ASSESMENT OF MULTIPLE ABIOTIC STRESS TOLERANCE MECHANISMS IN RICE (*Oryza sativa* L.)

by

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THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"Assesment of multiple abiotic stress tolerance mechanisms in rice (*Oryza sativa* L.)"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associate ship, fellowship or other similar title, of any other university or society.

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LIST OF ABBREVIATIONS

SOD	Superoxide dismutase
MDA	Malondialdehyde
DNA	Deoxyribo Nucleic Acid
SSR	Simple Sequence Repeats
FAO	Food and Agricultural Organization
IRRI	International Rice Reseach Institute
g/m^2	$grams/meter^2$
RARS	Regional Agricultural Research Station
PCR	Polymerase Chain Reaction
TE buffer	Tris-EDTA buffer
OD	Optical density
dNTP	Deoxynucleotide Triphosphates
ppm	Parts per million
CD	Critical Difference
SE(m)	Standard Error (Mean)
G	Genotype
T	Treatment

g	Gram
GxE	Genotype x Environment
%	per cent
^{0}C	Degree Celsius
µ moles/g tissue	micro moles/gram tissue
cm	Centimeter
cm^3	cubic centimeter
ml	Milliliter
µl	Microliter
Kg	kilo grams
mM	Millimolar
HT	High temperature
HS	Heat Stress
nm	Nanometer
bp	base pairs
U	Units
rpm	rotations per minute
et al.	and other co-workers
HSP	Heat shock proteins
ROS	Reactive oxygen species

POX	Peroxidase
CMT	Cell membrane thermo-stability
CMS	Cell membrane stability
CSI	Chlorophyll stability index
WS	Water Stress
HSF	Heat Shock Factor
HSP	Heat shock Protein
PEG	Poly Ethylene Glycol
NaCl	Sodium Chloride

Introduction

INTRODUCTION

Rice, (*Oryza sativa* L.) is the largest wetland food crop in the world belonging to the Poaceae family and the Oryzoidae subfamily. Asia accounts for 90% of the cultivation and consumption of rice. Rice is grown in a region of 164 million hectares worldwide with an annual output of 772.8 million tonnes (FAO, 2013). Globally, India ranks first in rice cultivation (43.92 million hectares) and second in rice production (91.61 million tonnes) (Pandey *et al.*, 2010).

In India, it is grown under varied agro-climate conditions and rice productivity is lower (2494 kg / ha) compared to China (6710 kg / ha). This lower level of productivity results from shortage of water, land, labour and other resources in addition to the stresses created by changing climate scenarios (Singh *et al.*, 2013).

Although the production of rice is rising, the rate of rise for the current demand is not adequate. We must therefore generate an extra 116 million tons of rice by 2035 (Kumar and Gautham, 2014). Farmers adopt better cultural and rice production management methods, but rice productivity does not increase as anticipated, due to multiple environmental stresses (Jagadish *et al.*, 2007), which include both biotic and abiotic factors.

Exposure of plants to unfavorable climate conditions such as extreme temperatures (heat, cold, freezing), drought (deficient rainfall, drying winds) and elevated salt contamination of the soil restrict plant growth, development and yield. The stresses, primarily drought, elevated temperature and salinity, pose significant reduction in the productivity of rice. Because excessive evaporation owing to drought and temperature stress leads to salt accumulation in soil, these stresses can also affect the crops together (Rachoski *et al.*, 2015). Plant resistance to a combination of two different abiotic stresses is unique and cannot be extrapolated straight from plant response to each individual stress (Mittler, 2006).

Limited knowledge of crop response to exceptionally elevated climatic conditions is an significant source of uncertainty in predicting the impacts of climate change on agriculture (Lobell *et al.*, 2012). To resist this extreme weather, crops have

developed many adaptive and tolerant mechanisms by creating short and long-term physiological and biochemical processes such as excess heat dissipation through evaporative cooling, preserving the integrity of the membrane, synthesis of HSPs, synthesis of metabolites and free radical scavengers (Wahid *et al.*, 2007). On the contrary, decreased availability of key elements such as iron, copper, zinc or manganese required for the function of various defense enzymes such as superoxide dismutase or ascorbate peroxidase could lead to increased oxidative stress in plants under various abiotic stresses (Mittler, 2006).

In addition to the fundamental variations between plant acclimatization reactions to distinct abiotic stress circumstances, when coupled, distinct stresses may involve conflicting or antagonistic reactions. For instance, crops open their stomata during heat stress to cool their leaves through transpiration. If heat stress is coupled with drought, however, crops would not be able to open their stomata and the temperature of the leaves would be higher (Rizhsky *et al.*, 2002). Similarly, salinity or heavy metal stress may present a comparable issue to crops when coupled with heat stress, as increased transpiration may lead to increased salt or heavy metals absorption (Mittler, 2002). Perhaps the most significant guideline for studying the combination of abiotic stress is to consider it as a new state of abiotic stress in crops and not just the sum of two distinct stresses (Mittler, 2006).

Better knowledge of the function and regulation of the responsive genes and their association with QTLs will make it possible to create a more structured design and selection for abiotic stress tolerance. Plant breeders should investigate further use of newly found QTLs to create rice varieties with higher salt tolerance and other abiotic stresses (Gregorio *et al.*, 2013). Primarily due to the quantitative nature of abiotic stress in the field, high quality phenotyping and genotyping data are essential for the identification of quantitative feature loci (QTLs)/genes and the development of markers for marker-assisted breeding, in particular for features that are difficult to screen (Vivitha *et al.*, 2017).

The magnitude of harm caused by a mixture of two distinct stresses to agriculture underlines the need to grow crops and plants with increased tolerance to

combination of distinct abiotic stresses, based on the restricted physiological, molecular and metabolic research conducted with crops undergoing two distinct abiotic stresses simultaneously

Simultaneous occurrence of multiple stresses, increases the deleterious effect, such that the effect considerably exceeds the simple additive effects of the action alone. Hence this programme envisages to screen selected rice varieties for multiple abiotic stress tolerance and to validate the genotypes with SSR markers linked to various stresses.

With these backgrounds the present study was carried out with following objective

- To study the multiple abiotic *viz.* drought, salinity and high temperature stress tolerance mechanisms in rice and to validate the identified QTLs for stress tolerance in rice.

Review of Literature

2. REVIEW OF LITERATURE

Rice (*Oryza sativa* L.) is both a cost-effective cereal and a staple dietary food. It is the main and most vital source of food for more than half of the population and more than 90% of the world's rice is grown and consumed in Asia, where 60% of the world's people live, as well as a major source of income for the rural population (Wani and Sah, 2014). It is the single most vital subsistence in the world and an essential food hotspot for over 33% of the total population. Rice accounts for 35 to 60% of the calories that 3 billion Asians have devoured.

In order to meet the estimated demand of an increasing global population, the world will need about 25% more rice by 2030 (Wani and Sah, 2014). One way to meet this challenge is to grow rice in more area, which is difficult in under developed countries due to increasing urbanization and population escalation.

Rice is produced in almost all states. Top three producer states are West Bengal, Punjab and Uttar Pradesh. Other rice growing states include Tamilnad, Andhrapradesh, Bihar, Jharkhand Uttarakhand, Chhattisgarh, Odisha, Uttar Pradesh, Karnataka, Assam and Maharashtra. It is also grown in Haryana, Madhya Pradesh, Kerala, Gujarat and Kashmir Valley. The statewise production of rice is depicted in Plate 1.

A quantum leap in rice yield has taken place over the past three decades because of the Green Revolution, although increased food production has not eliminated poverty and hunger. The increase in yields helped to prevent further disruption of food supply in Asia, unlike in some African countries, where lack of infrastructure led to a lack of benefits (Datta *et al*, 1997).

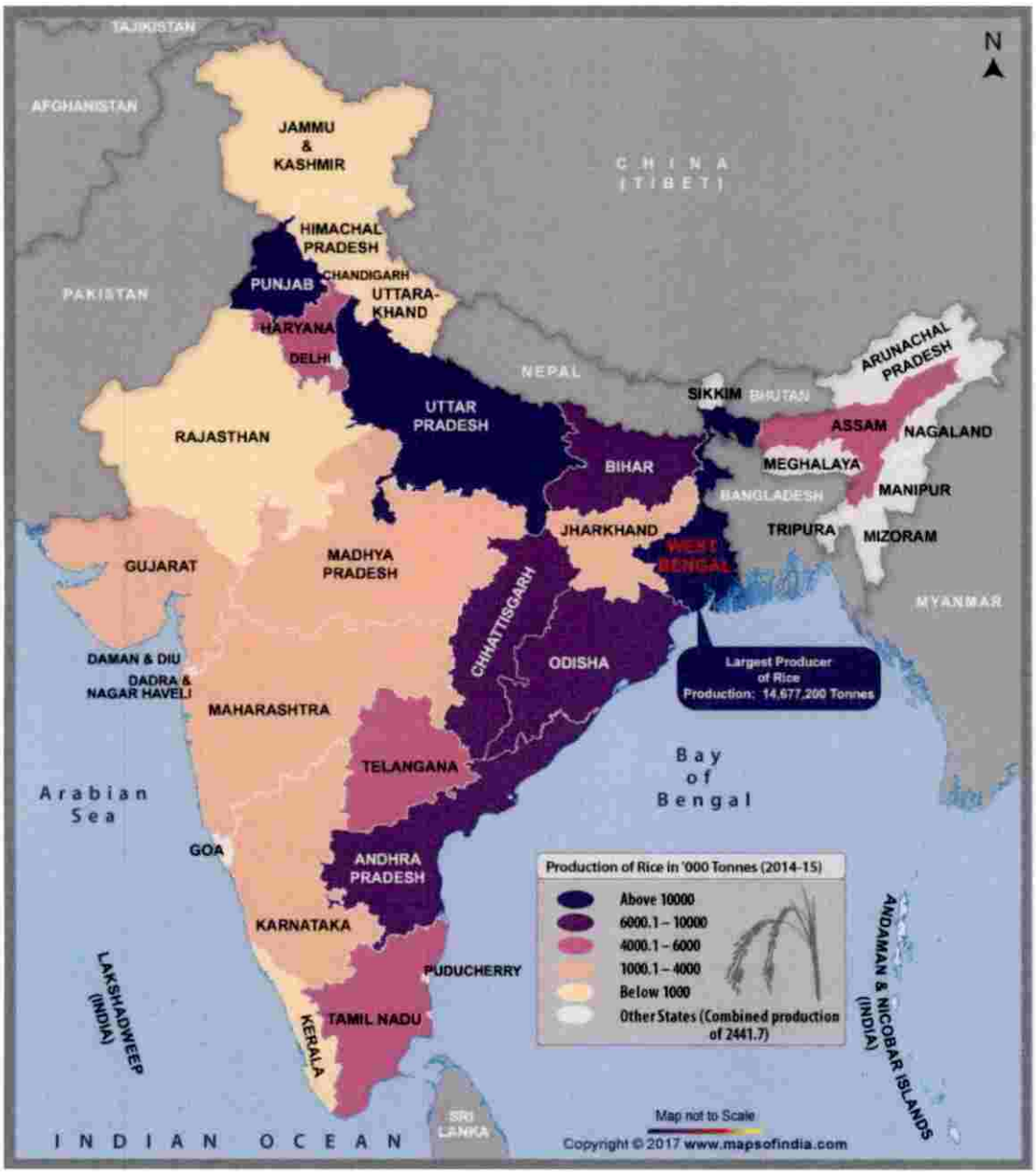


Plate 1. State wise production of rice in India (2014-15)

(Maps of India, 2017)

2.1 CLIMATE CHANGE AND CROP YIELD

World rice production is expected to increase by 1% per year to meet the growing food demand that may result from growth and economic development. Most of this increase should come from higher yields on existing crops. Higher yields depend on increasing the total biomass of crops.

