Comparative Study Analysis among Different Cultivars and Landraces of Rice under Salt Stress

Thesis Submitted to Midnapore City College for the Partial Fulfillment of the Degree of Master of Science (Agriculture) in Genetics and Plant Breeding

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Certificate

This is to certify that the project report entitled **Comparative study analysis among differentcultivars and landraces of rice under salt stress** submitted by **Subhadeep Patra, Roll-PG/VUWGP29/GPB-IVS No.-012; Subhadip Patra, Roll- PG/VUWGP29/GPB-IVS No.-013 and Sudip Gayen, Roll-PG/VUWGP29/GPB-IVS No.-015** to the Midnapore City College, Midnapore, West Bengal, India during the year of 2023 in partial fulfillment for the award of the degree of **M.Sc. (Agriculture) in Genetics and Plant Breeding** is a bona fide record of project work carried out by them under our supervision. The contents of this report, in full or in parts, have not been submitted to any other Institution or University for the award of any degree.

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Declaration

We do hereby declare that the present Master thesis entitled '*Comparative study analysis among different cultivars and landraces of rice under salt stress*' embodies the original research work carried out by us in the Department of Agriculture, Midnapore City College, Paschim Medinipur, West Bengal, India under the supervision of Dr. Anulina Manna, Assistant Professor, Department of Agriculture, Midnapore City College, Paschim Medinipur, West Bengal, India. No part thereof has been submitted for any degree or diploma in any University.

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ABSTRACT

Purba Medinipur (Lies between 21.9373° North Latitude and 87.7763° East Longitude) and South 24 Paraganas (Lies between 22.1367° North Latitude and 88.5565° East Longitude) both districts covering much coastal areas of West Bengal. Salt stress is an acute threat to plants, especially to field crops in irrigated and saline areas of the world. Rice is a relatively salt-tolerant crop which grows from upland to low land and dry land to water logged land, everywhere. Despite of salinity, rice is being cultivated in these areas. Rice is the second staple crop of the world after wheat and the second-most consumed cereal grain, after maize, and its production is strongly affected by salinity. For this reason, we select rice as a crop sample for this experiment. Therefore, to ensure food security, it is crucial to manage salt stress for sustainable rice production under saline conditions. Plant cytological, biochemical, and physiological characteristics play an important role in the adaptation of rice to saline environments. Further, the knowledge of the correlation among these characteristics is necessary to manage the salt stress and achieve optimal rice production. This experiment focuses on the response of rice in different salt concentration to salinity stress. The specimens had been observed under various morphological parameters and attributes also checked like chlorophyll content, cytological abnormalities and protein content etc. In conclusion, salt stress affects metabolism and physiology of rice and reduces the agronomic yield. Therefore, development of salt-tolerant genotypes from landraces by using modern breeding methodologies may be a prudent strategy to manage the salinity. Also seed treatment plays an important to adopt salt stress condition. Focused research on integration of different management options can lead to sustainable rice production in saline areas also providing specific variety for specific zone which may reduce the amount of barren land and contribute significantly to global food security.

Keywords: Salinity, Sustainable, Morphological parameters, Chlorophyll, Cytological abnormalities, Salt tolerant genotypes, Barren land.

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List of Abbreviations

BSA	Bovine Serum Albumin
CEC	Cation Exchange Capacity
cm	centimeter
CO_2	Carbon dioxide
CRD	Completely Randomized Design
DNA	Deoxyribonucleic Acid
EC	Electrical Conductivity
ESP	Exchangeable Sodium Percentages
FAO	Food and Agriculture Organization
Fig	Figure
GDP	Gross Domestic Product
HCl	Hydrochloric Acid
H_2O_2	Hydrogen Peroxide
mha	Million Hectare
mM	millimolar
MRR	Mitochondrial Retrograde Regulation
nm	Nanometer
PCA	Principle Component Analysis
pH	Potential of Hydrogen
ROS	Reactive Oxygen Species
RWC	Relative Water Content
SAR	Sodium Adsorption Ratio
TCC	Total Chlorophyll Content

Chapter 1. Introduction

1. Introduction

Agriculture sustainability can be defined as a state where increase of food production is at least proportional to population growth. According to Food and Agriculture Organization (FAO), the project world population by the year of 2050 will be 9.74 billion (http://esa.un.org/wpp). In the present scenario of sky-high population, increasing crop productivity shoulder to shoulder with burgeoning population is a challenge in 21st century. As a result of the rapid increase inthe global population, food production must be increased by 70% by the end of year 2050 (Hassan *et al.*, 2020). Abiotic stresses are responsible for a 50% reduction in crop production, imposing a serious threat to global food security (Acquaah, 2007; Seleiman *et al.*, 2019). Among the abiotic stresses salination brings major drawbacks in various countries in terms of agriculture. Soil salinity is the second major factor responsible for land degradation after soil erosion.

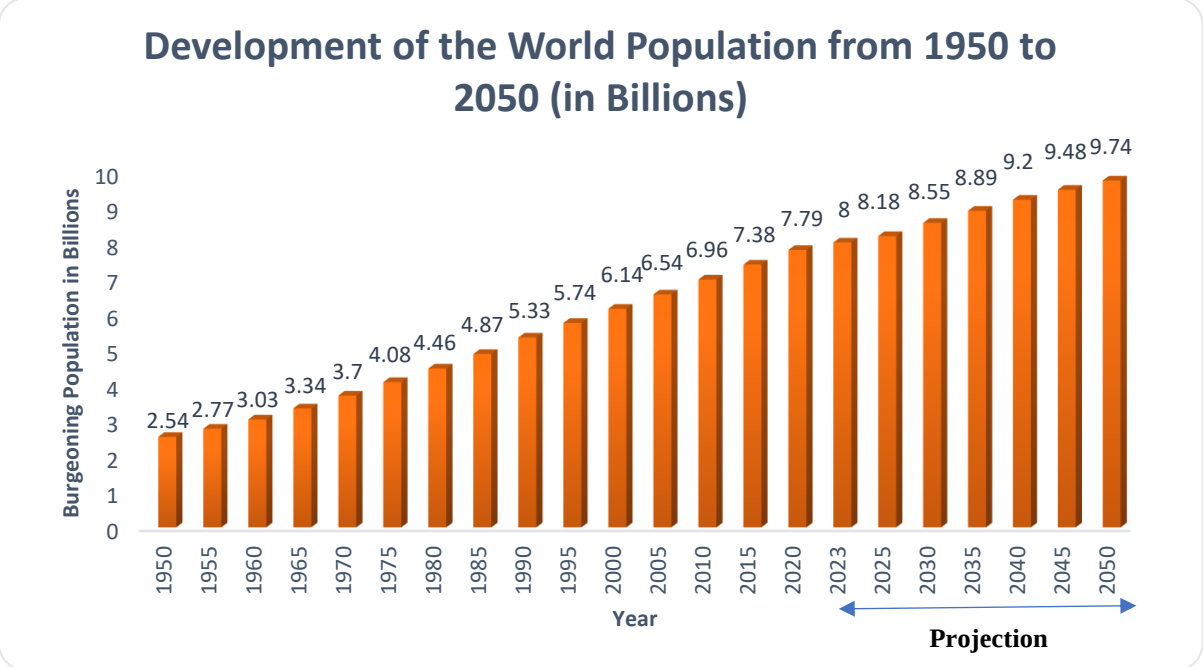

Fig 1. Estimated Global Population by 2050 (Source: International Data Base)

Salination of the soils is one of the major problems in not only in India but also all over the world.A soil is considered saline if the electrical conductivity of its saturated extract (ECe) is above 4 dS/m (US Salinity Laboratory Staff, 1954). Globally, more than 20% of soils are salt-affected and the extent of these soils is continuously increasing owing to anthropogenic activities and climate change (Munns & Tester, 2008; Ding *et al.*, 2021). Globally, 952.2 mha (7% of total land, 33% ofagriculture land) land throughout the world is salt-affected by either salinity (397 mha) or theassociated condition of sodicity (434 million ha) (Munns & Tester, 2008). In India the soil salinityproblem is becoming an important constraint on crop production particularly in arid and semi-aridregion. In India, 6.73 million ha land is salt affected out of which, 3.77 mha are covered with salinesoil and 2.96 mha with sodic soil (Singh *et al.*, 2013;

Arora *et al.,* 2016). Saline soil found in Indiain Gujrat, Bihar, Haryana, Rajasthan, Maharashtra, Odissa, Andhra Pradesh, Tamil Nadu, Uttar Pradesh, West Bengal etc. Only West Bengal occurs 0.44 mha saline soil (Sharma *et al.,* 2015). Inaddition, about two million ha of cropped land are deteriorating because of salinity every year (Rengasamy, 2006; Tuteja, 2007). As a result of increased salinization of agricultural land, it is projected that about 50 % of cropped land will be lost by the middle of the 21st century (Wang *et al.,* 2007). In arid and semi-arid regions, high temperatures during the summer season cause severeevaporation losses, which leaves behind large amounts of salts. However, the problem exists evenin some of the world's sub-humid and humid regions, especially in coastal areas.

Crop productivity is adversely affected by salinity. A significant reduction in productivity has been reported of wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) (Akbarimoghaddam *et al.,*2011; Nevo & Chen, 2010), maize (*Zea mays* L.) (Dorraji *et al.,* 2010; Farooq *et al.,* 2015), rice (*Oryza sativa* L.) (Thitisaksakul *et al.,* 2015), sunflower (*Helianthus annuus* L.), and sorghum (*Sorghum bicolor* (L.) Moench) (Zhao *et al.,* 2014). With the increasing global population and food demand, it is need of the time to sustain the production in the soils where its productivity is limited by alleviating different types of stresses.

"Rice is life" – this slogan of the International Year of Rice 2004, outlines the importance of rice in human life. Rice (*Oryza sativa* L.) is a staple food for a large part of the global human population approximately over 3 billion people all over the world;it is the second highest consumed staple food after wheat, the second-most consumed cereal grain after maize and the third most consumed food grains after wheat and maize. About half of the world population depends on rice for their survival. Rice is a primary food crop that provides a major portion of dietary carbohydrates consumed by nearly half of the world's population which provide >20 % of their calories per day. It is a rich source of energy and contains reasonable amounts of: protein 6- 10%, carbohydrates 70-80%, minerals 1.2-2% and vitamins (Riboflavin, Thiamine, Niacin and Vitamin E). Rice is growing globally in an area of 167.24 million hectares with a production of 769.65 million ton with an average productivity of 4.6 ton per hectare. India is the world's second largest producer of rice after China, accounting for 22% of the world rice production. In India, rice is cultivated in an area of 43.78 million hectare (20% of cropped area), with a productionof 168.5 million ton and productivity of 3.84 ton/ha and contributes 25% to agricultural GDP (FAOSTAT, 2017). India is the largest exporter of rice in the world. West Bengal is the largest rice producing state of India and contributing 14.24 % of total rice production of India. In West Bengal, rice is cultivated in an area of 5.8 million hectare, with a production of 15.57 million metric ton and productivity of 2.6 ton/ha. Rice occupies 53% of the total agricultural crop areas. It grows in this state in 3 different seasons' viz.,Aus (autumn rice),

Aman (winter rice) and Boro (summer rice).

