars, 96 hours treatment caused a reduction of sugar concentrations.

Reducing sugar (RS) of embryo axis (Plate 22.5): initially increased, then fell and again rose. Enhancement in Reducing Sugar concentration was caused by desiccation treatments in seedlings up to 72 hours germination. However, desiccation treatment of seedlings at 96 hours of germination caused reduction in Reducing sugar. Enhancement was more by
Effects of desiccation on
Invertase activity of (1) embryo axis and (2) cotyledon
Total sugar content of (3) embryo axis and (4) cotyledon
Reducing sugar content of (5) embryo axis and (6) cotyledon
24 hours desiccation, while reduction was more by 96 hours desiccation.

**Reducing sugar** content of cotyledon (Plate 22.6): increased with time. Desiccation treatment enhanced them considerably the enhancement being more by 2\(b\) hours desiccation.

**Non-Reducing sugar** content (NRS) of embryo axis (Table 5): of the control seedlings increased up to 48 hours of germination and then decreased. Desiccation treatment of 24 hours and 96 hours decreased the NRS content, and decrease was more by 96 hours of desiccation.

**Non-Reducing sugar** content (NRS) of cotyledon (Table 5): of the content seedlings slightly increased at 48 hours then decreased at 72 hours and again increased. Desiccation treatment of 24 hours and 96 hours decreased the NRS content and the decrease was more by 96 hours of desiccation. Desiccation treatment lowered the NRS content.

**Invertase activity** of embryo axis (Plate 22.1): continuously increased with time. It was severely depressed by 24 hours of desiccation treatment. 96 hours desiccation also depressed the enzymic activity.

**Invertase activity** of cotyledon (Plate 22.2): increased at 48 hours, then slightly fell and again increased almost two fold at 96 hours of germination. The response of the
<table>
<thead>
<tr>
<th>Germination hours</th>
<th>Embryo axis</th>
<th>Endosperm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Undesi.</td>
<td>24 hours</td>
</tr>
<tr>
<td>24</td>
<td>242.91</td>
<td>92.0</td>
</tr>
<tr>
<td>48</td>
<td>500.17</td>
<td>209.56</td>
</tr>
<tr>
<td>72</td>
<td>450.15</td>
<td>120.74</td>
</tr>
<tr>
<td>96</td>
<td>308.32</td>
<td>92.32</td>
</tr>
</tbody>
</table>

des, of embryo axis and cotyledon of *Guar* during germination
activity to desiccation was variable, while 24 hours desiccation treatment depressed the activity 96 hours desiccation treatment stimulated it.

Protein content of embryo axis (Plate 23.3): increased at 48 hours and subsequently it declined. Desiccation treatments caused a marked reduction of protein content; and the reduction was more by 24 hours desiccation.

Protein content of cotyledon (Plate 23.4): initially declined, increased at 72 hours and decreased again at 96 hours of germination. Desiccation treatments of 24 hours increased protein concentration, except in seedlings of 72 hours germination where it caused a decline; the 96 hours desiccation treatment raised protein content up to 48 hours of germination after which it caused a decrease in amount of protein content.

Protease activity of embryo axis (Plate 23.1): showed an increasing trend. Desiccation treatments at 24 hours and 48 hours germinated period, stimulated the protease activity, the stimulation being greater by 96 hours desiccation. Desiccation treatments, on the contrary, depressed the enzymic activity at 72 and 96 hours germination period and the depression was greater by 24 hours desiccation.
Effects of desiccation on

1. Protease activity of embryo axis
2. Cotyledon
3. Protein content of embryo axis
4. Cotyledon
5. Catalase activity of embryo axis
6. Cotyledon
7. Peroxidase activity of embryo axis
8. Cotyledon
Protease activity of cotyledons (Plate 23.2): initially increased, decreased at 72 hours, and again rose at 96 hours of germination. Desiccation treatments stimulated the protease activity and the stimulation was greater by 96 hours desiccation.