Temperature is the primary driving force that determines crop yield along with photoperiod. The optimum temperature ranges from 27 to 32°C for ordinary rice growth. High temperature impacts nearly all rice development phases, from emergence to maturation and harvesting. Flowering (anthesis and fertilization) and booting (microsporogenesis) are regarded to be the most temperature-sensitive developmental phases in rice. Exposure to 41°C for 4 hrs causes irreversible harm in rice crop and becomes totally sterile (Shah *et al.*, 2011).

Rice is particularly susceptible to drought stress during reproductive stages, where even mild stress can drastically reduce grain yield (Venuprasad *et al.*, 2007).

Young seedlings of rice are highly susceptible to salinity. Yield parameters such as panicle length, spikelet number per panicle, and grain yield after salt treatment have been considerably decreased (Zeng and Shannon, 2000)

2.2 EFFECT OF VARIOUS ABIOTIC STRESSES ON RICE

Abiotic stress will limit the productivity of crops and, in addition, limit the land for agriculture (Grennan, 2006).

Plants are sessile and sensitive organisms that throughout the life cycle encounter a range of environmental stresses. Plant development and yield are adversely affected by environmental abiotic stress conditions. Soil has been littered with environmental stresses in many regions of the planet over the past decade. Salt, cold, drought and ultraviolet light hinders growth and productivity of crops. It is expected that with global climate change can become very intense and frequent. On the other hand, it is calculated that by 2050 the planet's population will succeed in nearly ten billion, which can witness serious food crisis issues. Tolerant crops have to be developed to feed the growing population of the world. Perhaps the most important challenge facing trendy agriculture is to maintain crop yields below adverse environmental stresses. Plants have defense mechanisms to deal with environmental stresses such as drought, UV, high salinity, cold stress and attacks of microorganisms (Gill and Tuteja, 2010).

2.3 DROUGHT STRESS

Drought is the most vulnerable stress on rice production and most of the farmers varieties are susceptible to drought stress (Serraj *et al.*, 2011).

Blum (2011) reported that drought is the insufficient moisture content in the soil which results in reduced crop growth and development and also low yield. Rice's semi-aquatic phylogenetic origin makes it more vulnerable to drought.

Rice is one of the most important food crops needed in the lowland rice scheme, suffering drastically from drought. Dissecting the characteristics of importance and genomic regions that influence the response of drought tolerance and yield characteristics on grain yield can help breeders to understand the genetic mechanism of rice drought tolerance in case of drought tolerant varieties (Manickavelu *et al.*, 2006).

2.3.1 Physiological and biochemical changes in response to drought

Drought stress is one of the main abiotic stress affecting plant growth, productivity and has seen it as a severe threat to sustainable crop production under changing climate conditions. Drought produces a wide range of plant responses, ranging from cellular metabolism to changes in growth rates and crop yields. Knowledge of biochemical and molecular response to drought is needed to an integrated view of mechanisms for plant resistance to drought stress conditions. Limited water conditions gradually decrease the rate of CO_2 absorption by decreasing stomatal behavior. It reduces leaf size, increases extension and proliferation of roots, interferes with plant water supply, and reduces the efficiency of water consumption. It affects photosynthetic pigments and decreases the exchange of gas which leads to reduced production of crops. In addition, drought stress also enhances the generation of active oxygen species (Anjum *et al.*, 2011).

The primary impact of drought on plant is impaired germination. Plants have a well-developed escape mechanism against drought that is, short life cycle – plants complete their reproductive cycle before the onset of extreme stress conditions. Maintaining an optimum water level during stress results in avoidance of dehydration, mainly due to reduced water loss or increased root growth there by maximizing water absorption. Tolerance strategies in plants include increased root growth, rigid cell walls or small cells and efficient reactive oxygen species scavenging mechanisms (ROS) (Sairam and Saxena, 2000).

Many yield-determining pathways in plants respond to water stress. Grain yield of a plant is the combined result of expression and association of many plant growth elements. The water deficiency results in a serious drop in plant yield by disturbing the features of the leaf gas exchange, which not only restrict the supply and sink tissue, but also

impair the transfer of assimilation and dry matter portioning (Farooq *et al.*, 2009).

2.4 SALINITY STRESS

Amongst different abiotic stresses, soil salinity is a major stress that significantly reduces crop productivity worldwide. In many coastal, arid and irrigated systems, salinity is a serious problem and negatively affects cultivation (Kumar *et al.*, 2009). Despite the progress made in increasing plant productivity and resistance to various pesticides and diseases, however, it remains uncertain whether there is an increased salt tolerance of crop plants, as salinity affects almost every aspect of plant physiology and biochemistry. Rice yield is vulnerable to salinity, especially Asian rice (*sativa*) (Munns and Tester, 2008).

In India, the area under salt-affected soils is about 6.73 million ha with states of Gujarat (2.23 m ha), Uttar Pradesh (1.37 m ha), Maharashtra (0.61 m ha), West Bengal (0.44 m ha) and Rajasthan (0.38 m ha) together accounting for almost 75% of saline and sodic soils in the country. In most of the salt-affected environments, prevalence of poor quality (saline and sodic) waters is also noted. The states of Rajasthan, Haryana and Punjab, lying in the north-western arid part of the country, greatly suffer from the problem of marginal quality waters (Singh, 2009).

The salinity stress is estimated to affect half of arable soil by 2050 and will be a significant factor in the loss of arable soil during the coming decades. Salinity stress imposes ion toxicity and osmotic stress to the plants. In order to survive, the plants have evolved a sophisticated signal perception, transduction, and cellular and morphological response network. Both changes in gene expression and post-translation changes play an essential role in the response to salt stress (Chitteti and peng., 2007).

In general, during various organic processes throughout its life cycle, rice shows variability in sensitivity for excess salinity. At the germination stage it is thought of being comparatively tolerant to salinity, whereas the early reproductive stage of the young seed plant,i.e., The first salinity-sensitive stage of growth is racemic initiation and fertilization, which directly affect crop production (Zeng *et al.*, 2001).

There are a number of promising approaches to obtain rice cultivars that tolerate salt. Explore natural genetic variability by searching for pre-existing genotypes. In addition, many salt tolerant lines with breeding approaches are developed. In recent years, transgenic plant generation (to introduce new genes or change the expression of the prevailing genes) has also become an alternative technique in the effort to improve tolerance to salinity stress (Singh *et al.*, 2008).

2.4.1 Physiological and biochemical changes in response to salinity stress.

Salinity is another damaging stress known as the main bed problem in the arid and semi-arid areas (Almansouri *et al.*, 2001). In saline soils with varying reactions to species and cultivars, germination and spermatoophyte growth are reduced (Bliss *et al.*, 1986). In addition, salinity can associate the germination of seeds with Na^+ and Cl^- ions in the germination effect, by making an external dissemination potential that prevents water uptake or by causing venomous effects on the germinating seed (Khajeh Hosseini *et al.*, 2003).

The salinity changes a wide range of metabolic processes in growing plants and causes changes in the content of many enzymes and their activities. Secondary stresses, such as oxidative damage, may occur as a consequence of ion imbalance and hyperosmotic stresses, primary effects of salt stress. The

reduction of CO_2 attachment in photosynthesis by the Calvin cycle and a decrease in oxidized $NADP^+$ lead to a decrease in carbon reduction due to stress conditions. In photosynthesis electron transfer, when ferrodoxin is reduced, electrons can be transferred from PSI to oxygen to form superoxide radicals (O_2^-) through the Mehler reaction process, which triggers chain reactions generating more active reactive Oxygen species (ROS). Any cellular redox homeostasis imbalances may be called oxidant stress and result in the production of ROS by the univalent oxygen reduction. Salt stress enhances the production rate of ROS by increasing the electron oxygen supply such as superoxide radical (O_2^-). It is discovered that cytototoxic ROS, also produced in mitochondria and peroxisomes during a metabolic process, can destroy normal metabolism by oxidative damage to lipids, proteins and nucleic acids. Free radicals induced lipid peroxidation is also important for the deterioration of membranes (Amirjani, 2010).

2.5 TEMPERATURE STRESS

The irreversible harm to plant growth and development is mainly caused by high temperature stress. The optimal temperature ranges from 27 to 32°C for normal growth and development of rice plants (Satake and Yoshida, 1978). The response of rice plants differs to high temperature according to developmental and reproductive stages of rice. Chlorosis and reduced tillering results in high temperatures at the vegetative stage (Yoshida *et al.*, 1981).

Plants struggle to survive under various conditions of environmental stress, including HT. To some extent, a plant is able to tolerate heat stress through the physical change of the plant body and often through the production of metabolism signals. Plants modify their metabolism in response to HT in several ways and, in particular, by

producing compatible solutes which are able to manage proteins and cell structures. The plants retain their cell turgor by osmotic adjustment. Heat stress causes changes in the expression of genes which participate in direct protection against HT stress at molecular levels. These include osmoprotective expression genes, detoxifying enzymes, transporters and regulatory proteins (Hasanuzzaman *et al.*, 2013).

Rice is one of the largest staple food crops grown mainly in the Cauvery River Basin, which is also known as Madras rice bowl. The analytical results show a 41% decrease in rice productivity, with a temperature increase of 4°C (Geethalakshmi *et al.*, 2011).

2.5.1 Physiological and biochemical changes in response to temperature stress

The forecast temperature increase by 2-4°C at the top of the 20th century constitutes a serious threat to rice production. The severe effect of high temperatures at dark times is further devastating than daily or average daily temperatures. The steps like booting and blooming, which generally cause sterility, are most vulnerable to extreme temperatures. Humidity also plays an important role in increasing the spike sterility when the temperature is doubling. In response to temperature stress, there is a vital variation between rice germplasms (Shah *et al.*, 2011).

Temperature controls the photosynthesis process but, for some moment, the rise in air temperature above critical point leads to irreversible and undesirable factors in crop growth and development (Fahad *et al.*, 2013).

High temperature cell injury could include protein aggregation and denaturation, as well as the increase in lipid membrane fluidity. It may also disrupt motion of water, ions and organic solvents over the cell, disrupting photosynthesis and respiration of plants (Halford, 2009).