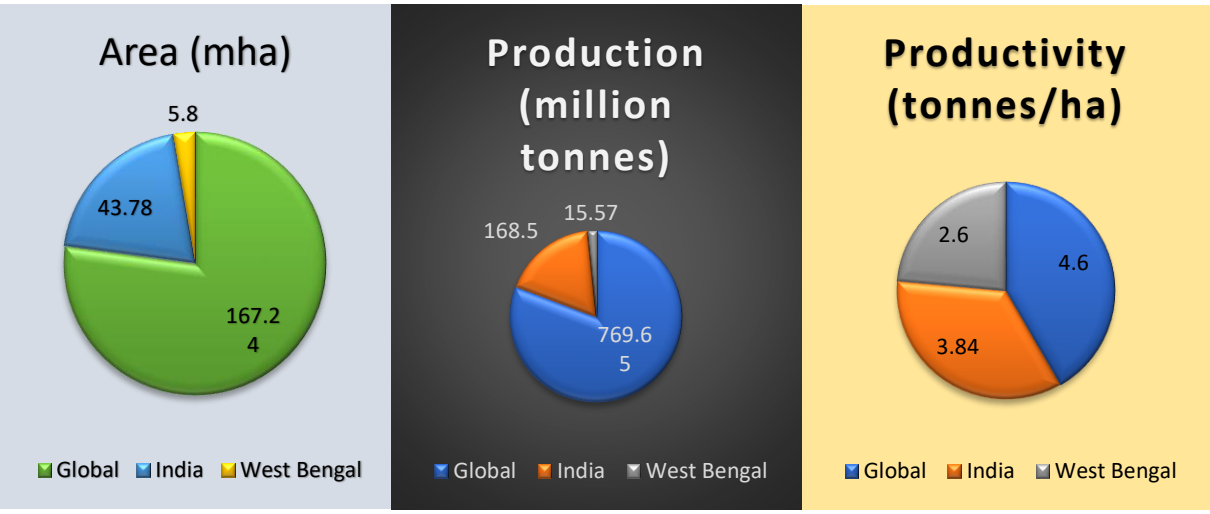

Fig 2. Area, Production and Productivity of rice (Source: FAOSTAT, 2021)

Rice is a relatively salt-sensitive crop with threshold salinity of 3 dS/m, above which yield loss occurs (Maas & Hoffmann 1977). Scientists indicated that rice yields decrease by 12% for every unit (dS/m) increase in EC above 3 dS/m (Maas & Grattan, 1999). The effect of salinity on rice depends on the amount of salinity, duration of exposure, cultivar, crop growth stage, water regime,soil physical properties, temperature, and solar radiation (Neue *et al.*, 1998, Ali *et al.*, 2013). However, rice productivity in many areas is affected by salinity stress, which originates from the accumulation of underground salt and is exacerbated by salt mining, deforestation, and irrigation (Akbar, 1986) and uses of fertilizers in huge amount. Rice is relatively tolerant of salt stress duringgermination, active tillering and grain filling and is sensitive during the early seedling and reproductive stages [panicle initiation (PI), anthesis and fertilization] (Zeng *et al.*, 2001, Singh *et al.*, 2008, Singh & Flowers, 2010).

The mechanism of salinity tolerance is still not very clear, but tolerant plants use a combination of mechanisms to overcome salinity stress. Salinity stress inhibits plant growth through many interruptions, such as osmotic effects; excessive uptake of toxic ions (Na^+ and Cl^-); inability of partitioning at the organ, tissue, and cell level; and poor regulation of antioxidants with impaired signal pathways (Munns, 2002, Singh & Flowers, 2010).

Therefore, the present study has been conducted to explore growth and physiological, biochemical, cytological changes in seedlings subjected to salinity stress in rice varieties differing in their levelof salt tolerance along with classification of rice varieties with diverse growth and physiological, biochemical, cytological parameters employing principal component analysis (PCA). The study conducted on rice seedlings of five genotypes, composed of tolerance and susceptible rice cultivars. The gained information may be used to assist in the

evaluation of relative field performance of different rice genotypes and characterization of contributing physiological, cytological traits that may be employed as reliable indicators for breeding and selection for salt tolerance.

Chapter 2. Literature Review

2. Literature Review

2.1. Salt Stress or Salinity:

The problem of salinity is the primary concern of arid and semi-arid regions all over the world dueto the high concentration of soluble salts in soil or water. Plants growing in these areas severely struggle for their proper growth and productivity. Salinity impairs the biological functions of plantsby disturbing the K^+ /Na^+ ratios as a decrement of in K^+ uptake, and an increment in Na^+ influx occurs in plant cells (Serrano & Rodriguez-Navarro, 2001). Salinity stress not only imposes ion toxicity to plants but also creates the water-deficit condition as a result of less water availability in solute saturated soil (Apse & Blumwald, 2002).

A saline soil defined as having a high concentration of soluble salts, high enough to affect plant growth. Salt concentration in a soil measured in terms of its electrical conductivity. A soil is considered saline if the electrical conductivity of its saturated extract (EC) is above 4 dS/m (USDASalinity Laboratory Staff, 1954). EC is the electrical conductivity of the 'saturated paste extract', that is, of the solution extracted from a soil sample after being mixed with sufficient water to produce a saturated paste. However, may crops are affected by soil with an EC less than 4 dS/m. The actual salinity of a rainfed field whose soil had an EC of 4 dS/m could be 8-12 dS/m.

Usually, two major types have been defined by using the EC and ESP characteristics and soil pH, salt affected soils are classified within salt affected soil, namely saline soils and sodicsoils. Saline soils contain neutral soluble salts sufficient to interfere seriously with plant growth. Sodic soils do not contain appreciable amount of soluble salts. (Sharma *et al.*, 2004).

Table 1. Keys to the degree of Salinity/ Sodicity (Mandal *et al.*, 2018):

Degree/ Classification	EC (dS/m)	Soil pH	ESP	SAR	Soil Physical Condition
Normal	< 4.0	6.5 – 7.3	< 15	6.5 – 7.51	Good
Saline	> 4.0	7.3 - 8.5	< 15	< 13	Normal to Poor
Sodic	< 4.0	> 8.5	> 15	> 13	Very Poor
Saline-Sodic	> 4.0	< 8.5	> 15	> 13	Poor

2.2. Characteristics of Salt-Affected Soils:

The salt-affected soils are classified into three groups depending on the nature and concentration of salts present in them:

- Saline soils (also called "white alkali" or "solonchak" soils): soils containing calcium, magnesium, and sodium as predominant exchangeable cations (Ca and Mg more than Na), andsulfate, chloride, and nitrate the predominant anions; sodium adsorption ratio (SAR) < 13; exchangeable sodium percentage (ESP) < 15 of total CEC; pH < 8.5; EC of saturation extract > 4.0 dS/m; white color due to white crust of salts on the surface; good permeability for water and air; salt problems in general; the salt concentration is enough to adversely affect the growth ofmost crop plants; mostly found in arid or semi-arid regions where less rainfall and high evaporation rates tend to concentrate the salts in soils; rarely found in humid regions (Kumar & Sharma, 2020).
- Sodic soils (also called "non-saline sodic soils" or "black alkali," or "solonetz"): soils highin exchangeable sodium compared to calcium and magnesium; sodium carbonate and sodium bicarbonate are the predominant salts; SAR > 13; ESP > 15; pH = > 8.5; EC of saturation extract < 4.0 dS/m; black color; poor permeability for water and air; soils formed due to exchangeof Ca2+ and Mg2+ ions by Na+ ions; sodium problems (Kumar & Sharma, 2020).
- Saline-sodic soils: these soils are transitional between saline and sodic soils; SAR > 13, ESP > 15, pH < 8.5; EC of saturation extract > 4 dS/m; air and water permeability depends on the sodium content; soils formed due to combined processes of salinization and alkalization; problems with sodium and other salts; leaching converts these soils into sodic soils (Kumar & Sharma, 2020).

2.3. Ingredients of Saline Soils:

The salt-affected soils contain excessive concentrations of either soluble salts or exchangeable sodium or both due to inadequate leaching of base forming cations. The major soluble mineral saltsare the cations: sodium (Na^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^+) and the anions: chloride (Cl^-), sulfate ($SO4^{2-}$), bicarbonate (HCO^{3-}), carbonate (CO^{2-}), and nitrate (NO^{3-}). Hyper-saline soil water may also contain boron (B), selenium (Se), strontium (Sr), lithium (Li), silica (Si), rubidium (Rb), fluorine (F), molybdenum (Mo), manganese (Mn), barium (Ba), and aluminum (Al), some of which can be toxic to plants and animals. Saline soils contain an excess of neutral soluble salts of chlorides and sulfates whereas alkali soils contain sodium carbonates/sodium bicarbonates. They lack nitrogen and calcium and have a low water-bearing capacity. The process of accumulation of salts leading to the formation of soils is known as salinization. (Tanji, 1990).

Table 2. Description of salt-affected soil characteristics (Mandal *et al.*, 2018):

Categories of salt-affected soils	**ECe (dS/m)**	**Salinity classes**	**pH range**	**ESP range**	**Sodicity Class**
Slightly Saline	4.0-8.0	Slight	Low	Traces	Low
Moderately Saline	8.0-30.0	Moderate	Low	Traces	Low
Strongly Saline	> 30.0	Strong	Low	Traces	Low
Slightly Sodic	Low	Low	8.5-9.5	< 15	Slight
Moderately Sodic	Low	Low	9.0-9.8	15-40	Moderate
Strongly Sodic	Low	Low	> 9.8	> 40	Strong
Slightly Saline, Slightly Sodic	4.0-8.0	Slight	8.5-9.0	< 15	Slight
Slightly Saline, Moderately Sodic	4.0-8.0	Slight	9.0-9.8	15-40	Moderate
Slightly Saline, Strongly Sodic	4.0-8.0	Slight	> 9.8	> 40	Strong
Moderately Saline, Slightly Sodic	8.0-30.0	Moderate	8.5-9.5	< 15	Slight
Moderately Saline, Moderately Sodic	8.0-30.0	Moderate	9.0-9.8	15-40	Moderate
Moderately Saline, Strongly Sodic	8.0-30.0	Moderate	> 9.8	> 40	Strong
Strongly Saline, Slightly Sodic	> 30.0	Strong	8.5-9.0	< 15	Slight
Strongly Saline, Moderately Sodic	> 30.0	Strong	9.0-9.8	15-40	Moderate
Strongly Saline, Strongly Sodic	> 30.0	Strong	> 9.8	> 40	Strong

2.4. Crop Adaptations to Salt Stress:

Based on the responses to high concentrations of salts, plants can be divided into two broad groups.These are following:

1) Halophytes
2) Glycophytes

Halophytes:

Group of plants able to grow even high saline conditions, which are otherwise called as salt

toleranttypes. The halophytes (salt lovers) that can complete their life cycle at salt concentrations exceeding300 mM (Flowers *et al.,* 1977).

Glycophytes or Nonhalophytes :

They are sensitive plants and unable to grow under saline conditions. Most of the cultivated crop species belong to glycophytes. Glycophytes begin to show the signs of growth inhibitions, leaf discoloration and loss of dry weight, when concentration of the salts reaches above the threshold level. Glycophytes (sugar lovers) which cannot complete their life cycle at salt concentrations exceeding 300 mM. However, at low salt concentrations (20 mM to 200 mM) there is an overlap of growth responses between the two groups (Greenway & Munns, 1980).

2.5. Rice:

Rice (*Oryza sativa* L.) is one of the most important cereal crops in tropical and temperate regions of the world. Rice is cultivated for more than 10,000 years as a major crop. There are two species of cultivated rice *Oryza sativa* and *Oryza glaberrima*. *Oryza sativa* (also known as Asian cultivated rice), is an economically important rice cultivated in majority of rice growing countries, particularly in Asian countries which together make up around 90% of the rice cultivation area in the world. Rice is a diploid (2n=24), annual, short-day plant, and normally self-fertilizes. It is believed that cultivated rice has evolved from one wild species *Oryza rufipogon* through long term domestication (Chang 1976) and the existing subspecies of cultivated rice are grouped into *indica*, *japonica*, and *javanica*. In Asia, cultivated rice consists of two major groups – *indica* (*Oryza sativa* subspecies. *Indica*) and *japonica* (*Oryza sativa* subspecies. *Japonica*). Among all common environmental stresses, salinity is a major factor decreasing the yield in rice cultivation in coastal areas and in irrigated farmlands. Problems associated with salinity are water deficit imposed by the greater osmolarity of the soil solution and the cellular damage inflicted by excessive ion accumulation in plant tissue. Rice is a glycophytes or non- halophytes.