Catalase activity of embryo axis (Plate 23.5): went on increasing up to 72 hours and then it declined. The enzyme activity was stimulated by desiccation treatments and stimulation was quite high by 96 hours desiccation.

Catalase activity of cotyledon (Plate 23.6): also exhibited a rising trend. It was also stimulated by desiccation treatment, however, unlike embryo axis, the stimulation was very high by 24 hours desiccation than by 96 hours.

Peroxidase activity of embryo axis (Plate 23.7): After an increase at 48 hours of germination, the activity declined at 72 hours and increased again at 96 hours. The activity was depressed by desiccation treatment in the case of seedlings at 24 and 48 hours germination. It was, however, stimulated in the embryo axis of seedlings at 72 and 96 hours germination.

Peroxidase activity of cotyledon (Plate 23.8): increased up to 72 hours of germination and decreased thereafter. It was stimulated by desiccation and the stimulation was
greater by 96 hours desiccation.

Statistical analysis of data (Table 9C):

The observations presented in this experiment have been analysed by the method of analysis of variance for protein, protease, total sugars, reducing sugar, invertase, catalase and peroxidase activities in both embryo axis as well as cotyledon. The summarized data for degree of freedom, variance and F. value are presented here for these estimations. The significance of data has been tested at 1% and 5% level.

The findings reported here are highly significant for the period of germination, desiccation treatment and its interaction with germination period and desiccation treatment are significant at 1% level. All data are statistically higher significant in embryo axis and cotyledon as far as germination period and desiccation treatment are concerned.
Experiment - III: Effect of low temperature on biochemical change during germination of Oat and Guar.

The seeds of Oat Var. N.P. Hyb. 1 and Guar Cv. Nau Bahar were germinated in Petridishes lined with filter paper (Whatman No. 1) in D.W. at room temperature (28±2°C) in normal daylight up to 96 hours of germination and subjected to low temperature treatment for two periods - 24 hours and 96 hours. The seedlings were placed into a freeze chamber (0±2°C) in a refrigerator up to 96 hours then taken out after 24 and 96 hours of low temperature period from freeze chamber. The following metabolites and enzymatic activities were estimated from embryo axis and endosperm/cotyledon separately.

For Oat, the following estimations were carried out.

**Starch content**: was absent in the case of the embryo axis throughout the experimental period. Starch content of endosperm (Plate 25.3) of the control seedlings went on declining as germination advanced. Low temperature treatment of 24 hours of germination caused a depletion of starch content which was further depleted by 96 hours treatment.

**Amylase activity** of embryo axis (Plate 25.1) increased with germination. Low temperature treatment depressed the enzymic
activity which was greater by 24 hours treatment than 96 hours treatment.

Amylase activity of endosperm (Plate 25.2): also went on increasing as germination advanced. The activity under low temperature was depressed, and the depression was much more at 96 hours treatment. The activity, however, was stimulated in the case of seedlings of 24 hours subjected to 24 hours low temperature.

Total sugar content of embryo axis (Plate 24.3): increased with time. Low temperature treatments enhanced sugar concentrations, the enhancement being more by 96 hours treatment.

Total sugar content of endosperm (Plate 24.4): the total sugars increased with time. Low temperature treatments raised sugar concentrations; 24 hours treatment caused more enhancement of sugar as compared to 96 hours.

Reducing sugar (RS) content of embryo axis (Plate 24.5): showed a rising trend. Low temperature treatments caused enhancement of Reducing Sugar concentrations, the enhancement being more by 96 hours treatment.

Reducing Sugar (RS) content of endosperm (Plate 24.6): also increased with time as in the embryo axis. Low temperature
OAT VAR. N.P. HYBRID

GERMINATION - HOURS

PLATE - 24

Effects of low temperature on
Invertase activity of (1) embryo axis and (2) endosperm
Total sugar content of (3) embryo axis and (4) endosperm
Reducing sugar content of (5) embryo axis and (6) endosperm
treatments enhanced Reducing Sugar content, but unlike embryo axis, 24 hours treatment caused more enhancement than 96 hours.