In future abiotic stress factors like high temperature, drought, salinity, and submergence are the risks in rice production.

Higher temperatures can adversely affect rice yields through two principal ways, namely

(i) high temperature cause spikelet sterility and adversely affect grain quality
(ii)increased night time temperatures that may reduce assimilate accumulation.

In rice HS induced spikelet infertility is attributed to the reduced swelling ability of pollen, which leads to poor thecae dehiscence (Matsui *et al.*, 2000).

In reproductive stages, rice is highly sensitive to heat. In rice production, high temperatures often coincide with other stresses, namely domestic drought or coastal submergence.

The majority of rice is currently grown in areas where current temperatures for rice production are already nearly optimal. Therefore, the mean temperatures or short episodes of high temperatures will increase in any way throughout sensitive stages, and the grain yield will also be super optimal and reduced (Ceccarelli *et al.*, 2010).

Heat damage takes place if more than 35°C is exposed on rice. Clear variations are determined in the case of temperature injuries at completely different stages of growth. The plant seems most likely to flower hot temperatures. The second most sensitive stage affected by temperature stress is nine days before blooming (Yoshida *et al.*, 1981).

Photosynthesis is more vulnerable to heat stress among physiological processes in plants (Yin *et al.*, 2010). Photosynthesis of rice leaves at temperatures above $35^{O}C$ has been reported to reduced (Taniyama *et al.*, 1988).

2.6 MULTIPLE ABIOTIC STRESSES

Plants are usually subjected simultaneous occurrence of different stresses under field conditions. Recent studies have found that the response of plants to a mixture of different abiotic stresses is exclusive and cannot be calculated directly from the simple learning of each of the different stresses. (Zandalinas *et al.*, 2018).

Abiotic stress conditions cause profound losses to agricultural production worldwide. Stress conditions like drought, salinity and temperature are the subject of intense analysis. However, crops and plants in the field are usually subject to a mixture of various abiotic stresses. Crops encounter a mixture of drought and other stresses such as heat or salinity in drought - affected areas (Mittler *et al.*, 2006).

Drought affects plant efficiency and productivity by causing cellular dehydration that decreases cytosolic and vacuolar volumes and stimulates the production of reactive oxygen species that influence cellular structures and metabolism negatively. High salinity, typically associated with water scarcity, creates ionic and osmotic stress, modifies the plasma membrane of plant cells which ultimately affects growth and yield parameters (Cominelli *et al.*, 2013).

Global warming reduces the yield of rice by increasing grain sterility and reducing the production of biomass. The yield of rice grain reduces by 10% when the night temperature rises by 1°C (Peng *et al.*, 2009).

Plant responses to entirely different stresses are extremely complicated and involve transcriptome, cellular, and physiological changes. Transcription factors, enzyme cascades and species of reactive elements are key elements of this cross – talk (Atkinson *et al.*, 2011).

2.7 STRESS TOLERANCE MECHANISM IN RICE

Plants growing under field conditions are typically exposed to a variety of abiotic stresses such as drought and salinity, which directly or indirectly affect plant metabolism and thus affect plant growth, development and ultimately productivity (Singh *et al.*, 2008).

Plants are non motile, so structural and metabolic modifications are the only way to safeguard them against multiple abiotic and biotic stresses. Plants can also experience distinct stresses at various phases of development and their protective mechanisms can differ in various tissue (Queitsch *et al.*, 2000).

Deep roots help the plants to avoid drought stress conditions by enabling the plants to absorb water from deeper layers of soil. Traits such as root development in rice, especially in terms of complete root dry matter, rooting depth was observed till flowering stage then declined sharply towards maturity (Yoshida and Hasegawa, 1982).

Rice responds by stomatal closure and leaf rolling to reduced water content similar to that of monocots. Leaf rolling in various species such as rice, corn, wheat and sorghum is regarded as a typical reaction to water deficit. It does not only result from the water deficit, however, but also from other abiotic stresses such as salt, temperature, heavy metals and UV radiation. The rolling reduces leaf area and transpiration and is therefore a potentially useful mechanism for preventing drought stress (Kadioglu *et al.*, 2011).

The adaptation of plants to environmental stress is governed by molecular network cascades. These activate mechanisms that respond to stress to restore homeostasis of damaged proteins and membranes. The plant abiotic stress tolerance is maintained by heat shock proteins (Hsp),

molecular chaperones and protein families involved in early embryogenesis (LEA) (Wang *et al.*, 2003).

Production of reactive oxygen species (ROS) is another detrimental effect which occurs in plants as a result of abiotic stress. The use of various antioxidatives and ROS scavengers can improve plant resistance to salt and drought by reducing oxidative damage. (Mittler *et al.*, 2002).

Glycine-betaine is a widely studied osmoprotectant, The introduction of Betaine aldehyde decarboxylase in to tobacco plants from the halophyte *Suaeda liaotungensis* resulted in the in the formation of invitro tobacco plantlets which were significantly tolerant to salinity stress (Li *et al.*, 2003).

Proline production in plants is associated with enhanced tolerance of plants under salt stress (Konstantinova *et al.*, 2002).

2.8 MORPHOLOGICAL, PHYSIOLOGICAL, BIOCHEMICAL CHANGES IN PLANTS IN RESPONSE TO ABIOTIC SRESSES

2.8.1 Shoot length

Under water deficit in potato, stem length was significantly influenced (Heuer and Nadler, 1995).

The length of the stem in soybean has been reduced under conditions of water deficit (Specht *et al.*, 2001).

Drought stress in the initial phase of plant growth and development is a key constraining factor. It has an impact on elongation as well as growth in expansion (Shao *et al.*, 2008). Rice is probably more prone to drought stress in crops as an immersed crop than most other species of plants.

In water deficit citrus plants, plant height decreased by up to 25 percent (Wu *et al.*, 2008).

2.8.2 Root length

Root growth is an important component of the adaptation of rice to drought-prone environments (Price *et al.*, 1997).

Changes occur in root morphology is a function of change in temperature. It is generally characterized by change root length and branching. Generally root growth increases with increasing temperature until an optimum level, above which the growth will be reduced (Mc Michael *et al.*, 1998).

2.8.3 Seedling vigour

Seedling vigour is the plant's ability to emerge from soil or water (Heydecker, 1960). In rice, the seedling vigour is heavily influenced by the cultivation method and by the seed temperature.

Due to the large environmental effects, seedling vigor is difficult to measure in the field (Adair, 1968).

2.8.4 Proline content

Proline is a reliable indicator of the environmental stress imposed on plants (Claussen *et al.*, 2005).

The two-principal organic osmolytes Glycine betaine (GB) and Proline accumulate in a variety of species as a response to environmental stresses such as drought, salinity, extreme temperatures, UV radiation, and heavy metals. While their actual roles in plant osmotolerance remain controversial, the positive effect of the two compounds in the mediating osmotic adjustment of plants grown under stress conditions are identified (Ashraf *et al.*, 2007)

Free proline accumulation is a typical reaction to salt stress (Parida *et al.*, 2008). Many plants have been observed to accumulate high amounts of proline

in exposed to drought or high salt levels in soil, sometimes by the sum of all other amino acids (Ali *et al.*, 1999). Proline functions as an osmoprotectant and plays a major role in osmotic balance, the protection of subcellular structures, enzymes and increased (turgor pressure) cellular osmolarity, which is necessary for cellular expansion under stress conditions (Sairam and Tyagi, 2004).

Proline is the only osmolyte to scavenge singlet oxygen and free radicals including hydroxyl ions, thus stabilizing proteins, DNA and membrane (Matysik *et al.*, 2002). The enzyme denaturation due to heat, NaCl and other stresses is reportedly decreased by proline. During stresses and regenerations, Proline serves as a source of carbon, nitrogen and energy (Kavi Kishor *et al.*, 2005). Due to these features of proline, greater proline accumulation is most often linked to the salt tolerance of plant species and different researchers have reported a higher accrual of proline of the salt-tolerant genotype than in their salt-sensitive counterparts, including wheat (Sairam *et al.*, 2005).

Many plant species have been documented with very high accumulation in cellular proline (up to 80% of amino acid pool under stress and 5% under normal conditions) because of increased synthesis and degradation during various stress conditions such as salt and drought. Upon sodium chloride stress, Arabidopsis represent up to 20% of the Free Amino Acid Pool. Although proline is known in stress conditions to confer osmotic tolerance.

2.8.5 Cell membrane stability

Physiological index which is widely used for the evaluation of drought and temperature tolerance is cell membrane stability (Sullivan, 1972).

Water deficit leads to severe metabolic dysfunction by membrane deterioration (Buttrose and Swift, 1975)

One of the main cellular targets common to various stresses is cell membrane (Levitt, 1980). The common damages in plants, such as freezing

heating, drought, (Blum and Ebercon, 1981) and salt are used as a measure of their tolerance.

Osmotic adjustment (OA) and maintenance of cell-membrane stability (CMS) are the strategies adopted by the plants in response to water stress

2.8.6 Ion homeostasis

The higher duration of dry periods in several countries across the earth and the problems occurring with high level of salinity in irrigated areas are the result of consecutive occurrence of abiotic stresses such as drought and salinity in cultivated lands. About half of all irrigation schemes are salinity affected. Drought and salinity produce nutrient disturbance and which ultimately affect the plant growth via nutrient disturbance. However, drought and salinity affect mineral nutrition of plants differently. Competition of Na+ and Cl– with other nutrients such as nutrients such as K^+, Ca^{2+}, and NO_3^-. water deficiency also affects nutrient uptake and impair translocation of some nutrients. (Hu *et al.*, 2005)

The homeostasis of intracellular ion levels is essential to living cells. In order to attain homeostasis cells maintain low levels of toxic ions and acquire essential ions, adequate ion flux regulation is needed. Plant cells are used to maintain typically high K^+ and low Na^+ concentrations in cytosol by primary active transport, mediated by H^+-ATPases and secondary transport mediated by channels and co-transporters. The proper functioning of many cytosolic enzymes and maintaining membrane potential is regulated by the Intracellular K^+ and Na^+ homeostasis (Zhu *et al.*, 2003).

Na^+ interferes with K^+ uptake. In cells high concentrations of Na^+ is toxic to enzymes. Excessive Na^+ must be extruded or divided into a vacuole to avoid cell death (Hasegawa *et al.*, 2000).

2.8.7 Plant height

Drought and heat stress reduce the growth of stem and plant height simultaneously. Changes in internal water conditions affect stem growth and shrinkage of stem diameter (Simonneau *et al.*, 1993).

Prasad *et al.* (2006) reported that plant growth and stem height were reduced under severe heat and drought stress

Wei *et al.* (2014) reported that coating soyabean seeds with mannitol increased leaf area as well as plant growth.

2.8.8 Spikelet fertility

Ekanayake *et al.* (1989) reported spikelet sterility in rice is largely influenced by water potential.

Enhanced CO_2 levels can aggravate this problem further, possibly due to reduced transpiration cooling. At temperatures higher than 35°C, spikelet sterility was significantly increased (Matsui *et al.*, 1997).

