EXPERIMENTAL
Plants species differ in their tolerance to total salts and to specific ions. Also, crops that may be highly tolerant at one growth stage may be sensitive during another stage. Hence, to find out the sensitive and tolerant stages both early stages of germination (Laboratory condition) and plant developmental stages (Field grown) were taken for different biochemical analysis. For the experimental convenience whole experiment was divided into two parts.

1. Effect of sodium chloride salinity on early seedling growth and biochemical changes of bajra (Pennisetum typhoides S & H)

Experiment 1

Effect of sodium chloride salinity on early seedling growth and biochemical changes of bajra (Pennisetum typhoides S & H)

Graded seeds of *Pennisetum typhoides* S & H var. B.J. 104 were washed thoroughly in water and surface sterilized with 0.1 percent HgCl₂ for 2-3 minutes and were placed directly in (9cm diametar) petridishes (30 each) having Monol-XI filter paper.
### Table 1: Physical data of NaCl used in germination experiment

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Electrical conductivity at 30°C mmhos/cm</th>
<th>Molarity</th>
<th>Meq/litre</th>
<th>$p^H$</th>
<th>Osmotic pressure (atm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4% NaCl</td>
<td>8.03</td>
<td>0.0684</td>
<td>68.48</td>
<td>6.50</td>
<td>2.64</td>
</tr>
<tr>
<td>0.8% NaCl</td>
<td>15.51</td>
<td>0.1368</td>
<td>136.36</td>
<td>6.75</td>
<td>5.28</td>
</tr>
</tbody>
</table>

### Table 1.B: Nature of the Soil and Water used in the Pot experiment

<table>
<thead>
<tr>
<th></th>
<th>Cl$^-$</th>
<th>HCO$_3^-$</th>
<th>CO$_3^-$</th>
<th>SO$_4^-$</th>
<th>Mg$^{++}$</th>
<th>Ca$^{++}$</th>
<th>Na$^+$</th>
<th>K$^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tap Water</strong></td>
<td>4.02</td>
<td>10.6</td>
<td>trace</td>
<td>1.2</td>
<td>1.8</td>
<td>4.2</td>
<td>9.8</td>
<td>trace</td>
</tr>
<tr>
<td><strong>Soil</strong></td>
<td>1.1</td>
<td>7.3</td>
<td>trace</td>
<td>trace</td>
<td>1.0</td>
<td>2.0</td>
<td>5.0</td>
<td>trace</td>
</tr>
</tbody>
</table>

ECC and $p^H$ are given in the Table-2.
Salinity treatment was given by adding known volume (5 ml) of aqueous solution of NaCl in two concentrations (0.4% and 0.8%). The physical data of NaCl is given in the Table 1 for ready reference. Distilled water was added to the control petridishes. The petridishes were kept in continuous light (1000 lux) at laboratory temperature 28 ± 2°C till 96 hours. The solutions were renewed every day to prevent the bacterial contamination.

Sampling was carried out after 24, 48, 72 and 96 hours of sowing. Three petridishes were sampled every time for each treatment. When the seedlings were of appropriate age, embryo axis was separated into root and shoot and their length was measured. In addition, fresh and dry weights of embryo axis and endosperm, as the case may be were determined. From this data water content of individual parts was calculated.

Each reading represents a mean of 10 replicates for root/shoot length and for fresh and dry weights, percent moisture content and for biochemical activities are mean of 3 replicate readings unless otherwise specified.

Common biochemical parameters were performed both in embryo axis and endosperm separately. List of the biochemical parameters carried out is given below:
1. **Carbohydrate metabolism**
   - Starch content
   - Amylase activity
   - Reducing Sugars Content
   - Invertase Activity

2. **Protein metabolism**
   - Total protein content
   - Soluble protein content
   - Amino acids content
   - Protease activity

3. **Phenolic compounds content**

4. **Nucleic Acid Metabolism**
   - Ribonucleic acid content
   - Ribonuclease activity

5. **Growth Regulators**
   - Gibberellin-like substances activity
   - Cytokinin-like substances activity
   - Abscisic-acid like substances activity

6. **Oxidizing Enzymes**
   - Peroxidase activity
   - Catalase activity
Experiment 2

Effect of sodium chloride salinity on growth and biochemical changes of bajra (Pennisetum typhoides S & H) leaf.

