Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 19s (2025)
https://www.jneonatalsurg.com

Physiological and anti-oxidative properties of wheat (Triticum aestivum) plant subjected to salt stress

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Cite this paper as: Ansul Sarangi, Manoja Das, Kambaska Kumar Behera, Nibedita Samal, (2025) Physiological and anti-oxidative properties of wheat (Triticum aestivum) plant subjected to salt stress. *Journal of Neonatal Surgery*, 14 (19s), 883-895.

ABSTRACT

India's lakes and oceans, adorned with nature's brushstrokes, offer a sanctuary for reflection, inspiration, and spiritual communion. One of the most remarkable Chilika Lake is its importance as a habitat of its surroundings. It has already been declared a Ramsar site under the Convention on "Wetlands of International Importance" due to its ecological significance. We collected water and soil from it and its surroundings, like Brahmagiri and Gadarodaanga, and observed its physicochemical properties like TDS, PH, conductivity and salinity. The details observations are recorded and are represented with graphs. We learned that water and soil impact the plants in this area, which could be due to salt stress. However, we cannot determine the actual salt concentration of the whole area for which we conducted experiments by taking a different concentration of NaCl solution in our laboratory and extracted antioxidant enzymes like Proline, Malondialdehyde(MDA), Soluble protein Peroxidase and Glutathione, observed to be increasing when the concentration of NaCl increases. Still, the concentration of antioxidant enzymes like catalase, Ascorbate peroxidase, and NADH peroxidase decreases in the leaves of newly grown wheat plants..

Keywords: *Triticum aestivum, anti-oxidative, physiological, salt stress, ecological significance*

1. INTRODUCTION

India is known for its diverse climatic regions, which include extreme conditions like drought, cold, high or low temperatures, nutrient deficiencies, excess or deficient water, high salinity, heavy metal presence, and ultraviolet radiation. These abiotic factors significantly impact global agriculture, causing over 50% reduction in potential yields for significant food and fodder crops (Wang et al., 2003). The main stressors include high temperature (40%), salinity (20%), drought (17%), low temperature (15%), and other forms of stress(Ashraf et al., 2008).Stress impacting seeds can significantly affect plant reproduction, productivity, agriculture, and biodiversity. The east coast of India is blessed with the largest brackish water lagoon in the subcontinent, the Chilika Lake. Due to its ecological significance, it has already been declared a Ramsar site under the Convention on "Wetlands of International Importance" (Arya et al. 2006). The lake is shallow, and because of the silting action of the Mahanadi River, the lake has been formed with a variety of habitats such as marshes, mudflats, fresh water, open water, as well as areas covered under coastal vegetation. In India, it is considered one of the hotspots for biodiversity.Over the past eight decades, scientific exploration has proposed that the physicochemical limitations of Chilika Lake have experienced numerous difficulties because of environmental variations and human-induced pressures. The hydrological conditions of the lake water have adversely affected aquaculture activities, resulting in the reduction of fish productivity and changes in the composition of avifauna species. Eutrophication has also occurred, leading to an overall biodiversity loss and degradation of the lake's ecosystem (Lester et al. 1975).

In this study, we collected water and soil from Chilika and its surroundings and observed its physicochemical properties. We concluded that due to the difference in salt concentration, the growth of plants shows a difference from average water concentration. Hence, we got the idea to observe plants' physical and biochemical properties in different salt concentrations in our laboratory.

Abiotic Stress

Global warming poses a grave threat to agriculture, especially wheat, with potential losses of up to 42 million tons per degree Celsius rise in temperature. Abiotic stresses like drought, salinity, and high temperatures significantly impact wheat productivity. Understanding these stresses is vital for effective improvement programs that rely on genetic variations through conventional breeding. Drought leads to water scarcity, salinity disrupts water uptake, and high temperatures cause physiological damage, all reducing yield (Arya et al. 2006). Conventional breeding plays a crucial role in leveraging genetic diversity to develop resilient wheat varieties. Crossbreeding for stress tolerance traits creates varieties suited to changing climates. Advanced biotechnological tools like marker-assisted selection and genetic engineering expedite this process. Marker-assisted selection identifies stress-resilient traits efficiently, while genetic engineering introduces specific stress-tolerance genes, enhancing wheat's ability to withstand adverse conditions. These approaches are essential for ensuring food security amidst climate challenges (Ahmad et al., 2019).

TDS, pH, salinity and conductivity:

Total Dissolved Solids (TDS) in the water represent the total concentration of dissolved substances, measured in milligrams per litre (mg/L) or parts per million (ppm). TDS includes organic matter and inorganic salts like magnesium, calcium, sodium, potassium (cations), and anions such as nitrates, carbonates, chlorides, sulfates, and bicarbonates (Ahmad et al., 2019).

The pH (Potential of hydrogen) value indicates the acidity or alkalinity of water, typically ranging from 6.5 to 8.5 in surface and groundwater. pH meters measure pH on a scale from 0 to 14, with 7 as neutral. They detect hydrogen ion concentration using glass, combination, or solid-state electrodes. Overall, pH meters play a crucial role in monitoring and controlling pH levels in various processes for optimal performance and safety (Ashraf et al., 2008).

Soil salinity, caused by natural processes like weathering and human activities such as irrigation, results in elevated salt levels in soil and water. Salts like K^+, Na^+, Ca^{2+}, Mg^{2+}, and Cl^- contribute to salinization. Effective irrigation management is crucial for mitigating salt buildup. Salinity in water bodies is measured in g/kg or g/L and serves as an indicator of water quality and ecosystem health.Monitoring and managing salinity are vital for maintaining soil fertility, water quality, and ecosystem balance (Lester et al. 1975).