Abdullah *et al.* (2001) reported that under the conditions of salinity stress spikelet fertility was reduced this is due to the accumulation of more sodium than potassium in floral parts and also due to the inhibition of starch synthetase in developing rice grains.

Selote *et al.* (2004) reported Drought-resistant N22 genotype showed less water stress-induced spikelet sterility when compared to the susceptible N118 genotype under upland conditions.

Jagadish *et al.* (2007) found that less than 1 hour of exposure to temperatures above 33.7 ° c was adequate to induce sterility in greenhouses experiments with both *indica* and *japonica* genotypes.

According to Rang *et al.* (2011) among the abiotic stresses temperature stress produces maximum spikelet sterility and also identified a strong relationship between spikelet fertility and pollen grains on stigma

2.8.9 Pollen viability

During anthesis, temperature above $35^{O}C$ with a duration of more than 1 hr may result in elevated sterility in rice. Abnormal anther dehiscence, impaired pollination and pollen germination under temperature stress leads to spikelet sterility (Jagadish *et al.*, 2009).

Pollen viability and pollen germinability are negatively affected by heat stress (Cross *et al.*, 2013).

2.8.10 Yield

Woperesis *et al.* (1996) reported that yield was decreased to below 200 g m^{-2} because of drought stress at mid tillering and panicle initiation stage. They also argued that the lower yield obtained in drought stressed plants was due to the larger percentage of unfilled grains.

Oh-e *et al.* (2007) found faster grain growth and a shorter duration for grain filling under high temperature stress.

2.8.11 Chlorophyll content

Reduced leaf internal CO_2 concentration under mild or moderate drought stress condition is reason for decreased rates of leaf photosynthesis (chaves, 1991).

Ommen *et al.* (1999) reported that Drought stress reduces leaf chlorophyll content. Drought stress has led to a major decline in the content of chlorophyll a, chlorophyll b and total chlorophyll.

Photosynthesis under drought condition is very much limited due to the metabolic impairment and mainly because of decreased photosynthetic pigment contents in sunflower (Reddy *et al.*, 2004).

Woperesis *et al.* (1996) reported drought stressed cotton has a decrease in chlorophyll content.

The ratio of chlorophyll ' a ' and ' b ' and carotenoids was altered by drought stress (Farooq *et al.*, 2009).

2.8.12 SOD action under stress

The decrease in SOD (Superoxide dismutase) and CAT (Catalase) activity in plants with increased temperature results was strongly related to the severe heat stress and thermal toleration of the rice crops. HT-sensitive cultivars show declined level of SOD and CAT than HT-tolerant cultivars in the same HT regimes (Karuppanapandian *et al.*, 2011).

ROS can be extremely reactive, particularly single oxygen and hydroxyl radical, and can oxidize several cellular components, such as proteins and lips, DNA and RNA (Cruz *et al.*, 2008).

The effect of mild and high drought stress on the activity of superoxide dismutase (SOD) was analyzed by (Sharm and Dubey, 2005). Together with the root crops of both rice cultivars (Malviya 36 and Pant 12), they noticed that total SOD activity increased markedly. The total activity of SOD in the roots was higher. The 20-day-old mild drought ((PEG-6000 of 17%) showed an increase in total root SOD activity of about 71% to 78% in root and a increase of 56% to 90% in shooting activity compared to control seedlings.

2.8.13 Malondialdehyde content

Lipid peroxidation is the process of generation of free radicals process in an organism. The final product of lipid peroxidation in cell is malondialdehyde. An increase in free radicals causes overproduction of MDA. Malondialdehyde level is commonly known as a marker of oxidative stress (Gaweł *et al.*, 2004).

Damanik *et al.*(2010) reported that three rice cultivars have lipid peroxidation levels measured as MDA content. The 4-day submergence of MR219-4 and MR219-9 cultivars showed a considerable variation in MDA production. The immersive intolerant MR219-9 had a significant increase in MDA production in tolerant FR13A cultivars compared to MR219-4, and MR219-9 culture cultivars, although it was significantly increased at eight days during submergence. Furthermore, no significant differences between FR13A and MR219-9 cultivars in the production of MDA were observed.

MDA and other aldehydes can affect proteins, DNA, RNA and other biomolecules via the basis additional reactions of Schiff but they are also powerful secondary messengers which upregulate many genes that are involved in the abiotic stress response of plants (Davey *et al.*, 2005).

2.10 MOLECULAR MARKERS

Genetic engineering and biotechnology have great potential for breeding crops because they are committed to speeding up the time required to produce crop varieties with desired characteristics. Using molecular techniques, the transfer of desired genes among varieties could now be speeded up and new genes of associated wild species can be introduced. Polygenic characters, which used traditional plant breeding processes before were hard to analyze, are now easily marked using molecular markers (Mohan *et al.*, 1997).

Molecular maps of crops are mainly constructed by Restriction fragment length polymorphisms micro- and mini-satellites and PCR based approaches (Winter and kahl, 1995). Genetic markers include three categories: based on visual identifiable traits (morphological and agronomic traits), based on gene product (biochemical markers), and based on DNA assay (molecular markers) (Sax. 1932).

The use of DNA polymorphisms by the ever-growing number of molecular technologies has a big role on research and development in plant genomes. In order to identify inter-and intra-specific genetic diversities and build molecular maps of crops using specifically developed mapping populations, micro-and mini-Satellite and PCR approaches are used nowadays.

Repetitive DNA sequences represent a large portion of the higher eukaryotic genomes. They can serve as highly informative genetic markers and allow the detection of longitudinal variation with the use of PCR technology (Powell *et al.*, 1996).

Markers based on DNA differs in to two hybridization based (RFLP) and PCR based markers (RAPD, AFLP, SSR, SNP, EST etc.). Microsatellite DNA based marker is the most widely used one , because of its easy use by simple PCR, followed by simple gel electrophoresis technique for allele size identification, large amount of data provided by its large number of alleles per locus (Madhumati, 2014).

2.11 QUANTITATIVE TRAIT LOCI

The complex features controlled by quantitative feature Locus (QTLs) include many important agronomic properties in crop plants, such as tolerance to

abiotic stresses. The isolation of these QTLs is very promising for the improvement of world agriculture (Ren *et al.*, 2005).

Most of the agricultural traits are quantitative, which means they are monitored by multiple genes with a small effect (QTLs). Mapping and isolating QTLs is important for the effective reproduction of marker assisted selection (MAS) crop production and for a better understanding of the molecular mechanisms behind the characteristics (Takagi *et al.*, 2014).

According to a study done by Lin *et al.* (1998) 98 BC 1F5 lines (backcross inbred lines) derived from Nipponbare backcrow (japonica)/Kasalath (indica)/Nipponbare have been genetically analyzed in order to detect quantitative trait loci (QTLs) controling seed dormacy. In order to build the framework link map, 245 RFLP markers were used. In chromosomes 3, 5, 7 (two regions) and 8, five putative QTLs were found which affected the dormitory of seeds. The phenotypical variations in each QTL ranged from 6.7 to 22.5 % and the five supposed QTLs explained about 48% of the total phenotypic variation in the BC1F5 Lines.

2.12 SSR MARKERS

Simple sequence repetition (SSR) is an essential element for genetic analysis in rice (*Oryza sativa* L.) because of its abundance, high polymorphism and simple tests on agarose gel electrophoresis (Singh *et al.*, 2010).

Simple sequence repeats of 2 to 6 bp motives are very important among the different types of markers, because of their relative abundance, multiple alleles, their co-dominant heritage, uniform genome coverage and simple reproductible tests. (Powell *et al.*, 1996).

Based on their repeat length and potential as informative genetic markers are categorized in to: class I SSRs with repeat lengths of 20 bp or higher, and class II SSRs with repeat lengths of 12–19 bp (Temnykh *et al.*, 2001).

2.12.1 SSR markers associated with heat tolerance in rice

Heat is one of the main factors limiting rice production significantly. A deeply rooted, heat and drought tolerant from rice cultivar is Nagina 22 (N22). The two-dimensional N22 and NH 219 gel electrophoresis leaf proteome test showed a distinct constitutive expression of the NH219 ambient growth ribulose bisphosphate carboxylase broad chain precursor (EC 4.1.1.39). Hot weather stress reduced all 11 characteristics in N22 and NH219 except plant height (poli *et al.*, 2013).

Buu *et al.* (2014) assessed for heat stress during flowering in a total of 310 BC2F2 lines from the cross OM5930/N22. The 264 polymorphic SSR map for the detection of linkage to the target characteristics has been created. The map was 2741,63 cm and the interval between two loci marker was 10,55 cm in average. The heat tolerance markers have been found mainly on 3, 4, 6, 8, 10 and 11 chromosomes. Each QTL explained the proportion of phenotype variation from 17.1% for RM 160 to 36.2% for RM 3586. There were four QTLs, explaining 13,1% and 31,0% of the overall phenotypic variation, for the filled grains per chromosome 4 on the intervals of RM 468 — RM 7076 and RM 241 — RM 26212, respectively.

Wei *et al.*, (2013) identified heat tolerant marker RM 242, RM7076, RM 3586, 26212 and RM 5749 were identified as polymorphic for heat by (Buu *et al.*, 2014).

Zhao *et al.*, (2006) reported polymorphic heat tolerance markers RM 3340, RM 447, RM 5545, RM 3701, and RM 336.

2.12.2 SSR Markers associated with drought tolerance in rice

QTL (Quantitative Trait Loci) is the regions that contain genes linked to a specific quantitative characteristic within the genome. Molecular marker approaches were widely used for rice, starting with the QTL analysis. For many characteristics associated with drought response, such as root characters, membranes, osmotic adaptation and morphological and physiological characteristics, QTLs have been identified where tolerance is measured as a drought outcome.

Venuprasad *et al.* (2001) worked in 3 different environments on IR64 x Azucena DH mapping rice population and detected 10-trait QTLs at a threshold. QTLs were distributed in Six chromosomes 1, 3, 4, 5, 6, and 7. On chromosomes 3, 4 and 5, each three QTLs of grain yield were detected.

Kanagaraj *et al.* (2010) reported that the genomic region RM 212–RM 302–RM 3825 on chromosome 1 is linked to drought resistance traits and may be useful in marker assisted breeding for drought resistance in rice.

Gomez *et al.* (2010) identified 22 QTLs, which individually explained 4.8–32.2% of the phenotypic variation in *indica* varieties.

2.12.3 SSR markers associated with salt tolerance in rice

Salinity tolerance is a complex, numerous genes-controlled genetic characteristic. The large effect of the environment and the low heredity of salt-tolerance make it difficult to plant selection for salt tolerance using conventional breeding procedures (Yeo and Flowers. 1986).

Thomson *et al.* (2010) confirmed the position of the Saltol QTL on chromosome 1 by an analysis of 100 SSR Markers on 140 IR29/Pokkali recombinant inbred lines (RILs).