Seeds of Pennisetum typhoides S & H. var. B.J. 104 were washed in tap water and sown in earthen pots (12"x 12") containing a mixture of soil and compost (3:1). After about 10 days from sowing the plants were thinned five per pot. After a preliminary screening of (0.1 to 0.8%) of different concentrations 0.2% (35mM) as a lower salinity level and 0.4% (70mM) as higher salinity level were selected for the present study. Salt treatment was given at the 20th day of sowing by adding sodium chloride in the form of solution to raise the soil salt concentration to 0.2% and 0.4% based on soil dry weight. A set of controls were maintained by adding tap water in place of NaCl solutions. Chemical nature of the soil and water used are given in the Table 18. At the 35th day of sowing a second dose of NaCl in the respective concentrations was given to maintain the same salinity level. The pots were kept in the natural day light and watered daily to a moisture content of 50-80% field capacity. Mature leaf was collected for different analysis according to the following stages.

Stage I 7 days after I NaCl treatment
Stage II 15 days after I NaCl treatment
Stage III  
7 days after II NaCl treatment  
(22 days after I NaCl treatment)

Stage IV  
15 days after II NaCl treatment  
(30 days after I NaCl treatment).

Plant height, leaf and root length were recorded at above given intervals. Fresh and dry weights of whole plant, root, shoot and leaf were taken. Moisture content was calculated on fresh weight basis. Growth data represents a mean value of 10 replicates. Fresh and dry weights and all biochemical changes are mean of 3 replicates.

Following biochemical estimations were done in leaf material at given 4 stages.

1. **Carbohydrate metabolism**
   - Starch content
   - Reducing sugars content
   - Invertase activity

2. **Photosynthetic pigments**
   - Total chlorophylls, chlorophyll-α, β and a/b ratio
   - Carotenoids content
   - Chlorophyllase activity

3. **Hill reaction activity**
   - DCPIP reduction activity
   - Ferricyanide reduction activity
4. **Protein metabolism**

   - Total Protein content
   - Soluble protein content
   - Amino acids content
   - Protease activity

5. **Phenolic Compounds content**

6. **Nucleic acid metabolism**

   - Ribonucleic acid content
   - Ribonuclease activity

7. **Endogenous growth regulators**
   (endogenous growth regulators activities were estimated in stem also)

   - Cytokinin-like substances activity
   - Gibberellin-like substances activity
   - Abscisic acid like substances activity

8. **Mineral constituents**

   - Potassium, Sodium, Calcium, Magnesium and Chloride.

**Experimental Methods**

I. **Fresh weight, dry weight and moisture content determination**
The plant material (embryo axis, endosperm, whole plant, leaf and root) as the case may be were separated at their respective germination or growth periods and it was weighed and designated as "Fresh Weight". The material was oven-dried at 80°C for 48 hours to obtain dry weights. Fresh weight and dry weights were expressed as mg/organ.

Percent moisture content was calculated on gram fresh weight basis.

II. Biochemical methods

1. Reducing Sugars

The method described by McCready et al., (1950) was employed to estimate reducing sugars. Weighed plant material was crushed and extracted with 80% ethanol for 24 hours. After centrifugation, the ethanol was evaporated and the residual sugars were dissolved in known volume of distilled water. This aliquot and anthrone reagent (0.2% anthrone in chilled 95% sulphuric acid) were mixed in 1:2 proportion in an ice-cold condition. This reaction mixture was heated on a waterbath at 100°C for 10 minutes with stoppers and cooled immediately. The optical density (O.D) was read at 660 nm. The following regression formula prepared with glucose as standard was used to calculate reducing sugars content in mg/g. dry or par organ.
\[ Y = 124.61 \times -3.25 \]

\( (Y = \text{concentration and } x = \text{OD}). \)

2. **Starch**

Weighed plant material was grounded in 80% ethanol and extracted twice. The residue was dissolved in known volume (20 ml) of 0.7% KOH and it was gelatinized in boiling water bath for 40 minutes. It was cooled and made to a known volume (Chinoy, 1939).