Non-reducing Sugar (NRS) content of embryo axis (Table 6a): of seedlings were lowered by 24 hours low temperature treatment. They were also lowered by 96 hours low temperature at 24 and 48 hours of germination but enhanced them manifold at 72 and 96 hours of germination.

Non-reducing sugar (NRS) content of endosperm (Table 6a): seedlings were enhanced by low temperature treatments; 96 hours treatment caused more enhancement.

Invertase activity of embryo axis (Plate 25.1) of control seedlings continuously fell with time. It was much depressed by low temperature treatments, there being no difference due to duration of low temperature i.e. 24 or 96 hours.

Invertase activity of endosperm (Plate 25.2): of control seedlings also exhibited a declining trend. It was also depressed by low temperature treatment.

Catalase activity of embryo axis (Plate 25.4): went on rising up to 72 hours and then declined. Low temperature treatment caused a depression of the enzymic activity, the depression being greater by 96 hours treatment.
## Table 6

Effects of low temperature on Non-Reducing Sugar Content
mg/gr. fr. wt. of embryo axis and endosperm of Oat Var. N.P.
Hybrid and Guar - embryo and cotyledon during germination

<table>
<thead>
<tr>
<th>Germination hours</th>
<th>Control 24 hours</th>
<th>Control 96 hours</th>
<th>24 hours low temp.</th>
<th>96 hours low temp.</th>
<th>Endosperm 24 hours low temp.</th>
<th>96 hours low temp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Oat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Embryo axis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>10.95</td>
<td>4.24</td>
<td>8.65</td>
<td>2.41</td>
<td>28.67</td>
<td>19.46</td>
</tr>
<tr>
<td>48</td>
<td>11.08</td>
<td>3.03</td>
<td>4.24</td>
<td>16.66</td>
<td>19.07</td>
<td>23.06</td>
</tr>
<tr>
<td>72</td>
<td>8.26</td>
<td>7.04</td>
<td>32.68</td>
<td>5.91</td>
<td>7.44</td>
<td>17.46</td>
</tr>
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<td>96</td>
<td>13.85</td>
<td>12.24</td>
<td>122.80</td>
<td>10.89</td>
<td>7.85</td>
<td>19.46</td>
</tr>
<tr>
<td>(b) Guar</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Embryo axis</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>50.48</td>
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<td>59.07</td>
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<td>35.46</td>
<td>15.86</td>
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<tr>
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<td>3.82</td>
<td>46.49</td>
<td>44.28</td>
<td>1.81</td>
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<tr>
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<td>28.67</td>
<td>3.44</td>
<td>0.60</td>
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<td>24.67</td>
<td>3.23</td>
</tr>
<tr>
<td>96</td>
<td>17.05</td>
<td>9.03</td>
<td>1.43</td>
<td>59.30</td>
<td>15.45</td>
<td>1.42</td>
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</table>
Effects of low temperature on
amyrase activity of (1) embryo axis and (2) endosperm
Starch content of (3) endosperm
Catalase activity of (4) embryo axis and (5) endosperm
Peroxidase activity of (6) embryo axis and (7) endosperm
Catalase activity of endosperm (Plate 25.5): also rose upto 72 hours and then declined. Low temperature stimulated the activity and stimulation was greater by 96 hours treatment.

Peroxidase activity of embryo axis (Plate 25.6): of control seedlings showed a slight rise as germination advanced. Low temperature treatment depressed the activity; 96 hours treatment caused more depression of the enzymic activity.

Peroxidase activity of endosperm (Plate 25.7): increased upto 72 hours of germination and declined thereafter. Low temperature treatment depressed the enzymic activity which was more by 24 hours of germination.

Statistical analysis of the data (Table 9A):

The result presented in this experiment have been analyzed by the method of analysis of variance for starch, amylase, total sugars, reducing sugar, invertase, catalase and peroxidase activity in embryo axis and endosperm. The summarised data for degree of freedom, variance and F. value are presented for above estimations. The significance of data has been tested at 1% and 5% level.