Mohammadi-Nejad *et al.* (2008) reported the major Salt tolerant Quantitative Trait locus (QTL) called Saltol has been mapped on chromosome 1 using Pokkali / IR29 cross recombinant F8 inbred lines (RILs) for low Na+, high K+ uptake and rice homeostasis. Among different markers RM 8094 identified as superior for genetic diversity analysis.

Krishnamurthy *et al.* (2014) reported genetic diversity of 57 rice genotypes with Saltol markers. Eight of the 21 SSR markers were polymorphic. The highest PIC value was found for RM 10843 and for RM 10871, followed by RM 10852 (0.81), RM 10713 (0.78), RM 10793 (0.74), RM 10748 (0.73) and RM 493 (0.71) and RM 3412 (0.52) respectively.

Materials and Methods

3.MATERIALS AND METHODS

The present study entitled "Assessment of multiple abiotic stress tolerance mechanisms in rice (*Oryza sativa* L.)" was conducted in Department of Plant Physiology, College of Agriculture, Vellayani and, Poly house and rain out shelter maintained by Instructional farm, College of Agriculture, Vellayani, Kerala Agricultural University during the years from 2018-2019. The main objective was to study the multiple abiotic *viz.* drought, salinity and high temperature stress tolerance mechanisms in rice and to validate the identified QTLs for stress tolerance in rice. The details of the materials used and methods adopted for this experiment as well as the procedures followed for laboratory analysis during the course of experimentation are described in this chapter.

3.1 SCREENING OF RICE VARIETIES FOR SINGLE STRESS TOLERANCE

3.1.1 Plant materials

Twenty rice genotypes were used for initial single stress tolerance evaluation using paper towel method

3.1.2 Location

The study was conducted at Department of Plant Physiology, College of Agriculture, Vellayani during 2018-19.

The rice accessions used in the present study consist of land races and improved local strains collected from RARS, Pattambi, Rice Research Station, Vyttila and NRRI, Cuttack, Orissa.

Table 1. List of rice genotypes selected for study

Sl. No.	Genotype
1	Chomala
2	Uma
3	PTB 35
4	PTB 60
5	PTB 39
6	PTB 55
7	PTB 30
8	PTB 7
9	CR Dhan 307
10	APO
11	Vyttila–3
12	Vyttila-4
13	Vyttila-5
14	Vyttila-6
15	Vyttila-7
16	Vyttila-8
17	Vyttila-9
18	Vyttila-10
19	N-22 (Nagina-22)
20	NL-44 (Nerica Line-44)

3.1.3 Methodology of imposing single stress using paper towel method

In this study seeds were placed in paper towel and subjected to individual drought, temperature and salinity stress using PEG 6000, incubator and NaCl respectively. Different levels of stress were given to the seeds using different concentration of PEG6000 (-1bar, -3bar, -5 bar and -7 bar water potentials) for inducing drought stress, salinity stress was induced using NaCl at concentrations of 100mM, 150mM, 200mM and 250mM NaCl and temperature stress was given by providing different temperature conditions (35°C, 40°C, 45°C and 50°C using an temperature controlled incubator). Measurements regarding the physiological and biochemical parameters were taken on 14th day of germination. The control plants were under well-watered condition till the 14th day. The selected three genotypes from each stress were subjected to combination of stress (Temperature and salinity, drought and salinity, temperature and drought, drought temperature and salinity). The selected tolerant genotypes from combination stress were subjected to pot culture experiment

3.1.4 EXPERIMENT 1 - PAPER TOWEL METHOD OF IMPOSING INDIVIDUAL DROUGHT, SALINITY AND TEMPERATURE STRESS

Three hundred paper towels having a size of 45 x 28 cm were used for this study. Seeds of 20 rice genotypes was placed in paper towel and given individual stress for a period of 14 days at different concentrations such as-1 bar, -3bar, -5bar and -7bar PEG6000 for inducing drought stress. Salinity stress was induced at concentrations of 100mM, 150mM, 200mM and 250mM NaCl. Temperature stress was given at 35°C, 40°C, 45°C and 50°C using an temperature controlled incubator.

Table 2. Particulars of paper towel method for screening individual stress levels

1) Crop	Rice (20 Genotypes)
2) Design	CRD
3) No. of treatments	200 Four individual Stress levels for each abiotic stress(Salinity, temperature and drought) and control 20 rice genotypes 2 replications

3.1.4.1Physological Parameters

3.1.4.1.1 Shoot length

Seedlings were selected from each replicate on 14th day of germination. The shoot length was measured from the tip of the primary leaf to the base of the hypocotyl and mean shoot length was expressed in centimetre.

3.1.4.1.2 Root length

Seedlings used for root length measurement, were also used for shoot length measurement and the root length was measured from the tip of the primary root to base of hypocotyl with the help of a scale and mean root length was expressed in centimetres

3.1.4.1.3 Seed vigour index

Vigor indices of the seedlings obtained from the germination test were calculated using the following formulae (Abdul-Baki and Anderson, 1973)

Vigor index: Seed germination per cent x seedling length (cm)

3.1.4.1.4 Seed germination percentage

Germination rate is estimated by using the following formula

$$\text{Germination Percentage} = \frac{\text{Seeds germinated}}{\text{Total seeds}} \times 100$$

3.1.4.2 Biochemical parameters

3.1.4.2.1. Cell membrane stability index

In accordance with the procedure described by Blum and Ebercon. (1981) the cell membrane stability index was calculated. Triple washing of samples was made in deionized water to remove electrolytes adhering to the surface from all treatments. A sample containing 10ml of deionized water was kept in the capped (20ml) vial and incubated at room temperature for 24 hours in the dark. The conductivity was measured by a conductivity meter. These vials were then autoclaved, killing the leaf tissue for 15 minutes, and releasing the electrolytes.The second reading of conductivity was taken after cooling. For all treatments, these two measurements have been performed individually. By using the following formula, the cell membrane stability index was calculated and expressed as a percentage.

$$CMS\ (\%) = [1-(T_1/T_2)/1-(C_1/C_2)] \times 100$$

Where, T and C refer to the stress and control samples respectively. The subscripts 1 and 2 refer to the initial and final conductance readings, respectively.

3.1.4.2.2. Proline content

As per the procedure described by Bates *et al.* (1973) proline content was determined. 0.5g of mid-leaf portion was homogenized with 3% aqueous sulphosalicylic acid having a volume of 10 ml and centrifugation was done at 3000 rpm for 15 minutes. Supernatant (2ml) was taken and blended with an equal amount of acid ninhydrin and glacial acetic acid. The mixture was kept at 100°C for one hour in water bath. By keeping it in ice bath for 10 min the reaction was terminated.The reaction mixture was mixed with 4ml toluene using vortex mixture for 15-20 seconds. The chromophore containing toluene was aspirated from aqueous phase, warmed to room temperature and the optical density was read at 520nm with toluene as blank. A standard curve was drawn using concentration verses absorbance.

The concentration of proline was determined from graph and expressed as

µg /g tissue = {[(µg proline / ml) x ml toluene] / 115.5} x (5 / g sample),

where, 115.5 is the molecular weight of proline.

3.1.4.2.3. Na+ - K+ ratio

As per the procedure described by Zasoski and Buraum (1977) Na+ - K+ ratio was calculated. Digestion of plant material was achieved by the addition of 10ml of Nitric acid and perchloric acid in the ratio 0f 9:4 on to the dried plant samples (1g). The mixture is allowed to stand overnight.

After that it was heated in sandbath for 3-4 hours at a temperature of 180 - 200°C till the mixture become transparent white. Then the digested sample was filtered through filter paper and made up to 100 ml with distilled water and Na+ and K+ was determined through flame photometric method. Flame photometry method is based on atomic emission method for the routine determination of Na+ and K+. As per the procedure described by Munns *et al.* (2010) concentration of ions is measured by flamephotometer method with appropriate standards

$$\text{Na+ concentration(ppm)} = \frac{\text{(flame photometer reading x 100/ weight of sample)}}{10000}$$

$$\text{K+ concentration(ppm)} = \frac{\text{(flame photometer reading x 100/ weight of sample)}}{10000}$$

$$\text{Na+ - k+ ratio} = \frac{\text{Na+ concentration}}{\text{K+ concentration}}$$

3.2 SCREENING OF PLANTS FOR MULTIPLE STRESS TOLERANCE

3.2.1Experiment -2 imposing multiple stress at a time

Combination of stresses such as Temperature and salinity, Salinity and drought, Temperature and drought, temperature, salinity and drought were given to selected genotypes from experiment 1. Observations were taken on 14thday of germination

Table 3. Particulars of paper towel for combination of stresses

1) Crop	Rice (9 Genotypes)
2) Design	CRD
3) No. of treatments	135 5 Combination of stresses 9 rice genotypes 3 replications

3.2.1.1Parameters

Parameters such as shoot length, root length, germination percentage and seedling vigour index were calculated

3.3 POT CULTURE EXPERIMENT OF SELECTED GENOTYPES

3.3.1 Location

The study was conducted in the rainout shelter and poly house of Instructional farm, College of Agriculture, Vellayani during 2018-19.

3.3.2 Preparation of potting mixture and transplanting

Earthen pots were filled with potting mixture prepared by mixing soil, sand, and FYM in the ratio of 3:2:1. Fifteen days old seedlings were transplanted to the pots at the rate of three seedlings. Thinning and gap

filling was done on 6th day after transplanting and one healthy seedling was maintained in each pot. Foliar spray of 19:19:19 mixture was given on seedlings in pro-trays and on 15th day after transplanting. Crop was applied with recommended dose of fertilizer as per package of practices of Kerala Agricultural University, Thrissur.

3.3.3 Methodology

In this study, plants were raised in earthen pots in polyhouse and rainout shelter. Separate set of plants with two replications were maintained for the two combinations of treatments (drought and salinity, temperature and salinity). Normal irrigation was done regularly for all the eight treatments up to the reproductive stage according to their duration. During the period of reproductive stage irrigation was withhold, stresses such as highest tolerated level of drought (D_h) and highest tolerated level of salinity (S_h) salinity stresses were given using -5bar PEG6000 and 250mM NaCl for one set of selected genotypes for 5 days during reproductive stages, similarly highest tolerated level of temperature (35°C) and highest tolerated level of salinity (250mM NaCl) stress were given to another set of selected genotypes from panicle initiation to maturity by transferring the plants in to temperature controlled poly house and 250mM NaCl respectively.

Plate 2.General view of experimental unit for drought (-5bar PEG6000) and salinity (250mM NaCl), temperature(35°C) and drought (-5bar PEG6000)

Plate 3. View of experimental unit with rice plants inside rain out shelter

Plate 4. View of experimental unit with rice plants inside automated (temperature (35°C) controlled) poly house facility

3.3.4. MORPHOLOGICAL PARAMETERS

3.3.4.1 Plant height (cm)

The plant's height was measured in centimeters from the base of the plant to the tip of the primary panicle.

3.3.5. YIELD PARAMETERS

3.3.5.1 Productive tiller number

The number of panicles bearing tillers was counted and recorded at the moment of harvest

3.3.5.2. Observations in Spikelet

In each treatment, the total number of filled and unfilled spikes were counted, with three primary tillers randomly selected from the target plants.