Known volume of this aliquot was mixed with 6 ml of iodine solution (2.5 g I\(_2\) and 25 g\(\text{m}\text{g}\)\_s\(\text{m}\) KI in one litre of 0.05 NHCl). The OD was read at 600 nm. The following regression formula was prepared by using known concentration of starch and starch content was expressed as mg starch/g. dry weight or per organ.

\[ y = 331.49 \times -2.34 \]

3. **Protein**

Weighed plant material was grounded in 80% ethanol and extracted twice. The residue was washed first with cold 5% H\(_2\)O\(_4\) and secondly with a mixture of ethanol : ether : chloroform (2:1:1) to remove acid soluble fractions and lipids. The residue was then washed with 1 M cold trichloracetic acid (TCA) and centrifuged.
The protein fraction was suspended in 1 N sodium hydroxide for one hour and centrifuged. The supernatant was made up to a known volume and used as aliquot. To a known volume of aliquot 0.5 ml of distilled water and 5 ml of reagent C prepared by mixing 50 ml of reagent A (2% sodium carbonate in 0.1 N NaOH) and 1 ml of reagent B (0.5% copper sulphate in 1% sodium potassium tartrate) were added and incubated for 10 minutes at room temperature. The colour was developed by adding 0.5 ml of 1 N folin phenol reagent and incubating for 30 minutes at room temperature (Lowry et al., 1951). The O.D was read at 660 nm. The following regression formula was prepared by using known concentrations of casein, and protein content was expressed as mg. protein/g. dry weight or per organ.

\[ Y = 303.11 \times -0.98. \]

For soluble protein seedlings were extracted in ice cold distilled water and protein was precipitated in 15% TCA. The precipitated protein was dissolved in 1 N NaOH and centrifuged. The protein present in the supernatant was measured according to Lowry et al., (1951).

4. **Ribonucleic acid**

RNA was extracted following the procedure of Bonner and Zeaawart (1962). The weighed plant material was crushed and extracted with 80% ethanol and centrifuged. The residue was washed
with 5% perchloric acid to remove acid soluble substances, mixture of ethanol : chloroform : ether (2:1:1) to remove lipids and finally with 1 M TCA as described in protein extraction method. The residue was dissolved in 0.3 M potassium hydroxide and incubated at 37°C for 42 hours. Later it was centrifuged. The supernatant was adjusted to pH 3.0 and made to a known volume. This was used as aliquot. RNA was analysed by the method of Markham (1955) using orcinol reagent (prepared by mixing 1% orcinol, conc. HCl, 10% ferric chloride in a ratio of 10 : 40 : 1). To a known amount of RNA aliquot 6 ml of orcinol reagent was added and heated at 100°C for 10 minutes. The following regression formula calculated using a standard RNA was employed to determine the mg. RNA/g. dry weight, or per organ.

\[ Y = 96.05 x -0.35. \]

5. Amino acids

The content of free amino acids was determined following the method of Harding and Maclean (1916).

The reaction system contained 0.5 ml of ethanol extract of the material, 1 ml of 10% pyridine and 1 ml of 2% ninhydrin reagent. The test tubes were stoppered and heated at 100°C on a waterbath for 30 minutes. Later it was cooled and diluted with
distilled water to a known volume. The OD of the violat blue colour developed was read at 570 nm. The following regression formula was prepared using isoleucine as standard and the amino acid content was expressed as mg/g dry weight or per organ.

\[ Y = 50.85x + 3.36 \]

6. **Total Phenols**

Total phenol content was determined by the method of Farks and Kirly (1962). The reaction mixture containing 0.5 ml of ethanol extract, 1 ml 20% sodium carbonate and 0.5 ml folin-phenol reagent, was heated on a waterbath for 10 minutes, cooled and diluted to a known volume with distilled water. The blue colour of the supernatant was measured at 660 nm. Phenol content was expressed as gallic acid equivalents, employing following regression formula:

\[ Y = 17.23x - 3.25 \]

7. **Free Proline**

The method described by Bates et al., (1973) was employed to estimate free proline. Weighed material was crushed and extracted in 3% sulphosalicylic acid. After centrifugation 2 ml of this aliquot was added to 2 ml of ninhydrin reagent and 2 ml of glacial acetic acid and boiled it for 1 hour at 100°C on
waterbath with stoppers, and the reaction was terminated in an ice bath. Then it was extracted against 6 ml toluene with a separating funnel. The absorbance of Toluene extract was read at 520 nm against a Toluene blank.

Proline content was expressed as g/g dry weight using the regression formula:

\[ Y = 37.64x - 0.49 \]

For enzymes, the plant material was macerated in ice-cold distilled water at 4-5°C and centrifuged. The supernatant was used as enzyme source except for lipase and catalase.