Soil electrical conductivity (EC) measures the soil water's ability to conduct electrical current, primarily through water-filled pores. It relies on electrolytic principles involving cations (Mg^{2+}, Ca^{2+}, Na^+, NH^{4+}, K^+) and anions (Cl^-, SO_4^{2-}, HCO_3^-, NO_3^-) from dissolved salts. EC is influenced by planting practices, irrigation, land use, and soil texture (Nanda et al., 2008). These methods aid in understanding soil properties and optimizing agricultural practices for sustainable crop production (Ashraf et al., 2008).

Seed germination:

Seed germination is pivotal for crop quality and yield. Understanding molecular mechanisms, especially in cereals and pulses, enhances productivity. In steppe regions, drought during planting and sowing seasons necessitates improving water use efficiency or drought tolerance in crops. Five critical events in germination include imbibition, light's effect, respiration, embryo axis development, and reserve mobilization regulated by growth regulators like gibberellins and auxins. These processes ensure successful germination and seedling growth, impacting agricultural and horticultural practices. Understanding and optimizing these events are vital for sustainable crop production and addressing water scarcity and environmental stresses in agriculture (Nanda et al., 2008).

Factors required for germination are: -

1. Water 2. Temperature 3. Aeration

4. Exposure to light and dark 5.Seed vitality 6. Genotype

7.Seed maturation 8.Seed dormancy

Germination test- It refers to a specific procedure that helps predict the potential seeds and obtain information about the planting value of the seeds.

Significance of seed test-

1. Sowing purpose 2. Seed certification purposes

3. Labelling purpose 4. Experiment purpose.

5. General home purpose 5. Seed act and law enforcement purpose.

2. MATERIAL AND METHODS

Plant material

Healthy as well as dried seeds of wheat (Triticum aestivum) were collected were selected for analysis and purchased from Seed bank Bhubaneswar. The seeds were preserved in a cool and dry place.

Method of germination tests: -

Roll towel method- It is done at home. By use of towel.

Petri plate method- Useful for small seeds. It can be used for seeds that require light as it is an easy procedure and common to lab and home.

Sand method- Used for bigger seeds.

Between paper method- No extra container required. Holds good for a variety of seed sizes. Easy procedure and seeds can be grown taller.

$$\text{Germination percentage} = \frac{\textbf{Number of seeds germinated}}{\textbf{Total number of seeds}} \times 100$$

The lab experiment was carried out using dry seeds of wheat (Triticum aestivum). The seeds were soaked in three different water samples collected from different areas (Chilika, Gadarodanga (32km from Chilika) and Brahmagiri (35km from Chilika) for 12 hrs and then germinated on Petri plates (Fig. 4). Then observation was taken.These seeds were used as the source material for germination under abiotic-stressed conditions and were analysed for antioxidant activity and again here we used some aseptic techniques for better result(Ashraf et al., 2008).

Aseptic techniques

Surface sterilization

Healthy and uniform seeds were selected carefully without any injury to the embryo portions and soaked in 0.05% solution of Teepol (a non- phytotoxic liquid detergent) for 3 minutes. The seeds were then dipped in 5% (W/V) solution of commercial bleaching powder [Calcium oxychloride. (CaOCl)] for 5 minutes followed by a dip in 0.1% (W/V) mercuric chloride ($HgCl_2$) solution for another 5 minutes. The duration of treatment of the seeds in the abovesterilant was standardized after several trials. Finally, the seeds were washed thoroughly for another 30 minutes with several changes in sterile double distilled water. All operations were performed under aseptic conditions in an Ultra-klenz horizontal laminar airflow cabinet (Klenzaids, India) with airflow of 0.46 m.s-2 allowing less than 4 particles of 0.5um diameter L-1 of air. Before working in the cabinet, the surface was cleaned with rectified spirit and the airflow was allowed to run for at least half an hour. The UV lamp of the cabinet was kept on for at least 20 minutes prior to inoculation to kill the remaining microbes, if any, on the surface (Nanda et al., 2008).

In vitro culture

Culture media

The formulation of Linsmaier and Skoog's (1965) basal medium (LS medium) is the most used medium for rice tissue culture (supplemented with 3% sucrose, 1.0 mgLhiamine HCl and 2.0 mgL phytohormone (2,4-D) were used throughout the investigation. The media was made semisolid with 0.8% agaragar (Qualigens Fine Chemicals Ltd., Bombay, Table-1).

Table 1 Composition of LS medium (Linsmaier and Skoog, 1965) for the experiment.

Constituents	Concentration in mg L^{-1}
$CaCl_2 .2H_2O$	440.000
$NH_4.NO_3$	1650.000
KNO_3	1900.000
KH_2PO_4	170.000
$MgSO4.7H_2O$	370.000
KI	0.830
$CoCl_2.6H_2O$	0.025
H_3BO_3	6.200

Na_2 MoO4. $2H_2O$	0.250
$MnSO_4.5H_2O$	22.300
$CuSO_4$. $5H_2O$	0.025
$ZnSO_4$. $7H_2O$	8.600
$FeSO_4$ $7H_2O$	27.850
Na_2-EDTA	37.250
Inositol	100.000
Thiamine HCI	1.000
Sucrose	30,000,000
2,4-D	2.000

Media preparation

The stock solution of inorganic salts and organic compounds were made with sterile double distilled water and were kept in closed glass bottles plus stored in a refrigerator. 2,4-D (Hi Media laboratories Pvt. Ltd., Bombay, India) stock solution was made in minimum volume of ethanol and diluted in sterile double distilled water to a definite volume and was stored in cold condition. 100 mg.L inositol and 30,000 mgL^1 sucrose were added to the medium. The solution was adjusted to pH 5.8 with 0.1N NaOH. Finally, the conical flasks were closed with cotton plugs (non-absorbent), wrapped with cheesecloth and were autoclaved for 20 minutes at 121°C and 15 p.s.i. These culture flasks were stored in the tissue culture room and were used within 2-3 days(Nanda et al., 2008; Ashraf et al., 2008).

