Materials and Methods
MATERIALS AND METHODS

The study was made with the cultivar Sonalika, Janak, UP 262 and WL 711. Seeds of the cultivar Sonalika of 1978 harvest were obtained from the University Experimental Farm, Baruipur. Seeds of cultivars Janak, UP 262 and WL 711 of 1978 harvest (harvested in April) were obtained from the Department of Agronomy, Bidhan Chandra Krishi Viswa Vidyalaya, Kalyani. The seeds were dried in the sun to an approximate moisture content of 10 per cent and stored in rubber-stoppered, 2.5-litre-capacity glass bottles which were opened from time to time for the use of seeds for treatment.

The methods adopted in the different experiments are described experimentwise under respective headings and subheadings. The experiments undertaken were as follows:

I. Determination of the pattern of decline in vigour and viability of important wheat cultivars under ambient condition.

II. Standardization of hydration-dehydration methods
   (a) Standardization of dipping-drying treatments for the maintenance of vigour and viability
   (b) Standardization of multiple dipping-drying seed treatment for the maintenance of vigour, viability and productivity
III. Effect of varietal response on hydration-dehydration treatment for the maintenance of vigour, viability and productivity

IV. Studies on the compatibility of insecticidal and fungicidal formulations with the hydration-dehydration treatment

V. Studies on the effect of seed treatment with micronutrient

VI. Studies on the effectiveness of hydration-dehydration in the alleviation of salinity stress

VII. Studies on dry dressing treatments with calcium oxychloride for the maintenance of vigour and viability

VIII. Physiological and biochemical studies on hydration-dehydration treatments
   (a) Studies on membrane permeability
   (b) Studies on enzymes
   (c) Lipid peroxidation in relation to ageing

I. LOSS OF VIGOUR AND VIABILITY OF WHEAT SEED UNDER AMBIENT CONDITIONS

   The seeds of cultivar Sonalika, Janak, UP 262 and WL 711 for this experiment were dried to a moisture level of about 9% in a drying cabinet. After drying, the seeds were stored in ordinary cloth bags which are generally used for small scale seed storage and kept in the laboratory in Calcutta under ambient temperature and humidity conditions. The average annual temperature and humidity of Calcutta ranges between 27°C and
Samples of seeds were tested for vigour and viability at monthly intervals. The vigour was measured as a length of the root and shoot of the seedlings.

**Germination test**

The glass plate method of seed germination standardized by Dasgupta *et al.* (1976) was adopted in all the experiments (Fig. 1). The seeds for germination test were placed over a moist blotter spread on a 15 cm x 15 cm ordinary glass plate of 2 mm thickness. A blotting paper sheets of 15 x 12 cm size were previously washed in running tap water and finally in distilled water and then placed over the glass plate leaving approximately 3 cm of the plate uncovered. The seeds were placed in straight line keeping the embryo downward and leaving a margin of 1 cm from the upper end of the blotter. Then the seeds were covered with a 2 cm x 15 cm polythene strip. To keep them in position, two 2 cm long, 0.75 diameter polythene tubings, longitudinally slit on one side, were fixed on the two sides of the glass plate securely holding the blotting sheet and the polythene strip. A rubber band was then fitted firmly across the tubings for keeping the seeds in position. Another band was similarly spread across the two split polythene tubings which were placed 4 cm above the bottom end of the plate to maintain a space between the plate and polythene packet into which the plate was
Figure 1. Arrangement for wheat seed germination on moist blotters spread on 15 cm x 15 cm glass plates enclosed in polythene envelops containing distilled water (upper figure) and placement of the plates on plastic rack at an angle of 66° (lower figure), the diagram shows the seedlings before recording the final germination percentage and root and shoot length.
POLYTHENE PACKET
GLASS PLATE
BLOTTING STRIP
POLYTHENE TUBING
RUBBER BAND
SEED
BLOTTING PAPER
DISTILLED WATER

GERMINATION PLATE WITH SEEDS ON MOIST BLOTTER

GERMINATION PLATE ARRANGED ON PLASTIC RACK
put for gemination. Then the plate was kept in a 22 cm x 18 cm polythene packet containing 10 ml of distilled water. The plate was then placed on a plastic holder at an angle of 60° for germination. The use of these plates over the petridish method ensures greater uniformity in germination test and enable better measurement of the shoot and root growth. The germination tests were terminated after 96 hour, as there was virtually no increase in germination percentage after the above mentioned hours at 20°C. The vigour and viability of the seeds were assessed on the basis of germination percentage and seedling growth.