Starch, amylase, total sugars, invertase, peroxidase and catalase activities are significant at 1% level except reducing sugar which is not significant in embryo axis for germination, while in endosperm significant at 1% level for
### Table 9

#### Analysis of variance of data of biochemical analysis in embryo axis and endosperm/cotyledon in oat and quen during germination in low temperature/destoration (laser) treatment

<table>
<thead>
<tr>
<th>Factor</th>
<th>D.F.</th>
<th>Variance (low temperature treatment)</th>
<th>F-value</th>
<th>Variance (low temperature treatment)</th>
<th>F-value</th>
<th>Variance (low temperature treatment)</th>
<th>F-value</th>
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<th>F-value</th>
<th>Variance (low temperature treatment)</th>
<th>F-value</th>
<th>Variance (low temperature treatment)</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
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<td></td>
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<tr>
<td><strong>SHBB10 AXIB</strong></td>
<td>1</td>
<td>Sugar</td>
<td>267.4</td>
<td>129.4</td>
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<td>19.2</td>
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<td>297.7</td>
<td>34.4</td>
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</tr>
<tr>
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<td>Sugar</td>
<td>158.0</td>
<td>87.9</td>
<td>15.2</td>
<td>0.5</td>
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<td>87.9</td>
<td>158.0</td>
<td>87.9</td>
<td></td>
</tr>
<tr>
<td><strong>O x Low temp.</strong></td>
<td>1</td>
<td>Sugar</td>
<td>279.6</td>
<td>162.4</td>
<td>15.2</td>
<td>0.5</td>
<td>357.1</td>
<td>19.2</td>
<td>357.1</td>
<td>19.2</td>
<td>357.1</td>
<td>19.2</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>2</td>
<td>Sugar</td>
<td>279.6</td>
<td>162.4</td>
<td>15.2</td>
<td>0.5</td>
<td>357.1</td>
<td>19.2</td>
<td>357.1</td>
<td>19.2</td>
<td>357.1</td>
<td>19.2</td>
<td></td>
</tr>
</tbody>
</table>

#### Significance

- * Significant at 1% level
- ** Significant at 5% level

---

### Table 8

#### Analysis of variance of data of biochemical analysis in embryo axis and endosperm/cotyledon in oat and quen during germination in low temperature/destoration (laser) treatment

<table>
<thead>
<tr>
<th>Factor</th>
<th>D.F.</th>
<th>Variance (destoration (laser))</th>
<th>F-value</th>
<th>Variance (destoration (laser))</th>
<th>F-value</th>
<th>Variance (destoration (laser))</th>
<th>F-value</th>
<th>Variance (destoration (laser))</th>
<th>F-value</th>
<th>Variance (destoration (laser))</th>
<th>F-value</th>
<th>Variance (destoration (laser))</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Protein</td>
<td>91.6</td>
<td>57.8</td>
<td>15.2</td>
<td>0.5</td>
<td>158.0</td>
<td>87.9</td>
<td>158.0</td>
<td>87.9</td>
<td>158.0</td>
<td>87.9</td>
<td></td>
</tr>
<tr>
<td><strong>O x Low temp.</strong></td>
<td>1</td>
<td>Protein</td>
<td>279.6</td>
<td>162.4</td>
<td>15.2</td>
<td>0.5</td>
<td>357.1</td>
<td>19.2</td>
<td>357.1</td>
<td>19.2</td>
<td>357.1</td>
<td>19.2</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>2</td>
<td>Protein</td>
<td>279.6</td>
<td>162.4</td>
<td>15.2</td>
<td>0.5</td>
<td>357.1</td>
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<td>357.1</td>
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<td>357.1</td>
<td>19.2</td>
<td></td>
</tr>
</tbody>
</table>

#### Significance

- * Significant at 1% level
- ** Significant at 5% level

---

### Table 7

#### Analysis of variance of data of biochemical analysis in embryo axis and endosperm/cotyledon in oat and quen during germination in low temperature/destoration (laser) treatment