2.6. Responces of Rice to Salt Stress:

Rice displays tolerance to salt in the developmental stages of germination, tillering, and maturity, but demonstrates susceptibility in the early stages of seedling and reproduction (Moradi & Ismail,2007; Zeng *et al.,* 2001). The unfavorable effects of low salt levels on rice plants are because of osmotic stress, nutritional disorder, and ionic toxicity (Grattan & Grieve, 1998; Wahome *et al.,* 2001), while the effects are due to nutritional imbalances and gathering of ions, especially Na^+ andCl^- ions under moderate to high salt stress (De Pascale *et al.,* 2003). Usually, salt-stressed plants suffer cyclical physiological, morphological, and biochemical alterations from appearance to ripeness (Läuchli & Grattan, 2007; Munns, 2008). Nevertheless,

salt-stressed plants' response is referred to as interacting causes, which including genotype, growth phase and shape, solution composition, and salt-induced stress concentration (Jenks *et al.,* 2007).

Understanding the mechanisms by which plants perceive and transmit signals of salt stress to cellular machinery to generate adaptive responses is important for classical breeding and biotechnology programs designed to improve salt tolerance (Roychoudhury *et al.,* 2008, 2009). Recent studies on model plants and crops have shown that these adaptive responses focus on complex signaling mechanisms which lead to an integrated physiological response to environmental stress (Hasegawa *et al.,* 2000; Zhu *et al.,* 2004). It is therefore vital to increase crop productivity to recognize morphological, physiological, and biochemical compounds acting as coordinators controlling multiple stress signaling pathways and to show how these enhance the physiology of whole plant stress.

2.6.1. Effects of Salt Stress on Rice Seed Germination:

A complex physiological and biochemical modification phenomenon, which leads to the activationof the embryo. Seed germination is a critical stage, which affects total dry matter and grain yield output (Parihar *et al*., 2015). Salt stress induced a delay in rice seed germination showing an adverseassociation between seed germination and salt stress (Shereen *et al.,* 2011).

2.6.2. Morphological Changes of Rice due to salt stress:

Plant height is a major feature of plant morphology, which under biotic and/or abiotic stress frequently results in alterations in growth and development. The hostile influence of salt stress in plant height has been reported in several studies. A clear negative impact of salinity on plant heightand shoot length with a gradual increase in solutions. The length of the root varies significantly even under salt stress. A rise in salt- stressed root length is attributed to the plant's capacity to enterdeeper soil field layers at low water potential over root elongation (Perez Alfocea *et al.,* 1996). Basically, the roots of saline-adapted plants show reduced cortex to shorten the distance between epidermis and stele.

2.6.3. Physiological Changes of Rice due to Salt Stress:

Salinity induced stress induces stomatal closure resulting in increased temperature of the leaves and reduced elongation (Rajendran *et al*., 2009; Sirault *et al.,* 2009). The physiological alterationsin salt-stressed plants comprise a range of responses, for example, higher Na^+: K^- ratio, lower stomatal conductance, lower photosynthesis, and increased accumulation of reactive oxygen species (ROS), which inhibits photosynthesis, and all these changes contribute to plant growth reduction. It may cause death of leaves and decrease in leaf area and ultimately reduce photosynthesis rate of plant (Amirjani, 2011). Salt stress has specific effects on plant

cell metabolism, particularly on leaf senescence. It can also injure the cells in transpiring leaves, and cause rice plant growth inhibition. The salt concentrate in the old leaves cause the leaves death, which is crucial for the survival of a plant (Munns *et al.*, 2006). Under salt stress, the accumulationof Na^+ ions disturb photosynthetic constituents (Davenport *et al.*, 2005). For many plant species, decreased stomatal conductance followed by decreased transpiration and CO2 assimilation is the immediate response of plants to a large range of salt stress (Ashraf 2001; Munns and Tester 2008;Romero-Aranda *et al.*, 2001). Generally, chlorophyll and stomatal conductance regulate photosynthesis, which are decreased in salinity condition (Dingkuhn *et al.*, 1992; Parida *et al.*, 2004). Salt stress also effect on relative water content (RWC) in rice. Relative water content (RWC) was introduced as a best criterion for plant water status, which afterwards, was used instead of plant water potential, RWC accurately reflects the balance between absorbed water by plant and consumed through transpiration (Ghogdi et al., 2012). Several studies reported that RWC was reduced when the salinity increased and the tolerant cultivars showed less reduction in the RWC (Jan et al., 2016; Hand et al., 2017; Hussein et al., 2017).

2.6.4. Biochemical Changes of Rice due to Salt Stress:

Salt stress toxic effects trigger the occurrence of mature and older leaves by diminished protein production and function of the enzymes (Carillo *et al.*, 2011; Munns, 2002; Munns & Tester, 2008).It is well known that salinity induces oxidative stress in plants at the subcellular level (Hernandez *et al.*, 1993, 1995, 2001; Mittova *et al.*, 2003, 2004). Different works have reported that salt stress induces an accumulation of hydrogen peroxide (H2O2) in different cell compartments, including chloroplasts, mitochondria and apoplastic space, which correlates with increases in some oxidativestress parameters, such as lipid peroxidation and protein oxidation (Acosta-Motos *et al.*, 2017; Hernandez *et al.*, 1993, 1995, 2001; Mittova *et al.*, 2003; Gomez *et al.*, 1999; Mittova *et al.*, 2002).In contrast, in leaf or root peroxisomes from salt-treated plants, no important changes in O2 or H2O2 concentration have been reported (Mittova *et al.*, 2003, 2004; Corpas *et al.*, 1993), althoughlipid peroxidation levels have increased in some cases. ROS production is a basic reaction to plantunder salt stress. ROS overload is toxic in the cell but can mediate the induction of stress tolerance mechanisms (Das & Roychoudhury, 2014; Lee *et al.*, 2003). If no protective mechanism exists, ROS are highly reactive and can harm all metabolic pathways through lipid, nucleic acid, and protein damage. Degradation of ROS by antioxidant compounds can be substantially affected by salinity (Banerjee & Roychoudhury, 2018; Carillo *et al.*, 2011; Foyer & Noctor, 2003).

2.6.5. Effect of Salt Stress on Rice Growth and Yield:

Salinity stress results in reduced rice growth and rice yield quantity and quality (Asch & Wopereis,2001; Hasanuzzaman *et al.,* 2009; Zeng & Shannon, 2000). Further, moderately salt stressdecreased rice yield by 25% in tolerant cultivars (Beecher, 1991). In addition, a reduction of 50% in seed germination and 80% loss in grain yield based on cultivar tolerance were reported when rice was grown in high salt stress condition (Asch & Wopereis, 2001). Yields decreased to reach zero in sensitive salt varieties when rice was grown in hot and dry seasons with salt stress, and drymatter accumulation decreased by 90% (Asch & Wopereis, 2001).

Different types of changes during different growth stages of rice due to the mischievous impact ofsalt stress have been well discussed in various researcher's paper. But there are no accurate data onthe genotypic and phenotypic relationship of rice under saline condition is available. No information about palynological changes was also found. Even the correlation between genotypic and biochemical changes, which help to adopt saline condition, are not well known. So, this experiment focuses on observing the changes due to salt stress and the correlation between them which helps in finding specific salt tolerant varieties for specific salinity areas.

The Study Area

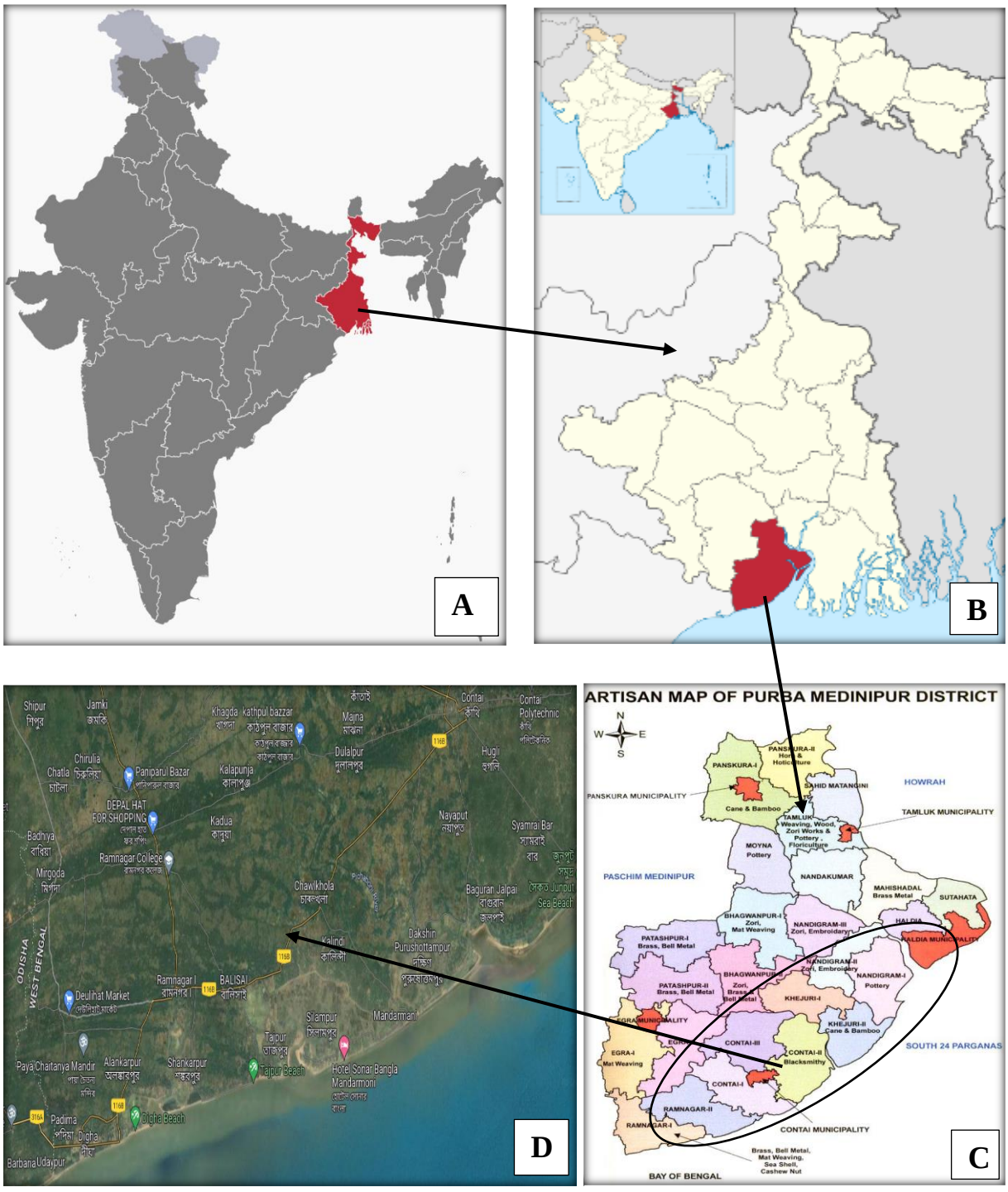

Fig 3: Study area: **A.** Map of India showing West Bengal state Courtesy: www.mapsofindia.com.