Aseptic transfer of plant material and culture condition

The sterilization of wheat (Triticum aestivum) seeds was done under aseptic conditions as described earlier. The inoculation and transfer of sterile plant materials was conducted under aseptic conditions in a laminar airflow cabinet. Twenty-five seeds were transferred into each culture flask containing 30 ml of previously sterilized nutrient medium. The culture flasks were plugged with cotton wool plugs under the laminar airflow cabinet. The cultures were kept under a continuous light irradiance of 35 µmol m-2. S-2 (provided from a bank of white, fluorescent tubes). The room temperature was maintained at 25±1°C(Ahmad et al., 2019).

Tissue sampling

Soaked wheat (Triticum aestivum) seeds were taken out from the flask and were transferred to the wet cotton bed kept on Petri disc. After one night, out of the 100 seeds,70 well-germinated seeds were chosen for further growth Fig. 1.

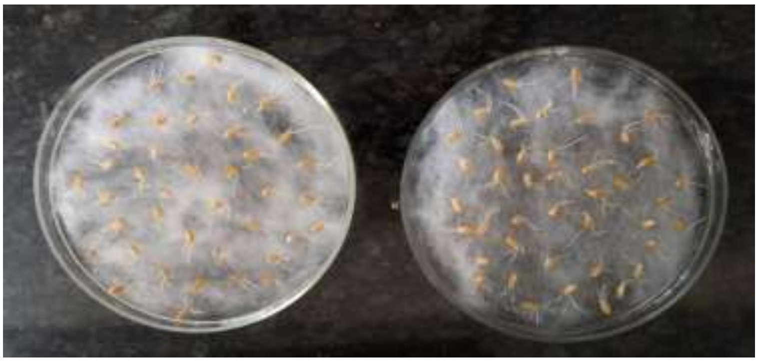


Fig. 1Germinated wheat seeds after tissue sampling.

Method for the determination of physiological properties of water:

The pH, salinity, TDS and temperature determine the physiological properties of water. These are analyzed with the help of the Systronics Water Analyzer 371 kit. Using the different instruments available in the kit, we calculate the different properties of water and the table in Table 1.

Method for the determination of physiological properties of soil:

For the soil, we must prepare the KCl solution in which the soil will be mixed to make it easy to determine the properties.

Preparation of 3M KCl solution:

22.365gm of KCl with 70ml of distilled water is taken.

Then it is mixed until perfectly dissolved.

The solution is made of 100ml by adding distilled water to it.

Soil particles are mixed with the solution, and the soil properties are analyzed by the Systronics Water Analyzer 371 kit, and observation was recorded in Table2.

Salt stress on non-leguminous seed Triticum aestivum:

Salt stress represents a major environmental limitation (Munns and Tester, 2008; Ahmad et al., 2019). It has a widespread impact, affecting approximately 80 million hectares of arable lands worldwide. The root system is the first to detect and respond to salt stress, leading to hindered plant growth. This stress arises from both reduced water availability due to osmotic effects and the toxicity of ions, resulting from an imbalance of solutes in the cytosol (Heath and Packer, 1968). In this section, we will specifically address the effects of salt stress on non-leguminous species.

In case of germination under salt stress, the sterilized seeds were germinated in cotton in a Petri disc containing Sodium Chloride (NaCl). The seeds were subjected to salt stress through application of NaCl. A calculated concentration of chemical was used to obtain 0.5mm, 1mm, 2mm, 5mm saltwater potential. For the corresponding control, seeds were geminated by supplementing normal tap water. After sampling the healthy and well germinated seedlings, it was dissected into embryo and endosperm and each part was analysed in third, fifth and seventh day of germination for its Morphological, enzymatic and biochemical changes (Ahmad et al., 2019).

Biochemical analyses

Extraction and evaluation of malondialdehyde (MDA) in stress:

The levels of Lipid peroxidation were assessed by quantifying malondialdehyde, which is a breakdown product resulting from the peroxidation of polyunsaturated fatty acids. The measurement was performed using the thiobarbituric acid reactive substance (TBA-RS) method (Thurman et al., 1972). The tissues subjected to different stresses were homogenized with 5% (w/v) trichloroacetic acid so also the homogenate was precisely implemented for the estimation of malondialdehyde.

3. RESULTS AND DISCUSSION

We collected sample water from three places that are from Chilika Lake, Gadarodanga and Brahmagiri in three different days determining as observation- 1, observation- 2 and observation- 3. We conducted an experiment to find out pH, TDS, salinity and temperature of water from these places.

Effect of physiological properties of water for the germination of wheat in and around Chilika Lake.