Spikelet fertility (%) was calculated by using the formula

$$\text{Spikelet fertility (\%)} = \frac{\text{Number of fertile spikelets}}{\text{Total number of spikelets}} \times 100$$

3.3.5.3. Pollen viability (%)

The viability of the pollen has been measured by dissolving of 2.5 g KI and 250 mg iodin made up to 125 ml using 1% iodine potassium iodide (IKI). Spikelets were collected in the glass slides shortly before the anthesis from each treatment and were crushed and stained with IKI. Fertile pollen, with fully stained grain, are fertile, untouched, shriveled, empty grains. Leica, a compound microscope, is used for visually counting the pollen grains. The viability of pollen was calculated using the following formula and as a percentage.

$$\text{Pollen viability} = \frac{\text{Number of pollen grains stained}}{\text{Total number of pollen grains}} \times 100$$

3.3.5.4. Yield per plant

The grain yield per plant was derived by taking the weight of filled grains in each panicle and expressed in grams.

3.3.5.5. Superoxide Dismutase

As per the method described by Beauchamp and Fridovich. (1971) SOD was estimated. In a pre-chilled pestle and mortar 1g of clean leaf tissue was grinded in 10 ml ice cold 50 mM potassium phosphate buffer, pH 7.8. The homogenate was centrifuged at 10000rpm for 10 min at 4°C and the assay was carried out using the supernatant. A 3 ml reaction cock tail was prepared by using 50 µl of crude enzyme extract, 50 mM potassium phosphate buffer, 13mM methionine, 2 µM riboflavin, 0.1 mM EDTA and 75 µM NBT, in duplicate. The volume was made equal by double distilled water. A solution without enzyme and NBT is set as blank to calibrate the spectrophotometer. Set another control having NBT but no enzyme as reference control. The tubes were exposed to 400 W bulb (4 x 100 W bulbs) for 15 min. absorbance was read immediately at 560 nm. The 50 % inhibition of the reaction between riboflavin and NBT in the presence of methionine was taken as 1 unit of SOD activity.

3.3.5.6. Malondialdehyde content

As per the procedure described by (Wang *et al.*, 2013) MDA was calculated

Extraction of MDA

Functional or senescent leaves (0.5 g) were taken and ground in 5 mL extraction solution of 5% TCA then the centrifugation was performed at 5000g for 15 min. The supernatant which is the MDA extraction solution, was stored at 4°C.

Measurement of MDA

According to the corrected TBA method (Hodges et al., 1999), the level of MDA was estimated. Two ml of extraction solution were mixed with 3 mL 0.5% TBA including 5% TCA vigorously. Then the mixture was heated at 95°c for 3o min in a boiling water bath and then cooled it to room temperature. Then it was centrifuged at 5000 g for 15 min, the OD value of supernatant was detected at 450, 532 and 600 nm. The concentration of MDA was determined using the formula.

CMDA (µmol mL−1) = 6.45 × (D532-D600)- 0.56×D450, where D450, D532 and D600 are the absorbencies at 450, 532 and 600 nm, respectively.

3.3.5. 7. Chlorophyll a/b ratio

. 5 g of leaf sample was taken and put it in a test tube containing DMSO and 80% Acetone and incubated over night at room temperature. All the pigments were extracted in the solution and record the absorbance at 645 and 663 nm using spectrophotometer. The chlorophyll content was analyzed using the formula

Chlorophyll a: 12.7(A663) – 2.69 (A645) x V/1000 x 1/fresh weight

Chlorophyll b: 22.9(A645) – 4.68 (A663) x V/1000 x 1/fresh weight

3.4 POLYMORPHISM STUDY USING REPORTED MICROSATELLITE SSR MARKERS FOR DROUGHT SALINITY AND TEMPERATURE

All the twenty rice genotypes were used for polymorphism analysis for reported microsatellite SSR markers belongs to drought, temperature and salinity.

3.4.1. Genomic DNA isolation

According to the procedure described by (Murray and Thompson.1980) plant samples are grinded in a prechilled mortar pestle using liquid N2 at room temperature. For each .1g of homogenized tissue 100µl of CTAB extraction buffer was used then the homogenate was incubated in water bath at 60°c.following the incubation centrifugation was performed at 14,000g. The supernatant was transformed into a new tube containing 5µl of Rnase solution and incubated at 32°c for 20 minutes. Equal volume of chloroform: isoamylalcohol (24:1) was added and vortexed for 5 seconds then centrifugation was performed at 14,000g and upper aqueous phase was transferred to new tube. TheDNA was precipitated using 0.7 volume of ice-cold isopropanol and incubated at -20°c for 15 minutes. Sample was centrifuged at 14,000g for 10 minutes discard the supernatant without disturbing the pellet and the pellet was subsequently washed with 500µl of ice-cold ethanol and remove subsequent ethanol by drying, dissolve the dna in 20µl T.E buffer.

3.4.2 Quantification and quality assessment of DNA samples

The DNA present in the sample was quantified by reading the absorbance at 260nm and 280nm in a spectrophotometer (ELICO, SL 21 UV-Vis spectrophotometer). The purity of DNA was checked by reading at 260nm and 280nm (OD 260/OD 280) was used as an estimate of the purity of the DNA samples. Pure preparations of DNA have 260 nm/ 280 nm OD ratio between 1.7 and 1.8 (Sambrook and Russell, 2000). Quality was assessed by using gel electrophoresis with 5µl of crude DNA sample on agarose gel (0.8%) and stained with ethidium bromide.

3.4.5. Dilution of DNA samples

After quantification the stock DNA samples were diluted to 30ng/µl of working solutions for PCR analysis. DNA dilutions were prepared by using the formula as follows:

$$M_1V_1 = M_2V_2$$

Where M_1 is the stock DNA concentration, V_1 is the volume of stock to be diluted, M_2 is the concentration of working solution and V_2 is the volume of working solution to be prepared. Then the appropriate volume from the stock was transferred to 0.5 ml micro-centrifuge tube, and the volume was made to 100µl using TE buffer. The DNA working solutions were stored at -20°C till further use.

3.4.6 PCR amplification using SSR primers

3.4.6.1 PCR reaction

PCR reaction was performed in a 20µl reaction mixture which consisted of,

a) Genomic DNA (25ng/µl) - 2.0µl
a) 10X Taq assay buffer A - 2.0µl
b) dNTPs mix (10mm each) - 1.5µl
c) Taq DNA polymerase (1U) - 0.3µl
d) Forward primer (10pM) - 0.75µl
e) Reverse primer (10pM) - 0.75µl
f) Autoclaved distilled water - 12.7µl

Total volume 20µl

PCR reaction was carried out using Master Cycler gradient 5331-Eppendorf version 2.30.31-09, Germany. The thermal cycling was carried out with the following programme

Initial denaturation – 94°C for 3 minutes

Denaturation – 94°C for 1 minute

Primer annealing – 53°C to 55°C for 1 minute } 35 cycles

Primer extension – 72°C for 1 minute

Final extention – 72°C for 5 minutes

Incubation – 4°C for infinity to hold the sample

3.4.7 Detection of polymorphism between the genotypes using SSR markers

PCR screening was carried out using Thirty reported microsatellite SSR markers linked to drought, salinity and temperature. combinations were screened by PCR and their sequence of primers are listed in Table 4. The PCR products were separated on agarose gel along with marker (100bp ladder) and 1X TBE buffer. Ethidium bromide is used for staining purpose. The gel profile was visualized using (Syngene G box documentation system). The documented SSR profiles were carefully examined for the polymorphism in banding pattern between the rice genotypes

Table 4. List of primers used for polymorphism analysis.

sl. No.	Marker	Forward primer	Reverse primer
1	RM 5749	GTGACCACATCTATATCGCTCG	ATGGCAAGGTTGGATCAGTC
2	RM 26212	GTCGCTCCTCTCCTCCAATCC	GCTCGCTGCTTCTAATCTCTTGC
3	RM 7076	CTCCACCAACAACTCGTATC	AAGCTATTCACAAGCAGCTC
4	RM 10793	GACTTGCCAACTCCTTCAATTCG	TCGTCGAGTAGCTTCCCTCTCTACC
5	RM 3412	AAAGCAGGTTTTCCTCCTCC	CCCATGTGCAATGTGTCTTC
6	RM 493	TAGCTCCAACAGGATCGACC	GTACGTAAACGCGGAAGGTG

7	RM 8094	AAGTTTGTACACATCGTATACA	CGCGACCAGTACTACTACTA
8	RM1287	GTGAAGAAAGCATGGTAAATG	CTCAGCTTGCTTGTGGTTAG
9	RM10843	CACCTCTTCTGCCTCCTATCATGC	GTTTCTTCGCGAAATCGTGTGG
10	RM349	TTGCCATTCGCGTGGAGGCG	GTCCATCATCCCTATGGTCG
11	RM 6100	TCCTCTACCAGTACCGCACC	GCTGGATCACAGATCATTGC
12	RM3042	CAAAAAGGAATCAATGTGAA	GGCTGTTGAGAGGTAGAGAA
13	RM7039	GCACATTTGCCATTCTACCG	GCCTTCCAGTGAGGTGACTC
14	RM256	GACAGGGAGTGATTGAAGGC	GTTGATTTCGCCAAGGGC
15	RM224	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG
16	RM243	GATCTGCAGACTGCAGTTGC	AGCTGCAACGATGTTGTCC
17	RM235	AGAAGCTAGGGCTAACGAAC	TCACCTGGTCAGCCTCTTTC
18	RM112	GGGAGGAGAGGCAAGCGGAGAG	AGCCGGTGCAGTGGACGGTGAC
19	RM241	GAGCCAAATAAGATCGCTGA	TGCAAGCAGCAGATTTAGTG
20	RM527	GGCTCGATCTAGAAAATCCG	TTGCACAGGTTGCGATAGAG
21	RM507	CTTAAGCTCCAGCCGAAATG	CTCACCCTCATCATCGCC
22	RM447	CCCTTGTGCTGTCTCCTCTC	ACGGGCTTCTTCTCCTTCTC
23	RM528	GGCATCCAATTTTACCCCTC	AAATGGAGCATGGAGGTCAC
24	RM454	CTCAAGCTTAGCTGCTGCTG	GTGATCAGTGCACCATAGCG
25	RM348	CCGCTACTAATAGCAGAGAG	GGAGCTTTGTTCTTGCGAAC
26	RM256	GACAGGGAGTGATTGAAGGC	GTTGATTTCGCCAAGGGC
27	RM490	ATCTGCACACTGCAAACACC	AGCAAGCAGTGCTTTCAGAG
28	RM232	CCGGTATCCTTCGATATTGC	CCGACTTTTCCTCCTGACG
29	RM226	AGCTAAGGTCTGGGAGAAACC	AAGTAGGATGGGGCACAAGCTC
30	RM208	TCTGCAAGCCTTGTCTGATG	TAAGTCGATCATTGTGTGGACC

RESULTS

4. RESULTS

The experiment was conducted to evaluate multiple abiotic stress tolerance mechanism in rice and to validate the identified QTLs linked to drought, salinity and temperature stress among 20 rice varieties, in the Department of Plant Physiology, College of Agriculture, Vellayani during 2018-19. The rice seeds were initially exposed to individual stresses such as drought, salinity and temperature using different concentration of PEG6000, NaCl and providing different temperature conditions using a temperature controlled incubator respectively. The selected three varieties from each highest tolerated stress level were subjected to combination of stress treatments. Then the selected plants from combination stress were subjected to pot culture experiment to analyze yield parameters under combined stress effect. The physio-morphological, biochemical and yield characters were recorded after stress imposition in both stress and control plants. Microsatellite markers linked to drought, salinity and temperature were used to validate the rice varieties tolerant to different abiotic stresses. The data were statistically analyzed and the results are presented in this chapter with suitable tables.