B. Map of West Bengal showing Purba Medinipur district Courtesy: www.d-maps.com.

C. Map of Purba Medinipur Courtesy: www.mapsofindia.com.

D. Map of the area surveyed in Purba Medinipur Courtesy: Googlemap.

The Study Area

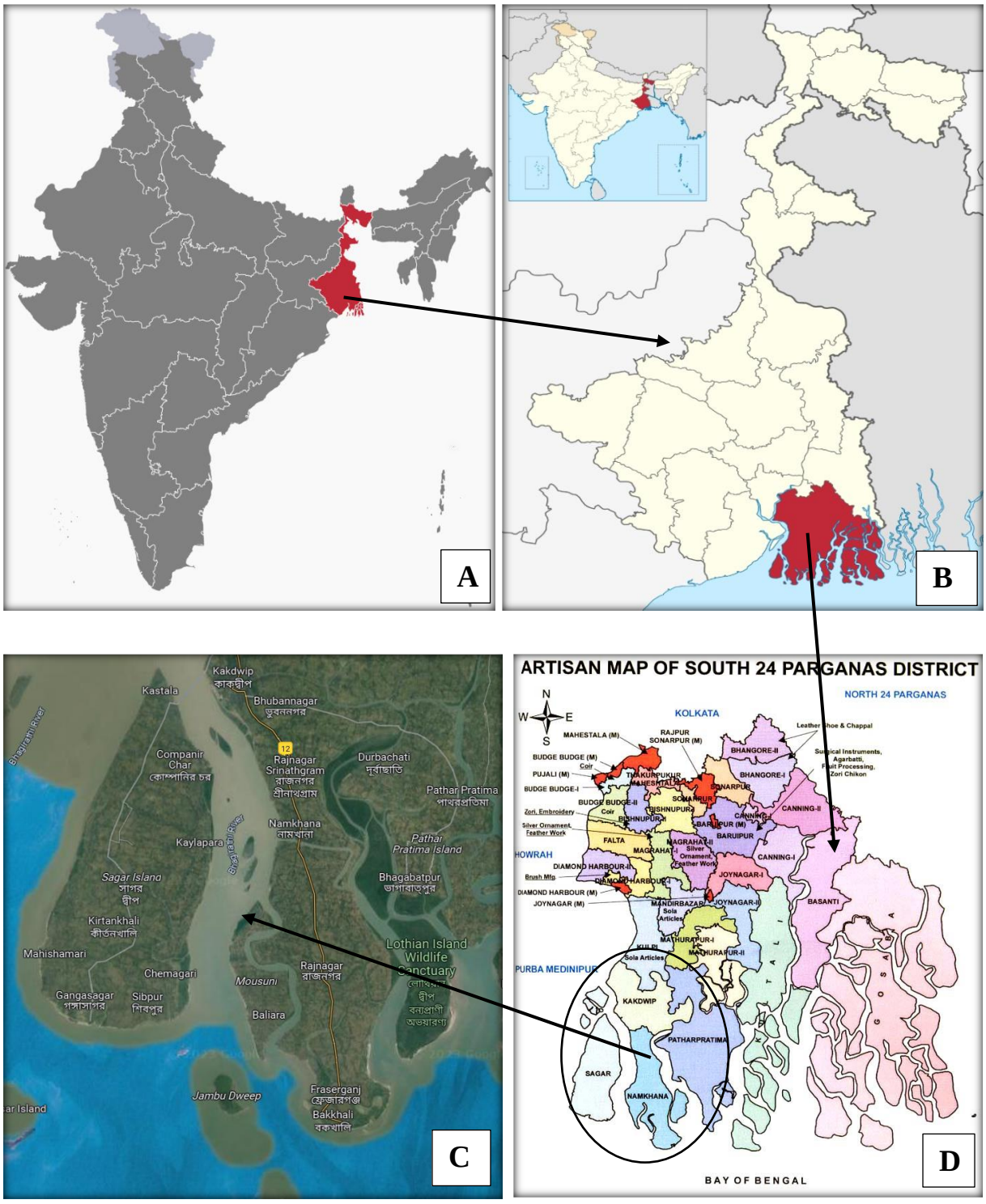

Fig 4: Study area: **A.** Map of India showing West Bengal state Courtesy: www.mapsofindia.com.

B. Map of West Bengal showing South 24 Parganas district Courtesy: www.d-maps.com.

C. Map of South 24 Parganas Courtesy: www.mapsofindia.com.

D. Map of the area surveyed in South 24 Parganas Courtesy: Googlemap

Chapter 3. Aims & Objectives

3. Aims & Objectives

Salt stress widely effects on rice plant at different growth stages. The experiment had been conducted on different rice cultivars of rice as well as landraces for their comparative study analysis of morphological, cytological, physiological, and biochemical screening as well as their co-relation. We aim to screen some salt tolerant genotypes which can show impressive productivity in these coastal regions by laboratory and field screening methods. For fulfillment of these study following objectives were set:

- Survey on some coastal areas of West Bengal for selection of rice cultivars as well as propersoil profiling.
- Comparative study on germination, morphological changes by salt stress ondifferent cultivars and landraces of rice.
- Comparative study on physiological and biochemical changes by salt stress on different cultivars and landraces of rice.

Chapter 4. Materials & Methods

4. Materials & Methods

To satisfy above objectives an experiment had been conducted among five rice cultivars.

4.1. Materials:

4.1.1. Crop Sample:

Table 3. Classification of Crop Sample:

Rice	
Family	Poaceae (Grass Family)
Genus	*Oryza* L.
Scientific Name	*Oryza sativa* L. (2n = 24)

4.1.2. Selected Cultivars:

Table 4. Required cultivars for experiments:

Source	**Cultivars name**	**Characteristics**
West Bengal State Seed Corporation Limited, Paschim Medinipur	IR 36	Salt susceptible
	IET 4786	Salt susceptible (Local check)
	IR 64	Moderately Salt susceptible
Bidhan Chandra Krishi Viswavidyalaya, Mohanpur	Kalanamak	Aromatic, salt tolerant
	Nonakathi	Salt tolerant landraces (110 – 120 days), semi-dwarf, N_2 responsive

4.1.3. Chemicals:

Table 5: Required chemicals for experiments:

Experiment	**Chemical Name**	**Trade Name**
Solution Preparation	Sodium chloride	Common Salt (NaCl)
Seed germination	Carbendazim	Bavistin
Relative Water Content	Deionized water	Distilled water
Chlorophyll content	Acetone	Acetone

Protein Content	Bovine Serum Albumimn	BSA
	A. Sodium carbonate in sodium hydroxide B. Copper sulphate in potassium sodium tartrate	Lowry's reagent
	Sodium molybdate + Sodium tungstate	Folin–Ciocâlteu reagent
	Trichloro acetic acid	TCA

4.1.4. Instruments & Glassroots:

Table 6. Required equipment and instruments for experiments:

Experiment	**Instruments**	**Glassware & equipment**
Preparation of Solution	Weighing machine	Beaker, volumetric flask
	EC meter	
Seed germination		Petri dishes, wet filter papers
Relative water content	Hot air oven	Petri dishes, Polythene bags
Chlorophyll Content	Mortar – Pestle	Bloating paper, Slide, Watch glass, Coverslip, Blade, Needle, Forceps, Scalpel, Dropper, Test tube, Centrifuge tube.
	Centrifuge machine	
	Spectrophotometer	
Protein content	Mortar – Pestle	Bloating paper, Slide, Watch glass, Coverslip, Blade, Needle, Forceps, Scalpel, Dropper, Test tube, Centrifuge tube.
	Centrifuge machine	
	Spectrophotometer	

4.2. Methodology:

4.2.1. Prepare of Solution:

1 M solution of NaCl prepared by measuring 58.44 g of NaCl (Molar mass of NaCl = 58.44g) and placing this amount of salt in 1 L volumetric flask and then filling the flask with distilled water to the graduation mark. Then divided into required concentration for experiment. One nutrient solution also prepared without NaCl (whose EC 0 dS/m) which one serve as control solution.

4.2.2. Seed germination and exposure to salinity stress:

For an establishment of seedlings, at first rice seeds surface sterilized and treated with 2% Bavistin for 8h. Then germinated on wet filter papers embeddedin Petri dishes which were poured by different salt concentration including control. After 15 days, seedlings of each rice cultivars which were subjected to different salt contration and nutrient solution without NaCl served as control (whose EC 0 dS/m) collected and observed. Sampling performed at the end of the experiments and physiological changes also evaluated. Scoring of visual salt stress injury and growth reduction of rice seedlings treated with different salt concentration performed using the Standard Evaluation System of rice (Gregorio *et al.*, 1997).

4.2.3. Germination Percent and Germination Index:

The germination percentage calculated by the formula:

$$GP\ (\%) = (S / T) \times 100$$

Where, S = The Number of Germination SeedT = Total Number of Seeds Sown

The germination index was calculated after complete germination using the following formula:

GI = (Germination percent in each treatment / Germination percent in control) x 100

4.2.4. Relative Water Content:

Randomly selected shoot tissues from control and each treatment had been sampled in polythene bags and sealed properly to minimize water loss. The midvein along with edge sections cutted out and the remaining tissue weighed immediately and the fresh weight (FW) had been recorded.Then, the samples had been hydrated to full turgidity by floating on deionized water in a closed Petridish for 4 h at room temperature. After 4 hours, excess moisture had been removed from the leaf surface and weighed immediately to obtain turgid weight (TW). Then the samples further packed in butter paper bags and oven dried at 80 °C for 24 h or at 60 °C for 1 week and the dry weight (DW) recorded (Whetherely, 1950).

The WC will be calculated using the following formula:

$$WC = [(FW-DW) / FW] \times 100$$

The RWC will be calculated using the following formula:

$$RWC = [(FW - DW) / (TW - DW)] \times 100$$

Where,

FW = Fresh Weight

DW = Dry Weight

TW = Turgid Weight

4.2.5. Estimation of Chlorophyll Content:

Chlorophyll content of the collected varieties had been estimated following the method of Arnon (Arnon *et al.*, 1949). A total of 500 mg leaf tissue will be grinded into a fine pulp with the additionof 10 mL of chilled 80% acetone. The extract then transferred to a vial and centrifuged at 5000 r/min for 5 min the process being repeated until the tissue became colorless. The supernatantwill be then transferred to a test tube. The volume will be made up to 10 mL with 80% acetone. The readings will be taken at 645 and 663 nm respectively in a spectrophotometer (UV10) against the solvent (80% acetone) as blank. The amount of chlorophyll calculated by using folling formula:

$$\text{Chlorophyll a (Chl a, mg/g)} = [12.7(A_{663}) - 2.69(A_{645})]$$

$$\text{Chlorophyll b (Chl b, mg/g)} = [22.9(A_{645}) - 4.68(A_{663})]$$

$$\text{Total Chlorophyll} = [\text{Chlorophyll a} + \text{Chlorophyll b}]$$

Where, A represents the absorbance at specific wavelengths;

4.2.6. Estimation of Protein Content:

Protein content estimated by Lowry method. For protein estimation after collecting fresh leaves, leaves were cleaned up by suing tap water and double distilled water to remove all the dust. 1 g of finely chopped leaves are taken in a morter and pestle. 20ml phosphate buffer of pH 7.4 was added in the sample and keep it pesting until a clear plant solution was observed. Then the solutions were centrifuged at 10000 rpm for 10 min and the final supernatants were collected in falcon tube. BSA was used as standard reagent for preparing the standard curve (Figure 13) against which the unknown concentration of proteins was estimated. Weight accurately 50 mg of BSA and dissolve in distilled water and make up to 50 ml in standardflask using distilled water. Working standards, 0.2, 0.4, 0.6, 0.8 and 1.0 mL, pipetted out into a series of test tubes.