The aforesaid method show the following advantages over the conventional petri-dish, glass vial and rolled towel methods of seed germination:

(i) greater uniformity in seedling growth, (ii) the seeds on the plate receive equal amount of water, (iii) capillary movement of water prevents water logging of the seed, (iv) during the period of germination there is no need of adding water over the initial 10 ml, (v) it is possible to observe the progress of germination from outside, (vi) little entangling of roots as it grows vertically down without coiling or curving on the paper, (vii) fungal contamination is less, (viii) the set up is much cheaper than the petri-dish method, (ix) the extra time required for placing the seeds on the plate is more than compensated by the time saved in
recording of root and shoot length of seedlings. In all the experiments on wheat seed germination except in the salinity experiments, this newly standardized glass plate method was followed.

II. STANDARDIZATION OF HYDRATION-DEHYDRATION METHODS

A. Standardization of dipping-drying treatment for the maintenance of vigour and viability

Dasgupta (1978) standardised the soaking-drying treatments for wheat and in the present study, the duration of soaking and other factors were the same as described by her. Dipping-drying treatments were however, standardised.

Five-month-old stored wheat seeds of cultivar Sonalika were dipped in double the volume of water for 2, 5 and 10 minutes respectively. Then the seeds were taken out and kept covered for 0, 0.5, 1, 2, 3, and 4 hours for slow imbibition of water with occasional stirring at room temperature. After that the seeds were dried back to its original weight in a drying cabinet over a current of dehumidified air at 35±1°C. The control seeds were not dipped but dried along with the treated seeds. Both the control and treated seeds were then transferred to a desiccator containing fused calcium chloride for 7 days for stabilizing the moisture content of the seeds to a low and uniform level. These treated and untreated seeds were
thereafter kept in perforated paper packets and subjected to accelerated ageing conditions at 100% RH and 40°C.

Accelerated ageing: The loss of vigour and viability of the seeds was studied under artificially controlled regimes of different temperatures and relative humidities. Relative humidity level 100 per cent was maintained by using water in glass desiccator and closing them properly with greased lids. A saturated solutions of calcium chloride was used to get 36 per cent relative humidity. For the maintenance of constant temperature the desiccators were kept in a B.O.D. incubator.

B. Standardization of multiple dipping-drying seed treatment for the maintenance of vigour, viability and productivity

Seeds of Sonalika were used for this experiment. The experiments were conducted in the laboratory as well as in the field to evaluate the treatment effects on vigour and viability test and field performance respectively.

Seeds were dipped in double the volume of water and dilute solutions of sodium phosphate (dibasic, $10^{-4}$M) for 2 minutes and kept covered for 3 hours at room temperature and then dried back to its original weight in a drying cabinet following the above mentioned method. The control seeds were not treated but dried along with the treated seeds. Seeds were treated from July to October. The seeds
received one, two, three or four dipping-drying treatment depending on the time of first treatment. Thus in October only one dipping-drying treatment could be given before taking the seeds to the field for sowing. The concentration of sodium phosphate and other chemicals were standardized by Dasgupta et al. (1978) from this laboratory.

After drying seeds were placed over fused calcium chloride for moisture stabilization for 7 days. After moisture stabilization, one portion of the seeds was kept in a perforated paper packet and subjected to accelerated ageing (100% RH and 40°C) and natural ageing under ambient conditions. Another portion was stored in glass bottle for the subsequent studies on the effects of treatments on field performance and productivity. In both the cases (i.e. A and B) germination tests were conducted following the previously mentioned methods.