8. Peroxidase:

The method described by George (1953) and Maehly (1954) was employed to assay peroxidase activity. The reaction mixture containing 2 ml enzyme aliquot, 2 ml 0.2 M phosphate buffer (pH 7.0) and 2 ml guaiacol reagent (0.22 ml guaiacol, in 100 ml distilled water) was taken into a cuvettes. The cuvettes were placed in the spectrocolorimeter. The wavelength was adjusted to 470 nm. A drop of 10 mM hydrogen peroxide (H₂O₂) was added to the reaction mixture with a glass rod. The OD was noted after 30 seconds and peroxidase activity was expressed as OD/min/mg protein.

9. Catalase activity:

Manometric method of Chance and Maehly (1955) was
employed to estimate catalase activity.

5 ml of 10 mM H$_2$O$_2$ was taken in a polyethylene vial. The vial was carefully placed in a reaction bottle containing 10 ml of enzyme extract (the seedlings were grounded in ice-cold DW with a pinch of Na$_2$Co$_3$) and 2 ml of 0.1 M phosphate buffer (pH 7.0). The bottle was plugged with an India rubber stopper through which it was connected to manometer. The reaction bottle was shaken for one minute at a constant speed so that the substrate in the vial and enzyme in the bottle would get mixed and oxygen was liberated. The difference of the water level in the manomater before and after the reaction was equal to the ml O$_2$ evolved as a result of enzyme activity which was expressed as ml O$_2$ evolved/minute/mg protein.

10. Amylase activity
(Paleg 1960)

0.5 ml of aliquot was mixed with 1 ml of citrate buffer (pH 5), and 0.1% starch (2ml) and incubated for 10 minutes at room temperature and enzyme activity was terminated by adding 1 ml of I$_2$ KI. After 5 minutes final volume was made to 25 ml. The difference in OD between reaction and blank was worked out using inactivated (by heating) enzyme (Malik and Singh 1980). Starch hydrolysed was calculated using the regression formula:
Amylase activity was expressed as mg starch hydrolysed/10 min/mg protein

11. **Invertase activity:**

The assay procedure was of Hatch and Glassiou (1963).

1 ml of enzyme aliquot, 1 ml of 0.25% sucrose and 1 ml of 0.1 M citrate buffer (pH 5.5) were taken into a test and incubated at 30°C for 30 minutes. The enzyme was inactivated by adding 2 ml of 5% perchloric acid. The amount of glucose released was estimated by anthrone method as in case of sugar estimation. The invertase activity was calculated and expressed as mg glucose released/hour/mg protein using glucose as standard.

\[ Y = 124.61 \times -3.25 \]

12. **Protease activity:**

The method described by Penner and Ashton (1967) was followed for protease activity.

1 ml of enzyme aliquot, 3 ml of 0.1 M phosphate buffer (pH 7.0) and 2 ml of 0.5 ml casein (pH 7.0) were taken into a test tube and incubated at 30°C for one hour. Later, 2ml
of the reaction mixture was mixed with 2 ml of 15% TCA. After 20 minutes the precipitated protein was discarded by centrifugation. The liberated tyrosine in supernatant was estimated using folin-phenol reagent (Lowry et al., 1951).

The following regression formula was calculated using tyrosine as standard. Protease activity was expressed in terms of mg tyrosine liberated/hour/mg protein.

\[ Y = 232.14 \times -0.71 \]

13. **Ribonuclease activity:**

The enzyme RNase was assayed following the method of Peach and Tracy (1964).

The assay system consisted of 1 ml 0.4% in 0.1 M acetate buffer (pH 5.0), 1 ml enzyme extract and 1 ml 0.1 M acetate buffer (pH 5.0). The reaction was carried out for 30 minutes at 24°C. The enzyme activity was stopped by adding 1 ml of 0.75% uranyl acetate in 25% HClO₄ (W/V). The content was centrifuged for 15 minutes at 1500 rpm. The ribose sugar in the supernatant was analysed by orcinol method (Markham, 1955) as described for RNA.

RNase activity was expressed as mg ribose released/hour/mg protein. The following regression formula was prepared
using ribose sugar as standard:

\[ Y = 330.19 x - 12.02 \]

III. Photosynthetic Pigments

1. Chlorophylls:

Chlorophyll pigments in the leaves were estimated following the method of Arnon (1949).

Freshly harvested (100 mg) leaf material was crushed and extracted thrice in chilled 80% acetone. The volume of acetone extract was made to a standard mark (25 ml). The absorbance of the acetone extract was measured at 645 and 663 nm in a spectrophotometer using acetone as a blank. The amount of pigments were calculated from the following formula and expressed as mg/g f weight.