4.1. EFFECT OF DIFFERENT ABIOTIC STRESS ON PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS

4.1.1. Germination percentage at different level of drought stress

Results showed that there is significant difference for seedling vigour index between treatment and control varieties. Out of 20 rice varieties 14 were found to have 100% germination at -1 bar PEG6000. The least germination percentage was shown by NL-44 (40%) followed by N-22 (60%). 100% germination was observed for all varieties under control condition. Out of 20 rice varieties 16 varieties were germinated at -3 bar PEG6000. Among 16 varieties PTB-35, PTB-60, PTB-7, Vyttila-9, Vyttila-10 were found to be having 100% germination. The

least germination was found in PTB-39 (40%), Vyttila–3 (40%) and Vyttila–6 (40%). Out of 20 varieties five varieties were germinated at -5bar PEG6000 maximum germination percentage was found in PTB-7 (100%) followed by PTB-60 (60%) and PTB-35 (60%), minimum germination percentage was found in PTB-39. All the varieties under control showed 100% germination. None of the varieties were germinated at -7 bar PEG6000. Germination percentage under different levels of drought stress is shown in table 5.

Table 5. Germination percentage (%) of seedlings under various drought stress condition.

Sl. No.	Variety	-1 bar PEG6000	-3 bar PEG6000	-5bar PEG6000	Control
1	Chomala	100 (5.67)	0 (.573)	0 (.573)	100(5.67)
2	MO-18	100 (5.67)	60 (4.44)	0 (.573)	100(5.67)
3	PTB-35	100 (5.67)	100 (5.67)	60 (4.44)	100(5.67)
4	PTB-60	100 (5.67)	100 (5.67)	60 (4.44)	100(5.67)
5	PTB-39	80 (5.12)	40 (3.62)	30 (3.09)	100(5.67)
6	PTB-55	90 (5.40)	60 (4.44)	0 (.573)	100(5.67)
7	PTB-30	80 (5.12)	60 (4.44)	50 (4.03)	100(5.67)
8	PTB-7	80 (5.12)	100 (5.67)	100 (5.67)	100(5.67)
9	CRdhan307	100 (5.67)	80 (5.67)	0 (.573)	100(5.67)
10	Apo	100 (5.67)	0 (.573)	0 (.573)	100(5.67)
11	Vyttila-3	100 (5.67)	40 (3.62)	0 (.573)	100(5.67)
12	Vyttila-4	100 (5.67)	60 (4.44)	0 (.573)	100(5.67)
13	Vyttila-5	100 (5.67)	60 (4.44)	0 (.573)	100(5.67)
14	Vyttila-6	100 (5.67)	40 (3.62)	0 (.573)	100(5.67)
15	Vyttila-7	100 (5.67)	55 (4.05)	0 (.573)	100(5.67)

16	Vyttila-8	100 (5.67)	0 (.573)	0 (.573)	100(5.67)
17	Vyttila-9	100 (5.67)	100 (5.67)	0 (.573)	100(5.67)
18	Vyttila-10	100 (5.67)	100 (5.67)	0 (.573)	100(5.67)
19	N-22	60 (4.44)	0 (.573)	0 (.573)	100(5.67)
20	NL-44	40 (3.62)	0 (.573)	0 (.573)	100(5.67)
	Mean	(5.418)	(3.667)	(1.514)	
		C.D. (5%)	SE(m±)		
	G	0.221	0.079		
	T	0.099	0.035		
	G X T	0.443	0.157		

*Values in ( ) are angular transformed values

4.1.2. Shoot length at different level of drought stress

Result showed that there is significant difference for shoot length between treatment and control varieties.

Drought stress was given to rice seeds at different concentrations of (-1bar, -3 bar, -5bar, -7bar) PEG6000 for initial screening of 20 rice varieties. Germination of seeds were occurred at -1bar, -3bar, -5bar and seeds were not germinated at -7bar. The shoot length was found to be negatively affected by the increase in concentration of PEG6000. Among 20 rice varieties maximum shoot length was observed in PTB-7 (8.48cm) followed by PTB-60 (8.00cm) at -1bar PEG6000, the lowest was recorded in NL-44 (2.17cm). In control condition maximum shoot length was recorded in PTB-7 (11.43cm) and lowest was recorded in N-22 (5.95cm). The mean shoot length was found to be 5.55 cm and 7.69 cm for stress and control plants respectively.

Shoot length was found to be reduced in all varieties at -3bar PEG6000 than that of -1bar PEG6000, maximum shoot length was observed in PTB-7 (3.06 cm) followed by PTB-60 (2.44cm) and PTB-35 (2.32cm). The mean shoot length of seeds under -5bar PEG6000 was found to be 0.67cm. Among 20 rice varieties only five varieties were found to be germinated at drought stress of -5bar PEG6000. Among those five varieties maximum shoot length was observed in PTB-60 (1.25cm) followed by PTB-7 (0.33cm) and PTB-35 (0.33cm). Varieties were not germinated at -7 bar PEG6000. The results related to shoot length at-1 bar PEG6000, -3 bar PEG6000, -5bar PEG6000 and Control are presented in table 6.

Table 6. Shoot length (cm) of seedlings under various drought stress condition

Sl. No.	Variety	-1bar PEG6000	-3bar PEG6000	-5bar PEG6000	Control
1	Chomala	5.39	0.00	0.00	7.08
2	MO –18	5.70	1.17	0.00	6.76
3	PTB-35	7.01	2.32	0.30	8.04
4	PTB-60	8.00	2.44	1.25	10.52
5	PTB-39	4.50	0.37	0.00	7.44
6	PTB-55	5.69	0.30	0.00	7.76
7	PTB-30	5.20	0.14	0.00	6.94
8	PTB-7	8.48	3.06	0.33	11.38
9	CRdhan307	7.05	0.58	0.00	8.26
10	Apo	4.30	0.00	0.00	6.04
11	Vyttila-3	7.50	0.53	0.00	8.72
12	Vyttila-4	6.31	0.28	0.00	7.32
13	Vyttila-5	5.62	0.31	0.00	7.02

14	Vyttila-6	5.25	0.29	0.00	6.66
15	Vyttila-7	4.92	0.38	0.00	7.32
16	Vyttila-8	4.00	0.00	0.00	9.16
17	Vyttila-9	5.47	0.25	0.00	7.08
18	Vyttila-10	5.48	1.16	0.00	7.86
19	N-22	3.07	0.00	0.00	5.9
20	NL-44	2.17	0.00	0.00	6.16
	Mean	5.55	0.67	0.09	7.67
		C.D. (5%)	SE(m±)		
	G	0.03	0.011		
	T	0.013	0.005		
	G X T	0.059	0.021		

4.1.3. Root length at different level of drought stress

Result showed that there is significant difference for root length between treatment and control varieties.

Maximum root length was observed in PTB-35 (23.11 cm) at -1bar PEG6000 followed by CRdhan307 (20.14cm) and PTB-7 (19.14cm). The mean shoot length of varieties under -1bar PEG6000 was found to be 15.66 cm. Shoot length was found to be reduced for all varieties at -3bar PEG6000 compared to that of -1bar PEG6000, maximum root length was observed in PTB-7 (17.52cm) followed by PTB-60 (15.62cm) and PTB-35 (15.06cm) and minimum root length was found in Vyttila–3 (3.38cm). The mean root length of seeds under -3bar PEG6000 was recorded as 6.80 cm.

Only five varieties were germinated at -5bar PEG6000 among which maximum root length was shown by PTB-7 (9.65cm) followed by PTB-35 (5.48cm) and minimum root length was shown by PTB-39 (1.8c.m). The mean root length of varieties at -5bar PEG6000 was found to be 1.19. Varieties were not germinated at -7bar PEG6000. Results regarding root length at -1bar PEG6000, -3 bar PEG6000, -5bar PEG6000 and Control are presented in table 7.

Table 7. Root length (cm) of seedlings under various drought stress condition.

Sl. No.	Varieties	-1bar PEG6000	-3bar PEG6000	-5bar PEG6000	Control
1	Chomala	18.26	0.00	0.000	19.77
2	MO – 18	16.37	6.80	0.000	18.82
3	PTB-35	23.11	15.06	5.480	25.82
4	PTB-60	18.10	15.62	5.180	20.5
5	PTB-39	13.28	5.640	1.080	18.04
6	PTB-55	15.97	7.860	0.000	19.66
7	PTB-30	13.82	7.080	2.500	19.79
8	PTB-7	19.14	17.52	9.650	26.83
9	CRdhan307	20.14	10.74	0.000	21.5
10	Apo	14.34	0.000	0.000	15.96
11	Vyttila-3	15.32	3.38	0.000	23.45
12	Vyttila-4	14.05	4.820	0.000	21.97
13	Vyttila-5	15.28	6.480	0.000	21.4
14	Vyttila-6	15.34	5.020	0.000	23.2
15	Vyttila-7	15.47	7.600	0.000	20.14
16	Vyttila-8	16.36	0.000	0.000	18.59

17	Vyttila-9	15.50	11.54	0.000	21.28
18	Vyttila-10	17.24	11.04	0.000	22.11
19	N-22	10.35	0.000	0.000	16.31
20	NL-44	5.790	0.000	0.000	17.65
	Mean	15.66	6.810	1.195	20.64
		C.D. (5%)	S.E.(m±)		
	G	0.089	0.032		
	T	0.04	0.014		
	G X T	0.178	0.063		

4.1.4. Seedling vigour index at different level of drought stress

Result showed that there is significant difference for seedling vigour index between treatment and control. Maximum seedling vigour index was observed in PTB-35 (2660) followed by CR dhan 307 (2366) and PTB-60 (2208) at drought stress of -1bar PEG6000. The minimum seedling vigour index was found in NL-44 (275.2). The mean seedling vigour index of treatments were 1746 and that of controls were 2,447.