Field performance: Field trials were conducted to study the effects of multiple dipping-drying treatment on crop yield. The experiments were taken up at the University Experimental Farm, Baruipur, 24 Parganas during the rabi season, using completely randomized block design with 3 replications for each treatment. The plot was divided into 90 subplots
(30 hydration-dehydration treatments x 3 replications each) of 2.5 m x 4 m in size (Fig. 2). The basal dose of N:P:K at the rate of 30:40:40 kg/hectare was added. During final land preparation 3/4 of the total nitrogen and total amount of phosphate and potassium were added and rest of the nitrogen was added in two split doses, one at tillering and another at panicle emergence stage. Seeds were sown at the rate of 100 kg per hectare and sowing was done on 2nd week of December in all three consecutive years during this study. The seeds were sown, giving a space of 20 cms between the rows and post-sowing irrigation was done on the same day. The crop received a total of 4 irrigation, viz. at peak tillering, at flag leaf emergence and finally at grain filling stages.

Data on plant population was counted in each replication after 15 days of sowing. Number of tillers were counted replicationwise, at the flowering stage. The crop was harvested in the first week of April. Grain weight per unit area, 1000 grain weight, length of ear head and number of seeds per panicle were recorded replicationwise for each treatment.

III. EFFECT OF VARIETAL RESPONSE ON HYDRATION–DEHYDRATION TREATMENT FOR THE MAINTENANCE OF VIGOUR, VIABILITY AND PRODUCTIVITY

To study the varietal response on seed treatment, four cultivars
Figure 2. Layout (randomised block design, RBD) of Experiment II(B) showing the distribution of different dipping-drying treatments.

<table>
<thead>
<tr>
<th>Months of first treatment</th>
<th>July</th>
<th>August</th>
<th>September</th>
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<tr>
<td>First dipping</td>
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<tr>
<td>1-C</td>
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<td>22-C</td>
<td>28-C</td>
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<td>17-W</td>
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<td>6-P</td>
<td>18-P</td>
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<tr>
<td>7-C</td>
<td>19-C</td>
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</tr>
<tr>
<td>8-W</td>
<td>20-W</td>
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<td>Fourth dipping</td>
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<tr>
<td>11-W</td>
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<tr>
<td>12-P</td>
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</table>

C, W and P indicate control, water and sodium phosphate (dibasic, 10^-4 M) respectively.

The numbers in parentheses viz. (1), (2) and (3) represent the three replications.
LAYOUT OF FIELD EXPERIMENT II(B)

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<td>7 (2)</td>
<td>20 (3)</td>
<td>11 (3)</td>
</tr>
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</table>

IRRIGATION CHANNEL

4.0m 1.2m 40cm

29.2m

43.9m

2.5m

4m
viz. Sonalika, Janak, UP 262 and WL 711 were used. After collection, seeds were dried in the sun to an approximate moisture content 10 per cent and stored in rubber-stoppered glass bottle till use.

It was observed that in wheat seeds kept in ordinary uncontrolled conditions in the laboratory in Calcutta, signs of seed deterioration were evident in 4-5-month-old lot, specially when it passed through the hot and humid monsoon months. The efficacy of mid-storage hydration-dehydration treatments were noted by Dasgupta (1978) from this laboratory. Excepting that observation, it is also provided in the appendix employing cv. Sonalika.

To check the rapid decline in germinability hydration-dehydration seed treatment was adopted. Hydration was done in three different ways: (i) soaking in water for 2 hours (ii) dipping the seeds for 2-3 minutes (iii) keeping the seeds in saturated atmosphere (100% RH at room temperature). In all the cases, seeds after hydration were dried back to the original weight.

(1) Soaking-drying: In this method wheat seeds were soaked in double the volume of water or dilute solutions of sodium chloride (10^{-3} M) and sodium phosphate (dibasic, 10^{-4} M) for 2 hours at room temperature (28±2°C) with occasional stirring. After decanting off the excess amount of water, the seeds were surface-dried with blotting papers and
then dried back to their original weights in a drying cabinet in a current of hot air at 35±1°C. The control seeds were not soaked but dried along with the treated seeds. After drying, the seeds (treated as well as the control) were stored in a desiccator over fused calcium chloride for 7 days for stabilizing the moisture content of the seeds to a low and uniform level.