Effect of pH of water on germination of wheat seeds

Understanding the intricate relationship between salinity, ROS production, and seed germination is crucial for developing effective strategies to ensure better crop establishment and yield in saline-affected regions.The pH of water can significantly influence seed germination in wheat seeds, and its connection with ROS is noteworthy (Mohanty, 2012).

pH of Water and Seed Germination:

Wheat Seeds: Wheat seeds generally germinate well in a pH range of 6.0 to 7.5. Water pH within the favorable range supports wheat seed germination. pH influences ROS levels, impacting germination. Extreme pH triggers ROS production, affecting seed germination negatively. ROS, when within tolerance, regulates germination; excess ROS impairs it(Lowry et al., 1951). Manipulating water pH indirectly affects ROS, crucial for understanding and enhancing crop resilience. As per the readings made by taking the sample water it is observed that:

pH of all the three days remained in the range of 6.85 to 7.49 in Chilika Lake. Thus, thesample shows that the average pH in Chilika Lake is around 7.10, the data the average pH in Gadarodanga is around 6.26. and the average pH in Gadarodanga is around 6.29. From the above data it can be noted that the water in Chilika Lake is neutral and that to in Gadarodanga and Brahmagiri is slightly acidic but can be consumed, as the ideal pH level of drinking water ranges between 6-8.5(Zor and Selinger, 1996).

Effect of TDS of water on germination of wheat seeds

Total Dissolved Solids affect wheat seed germination, preferring moderate levels. High TDS induces salinity, impeding germination, while low TDS lacks nutrients. TDS levels influence ROS production; high TDS triggers ROS overproduction, causing oxidative damage and hindering germination. Understanding this complex relationship aids in managing irrigation to prevent detrimental effects and exploring treatments to mitigate ROS-induced damage, crucial for improving crop yield in regions with high TDS water (Ahmad et al., 2019). As per the readings made by taking the sample water it is observed that:

TDS of water in all three days remained in the range of 152 to 161 in Chilika Lake. Thus, it can be assumed that the average TDS of water in Chilika Lake is 155, the average TDS of water in Gadarodanga is 387.33 and the average TDS of water in Brahmagiri is 206.66. From the above noted case study it is observed that TDS is maximum in Gadarodanga and minimum in Chilika Lake. However as per TDS the water in Gadarodanga is not fit for drinking (Marshall and Worsfold, 1978).

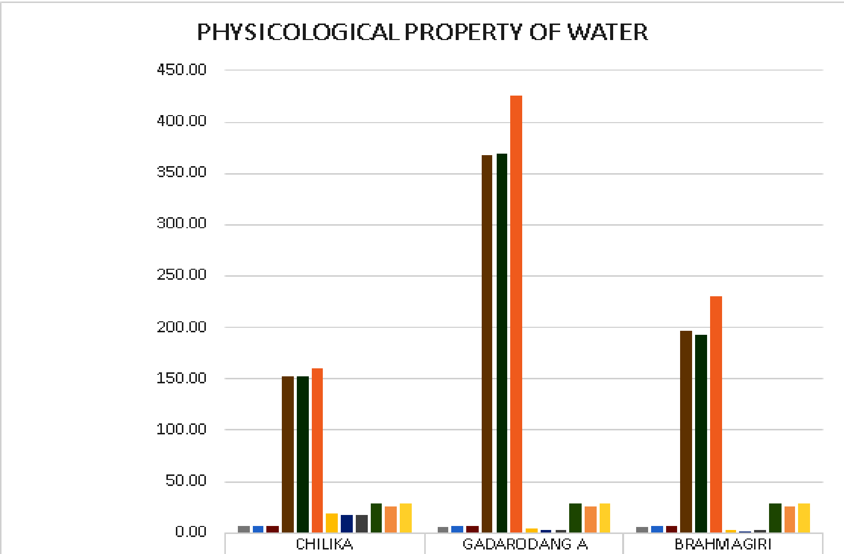
Effect of salinity of water on germination of wheat seeds

High salinity impedes wheat seed germination by limiting water uptake and inducing ROS production. ROS accumulation damages cellular components, affecting seed viability. Researchers explore antioxidant enzymes like catalase and superoxide dismutase to counteract oxidative stress, crucial for improving germination rates under saline conditions.As per the readings made by taking the sample water it is observed that:

The salinity of water in all three days remained in the range of 17.1 to 18.4 in Chilika Lake. So, it can be assumed that the average salinity in Chilika Lake is 17.56, the average salinity in Gadarodanga is 3.50 and the average salinity in Brahmagiri is 2.33.From the above noted case study it is observed that salinity is maximum in Chilika Lake and similar in Gadarodanga and Brahmagiri. The salinity is more in Chilika Lake because of the sea water that gets mixed with the water of Chilika. However, none of the waters is ideally fit for drinking(Aebi, 1983).

Effect of temperature of water on germination of wheat seeds

Water temperature affects wheat seed germination; 15-30°C is ideal. High temperatures accelerate ROS production, influencing germination. ROS, crucial for germination, can damage cells if excessive. Optimal water temperature ensures successful germination by managing ROS levels. Studying antioxidants may mitigate ROS damage, improving crop yield(Kar and Feierabend, 1984).The overall physiological property (Fig. 2) of the water in and around Chilika Lake is arranged in a single tabular form.



	CHILIKA	GADARODANGA	BRAHMAGIRI
PH OBS-1	6.97	6.08	6.2
PH OBS-2	6.85	6.33	6.37
PH OBS-3	7.49	6.37	6.32
TDS OBS-1	153	368	197
TDS OBS-2	152	369	193
TDS OBS-3	161	425	230
SALINITY OBS-1	18.4	4.24	2.34
SALINITY OBS-2	17.1	3.03	2.14
SALINITY OBS-3	17.2	3.24	2.52
TEMPERATURE OBS-1	28.5	28.5	28.5
TEMPERATURE OBS-2	25.2	25.2	25.2
TEMPERATURE OBS-3	29	29	29

Fig. 2 Physiological property of water.