- Total chlorophylls \((20.2 \times OD \text{ at } 645) + (8.02 \times OD \text{ at } 663)\)
- Chlorophyll - a \((12.7 \times OD \text{ at } 663) + (2.69 \times OD \text{ at } 645)\)
- Chlorophyll - b \((22.9 \times OD \text{ at } 645) - (4.86 \times OD \text{ at } 665)\)

2. Carotenoids:

Carotenoid content was estimated by the method of Jenson and Jensen (1970).
Known weight (1 gram) leaf material was cut into small pieces and macerated with chilled acetone in a mortar. The extract was centrifuged. The residue was reextracted with acetone and centrifuged. The procedure was repeated until no more pigment was extracted. The above supernatant was combined and the acetone was removed in vacuo. The residue was dissolved in petroleum ether (B.P. 40-60°C). Equal volume of 10% methanolic KOH was added. The beakers were tied with polyethylene cover and allowed to stand for two hours. The contents were taken into a separating funnel. Chlorophylls which dissolved in methanolic KOH were removed and the petroleum ether layer was partitioned against two volumes of 5% NaCl solutions. Petroleum ether extract was washed thrice with 5% NaCl solution to remove methanol. The petroleum ether extract was evaporated.

The dried carotenoids were dissolved in a known volume of 80% acetone and the absorbance was taken in a spectrophotometer at 445 nm. Carotenoid content was calculated from the following formula

\[ C = d \cdot v \cdot f \cdot \frac{10}{2500} \]

where

- \( C \) = mg carotenoids
- \( d \) = optical density
- \( v \) = total volume in ml
- \( f \) = dilution factor

Values were expressed as g/g.f. weight.
3. **Chlorophyllase activity**

Method of Holden (1961) was adopted for chlorophyllase activity estimation.

**Enzyme Preparation**

Freshly harvested leaves were ground in a pre-cooled mortar with chilled acetone. The suspension was filtered on Buchner funnel. The residue was washed with chilled acetone several times to remove chlorophyll pigments. Residue was dried at room temperature and the dried powder was used as an enzyme source.

**Substrate Preparation**

The substrate was prepared by grinding the fresh leaves in acetone (4ml/g leaf) filtered rapidly through Buchner funnel and then stored the extract in deep freeze for several days before use. Carotene which precipitated out during storage was removed by filtration and the chlorophyll in it was estimated according to the method of Amin (1949) as described earlier.

**Enzyme Assay**

Weighed (250 mg) acetone powder was incubated at room temperature in the dark with 8 ml of chlorophyll solution
and 4 ml of sodium citrate solution; the final concentration of acetone being about 50% and of citrate buffer 0.04 M. The suspension had a pH of 8.0. After incubation for 24 hours, the powder was filtered off on a Buchner funnel and washed with acetone until all the pigments were extracted. To the filtrate 10 ml of 2% NaCl solution was added and the volume was made up to 50 ml with 80% acetone. Half the solution was kept for the determination of total chlorophyll present. The other half was shaken twice with 10 ml of light petroleum ether (40-60°C) to extract unchanged chlorophyll. The light petroleum was discarded and the acetone layer containing chlorophyllide was again made up to 25 ml with 80% acetone. The absorption of the two solutions was determined at 645 nm and 663 nm. The amount of total chlorophyllides formed was calculated by the formula (mentioned earlier) for the total chlorophyll estimation and the activity was expressed as

\[ \text{mg chlorophyllides/250 mg acetone powder/24 hrs.} \]

IV. Hill Reaction activity:

**Isolation of chloroplasts:**

Procedure of Raghavendra and Das (1976) was adopted for chloroplasts isolation and Hill reaction activity.
Fully grown young leaves (one gram) were taken (mid rib removed) and washed with distilled water chilled to 0°C cut into small pieces ground in pre-chilled mortar and pestle in the following grinding media.

- 50mm Phosphate buffer (pH 7.4)
- 0.33 M sorbitol
- 1 mM EDTA
- 1 mM MgCl₂
- 5 mM DTT
- 0.5% BSA (Bovine Serum Albumin)

This homogenate was filtered through double layer muslin cloth and centrifuged at 600 x g for 15 minutes, the residue (debris) was discarded, again centrifuged the supernatant at 300 x g for 20 minutes. The chloroplast pellets were taken and resuspended in the above media without EDTA and stored in refrigerator till the experiment started.