Sample extracts of 200 and 250 µL also pipetted out into other test tubes. Then 4.5 ml of reagent 1 (48 ml of 2% sodium carbonate in 0.1N sodium hydroxide + 1ml of 1% sodium potassium tartrate + 1ml of 0.5% copper sulphate) was added to the sample extracts and incubated for 15 min. After this, 0.5 ml of freshly prepared reagent 2 (1-part Folin Ciocalteau: 1 part water) was mixed with each sample and left for 30 min of dark incubation. After that the absorbance was measured at 660 nm (Lowry *et al.,* 1951). The amount of protein was calculated as:

Protein content (mg/g) = (Concentration of protein × Initial buffer × dilution factor / Supernatant volume × Sample weight) × 100.

4.2.7. Statistical Analysis:

The experiments were arranged in a completely random design (CRD) with three replications. Salt tolerance index was defined as the observed value of a target trait under a given salinity level divided by the mean value for that trait under the control (Zeng *et al.,* 2002). All the experimental measurements were performed in triplicates and expressed as the mean ± standard deviation. The magnitude of the means, standard curve, standard errors, and standard deviations were calculated by using MS Excel 2016 software.

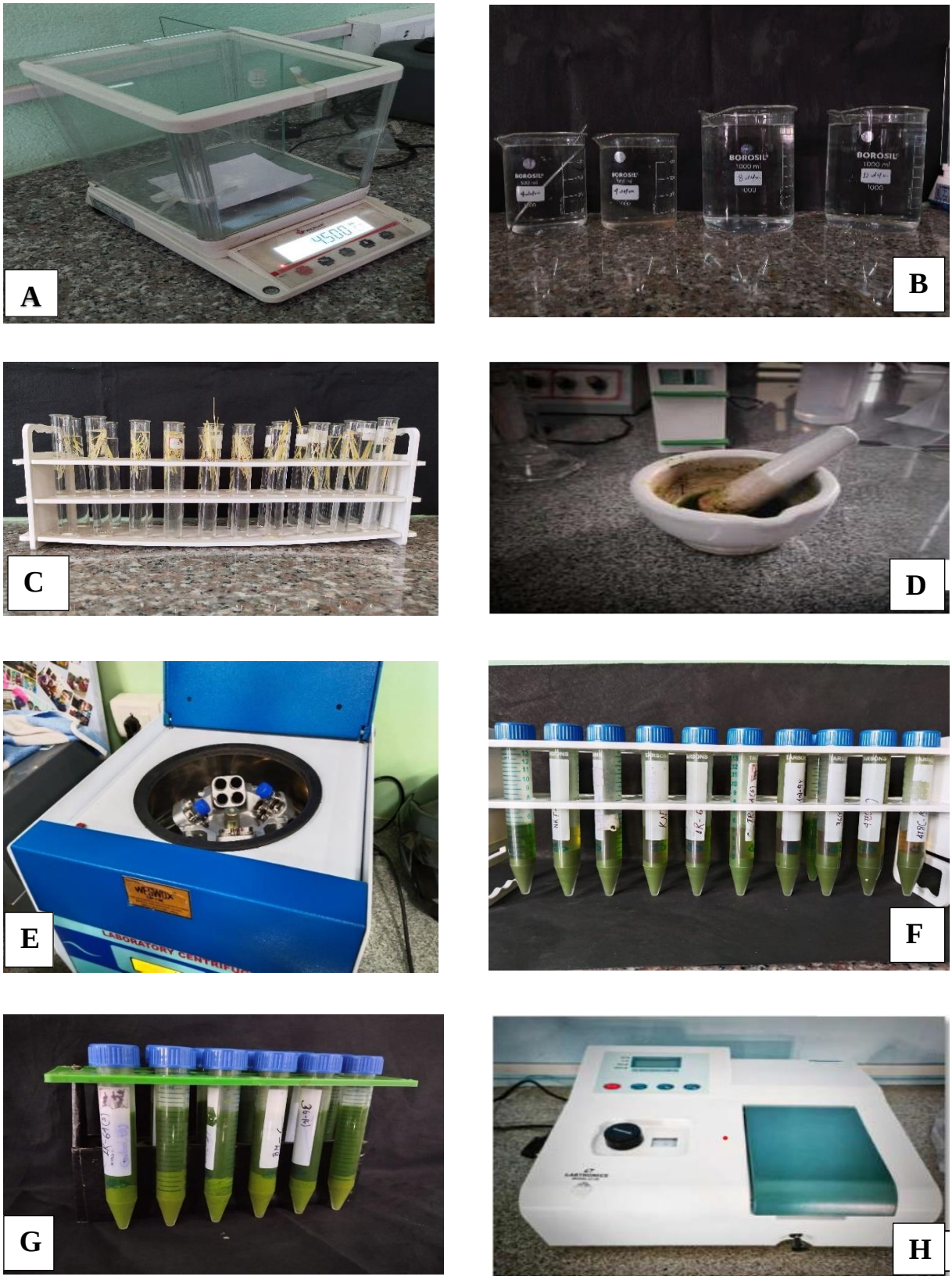

Fig 5. Instruments and samples: (A) Weight machine; (B) Different salt concentrations; (C) Leaf samples dipped in distilled water; (D) Mortar and pastel; (E) Samples ready for centrifuge; (F) Supernatant for chlorophyll estimation; (G) Supernatant for protein estimation; (H) Spectrophotometer.

Chapter 5. Results

5. Results

Observations were taken from both treated and non- treated plants. This study shows several morphological, biochemical, physiological and cytological changes occurred in treated plants as compared to normal plants. These following observations were noted here:

5.1. Soil Profiling:

At first, we surveyed in 4 blocks of South 24 Parganas (Kakdwip, Namkhana, Sagar, Pathar Pratima) and 6 blocks of Purba Medinipur (Contai-1, Contai-2, Ramnagar-1, Ramnagar-2, Udaypur-West Bengal and Udaypur-Odisa. Collected soil sample from surveyed blocks cultivated land and tested in laboratory. Also created profile of all collected soil samples.

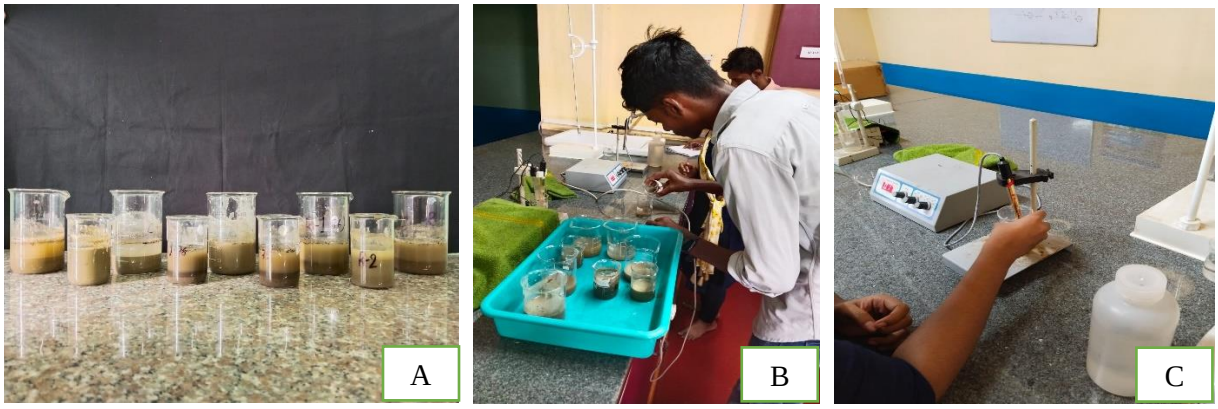

Fig 6. Testing of soil samples: (A) Precipitation of collected soil samples; (B) Testing of pH of collected soil samples; (C) Testing of EC of collected soil samples.

Table 7. Soil Characteristics of Surveyed Blocks:

SL No.	District	Block	pH	ECe (dS/m)	Fresh Weight (gm)	Dry Weight (gm)	Moisture Content (%)
1	South 24 Paraganas	Kakdwip	8.87	9.5	100	75	33.33
2		Namkhana	7.83	3.4	100	86	16.28
3		Sagar	8.26	10.1	100	92	8.70
4		Pathar Pratima	8.69	6.7	100	79	26.58
5	Purba Medinipur	Contai 1	7.41	3.7	100	94	6.38
6		Contai 2	7.6	3.3	100	85	17.65
7		Ramnagar 1	8.88	3.8	100	80	25.00
8		Ramnagar 2	9.52	3	100	79	26.58
9		Udaypur (West Bengal)	8.43	5.2	100	76	31.58
10		Udaypur (Odisha)	8.41	5.9	100	78	28.21

After observation of different soil sample from surveyed blocks, we select 3 concentrations as an average of all 10 soil samples. Then the prepared NaCl solution divided into 40 mM, 80 mM and 120 mM NaCl solution (which are equivalent to about EC 4, 8 and 12 dS/m, respectively). One nutrient solution also prepared without NaCl (whose EC 0 dS/m) which one serve as control solution. These were treated like 4 dS/m as T1, 8 dS/m as T2, 12 dS/m as T3 and control as T0.

5.2. Germination:

At first, we take 20 seeds of 5 cultivars for each treatment. Then treated with 2% Bavistin for 8h. Then 20 seeds of 5 cultivars were given in each treatement including control for germination. We counted number of germinated seeds at 2 days interval. Then at the end of 6 days we counted how many seeds germinated and average with previous data. Afterthat calculated germination percentages which presented as tabular form in table no. 8.

Table 8. Effect of salt stress on germination percentage of different rice cultivars:

Germination Percentage (%) [Mean ± Standard deviation]					
Treatments	**IR 36**	**IET 4786**	**IR 64**	**Kalanamak**	**Nonakathi**
T0	98.33 ± 1.67	86.67 ± 1.67	96.67 ± 1.67	76.67 ± 1.67	98.33 ± 1.67
T1	88.33 ± 1.67	81.67 ± 1.67	80.00 ± 2.89	55.33 ± 2.60	100.00 ± 0.00
T2	86.67 ± 1.67	75.67 ± 2.33	75.00 ± 2.89	40.00 ± 1.15	96.67 ± 1.67
T3	76.67 ± 1.67	69.67 ± 2.91	65.00 ± 2.89	32.00 ± 1.53	85.00 ± 2.89

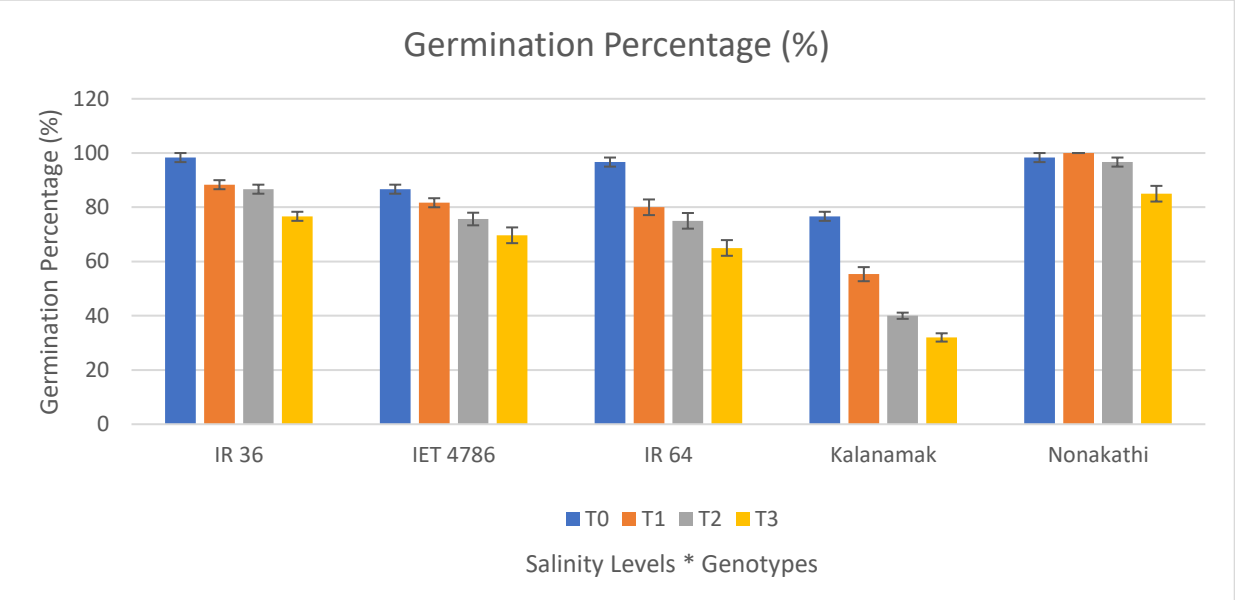

Fig 7: Interaction effect of salt stress and genotypes on the germination percentage of rice.