We collected sample soil from three places that are from Chilika Lake, Gadarodanga and Brahmagiri in two different days determining as observation- 1 and observation- 2. We conducted an experiment to find out pH, TDS, salinity of soil from these places.

Effect of pH of soil on germination of wheat seeds

Soil pH affects wheat seed germination (pH 6.0-7.5). Extremes induce ROS imbalance, hindering germination. Optimal pH prevents ROS accumulation, supporting germination. Crucial for agriculture, managing soil pH aids seed growth. ROS regulation is vital for seed vigor and yield improvement(Ahmad et al., 2019).

As per the readings made by taking the sample soil it is observed that:

The pH of the two days remained in the range up 7.1 to 7.28. Thus, it can be drawn from this sample that the average pH of soil in Chilika Lake is around 7.19, the average pH of soil in Gadarodanga is around 6.71 and the average pH of soil in Brahmagiri is around 7.02.From the above noted case study it is observed that pH of Chilika Lake is a bit alkaline and that of the Gadarodaanga is slightly acidic but the pH of Brahmagiri is neutral in nature. Hence, soil of all the areas is fit for agriculture (Nakano and Asada, 1981).

Effect of TDS of soil on germination of wheat seeds

Total Dissolved Solids (TDS) in soil, particularly those related to salinity, can significantly impact the germination of seeds, including wheat seeds.

Wheat seeds thrive in moderate TDS soils; high TDS causes salinity, hindering water uptake and germination. Low TDS leads to nutrient deficiency, impeding metabolic processes. TDS impacts water and nutrient availability crucial for germination. Managing soil salinity and nutrient balance through amendments and irrigation is vital for successful wheat seed germination(Kar and Feierabend, 1984).

As per the readings made by taking the sample soil it is observed that:

The TDS for the two days remained in the range up 455 to 510. Thus, it can be drawn from this sample that the average TDS of soil in Chilika Lake is around 482, the average TDS of soil in Gadarodanga is around 408.5 and the average TDS of soil in Brahmagiri is around 394. From the above noted case study it is observed that TDS of soil in Chilika Lake is maximum and that of Bramagiri is minimum. However, the TDS of soil is fit for agriculture(Church and Galston, 1988).

Effect of salinity of soil on germination of wheat seeds

Salinity, which refers to the presence of high levels of soluble salts in soil, can significantly impact the germination of seeds, including wheat seeds.

High soil salinity affects wheat seed germination by hindering water uptake and causing osmotic stress, delaying or reducing germination rates. Managing salinity involves proper irrigation, salt-tolerant varieties, organic soil amendments, and site selection. Understanding seed sensitivity to salinity informs soil management decisions, crucial for successful germination and crop growth in saline conditions. Providing an optimal environment for germination supports healthy seedling establishment, ensuring robust crop development despite salt-induced challenges.

As per the readings made by taking the sample soil it is observed that:

The Salinity of the two days remained in the range up 4.92 to 4.94. Thus, it can be drawn from this sample that the average Salinity of soil in Chilika Lake is around 4.93, the average Salinity of soil in Gadarodanga is around 5.89 and the average Salinity of soil in Brahmagiri is around 4.35.From the above noted case study it is observed that salinity is very steady in Chilika Lake, moderately steady in Brahmagiri but fluctuates in Gadarodanga. However, maximum crops can adopt salinity that ranges near to5; hence agricultural crops can grow comprehensively (Gamble and Burke, 1984). The details observations are as below along with the graph (Fig. 3).

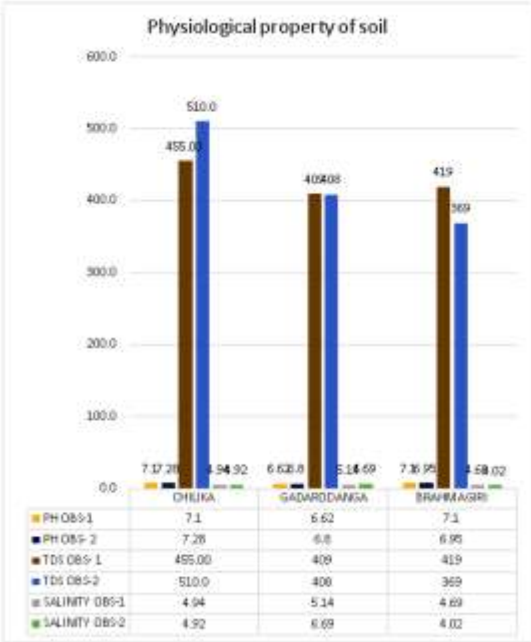


Fig. 3 Physiological property of soil in Chilika, Gadaarodanga and Brahmagiri.

Seed germination of wheat:

The water and soil of Chilika Lake, Gadarodanga and Brahmagiri are collected and brought to a laboratory in which germination of wheat is carried out. Two types of experiments are done.The first experiment is carried out taking only water from the three sources i.e. from Chilika Lake, Gadarodanga and Brahmagiri which is as follows.

Taking a sample size of 30 seeds the experiment is carried out and seeds are germinated by taking water from the 3 places.It is observed that in Chilika Lake germination of wheat is 23 out of 30. Hence the germination percentage is 77%. It shows clearly that wheat is more registrant to saline water i.e. in Chilika Lake.Similarly, in Gadarodanga germination of wheat is 27 out of 30. The germination percentage of wheat is 90%. Hence in Gadarodanga the wheat likeChilika shows more registrant to salinity.In Brahmagiri, the germination of wheat is 28 out of30 (Table 2 and Fig. 4). The germination percentage is 93% in wheat(Kar and Feierabend, 1984; Budak et al., 2013).This type of phenomenon could be observed due to extreme salinity that inhibits germination of seed due to osmotic potential that goes to a low level created around seed which prevents uptake of water(Rosegrant et al., 1995).