<table>
<thead>
<tr>
<th>Factor</th>
<th>D.F.</th>
<th>Variance (destoration (laser))</th>
<th>F-value</th>
<th>Variance (destoration (laser))</th>
<th>F-value</th>
<th>Variance (destoration (laser))</th>
<th>F-value</th>
<th>Variance (destoration (laser))</th>
<th>F-value</th>
<th>Variance (destoration (laser))</th>
<th>F-value</th>
<th>Variance (destoration (laser))</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Protein</td>
<td>91.6</td>
<td>57.8</td>
<td>15.2</td>
<td>0.5</td>
<td>158.0</td>
<td>87.9</td>
<td>158.0</td>
<td>87.9</td>
<td>158.0</td>
<td>87.9</td>
<td></td>
</tr>
<tr>
<td><strong>O x Low temp.</strong></td>
<td>1</td>
<td>Protein</td>
<td>279.6</td>
<td>162.4</td>
<td>15.2</td>
<td>0.5</td>
<td>357.1</td>
<td>19.2</td>
<td>357.1</td>
<td>19.2</td>
<td>357.1</td>
<td>19.2</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>2</td>
<td>Protein</td>
<td>279.6</td>
<td>162.4</td>
<td>15.2</td>
<td>0.5</td>
<td>357.1</td>
<td>19.2</td>
<td>357.1</td>
<td>19.2</td>
<td>357.1</td>
<td>19.2</td>
<td></td>
</tr>
</tbody>
</table>

#### Significance

- * Significant at 1% level
- ** Significant at 5% level
The interaction between germination period and low temperature are significant at 1% level in starch, amylase, invertase and catalase activity in embryo axis and endosperm while reducing sugar is at 5% level significant in embryo axis and endosperm; in peroxidase at 5% level for embryo axis. While for total sugar was not significant in endosperm. Thus, low temperature treatment is significantly effective in metabolic activities.

For Guar, the following metabolites and enzymic activities were estimated from embryo axis and cotyledon separately.

**Total sugar content of embryo axis (Plate 26.3)**: of the seedlings decreased up to 72 hours of germination and then slightly increased. Low temperature treatment at 24 hours caused a decrease in the sugar content of all the seedlings. The low temperature treatments of 96 hours enhanced sugar in the case of seedlings of 24 and 48 hours of germination; this treatment lowered the sugar, in the case of embryo axis of seedlings of 72 and 96 hours.

**Total sugar content of cotyledon (Plate 26.4)**: As in the case of the embryo axis, total sugars of the cotyledon declined up to 72 hours and then increased. 24 hours of low temperature treatment enhanced sugars at 24 and 48 hours while lowered them at 72 and 96 hours germination. Low
temperature treatment of 96 hours also caused a decrease in sugar concentrations and decrease was greater than by 24 hours low temperature treatment. However, seedlings of 24 hours germination had more sugars during this treatment.

Reducing sugar content of embryo axis (Plate 26.5): of the control seedlings decreased upto 72 hours of germination and then increased. Low temperature treatment of 24 hours enhanced the Reducing Sugar content upto 72 hours of germination while decreased them in 96 hours seedling. 96 hours low temperature treatment showed enhanced sugars in comparison to control seedlings as well as 24 hours low temperature treatment.

Reducing sugar content of cotyledon (Plate 26.6): of control seedlings also declined as in the embryo axis upto 72 hours and then remained steady. The low temperature treatment of 24 hours enhanced sugar content at 24, 48 and 72 hours and decreased at 96 hours of germination. There was further enhancement of Reducing Sugar content of 96 hours low temperature treatment.

Non Reducing sugar content of embryo axis and cotyledon (Table 7b): seedlings were decreased by low temperature treatments, 96 hours treatment caused more decrease.