5.3. Morphological:

After germination seedlings started to grow on different salt concentration and on control also. First of all, to observe the effect of salt stress, we selected root and shoot length as primary parameter.

5.3.1. Root Length:

To check the salt stress on root length, we measured the length of root at 5 days intervals. After 15 days we measured root length and averaged with previous data which helped us to calculate vigour index of all cultivars under different salt stress condition including control. Those data presented as tabular form in table no. 9.

Table 9. Effect of salt stress on Root Length (cm) of different rice cultivars:

Root Length (cm) [Mean ± Standard deviation]					
Treatments	**IR 36**	**IET 4786**	**IR 64**	**Kalanamak**	**Nonakathi**
T0	16.37 ± 0.26	14.37 ± 0.30	14.63 ± 0.27	11.17 ± 0.18	15.4 ± 0.26
T1	14.67± 0.24	10.03 ± 0.29	12.87 ± 0.15	9.77 ± 0.24	11.80 ± 0.17
T2	9.17 ± 0.18	8.23 ± 0.30	9.80 ± 0.17	6.83 ± 0.20	10.97 ± 0.44
T3	7.07 ± 0.15	6.83 ± 0.18	8.30 ± 0.26	6.17 ± 0.20	7.77 ± 0.20

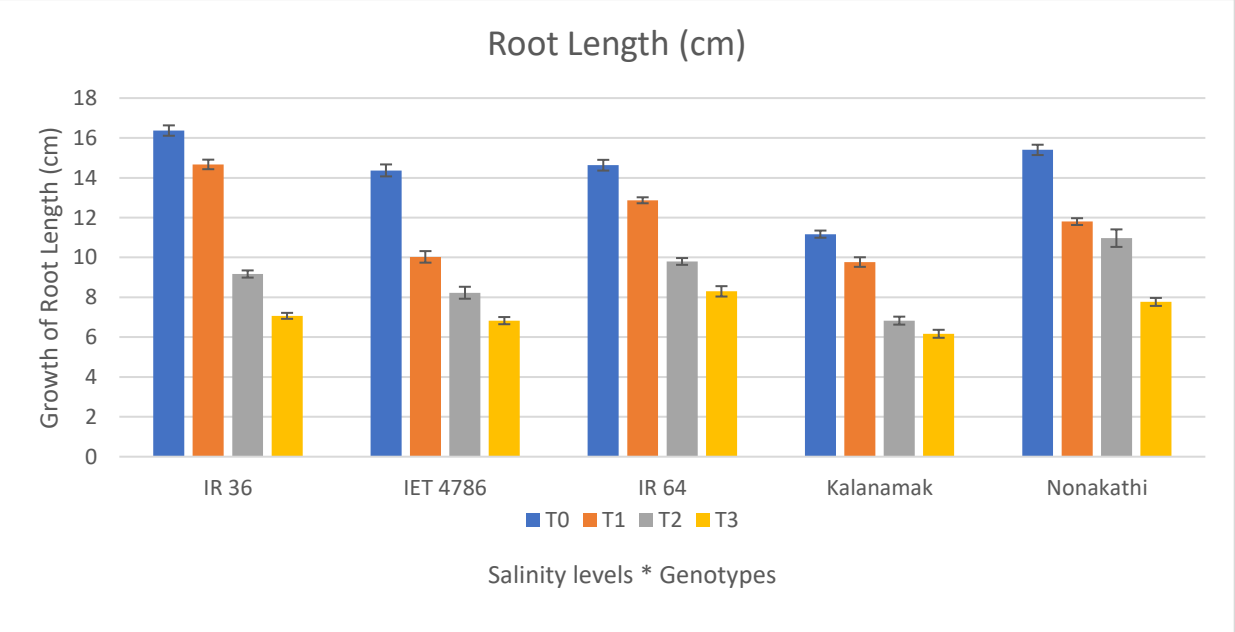

Fig 8: Interaction effect of salt stress and genotypes on the root length of rice.

5.3.2. Shoot Length:

To check the salt stress on shoot length, we measured the length of shoot at 5 days intervals. After 15 days we measured shoot length and averaged with previous data which helped us to

calculate vigour index of all cultivars under different salt stress condition including control. Those data presented as tabular form in table no. 10.

Table 10. Effect of salt stress on Shoot Length (cm) of different rice cultivars:

Shoot Length (cm) [Mean ± Standard deviation]					
Treatments	**IR 36**	**IET 4786**	**IR 64**	**Kalanamak**	**Nonakathi**
T0	19.63 ± 0.32	15.13 ± 0.41	21.27 ± 0.80	10.33 ± 0.24	17.07 ± 0.61
T1	16.27 ± 0.27	14.33 ± 0.22	14.13 ± 0.45	8.70 ± 0.31	14.77 ± 0.44
T2	13.47 ± 0.35	12.33 ± 0.23	11.60 ± 0.29	6.70 ± 0.30	14.80 ± 0.21
T3	11.63 ± 0.24	11.43 ± 0.23	7.53 ± 0.26	5.50 ± 0.35	11.47 ± 0.35

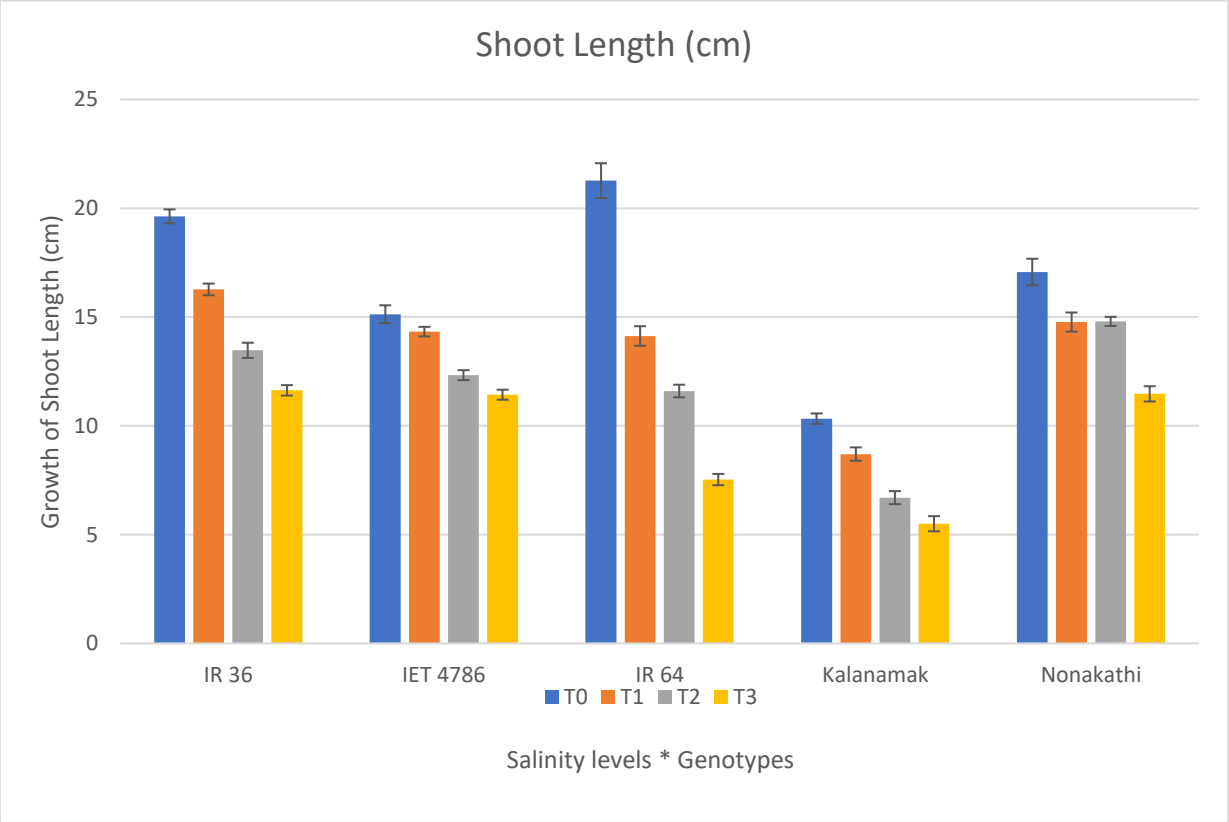

Fig 9: Interaction effect of salt stress and genotypes on the shoot length of rice.

5.4. Physiological:

Affter observation of morphological effect we turned to look at the physiological effect. Here we worked with several parameters like water content, relative water content and chlorophyll content etc.

5.4.1. Water Content:

After 21 days, collect 0.08 g leaf sample from each treatment including control of every cultivers. Then measure the fresh weight. Afterthat, the samples further packed in butter paper

bags and oven dried at 80 °C for 24 h and the dry weight (DW) recorded. Then calculated water content of plants taken from each treatment, which presented as tabular form in table no. 11.