Table2 Number of seeds germinated taking water from the different places in and around Chilika Lake (having sample size of 30 seeds).

PLACE NAME	SEED GERMINATION (Wheat)
CHILIKA	23/30
GADARODANGA	27/30
BRAHMAGIRI	28/30

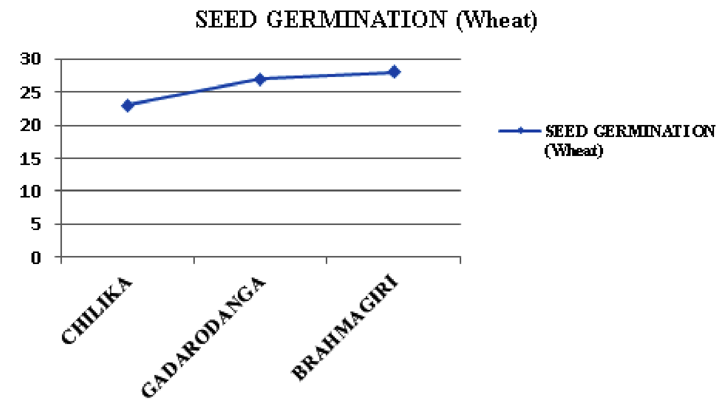


Fig.4 Seed germination taking water from the different places in and around Chilika Lake.

The second experiment, taking the sample size of 30 seeds, the experiment is carried out and seeds are germinated by taking soil from the 3 places.

It is observed that in Chilika Lake germination of wheat is 12 out of 30. Hence the germination percentage is 40%. It shows clearly that wheat is more registrant to saline soil i.e. in the soil found in Chilika Lake so it can be very hard for agriculture.Similarly, in Gadarodanga germination of wheat is 25 out of 30.The germination percentage of wheat is 83%. Hence in Gadarodanga's soil the registrant to salinity in wheat is very minimal, hence can be adopted for agriculture(Budak et al., 2013; Marshall and Worsfold, 1978).In Brahmagiri, the germination of wheat is 27 out of 30 (Table 3 and Fig. 5). The germination percentage is 90% in wheat. It shows that wheat adapts to the soil of Brahmagirivery well and is suitable for agriculture.

Table 3Seed germination taking soil from the different places in and around Chilika Lake

PLACE NAME	SEED GERMINATION (Wheat)
CHILIKA	12
GADARODANGA	25
BRAHMAGIRI	27

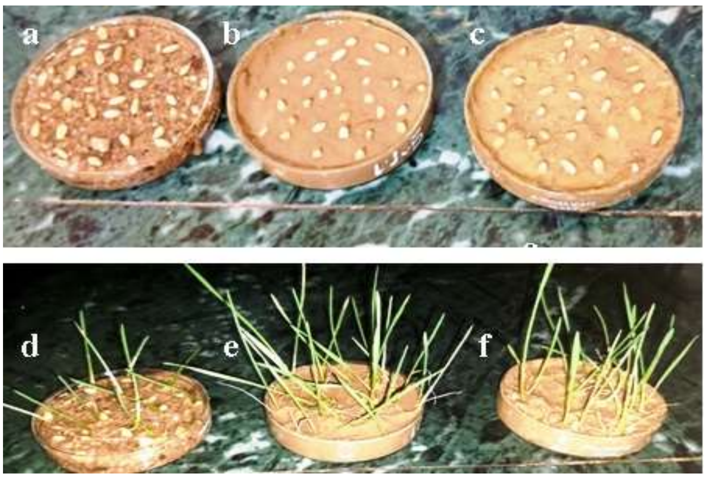


Fig. 5 Wheat seeds before germination take soil from all 3 places (a, b, c) and after germination take soil from all 3 places (d, e, f).

Properties of water:

The water that is found in and around Chilika Lake has a specific property. While collecting samples in all the three places that is in Brahmagiri, Gadarodaanga and Chilika Lake. The tabular form of properties of water is stated as below Table 13:

Table4 Property of water.

	BRAHMAGIRI	GADARODANGA	CHILIKA
TEMPERATURE (MORNING TIME)	34^0C	33^0C	31^0C
COLOUR	CLOUDY	CLOUDY	VERY CLOUDY
ODOUR	NO ODOUR	FOUL SMELL	LESS SMELL
TURBIDITY	LESS TURBID	LESS TURBID	HIGH TURBID

Properties of soil:

The soil that is found in and around Chilika Lake is collected and tasted with some physical parameters. The samples are also collected from Brahmagiri, and Gadarodanga. The tabular form is below Table 14.

Table 5 Property of soil.

	BRAHMAGIRI	GADARODANGA	CHILIKA
Texture	Clay content is more	Loamy	Sandy and contains pebbles
Water holding capacity	High	Medium	Very less
colour	Deep brown	Deep brown	Golden brown

Treatment and growth parameters after using aspectic techniques

The results of the experiment conducted are stated with the help of geometric expressions like tables and graphs. The research was carried out by enumerating the growth parameters of the plant's total growth in 3rd, 5th and 7th day(Church and Galston, 1988). Along with the calculation of effect in growth parameter of antioxidants, is represented in the form of graphs according to the significant days of the growth of the non-leguminous plant Triticum aestivum with proper description (Figs. 7, 8).