(ii) Dipping-drying: Seeds were dipped in double the volume of water or dilute solutions of sodium phosphate (dibasic, 10⁻⁴M) for 2 minutes and then kept covered for 3 hours at room temperature followed by drying back to its original weight in the usual manner.

(iii) Moisture equilibration-drying: Seeds were equilibrated with an atmosphere of 100% relative humidity. Dasgupta (1973) standardized the duration of equilibration and noted that 20-30 hour was the best for this type of hydration treatment in wheat. In this method wheat seeds were placed in monolayer in small non-absorbent paper trays placed on a raised platform in an enamel tray containing 200 ml of distilled water, covered with a heavy glass plate and lined internally on all sides with thick layers of moist blotters. Moisture equilibration was done at room temperature. After equilibration, the hydrated seeds along with the control were dried back to its original weight following the previous methods.
Moisture equilibration-drying treatment was not done for this experiment but was given in the biochemical portion (Expt. 8).

The seeds (i.e. treated and untreated) were then subjected to different types of accelerated ageing conditions (100% RH and 40°C and; 30% RH and 40±1°C) as well as natural ageing under ambient warm-humid conditions. The germination tests were performed after suitable intervals to evaluate the effect of seed treatment on the maintenance of vigour and viability under different conditions of storage.

**Moisture content**: Moisture content was measured following the ISTA method, 1976. Five grammes of seed sample was taken in the evenly distributed over the surface of the previously weighed container (glass vial) and placed rapidly in an electrical heated oven, with adequate ventilation and thermostatic control. The temperature of the oven was maintained at a temperature of 130°C. The drying period begins at the time the oven reaches to the required temperature. Drying was done for 2 hours at that temperature. After drying, the vial was rapidly taken out and covered immediately with the lid (which was also weighed previously) and placed in a desiccator to cool for 30-45 minutes. After cooling, weight of the container with its lid and contents was taken. If \( M_1 \) is the weight of the
vial and its lid, $M_2$ is the weight of the vial with lid and contents before drying and $M_3$ is the weight of the vial with lid and contents after drying then the moisture content of the seed calculated on the dry weight basis expressed in percentage is $(M_2-M_3) \times \frac{100}{(M_3-M_1)}$.

The determination was made in duplicated.

Factors influencing the effectiveness of seed treatment

(i) Age of the seed, (ii) volume of water, (iii) duration of soaking, (iv) concentration of chemical.

It has been observed earlier that harvest fresh seeds by soaking-drying treatment adversely effect storability. The optimum time of treatment, volume of water, duration of soaking and concentration of chemicals were standardized by Dasgupta (19??) from this laboratory.

Field performance: The field experiment was carried out at the University Experimental Farm, Baruipur, 24 Parganas, during the rabi season of the three consecutive years, using completely randomized block design with three replications for each treatment.

After final preparation of the land, all the varieties of wheat seed was sown in each sub-plot. The plot size, fertilizer, amount of seed, irrigation and other agronomical practices were done following
the same method, which were discussed earlier in the previous experiment of this thesis work.

Data on plant population, tiller number and grain yield per unit area and other yield attributes like 1000-grain weight, length of the ear and number of grains per ear were recorded like previous experiment.

IV. STUDIES ON THE COMPATIBILITY OF INSECTICIDAL AND FUNGICIDAL FORMULATIONS WITH THE HYDRATION-DEHYDRATION TREATMENTS

Seeds of the cultivar Sonalika obtained from the Calcutta University Experimental Farm, Baruipur, were used in the present study. After harvest, the seeds were dried in the sun to an approximate moisture content of 10% and then stored in rubber stoppered glass bottles until treatment. The laboratory and field experiments were conducted over a period of three years; 1978-79, 1979-80 and 1980-81.