Table 11: Effect of salt stress on water content of different rice cultivars:

Water Content [Mean ± Standard deviation]					
Treatments	**IR 36**	**IET 4786**	**IR 64**	**Kalanamak**	**Nonakathi**
T0	80.92 ± 0.58	85.88 ± 0.71	73.13 ± 6.56	67.13 ± 0.33	80.92 ± 0.58
T1	80.46 ± 0.40	77.04 ± 0.54	76.71 ± 5.57	80.17 ± 5.63	85.46 ± 0.33
T2	81.00 ± 0.19	78.96 ± 1.51	78.38 ± 1.38	76.75 ± 5.63	66.42 ± 1.29
T3	68.79 ± 1.93	84.08 ± 1.12	72.67 ± 4.04	76.88 ± 5.63	68.79 ± 1.93

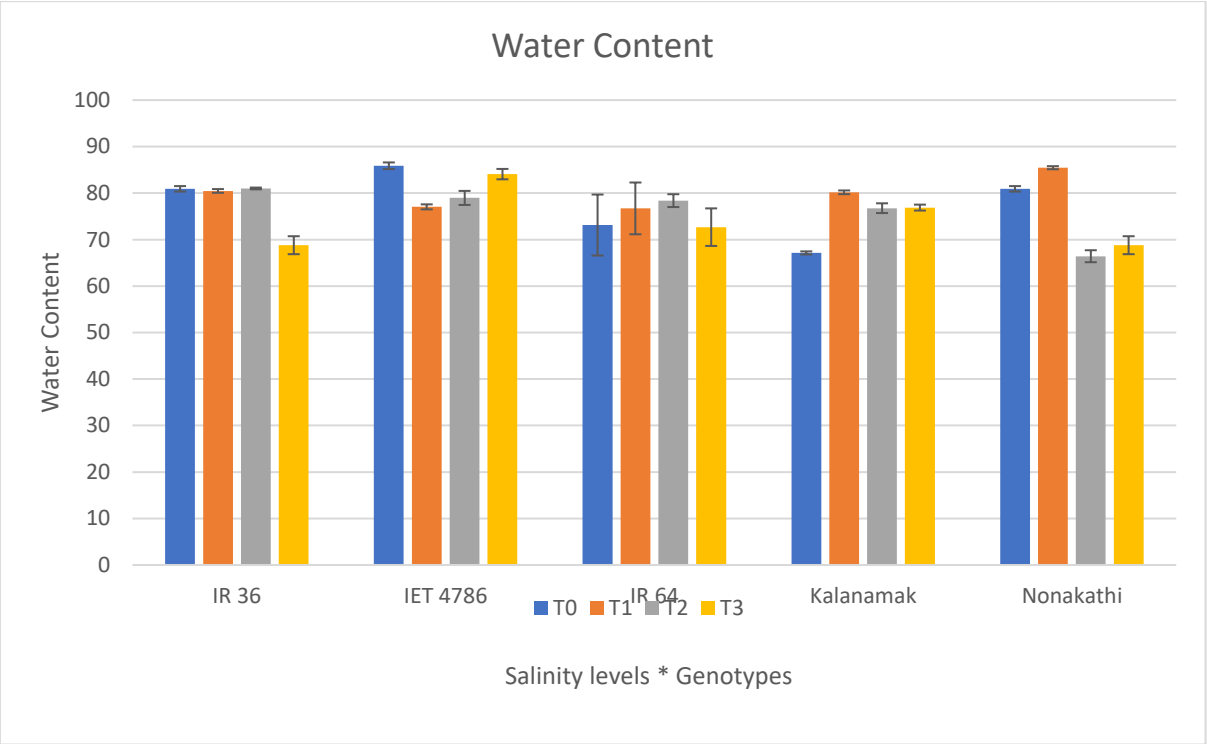

Fig 10: Interaction effect of salt stress and genotypes on the Water content of rice.

5.4.2. Relative Water Content:

Just like measuring water content, after 21 days, collect 0.08 g leaf sample from each treatment including control of every cultivers. Then measure the fresh weight. After measuring fresh weight, we dipped those leaf samples under distilled water for 4 h at room temperature and after 4 h excessive moisture had been removed and measured turgid weight of all samples. Afterthat, the samples further packed in butter paper bags and oven dried at 80 °C for 24 h and the dry weight (DW) recorded. Then calculated relative water content of plants taken from each treatment, which presented as tabular form in table no. 12.

Table 12: Effect of salt stress on relative water content of different rice cultivars:

Relative Water Content [Mean ± Standard deviation]					
Treatments	**IR 36**	**IET 4786**	**IR 64**	**Kalanamak**	**Nonakathi**
T0	90.32 ± 2.25	92.59 ± 1.71	85.37 ± 2.37	86.01 ± 4.55	75.69 ± 2.42
T1	85.43 ± 1.35	73.69 ± 2.44	73.65 ± 2.68	83.44 ± 1.22	68.01 ± 2.33
T2	73.85 ± 1.49	57.38 ± 4.04	73.18 ± 1.01	65.07 ± 1.39	63.26 ± 1.32
T3	73.32 ± 1.63	51.47 ± 0.86	59.28 ± 2.37	56.73 ± 0.73	60.58 ± 2.92

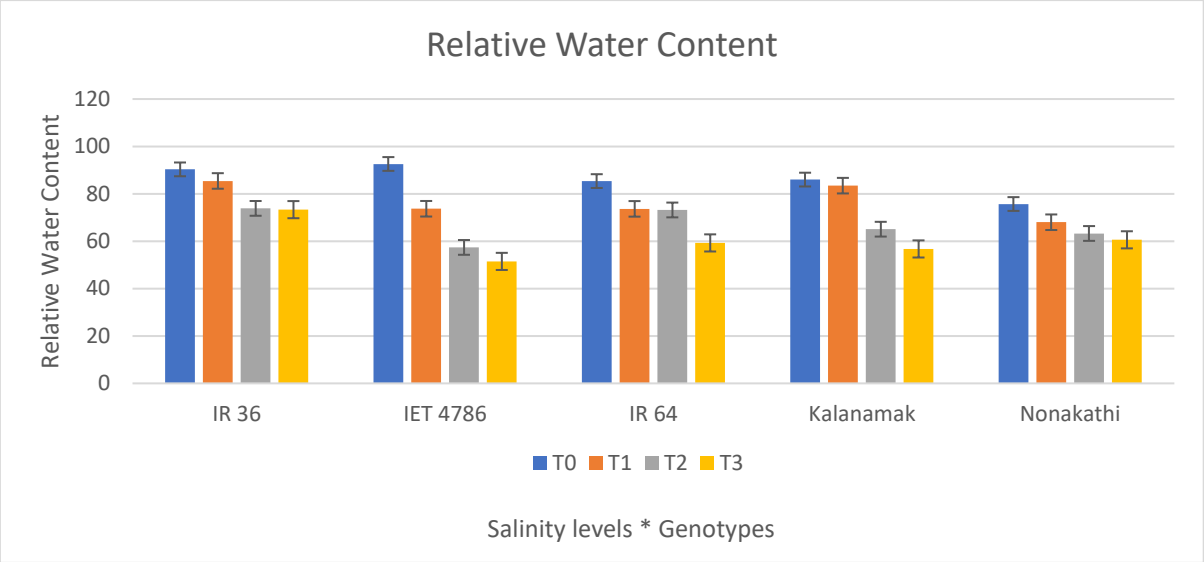

Fig 11: Interaction effect of salt stress and genotypes on the relative water content of rice.

5.4.3. Total Chlorophyll Content:

By using Arnon method, recorded absorbance at 645 nm and 663 nm wavelengths of spectrophotometer of prepared supernatant of each sample from each treatment of every cultivar. Then calculated total chlorophyll content of each sample from chlorophyll a and chlorophyll b, those were presented as tabular form in table no. 13.

Table 13: Effect of salt stress on total chlorophyll content of different rice cultivars:

Total Chlorophyll Content [Mean ± Standard deviation]					
Treatments	**IR 36**	**IET 4786**	**IR 64**	**Kalanamak**	**Nonakathi**
T0	45.53 ± 0.32	15.69 ± 0.15	35.52 ± 0.64	20.24 ± 0.42	49.53 ± 0.43
T1	35.20 ± 0.11	11.63 ± 0.33	30.72 ± 0.15	17.62 ± 0.25	44.35 ± 0.12
T2	25.89 ± 0.45	9.19 ± 0.11	23.77 ± 0.29	12.75 ± 0.18	35.23 ± 0.13
T3	19.49 ± 0.23	7.42 ± 0.33	17.30 ± 0.36	8.74 ± 0.13	29.64 ± 0.46

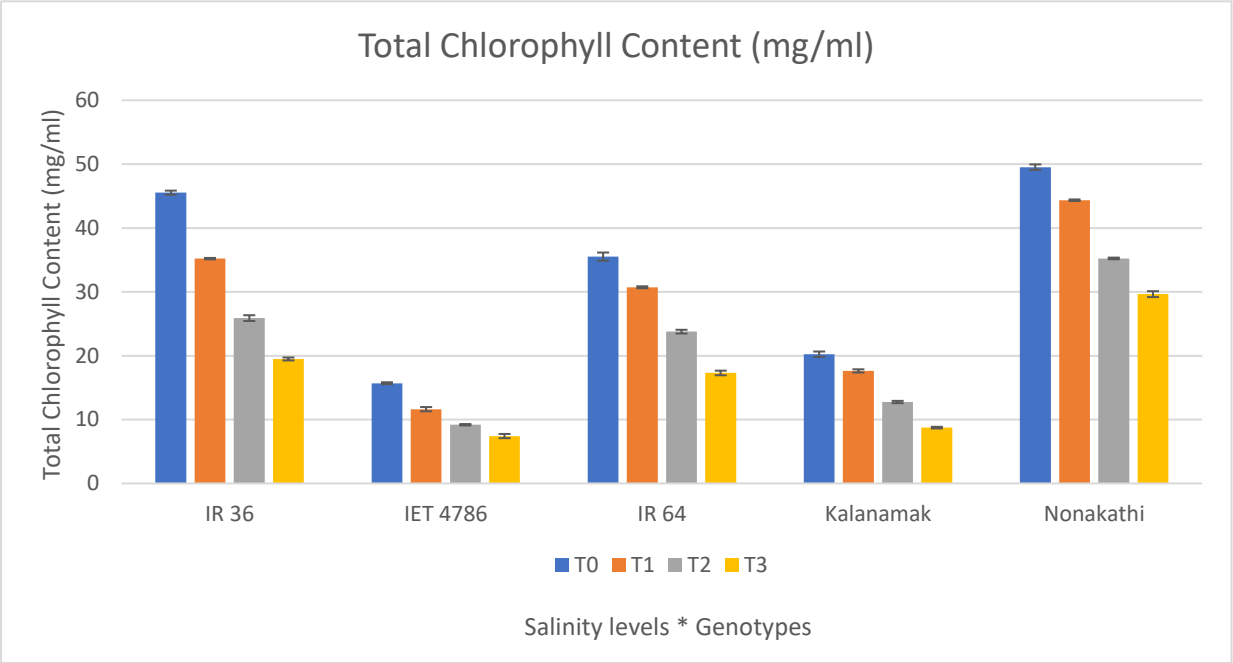

Fig 12: Interaction effect of salt stress and genotypes on the total chlorophyll content of rice.

5.5. Biochemical:

After observed physiological effect, we also checked the biochemical effect due to impact of different salt concentrations. Here we used protein content of each sample as an aid to biochemical examining.

5.5.1. Protein Content:

Here we observed protein content in the leaves of each sample from each treatment of every variety by using Lowry method. Made a BSA standard solution and then divided into different concentrations. Take absorbance of different BSA concentration by using spectrophotometer. Made a standard curve of BSA by using following data presented in table no. 14. Calculated protein content of every sample by using absorbances of samples supernatant and standard curve. The data of protein content presented as tabular form in table no. 15.