**Dichlorophenol indophenol (DCPIP) reduction:**

The reduction of the dye occurred in the following manner. A total volume of 6 ml contained following chemicals:
50mM Phosphate buffer (pH 7.5)
20mM DCPIP
1 mM MgCl₂
20mM NaCl
and chloroplasts suspension

Blank was maintained for each and one set was kept in illumination for known time (10 minutes) and the other set was kept in darkness. The samples were read at 620 mm spectrophotometer before and after illumination.

**Ferricyanide reduction:**

The reaction mixture for ferricyanide (in total volume of 6 ml) contained:

15 mM tris - Hcl buffer (pH 7.8)
0.5 mM ferricyanide
1 mM MgCl₂
20 mM NaCl
and chloroplasts suspension

Above samples were kept for illumination (10 minutes) at the end of illumination 1 ml of 20% TCA was added. The absorbance of the solution was measured at 420 mm after centrifuging to remove the protein.

Difference in OD was calculated for 10 minutes/mg chloroplasts
V. Extraction and Estimation of endogenous growth regulators

Endogenous Gibberellin-like and Cytokinin-like promoters and Abscisic acid-like inhibitors were extracted from the leaves and stem of control and NaCl grown plants and their biological activities were assayed.

Gibberellin Like substances:

Extraction:

Gibberellin like substances were extracted following the method of Murakami (1966). 25 g. of plant material was macerated and extracted with 70% acetone in dark and cool condition for 24 hours. The acetone was decanted into another beaker and the residue was reextracted with fresh acetone for four hours. The combined acetone extract was filtered and the filtrate was evaporated in vacuo. The resulting aqueous phase was adjusted to pH 8.0 with Na₂CO₃ and partitioned thrice against ethyl acetate. The three ethyl acetate fractions were collected as basic fractions. The aqueous phase was adjusted to pH 2.5 with H₃PO₄ and partitioned thrice with ethyl acetate. The ethyl acetate fractions were cooled which served as acidic fraction.

The acidic and basic fractions were combined and evaporated to dryness. The residue was dissolved in 1 ml of ethyl alcohol.
Chromatography.

The alcohol extract was loaded on Whatman No 1 chromatographic paper and separated with solvent system of isoproplol : 25% NH\textsubscript{4} OH : Water (10:1:1 V/V). The dried chromatogram was cut into ten equal strips and each strip was separately eluted in ethanol. The ethanol was evaporated and residues were assayed for gibberellin like substances using barley endosperm bioassay.

**Barley Endosperm bioassay**: (Jones and Verner, 1967).

The endosperm halves of barley seeds were washed under running tap water and distilled water, and allowed to imbibe for 24 hours. 3 ml of 0.1 M citrate buffer (pH 5.5) was added to the petridishes in which the chromatographic strips were eluted and dried. The imbibed endosperm halves were placed in petridishes (6 per dish) and kept under continuous light at 27°C for 48 hours. These endosperm halves were analysed for amylase activity (Palag, 1960). The observed values were expressed in terms of percent of control.

**Cytokinin like substances**: The extracting material was same as in case of gibberellins. The procedure employed was of Vanstandan (1978).

**Extraction**: 100 g. of fresh material was macerated and extracted with 80% ethanol in a cool and dark condition for 48 hours. After
Filtration, the residue was re-extracted twice with smaller quantities of 80% ethanol. The ethanolic extracts were combined and evaporated in vacuo at 35°C. The residue was redisolved in 80% ethanol pH of which was adjusted to 2.5 with HCl. The extract was filtered and the filtrate was passed through a Dowex 50w - X8 (H+ from 200 - 400 mesh) column of 2 x 15 cm at a flow rate of 10 ml/hour. Then the column was washed with 80% ethanol and cytokinin like substances were eluted with 150 ml of 5 N NH₄OH (V/V). The ammonia was evaporated and residue was dissolved in 2 ml of 80% ethanol. The ethanolic extract was loaded on a Whatman No.1 chromatographic paper. The chromatographic procedure was as described for gibberellins.