Method of seed treatment: The seed treatment was done according to the method described earlier by Basu and Dasgupta (1974) and Basgupta et al. (1976). Treatments were given to seeds which were stored in rubber stoppered glass bottles for 5 months under ambient conditions. The seeds were soaked in double the volume of water or solution of sodium phosphate (dibasic, $10^{-1}$M) for 2 hr at room temperature followed by drying back to the original weight at 35±1°C in a current of air in a drying cabinet.
Besides soaking-drying seeds were also treated by the dipping-drying method. For this, seeds were dipped in water or dilute solution of sodium phosphate (dibasic, \(10^{-6}\%\)) for 2 min, taken out and then kept covered for 3 hr for effective penetration of the surface water into the seed. After this, seeds were dried back to the original weight in the same way as above. The control seeds were not soaked or dipped but dried along with the other treatment.

After moisture stabilization over fused calcium chloride, seeds were dry-dressed with ceresan (2 g per kg), dithane M-45, thiram and captan (each at 2.5 g per kg). The insecticide, malathion 50\% E.C., was applied as a light spray (1:300 aqueous solution, at 20 ml of solution sprayed per m\(^2\) of seed spread in a monolayer) followed by drying.

**Aging Condition**: After treatment, the seeds were subjected to accelerated ageing at 100\% RH and 40\(^\circ\)C and, 36\% RH and 40\(^\circ\)C. Ageing under ambient conditions was done by placing the seed of the different treatment in perforated paper packets, in cloth bags. For field trials, treated and untreated seeds were stored in polythene bags.

**Germination test**: After requisite ageing, germination tests were carried out following the glass plate method, which is described earlier in the present thesis. Data on germination percentage, root and shoot length were
recorded after 5 days. For root length the total of all the seminal roots per seedling were taken.

Field experiments: The field experiment was carried out in the University Experimental Farm, Baruipur using completely randomized block design with three replication for each treatment.

After preparation of the land, the plot was divided into 105 sub-plots (for 5 hydration-dehydration treatments x 7 seed protectants x 3 replication) of 2.5 m x 4 m in size (Fig.3). A fertilizer dose of N:P:K at the rate of 80:40:40 kg per hectare was given. During final land preparation three-fourth of the total nitrogen and total amount of phosphate and potassium were added and rest of the nitrogen was supplied in two split doses; one at peak tillering and another at the panicle emergence stage. Seeds were sown at the rate of 100 kg per hectare and sowing was done in the first week of December, giving a space of 20 cm between the rows. The post-sowing irrigation was done on the same day. Thereafter the crop received 3 more irrigations; at peak tillering, at flag leaf emergence and finally at the grain filling stage. Data on plant population was recorded for each replication after 2 weeks of sowing. Number of effective tillers was counted at the post-flowering stage. After harvest, grain yield per
Figure 3. Layout (RED) of Field Experiment IV showing the distribution of various treatments.

Abbreviations: C-Control, WS-Water soaking, SP-Sodium phosphate soaking (dibasic, $10^{-4}$), WD-Water dipping and PD-Sodium phosphate dipping (dibasic, $10^{-4}$).

CS-ceresan, D-dithane 5-45, T-thiram, CA-capten and M-malathion 50% E.C.

The numbers 1, 2 and 3 represent the three replications.
### Layout of Field Experiment IV

**Diagram:**

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<table>
<thead>
<tr>
<th>WD</th>
<th>C+M+D</th>
<th>C+M+D</th>
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<tbody>
<tr>
<td>C+T</td>
<td>WD+D</td>
<td>PD+T</td>
</tr>
<tr>
<td>WD</td>
<td>WD+CA</td>
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**Legend:**

- WD: WD
- C+M+D: C+M+D
- C+M: C+M
- WD+D: WD+D
- PD+T: PD+T
- WD+CA: WD+CA
- WD+D: WD+D
- WD: WD
- C+T: C+T
- G+T: G+T
- WD+M+D: WD+M+D
- C+CE: C+CE
- PD+M+D: PD+M+D
- PS+GA: PS+GA

**Additional Notes:**

- The layout is designed to demonstrate various experimental combinations and treatments.
- The diagram includes a grid with specific plant treatments and their locations.
- The grid is oriented with north at the top and south at the bottom.
- The scale is indicated as 34.4 m and 43.9 cm, with a grid size of 4 m, 1.2 m, and 40 cm.
plot, 1000-grain weight and other yield attributes were recorded replication-wise for each treatment.