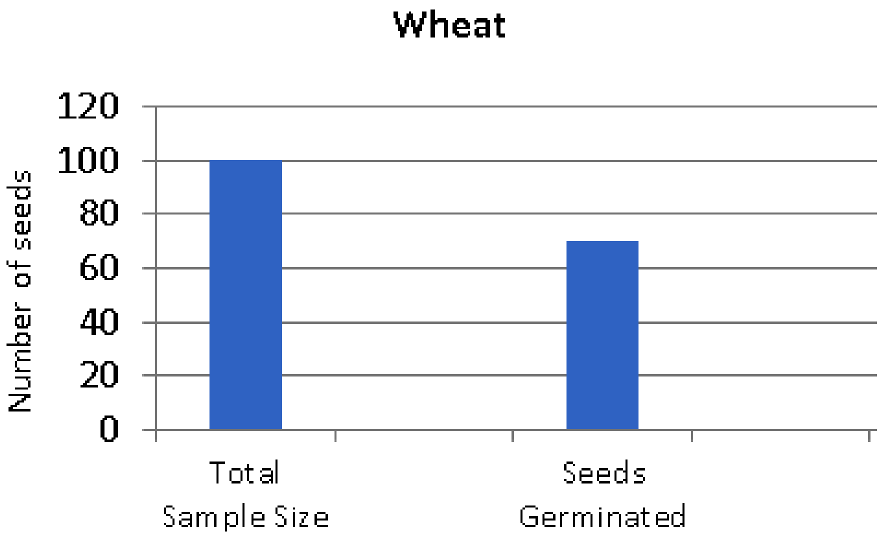


Fig. 6Germinated seeds

Growth Parameter:

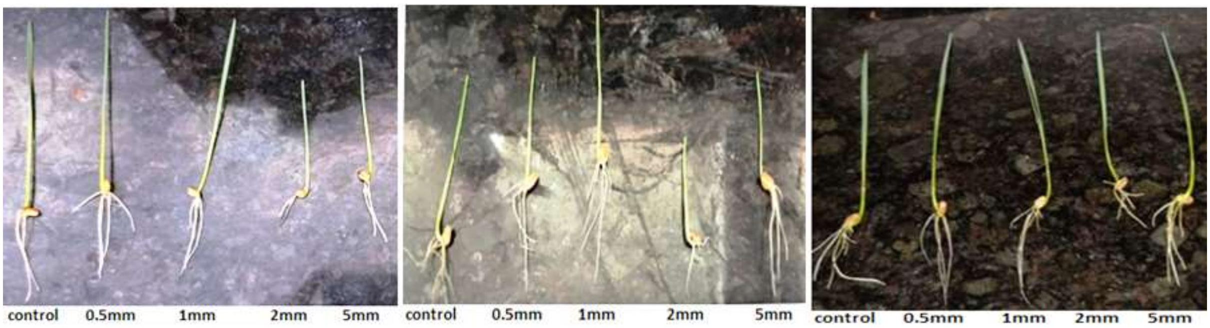
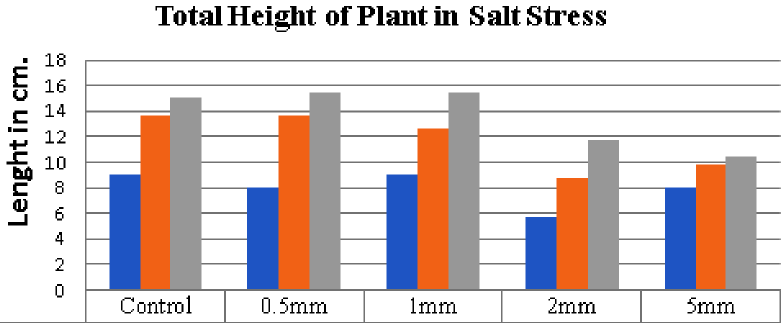


Fig. 7Growth of *Triticum aestivum*(Wheat) seedlings after 3rd, 5th and 7th day in salt stress



	Control	0.5mm	1mm	2mm	5mm
Day-3	9	8	9	5.7	8
Day-5	13.7	13.6	12.6	8.8	9.8
Day-7	15	15.5	15.4	11.7	10.5

Fig. 8 Growth of *Triticum aestivum* (Wheat) seedlings in salt stress

Extraction and estimation of enzymes in wheat plant

Proline, an Osmo protectant, is usually synthesized in plant tissues subjected to a variety of stresses. Proline level increased almost linearly during normal growth of plant but under NaCl induced salt Stress in different conc. the increase was higher than in the Control in Fig. 9.In this way with NaCl on Salt stress lipid Peroxidation level measured as MDA accumulation, increased different concentration of treated samples during investigation (Rosegrant et al., 2020). There is no huge difference in Lipid peroxidation level between the control and treated samplesFig. 9.

The rate of peroxide formation was measured in different concentration in different Stress. It was observed that the rate increased in salt stressed than control. So that in Stressed tissue, peroxidase activity gradually increased up to 5mm. Conc. of plant growth Fig. 9.

As the stress concentration increased, protein content also increased gradually. It was found to be increased as a function of different concentration per leaf Fig. 9.

SOD primarily acts to catalyze the dismutation (conversion) of superoxide radicals (O_2^-) into oxygen (O_2) and hydrogen peroxide (H_2O_2). Thus, in abiotic stressful condition in the treated non-legume leaf sample (Sahu and Antaryami, 2005), SOD activity showed an initial rise followed 7th day of plant leaf than the control when expressed on protein basis Fig. 9.