Table 14. Concentrations of standard BSA samples:

BSA (mg/ml)	Concentrations (µg/ml)	Absorbance (660 nm)
0.2	200	0.151
0.4	400	0.269
0.6	600	0.421
0.8	800	0.466
1.0	1000	0.598

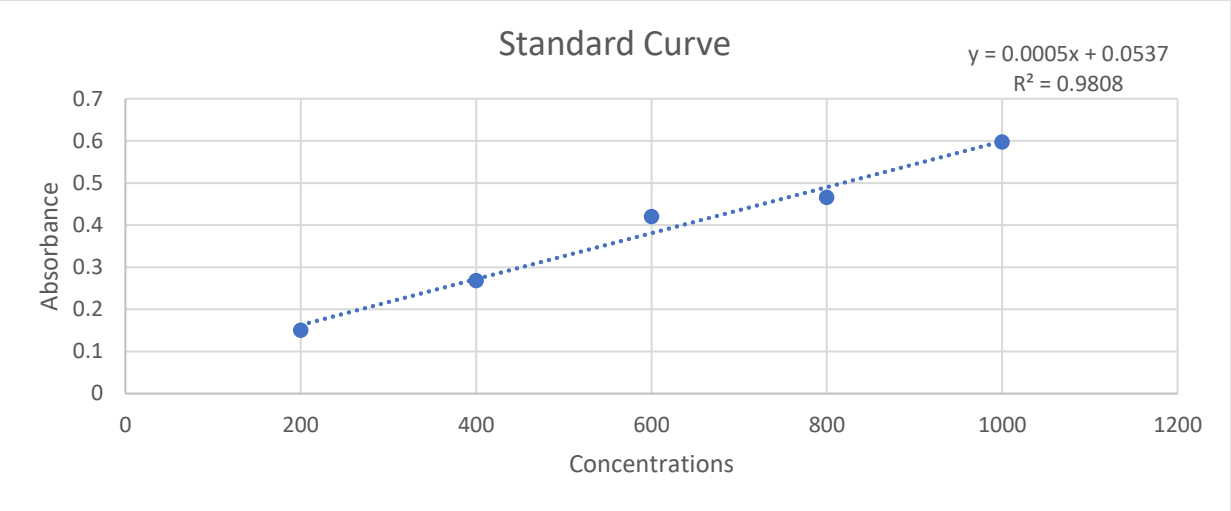

Fig 13. Standard graph for protein estimation using BSA solutions.

Table 15: Effect of salt stress on protein content of different rice cultivars:

Protein Content [Mean ± Standard deviation]					
Treatments	**IR 36**	**IET 4786**	**IR 64**	**Kalanamak**	**Nonakathi**
T0	4.83 ± 0.04	4.49 ± 0.07	4.80 ± 0.07	4.73± 0.05	4.76 ± 0.11
T1	4.49 ± 0.03	4.26 ± 0.07	4.31 ± 0.03	4.39 ± 0.06	4.30 ± 0.03
T2	4.16 ± 0.04	3.65 ± 0.03	3.85 ± 0.07	3.87 ± 0.03	4.01 ± 0.07
T3	3.86 ± 0.05	3.10 ± 0.12	3.36 ± 0.09	3.46 ± 0.45	4.44 ± 0.10

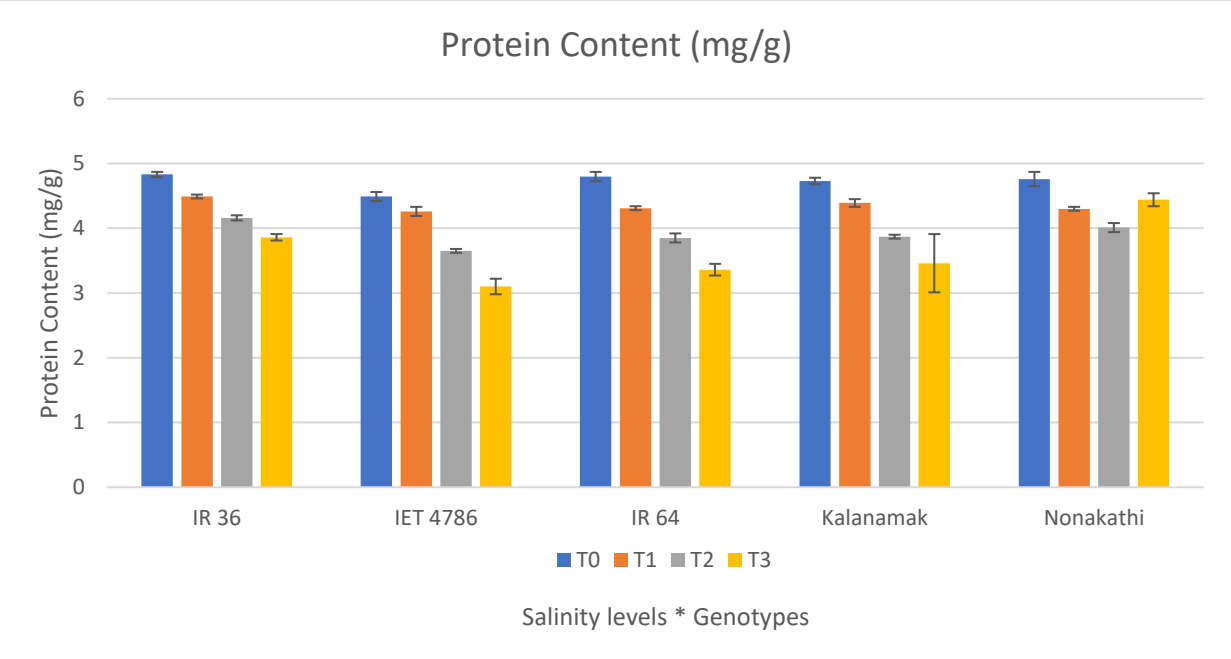

Fig 14: Interaction effect of salt stress and genotypes on the protein content of rice.

Chapter 6. Discussion

6. Discussion

In this experiment we worked on rice. The botanical name of rice is *Oryza sativa* L., chromosome no. 24 and belongs from the Poaceae family. Here we used four cultivated varieties of rice, those are IR 36 as V1, IET 4786 as V2, IR 64 as V3 and Kalanamak as V4. Also, we used one landrace i.e., Nonakathi as V5. The study entitled "Comparative study analysis among different cultivars and landraces of rice due to impact of salt stress". Here we try to find out the difference of morphological, physiological, and biochemical changes between stressed and normal plants.

At first surveyed report and soil profile helps us to know about pH, EC, and moisture content of collected soil samples from different blocks of Purba Medinipur and South 24 Parganas districts.

Parihar *et al*., 2015, showed that seed germination is a critical stage, which affects total dry matter and grain yield output. Salt stress induced a delay in rice seed germination showing an adverseassociation between seed germination and salt stress. During germination we show in this experiment that due to increase of salinity level germination percentages of IR 36, IET 4786, IR 64 and Kalanamak became decreased mostly as compare with control. While Nonakathi shows eminent result to salt stress than other varieties. Germination percentages of Nonakathi seeds also decreased for increasing salt stress but not like other varieties.

According to Perez Alfocea *et al.,* 1996, a rise in salt- stressed root length is attributed to the plant's capacity to enterdeeper soil field layers at low water potential over root elongation. Basically, the roots of saline-adapted plants show reduced cortex to shorten the distance between epidermis and stele. In this study we found similar results. For root and shoot length reduction, all varieties reduced root and shoot length for increasing salinity level gradually excluding Nonakathi as compare to control. Nonakathi shows tolerance in reduction of root and shoot length for increasing salinity level.

For water content, we know that due to increasing of salinity level water content became decreased. IR 64 and Kalanamak show more water content in stress condition as compare to control. IET 4786 shows increasing of water content with increasing of salinity level. While IR 36 and Nonakathi shows significant result to salt stress.

Jan et al., 2016; Hand et al., 2017 and Hussein et al., 2017, reported that RWC was reduced when the salinity increased and the tolerant cultivars showed less reduction in the RWC. For relative water content, we also know that due to increasing of salinity level relative water content significantly decreased. In this present study IR 36, IET 4786, IR 64 and Kalanamak varieties showed similar results while Nonakathi shows some different as compared to control. The decreasing level of relative water content of Nonakathi is quite low as compared to control.

While the rwc is decreased in all cultivars for increasing salinity level.

Ali et al., 2004 showed that the chlorophyll content (chlorophyll a, b, tcc) of rice leaves was generally reduced under high salinity. However, the present study showed that the reduction in chlorophyll content was variety specific and some cultivars like Nonakathi followed by IR 64 and IR 36 showed comparatively lesser quantum of negative variation in chlorophyll content thus indicating their potential to grow and perform moderately well even under higher levels of salt stress. IET 4786, Kalanamak mostly affected by increasing salinity level.

According to Carillo *et al.*, 2011, salt stress toxic effects trigger the occurance of mature and older leaves by diminished protein production. We found similar trends in this experiment. In this present study, due to increasing of salinity level, protein content of IR 36, IET 4786, IR 64 and Kalanamak became gradually decreased as compare to control. While in Nonakathi, protein content at 12 dS/m shows high than 4 dS/m and 8 dS/m but low as compare to control.

This discussion showed an efficient result with respect to previous researchers report which ensures this paper's acceptances to breeders, researchers, students and public.

Chapter 7. Conclusion

7. Conclusion

This experiment is a simple and efficient technique for screening rice seedlings for salinity tolerance or susceptible with a high degree of precision among IR 36, IET 4786, IR 64, Kalanamak and Nonakathi; however. The outcome shows inhibition and retardation of germination rate, morphological changes like reduction of radical or root and shoot or coleoptiles extension and seed become swollen. From this study, it concluded that, salinity affects the seedling survivability and also exhibits the reduction in relative water content and dry matter accumulation. Although both shoot and root morphological growth and developmental parameters are important indicators of salinity tolerance in rice, this study identified that root parameter are better predictors of salinity tolerance, and physiological parameters are non-predictive. Genotypes which can maintain a deep, well developed and extensive root system will help plants cope under stress conditions by taking up water and nutrients from the soil and efficiently storing them for a longer period for plant survival as compared to genotypes with poorly structured and less vigorous root systems. Salt-induced changes occurring during the vegetative stage of the rice plant may also affect the rice grain composition and quality. Physiological characters like chlorophyll Content decreased due to increase of salt stress. Also, in protein content estimation shows varience. Among 5 cultivars, Nonakathi shows more tolerance followed by IR 36 and IR 64. While IET 4786 and Kalanamak shows susceptibility to salt stress. It must be compared with results from future field studies to determine final utility. Also, the use of fertilizer is not mandatory for cultivation on saline soil, which is also a main reason of increasing salinity level in soil.

Therefore, this study clearly compared the effects of salt stress on different rice cultivars i.e., IR-36, IR-64, IET-4786, Kalanamak, and Nonakathi. Where, Nonakathi shown tolerance, IR-36, IR-64 shown moderately tolerance and IET-4786, Kalanamak shown susceptibility.

Chapter 8. Future Scope

8. Future Scope

Salinity as an abiotic stress is the second most devastating phenomenon after draught and interfering with the production and yield of rice globally. Soil EC, much like pH, is a good overall indicator of soil fertility. It can be used to show the capacity of the soil to store nutrients, as an indicator of soil texture and as an indication of an excess of soil nutrients (e.g., excessive sodium levels leading to salinity). Good soil fertility management practices will contribute to maintaining optimal EC levels. The two most important things to take into account are that in low EC soils, nutrient shortages should be addressed and in high EC soils, nutrient build up should be addressed. Rice is the major food crop cross across several countries globally. With the increased population worldwide the demand for rice is also increasing in accordance. Rice, a glycophyte, by nature is susceptible to salinity and show wide and vivid response against the detrimental effects of increased salt accumulation. The plant defense system in rice includes arrest and alleviation of the harmful effects of salt toxicity at physiological, biochemical, and molecular levels. A comparative study of the effect of salinity and the response of the crop in turn has been illustrated here. Due to the polygenic nature of the stress, it has been very meticulous to comment about the exact mechanism by which mitigation of the same is achieved. Breeding for salinity tolerance in rice requires reliable and rapid screening techniques which can keep pace with the large amount of breeding materials and germplasm collection to be used as donor parents. The quantitation of salinity resistance in the field poses serious difficulties owing to high field heterogeneity and genotype/environment interactions. Knowledge from this study can help rice breeders and other scientists screen and select salinity tolerant rice breeding varieties for tolerant cultivars development and related research. If these tolerance varieties will be cultivated in salt affected area, excessive use of fertilizers will be avoided easily. This screening method can be used by farmers to screen high yielding commercial cultivars for salinity tolerance at an early stage before taking a potential risk of sowing them in large acreage in salt prone areas.

Chapter 9. References

9. References

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