V. STUDIES ON THE EFFECT OF SEED TREATMENT WITH MICRONUTRIENT TO EVALUATE THE TREATMENT EFFECTS ON VIGOUR, VIABILITY AND PRODUCTIVITY

Seeds of cultivar Sonalika were employed for this study. After harvest, seeds were thoroughly dried in the sun and stored in the rubber-stoppered glass bottle, before treatment. The treatments were given to 5-month-old (mid-storage) and 10-month-old (pre-sowing i.e., just before sowing in the field) seeds.

Method of seed treatment: Seeds of cultivar Sonalika were dipped in water and dilute solutions of micronutrients viz., copper sulphate (2 x 10^{-4} M), zinc sulphate (10^{-3} M), ferrous sulphate (10^{-3} M), manganese sulphate (2 x 10^{-3} M), boric acid (10^{-3} M) and ammonium molybdate (10^{-4} M) for 2 minutes and kept covered for 2 hr and then dried back to its original weight following the same technique as described earlier. The mid-storage and pre-sowing treatments were done in the months of August and December respectively. Control seeds were not dipped but dried along with the treated one.
Ageing condition: After moisture stabilization, seeds were subjected to accelerated ageing (100% RH and 40±1°C and, 36% RH and 40±1°C) and natural ageing under ambient conditions. For field trials treated and control seeds were stored in rubber-stopper glass bottles.

Germination test: Germination tests were done following the glass plate method, as described earlier. Data were recorded after germination for 5 days. The vigour of seedlings were measured by root and shoot length. For root length the total of all the seminal roots per seedling were taken.

Field performance: Field trials were conducted in the Experimental Farm of the Calcutta University, during the rabi season using completely randomized block design with 3 replications for each treatment (Fig. 4). The fertilizer dose and application, rate of seed per hectare, size of the plot and other agronomical and cultural practices were the same as in the previous experiments. Data on plant population, number of tillers per unit area, grain yield per plot and other yield attributes viz. 1000-grain weight, number of grains per ear and length of the ear were recorded and discussed in the result section.

VI. STUDIES ON THE EFFECTIVENESS OF HYDRATION-DEHYDRATION IN THE ALLEVIATION OF SALINITY STRESS

Seeds of cultivar Sonalika were used for this experiment. After
Figure 4. Layout (RBD) of Field Experiment V showing the distribution of different treatments.
Abbreviations: C-Control, W-Water dipping, Cu-Copper sulphate \(2 \times 10^{-4}M\), Zn-Zinc sulphate \(10^{-3}M\), Fe-Ferrous sulphate \(10^{-3}M\), Mn-Manganese sulphate \(2 \times 10^{-4}M\), B-Boric acid \(10^{-3}M\) and Mo-Ammonium molybdate \(10^{-4}M\).

(M) - Mid-storage seed treatment, (P) - Pre-sowing seed treatment
The numbers 1, 2 and 3 represent the three replications.
LAYOUT OF FIELD EXPERIMENT IV

19.6 m

Cu, Mn, \( b_2 \) © c\(^3 \)

Fe, © Zn \(^2 \) © c \(^2 \) ® Zn \(^3 \) @

Fe, © W, © Mn\(^3 \) © Mo\(^3 \) ®

Cu, © Mo\(^2 \) © ... © Ma\(^2 \) Cu

Zn, © W, w\(^2 \) © b \(^3 \) ©

Mo, © Mn\(^2 \) (P). Cu, © Fe \(^3 \)

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4 m 40 cm 1.2 m
harvest, seeds were stored in rubber stoppered glass bottles and treatments were given to 5-month-old seeds.