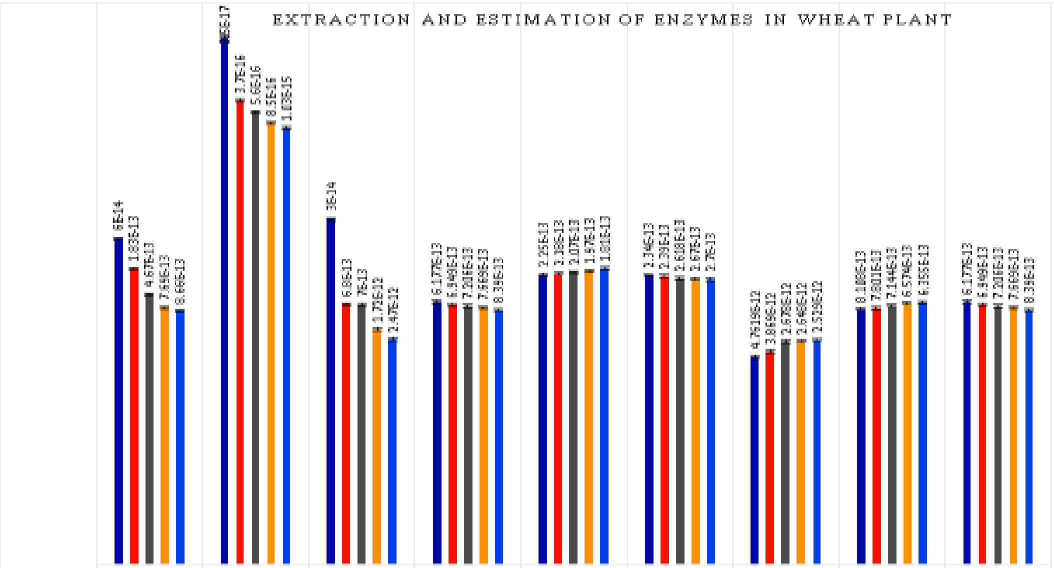
Catalase is the key enzyme, which is directly responsible for the scavenging of H_2O_2. However, in the Salt stress treated plan to Catalase activity was lower than the Control on the 7th day of plant growth Fig. 9.

Peroxidase is the ubiquitous enzyme and reported to be present in all genera plants. In Stressful environmental conditions, the production of reactive oxygen species increases and the Approx activity was increased Fig. 9.

Due to depletion of ascorbate in the enzyme activity, the data expression per protein showed a decreasing trend. In Stressful environmental conditions, the production of reactive oxygen species decreases, and the approx. activity was decreased Fig. 9.

When express also showed as protein basis, the activity also showed a similar decreasing trend in salt stress. The data expression per protein basis also showed almost same phenomenon (Budak et al., 2013; Gamble and Burke, 2013) and there was no huge amount difference between the NADH-peroxidase activity of control and treated plant leaf Fig. 9.

GR activity increased in treated plant leaf during the period of investigation when expressed in protein basis, enzyme activity showed an increasing trend. This trend was also observed when the activity was expressed per protein basis Fig. 9.



	PROLINE per mg fw-1	MDA per mg fw-1	SOLUBLE PROTEIN per mg fw-1	SUPEROXIDE DISMUTASE per mg fw-1	CATALSE per mg fw-1	PEROXIDASE per mg fw-1	ASCORBATE PEROXIDASE per mg fw-1	NADH-PEROXIDASE per mg fw-1	GLUTATHIONE-REDUCTASE(SALT STRESS) per mg fw-1
Control	6E-06	3.5E-09	0.000003	6.177E-05	0.0000225	0.0000234	0.00047619	0.00008108	6.177E-05
0.5mm	0.0000183	3.7E-08	6.8E-05	6.949E-05	0.0000218	0.0000239	0.0003869	7.801E-05	6.949E-05
1mm	0.0000467	5.6E-08	7E-05	7.206E-05	0.0000207	0.00002618	0.0002678	0.00007144	7.206E-05
2mm	7.69E-05	8.5E-08	0.000172	7.669E-05	0.0000197	0.0000267	0.0002648	6.574E-05	7.669E-05
5mm	8.66E-05	0.000000103	0.000247	8.39E-05	0.0000181	0.000027	0.0002529	6.355E-05	8.39E-05

Fig. 9Extraction and estimation of enzymes in wheat plant

4. CONCLUSION

Germination of wheat is carried out by taking the water and soil from Chilika Lake, Gadarodanga and Brahmagiri. The water and soil of Gadarodanga and Brahmagiri are the same but the physical and chemical properties of Chilika are very different. It was observed that plants showed different behavior during germination when the salt concentration of water changes. Thus, we conducted laboratory experiments on one of the abiotic stresses that is salt stress by taking different concentration of NaCl solution with same species of wheat and extracted enzymes from the leaves of the plant that are allowed to grow under artificial light without soil for seven days and studied its enzymatic activities.

In this study some of the antioxidant enzymatic activities were found to be increasing and decreasing during different concentrations of NaCl induced salt stress. The concentration of antioxidant enzymes like Proline, Malondialdehyde (MDA), Soluble protein Peroxidase and Glutathione was observed to be increasing when the concentration of NaCl increases but concentration of antioxidant enzymes like catalase, Ascorbate peroxidase, NADH peroxidase decreases.

ACKNOWLEDGEMENT

The authors would like to thank Gandhi Institute of Engineering and Technology (GIET) University (https://www.giet.edu/) Gunupur, Odisha, for its support in the present work.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding: No funding

Data availability: The datasets generated and/or analysed during the current study are not publicly available [Due to institutional policy] but are available from the corresponding author on reasonable request.

Author Contributions

All authors contributed to this research through conception and design, material preparation, data collection, and analysis, or the first draft of the manuscript. All authors read and approved the final manuscript

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