Bioassay for cytokinins: Fletcher et al., (1982)

Sterilized Cucumis sativus L seeds were germinated in dark at 27°C. On the sixth day the cotyledons were excised in dim green light and placed in petridishes (6/dish) in which the chromatogram strips were eluted. 3 ml of 40 mM Kcl was added to each petridish. These petridishes were exposed to a light (500 W tungsten bulb) for three hours. After the light treatment, the chlorophyll, as developed in each set of cotyledons was estimated (Arnon, 1949). Cucumber seeds soaked in 40 mM Kcl avoiding the eluted cytokinin like substances, served as control. Kinetin was used as standard.
Abscisic Acid Like Inhibitors:

Method of Lenton et al., (1971) with slight modifications was adopted for Abscisic acid extraction.

**Extraction:**

About 100 g of the freshly harvested leaf and stem material was macerated at 5°C in cold methanol. The supernatant was filtered and the residue was reextracted with an additional 500 ml methanol and filtered. The methanolic extract after filtration were pooled up and the methanol was evaporated in vacuo. The aqueous solution was frozen, thawed and centrifuged to remove suspended material. The supernatant was adjusted to pH 3.5 with 2 N HCl and extracted thrice with equal volume of diethyl ether. All the ether extracts were pooled up and extracted four times, alternatively with one quarter its volume of 5% sodium bicarbonate solution and one quarter of its volume water. All the aqueous extracts were combined and adjusted to pH 3.5 and reextracted with equal volumes of diethyl ether and all the ether extracts were pooled up and dried over anhydrous sodium sulphate. The residue after evaporation was taken in known volume of 80% ethanol and stored in deep freeze until further use.

**Chromatography:**

A known volume of this extract was loaded onto a What
man No 1 Chromatography paper and separated with iso propanol: 25% NH₄OH:water (10:1:1 V/V). The chromatograms were dried and divided into 10 equal Rf strips and eluted in ethanol. The ethanol was evaporated and the residues were assayed for abscisic acid like inhibitor activity using wheat coleoptile bioassay:

**Bioassay:**

The straight growth wheat coleoptile bioassay was carried out according to Nitsch (1956) as modified by Rudnicki (1969) for abscisic acid like inhibitors. Wheat seeds were sown in petridishes (9 cm diameter) having monolayer filter paper, in dark for 60 hours at 25°C. Ten subapical sections of 5 mm length were cut in dim green light and transformed to abscisic acid like inhibitors containing petridishes which were dissolved in 0.5% sucrose and incubated for 24 hours in dark. Sucrose solution alone was used for control. After 24 hours the length of the coleoptiles were measured up to nearest mm.

VI. **Estimation of mineral constituents:**

Oven dried ground plant material was transferred to 50 ml jaldal flasks containing triple acid mixture (HNO₃ : HClO₄ : H₂SO₄ (10:1:1 V/V) and heated until the fumes subsidized leaving the digest colourless. The digest was made to a known volume (50 ml).
Sodium (Na\(^+\)), Magnesium (Mg\(^{2+}\)), Calcium (Ca\(^{2+}\)), and Potassium (K\(^+\)) were estimated by Systronic digital (Model III) Flame Photometer.

**Chloride:**

Chloride (Cl\(^-\)) was estimated by titration with silver nitrate; USDA, (1954).

To the digested leaf material a pinch of Na\(_2\)CO\(_3\) and 4 drops of reagent A (5% Potassium chromate solution) was added and titrated against silver nitrate (0.005 N) till the sample turns reddish brown colour. The titration blank was also carried out. The difference was taken as chloride reading. Values were expressed as mg/g. dry weight.

**VII. Soil pH and Electrical conductivity:**

Soil samples were collected before and after NaCl treatments from both control and salinized pots. The soil was air dried and made to a fine powder. U.S. Salinity Laboratory Staff report (1954) method was adopted for soil saturation extractions.

20 g soil powder was taken in 200 ml conical flask and added 100 ml of distilled water. After thorough mixing
### Table 2: $^pH$ and Conductivity of Soil Saturation Extract

<table>
<thead>
<tr>
<th>Days after sowing</th>
<th>Sample</th>
<th>No. of treatments</th>
<th>Days after first salt treatment</th>
<th>$^pH$</th>
<th>Conductivity (EC) $30^\circ C$ mmhos/cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>8.20</td>
<td>3.14</td>
</tr>
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I T = First Salt Treatment  
II T = Second Salt Treatment
the samples were allowed to stand for 24 hours. These soil solutions were filtered and supernatant was used for pH and electrical conductivity determinations.

**Statistical analysis**

Analysis of variance was employed to draw the relationship among growth characters and biochemical analysis due to salt treatment and days and their interaction.