**Method of seed treatment:** Seeds were soaked in double the volume of water or solutions of sodium chloride (10^{-3}M), sodium phosphate (dibasic, 10^{-4}M) for 2 hr at room temperature, followed by drying back to its original weight at 35±1°C in a current of air in a drying cabinet. The control seeds were not soaked but dried along with the other treatment. After moisture stabilization over fused calcium chloride for 7 days, seeds were stored in rubber stoppered glass bottles under ambient conditions. After 8 months seeds were placed on saline solution to judge the tolerance capability of soaked-dried seeds on germinability and growth of the seedlings. The saline concentration were 0 M, 0.05 M, 0.1 M and 0.2 M. For saline solution, sodium chloride salts were used.

**Germination test:** For this experiment, germination tests were done following the petri-dish method. In petri-dishes clean absorbent cotton were placed in a uniform layer. The different concentration of saline (0 M, 0.05 M, 0.1 M and 0.2 M) solution were made from sodium chloride salt. After this, 15 ml of solution were added separately in each petri-dish. Single concentration was given to four hydration-dehydration treatment and ultimately 16 petri-dishes were (4 hydration-
dehydration treatment x 4 saline concentration) used for this experiment. Then the treated and untreated seeds (3-month-bottle stored under ambient condition) were placed for germination. 100 seeds were taken in each petri-dish of each treatment. Then the petri-dish were covered by the same size lid and placed for germination at 20±1°C for 7 days. In between the germination period, after 3 days 10 ml of extra solutions were added in the respective petri-dish. Data were recorded after germination for 7 days. Germination percentage were counted and vigour of seedlings were measured by root and shoot length. For root length the total of all the seminal roots per seedling were taken.

In this experiment, for germination, glass plate method were not used because a large amount of saline salts would be deposited on the upper surface of the blotting paper. In glass plate method water are transported on the upper surface through capillary movement. The saline are transported faster through capillary system and deposited on the upper surface of the blotting sheet and hampered the uniformity of the salt solution throughout the germination process.

VII. STUDIES ON DRY DRESSING TREATMENTS WITH CALCIUM OXYCHLORIDE FOR THE MAINTENANCE OF VIGOUR AND VIABILITY

Seeds of cultivar Sonalika were employed for this experiment.
Freshly harvested seeds were dried thoroughly in the sun to an approximate moisture content of 10 per cent and stored in a refrigerator, in a rubber-stoppered glass bottle.

After six months of storage, seeds were dry-dressed with calcium oxychloride (Bleaching powder) at the rate of 2, 5 and 10 g per kg of seed and kept in the stoppered glass bottles at room temperature.

One day after the treatments, the seeds were subjected to accelerated ageing at 100% RH and 40°C and natural ageing in perforated paper packets kept in cloth bag.

**Germination tests:** Germination tests were conducted using the glass plate method, which has been described earlier in the previous experiments.

**VIII. PHYSIOLOGICAL AND BIOCHEMICAL STUDIES ON HYDRATION-DEHYDRATION TREATMENTS**

The following physiological and biochemical studies were undertaken to elucidate the effect of seed treatment on the maintenance of vigour and viability.

a) Membrane functions as evidenced by membrane permeability,
b) Studies on the activity of dehydrogenase and amylase enzymes,
c) Studies on the estimation of lipid peroxidation.

(a) Membrane permeability

(i) Electrical conductivity of seed leachate: For studying membrane permeability of the treated and untreated seeds of different physiological ages, the electrical conductivity of the seed leachate was recorded in a Toshniwal conductivity bridge (cell constant 0.656) at 30°C following the method of Anderson et al. (1964); 50 uniform seeds per treatment were soaked in 25 ml of distilled water for 30 minutes.