Invertase activity of embryo axis (Plate 26.1): of control
GUAR CV. NAU BAHAR

GERMINATION-HOURS
PLATE - 26

Effects of low temperature on
Invertase activity of (1) embryo axis (2) Cotyledon
Total sugar content of (3) embryo axis (4) Cotyledon
Reducing sugar content of (5) embryo axis (6) Cotyledon
seedlings continuously declined; although the decline was slight. Low temperature treatment of 24 hours duration depressed the enzymic activity. 96 hours low temperature treatment also depressed the enzymic activity but the depression was lesser than by 24 hours low temperature treatment.

Invertase activity of cotyledon (Plate 26.2): of control seedlings went on declining up to 72 hours and then increased. Low temperature treatment of 24 hours and 96 hours depressed the activity. The depression being greater by 24 hours treatment.

Protein content of embryo axis (Plate 27.3): of the control seedlings declined as germination advanced. When the seedlings were subjected to low temperature treatment of 24 hours, protein content fell substantially as compared to their respective period of germination. There was also a fall in protein content of seedlings subjected to 96 hours of low temperature treatments which, however, was less than at 24 hours low temperature treatment.

Protein content of cotyledon (Plate 27.4): of control seedling fluctuated. 24 hours low temperature treatment had no conspicuous effect on protein content of seedlings at 24 and 48 hours germination but the low temperature treatment lowered protein content of seedlings at 72 and
96 hours germination. 96 hours low temperature treatment reduced protein content of cotyledon.

**Protease activity of embryo axis (Plate 27.1)**: of control seedlings increased with germination period. The activity was stimulated at 24 hours of low temperature treatment of control seedlings of 24 and 96 hours of germination. The activity was depressed, however, in seedlings of 72 and 96 hours. Low temperature treatment of 96 hours stimulated the protease activity.

**Protease activity of cotyledon (Plate 27.2)**: of the control seedlings increased at 48 hours, decreased at 72 hours and again increased at 96 hours of germination. The activity was stimulated at 24 hours of low temperature treatments, except in the case of seedlings at 96 hours. There was a further enhancement of protease activity by 96 hours of low temperature treatment.

**Catalase activity of the embryo axis (Plate 27.5)**: of the control seedlings increased up to 72 hours of germination and declined thereafter. Low temperature treatment stimulated the catalase activity and the stimulation was greater by 96 hours.

**Catalase activity of the cotyledon (Plate 27.6)**: of the control seedlings registered a consistent rise with
Effects of low temperature on:
1. Protease activity of embryo axis  
2. Cotyledon  
3. Protein content of embryo axis  
4. Cotyledon  
5. Catalase activity of embryo axis  
6. Cotyledon  
7. Peroxidase activity of embryo axis  
8. Cotyledon
germination period. Low temperature treatment stimulated the activity, the stimulation being greater by 24 hours.

**Peroxidase activity of embryo axis (Plate 27.7):** Of control seedlings went on declining up to 72 hours and then remained steady. The low temperature treatment stimulated the enzymic activity and the stimulation was greater by 96 hours low temperature treatment. In the case of 96 hours seedlings, the stimulation was more by 24 hours low temperature than 96 hours low temperature treatment.

**Peroxidase activity of cotyledons (Plate 27.8):** Of seedlings showed enhancing trend up to 72 hours, after which it declined. Low temperature response was variable. A stimulation was caused by low temperature treatment. The stimulation was greater by 96 hours low temperature treatment.

**Statistical analysis of the data (Table 9B):**

The observations presented in this experiment have been analysed so as to confirm this finding statistically for protein, protease, total sugars, reducing sugar, invertase, catalase and peroxidase activity in both embryo axis as well as cotyledon. The summarized data for degree of freedom, variance and F.value are presented for above estimations. The significance of data has been tested at 1% and 5% level.
Protein, protease, total sugars, reducing sugar, invertase, peroxidase activities are significant at 1% level except catalase which is at 5% level in embryo axis for germination period. While in cotyledon significant was at 1% level for all estimations. Low temperature treatment are significant at 1% level for all estimations in embryo axis and cotyledon. Interaction between germination period and low temperature treatment are significant at 1% level in all data in both embryo axis as well as cotyledon except invertase where, it is not significant on the whole low temperature treatment is significantly effective.