(ii) Leaching of sugar: The amount of sugar leached out of the seed was also recorded colorimetrically following the method of McCready et al. (1950) with minor modifications. 76 ml of pre-cooled leachate were taken in a hard glass test tube and 4 ml of cold freshly prepared anthrone reagent (0.2% anthrone in 95% sulphuric acid) were added and kept in cold for 30 minutes for the bluish green colour development which was measured on a Hilger Biochem photo-electric absorptiometer at 580 nm.

(iii) Leaching of amino acid: The amount of amino acid in the seed steep water was estimated following the method of Moore and Stein (1948)
with minor modifications. Seeds were soaked for 4 hr and to 4 ml of the seed-steep water, 0.5 ml of 0.1/ acetic acid-sodium acetate buffer (pH 5.3) and 1 ml of 1% ninhydrin solution in dioxane were added and the reaction mixture was heated for 15 minute in a water bath at 100°C for the colour development. The colour intensity was measured at 610 nm.

(b) Enzymatic studies

(i) Assay of dehydrogenase activity: The dehydrogenase activity of the treated and untreated seeds of different ageing conditions was estimated following the method of Kittock and Law (1967) with minor modifications. Hundred seeds per treatment were placed for germination on petri-dish at 20±1°C. After 24 hr, wheat embryos (sprouted) were separated out and 10 such uniformly sprouted embryos were placed in a 10 ml capacity glass vial and incubated with 5 ml of 0.2% solution of tetrazolium chloride for 3 h at 30°C. After incubation, the tetrazolium solutions were decanted off and the embryos were thoroughly washed with distilled water. Six ml of methyl cellosolve (2-methoxy ethanol) were added and the reading was taken on an AIMIL Hilger Biochem Absorptiometer at 470 nm after 6 h.

(ii) Assay of amylolytic activity: Amylolytic activity of the seeds were measured following the method of Basu et al. (1970) with minor modifications. Fifty seeds per treatment were taken and placed for
germination at 20±1°C. After 24 h of germination the enzyme were extracted from eight uniform sprouted embryonised half portion of the seed with phosphate buffer pH 6.5 (0.02 M). The ground material were strained through a muslin cloth and the final volume of the extract were made up 8 ml with phosphate buffer pH 6.5 (0.02 M). This extract were then centrifuged for 10 min at 5,000 rpm. The clear supernatant were separated discarding the residue. The incubation mixture consisted of the following: 1 ml of 0.2% starch solution, 1 ml of 2.5% CaCl₂, 0.5 ml of enzyme extract and 0.5 ml of phosphate buffer pH 5.0 (0.01 M). The mixture were incubated for 20 min at 35°C. The residual starch were estimated colorimetrically by developing blue colour with a standard iodine solution. The amylase activity was measured by the extent of hydrolysis of the starch solution. The blue starch iodine colour were read on a AIMIL-Hilger-Biochem Absorptiometer at 610 nm.

(c) Estimation of lipid peroxidation

Lipid peroxidation formation before and after accelerated ageing was studied by the thiobarbituric acid (TBA) colour reaction as outlined by Bernheim et al. (1948) with minor modification. Five millilitres of
1% TBA solution were added to 500 mg of dry wheat powder in a hard-
glass test tube and then 2 ml of 1(N) H₂SO₄ were added. The mixture
was heated for 30 min in an oven at 105°C. After cooling 5 ml of
methyl cellosolve (2-methoxy ethanol) were added and the mixture was
thoroughly shaken. It was then centrifuged for 10 min at 5,000 rpm.
The absorbance of the clear supernatant was read on an Aminco-
Berg Biochem Absorptiometer at 520 nm.

STATISTICAL ANALYSIS

The data obtained from laboratory germination tests and field
experiments were analysed statistically to evaluate the effects of
hydration-dehydration treatments, fungicidal and insecticidal dressings,
and their interactions and varietal response on seed treatment and their
interactions etc. on germinability and growth of seedlings, yield and
various yield attributes, following the analysis of variance technique
(Fisher, 1948). Before analysis, the germination percentage data were
transformed to respective angles (arc-sin); the L.S.D. values for
viability, whenever they have been given, are also in angles and are
meant for the comparison of germination angles only.