Maximum seedling vigour index was reported in PTB–7 (1904) followed by PTB-60 (1682) and PTB-35 (1622) at drought stress of -3bar PEG6000. The mean seedling vigour index was identified as 573.4 for treatments 2,447 and for controls. Maximum seedling vigour index was reported in PTB–7 (998.0) followed by PTB-60 (385.8) and PTB-35 (346.8) at -5bar PEG6000.The mean seedling vigour index was identified as 94.39 for treatments and 2,447 for controls. Varieties were not germinated at -7bar PEG6000. Results obtained for seedling vigour index under different levels of drought stress are presented in table 8.

Table 8. Seedling vigour index under varied drought stress condition

Sl. No.	Variety	-1 bar PEG 6000	-3 bar PEG 6000	-5bar PEG6000	Control
1	Chomala	2,095	0.000	0.000	2,213
2	M0-18	1,921	431.0	0.000	2,070
3	PTB-35	2,660	1,622	346.8	2,822
4	PTB-60	2,208	1,682	385.8	2,392
5	PTB-39	1,240	232.0	32.40	1,708
6	PTB-55	1,950	480.0	0.000	2,152
7	PTB-30	1,312	428.0	124.8	1,819
8	PTB-7	1,867	1,904	998.0	2,559
9	CRdhan307	2,366	881.0	0.000	2,464
10	Apo	1,647	0.000	0.000	1,772
11	Vyttila-3	1,908	140.0	0.000	2,344
12	Vyttila-4	1,719	296.0	0.000	2,563
13	Vyttila-5	1,806	397.0	0.000	2,491
14	Vyttila-6	1,797	206.0	0.000	2,653
15	Vyttila-7	1,791	438.0	0.000	2,380
16	Vyttila-8	1,836	0.000	0.000	2,317
17	Vyttila-9	1,821	1,166	0.000	2,482
18	Vyttila-10	1,996	1,161	0.000	2,604
19	N-22	712.2	0.000	0.000	1,926
20	NL-44	275.2	0.000	0.000	2,073
	Mean	1,746	573.4	94.39	2,447
		C.D. (5%)	S.E. (m±)		

	G	340.545	120.774		
	T	152.296	54.012		
	G X T	681.09	241.547		

4.1.5. Proline content at different level of drought stress

The proline content was significantly increased in varieties under drought stress than that of their corresponding control. Among the varieties the maximum proline content was reported in PTB-7 (36.42 µg/g tissue) followed by PTB-60 (35.199 µg/g tissue) and PTB-35 (35.1µg/g tissue) at -1bar PEG6000. The mean proline content of treatments was 25.25 µg/g tissue. PTB-7 recorded maximum proline content (37.70 µg/g tissue) followed by PTB – 35 (37.28 µg/g tissue) and CRdhan307 (32.66 µg/g tissue) at -3bar PEG6000. The mean proline content of treatments was 20.98 µg/g tissue.

Maximum proline content was observed in PTB-7 (43.234 µg/g tissue) followed by PTB-35 (42.557 µg/g tissue) and PTB-60 (38.345 µg/g tissue) at -5bar PEG6000. The mean proline content of treatments were 9.520µg/g tissue. Results related to proline content at different levels of drought stress are represented in Table 9.

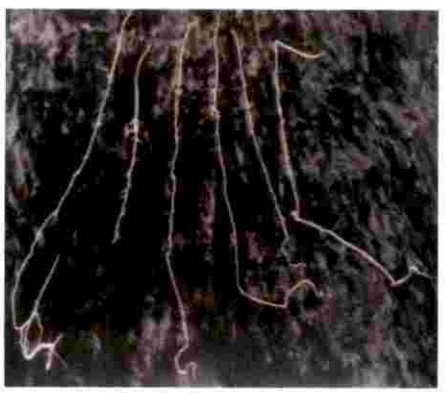
Table 9. Proline content (µg/g) under various drought stress.

Sl. No.	Variety	-1bar PEG6000	-3bar PEG6000	-5bar PEG6000	Control
1	Chomala	20.04	No germination	No germination	6.320
2	MO-18	22.91	27.15	No germination	6.691
3	PTB-35	35.10	37.28	42.55	12.67

4	PTB-60	35.19	36.36	38.34	11.62
5	PTB-39	27.95	27.99	34.39	14.55
6	PTB-55	29.50	29.91	No germination	15.80
7	PTB-30	29.57	23.90	31.87	17.28
8	PTB-7	36.42	37.75	43.23	6.022
9	CRdhan307	30.94	32.66	No germination	13.47
10	APO	23.03	No germination	No germination	11.99
11	Vyttila-3	28.14	29.33	No germonation	12.35
12	Vyttila-4	17.27	18.78	No germination	16.82
13	Vyttila-5	21.62	22.91	No germination	18.36
14	Vyttila-6	27.32	31.83	No germination	20.76
15	Vyttila-7	19.11	20.65	No germination	16.69
16	Vyttila-8	20.47	No germination	No germination	16.93
17	Vyttila-9	21.32	23.03	No germination	18.23
18	Vyttila-10	19.99	19.99	No germination	16.42
19	N-22	19.94	No germination	No germination	16.01

20	NL-44	19.14	No germination	No germination	15.86
	Mean	25.25	20.98	0.000	16.82
		C.D. (5%)	S.E. (m±)		
	G	1.776	0.63		
	T	0.794	0.282		
	G X T	3.552	1.26		

The varieties PTB -7, PTB – 60 and PTB -35 were selected as drought tolerant varieties



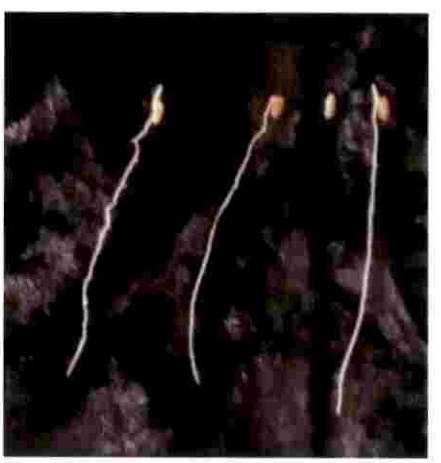
Control

PTB-7

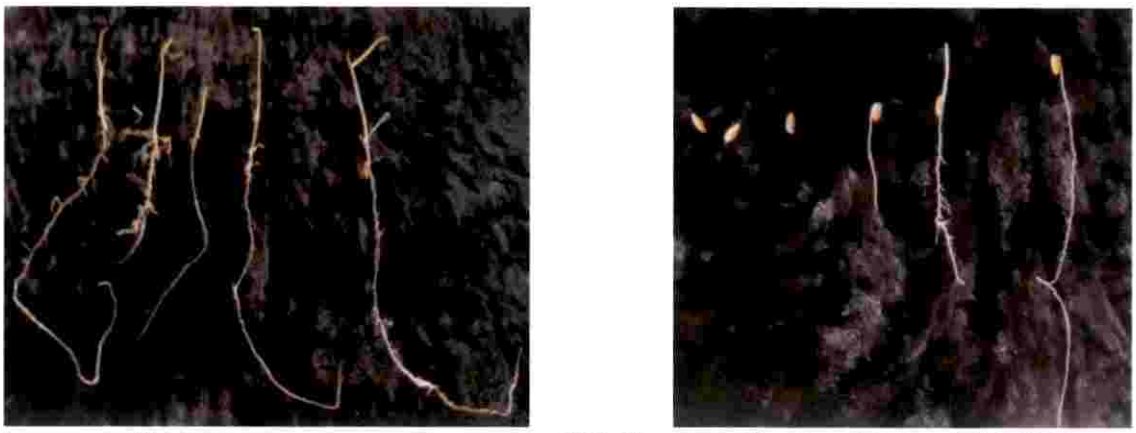
Treatment (-5bar PEG6000)

Control

PTB -60



Treatment (-5bar PEG6000)



Control PTB-35 Treatment (-5bar PEG 6000)

Plate 5. Growth pattern of selected varieties at -5bar water potential

4.1.6. Germination percentage at different level of salinity stress

All varieties except Chomala (80%) recorded 100% germination at 100mM NaCl. Out of 20 varieties nine recorded 100% germination at 150mM NaCl. The mean germination percentage under stress condition was 80.5%. The results showed 100% germination in eight varieties under salinity stress level of 200mM NaCl. The varieties under 200mM salinity recorded a mean germination percentage of 80.5%. Out of 20 varieties 18 varieties showed germination at 250mM NaCl. The maximum germination percentage is observed as 100%. Rice varieties MO-18, PTB-30, PTB-7, CRdhan307, Vyttila–3 and Vyttila–9 showed 100% germination. The minimum germination percentage was observed in Vyttila–5 (40%). The varieties under 250mM NaCl recorded a mean germination percentage of 65.5%. The results regarding to germination percentage at 100mM NaCl, 150mM NaCl, 200mM NaCl , 250mM NaCl and Control are represented in table 10.

Table 10. Germination percentage (%) of seedlings under various salinity stress.

Sl. No.	Variety	100mM NaCl	150mM NaCl	200mM NaCl	250mM NaCl	Control
1	Chomala	90 (5.40)	70 (4.785)	70 (4.785)	0 (.573)	100 (5.67)
2	MO-18	100 (5.67)	100 (5.67)	100 5.67)	100 (5.67)	100 (5.67)
3	PTB-35	100 (5.67)	100 (5.67)	80 (5.12)	100 (5.67)	100 (5.67)
4	PTB-60	100 (5.67)	80 (5.12)	80 (5.12)	100 (5.67)	100 (5.67)
5	PTB-39	100 (5.67)	60 (4.44)	80 (5.12)	100 (5.67)	100 (5.67)
6	PTB-55	100 (5.67)	100 (5.67)	100 (5.67)	100 (5.67)	100 (5.67)
7	PTB-30	100 (5.67)	100 (5.67)	100 (5.67)	100 (5.67)	100 (5.67)
8	PTB-7	100 (5.67)	100 (5.67)	100 (5.67)	100 (5.67)	100 (5.67)
9	CRdhan307	100 (5.67)	80 5.12)	80 (5.12)	100 (5.67)	100 (5.67)
10	Apo	100 (5.67)	100 (5.67)	100 (5.67)	90 (5.40)	100 (5.67)
11	Vyttila-3	100 (5.67)	100 (5.67)	100 (5.67)	100 (5.67)	100 (5.67)

12	Vyttila-4	100 (5.67)	100 (5.67)	100 (5.67)	0 (.573)	100 (5.67)
13	Vyttila-5	100 (5.67)	60 (4.44)	60 (4.44)	40 (3.625)	100 (5.67)
14	Vyttila-6	100 (5.67)	60 (4.44)	60 (4.44)	60 (4.44)	100 (5.67)
15	Vyttila-7	100 (5.67)	60 (4.44)	60 (4.44)	20 (2.326)	100 (5.67)
16	Vyttila-8	100 (5.67)	60 (4.44)	60 (4.44)	60 (4.44)	100 (5.67)
17	Vyttila-9	100 (5.67)	100 (5.67)	100 (5.67)	100 (5.67)	100 (5.67)
18	Vyttila-10	100 (5.67)	60 (4.44)	60 (4.44)	60 (4.44)	100 (5.67)
19	N-22	100 (5.67)	60 (4.44)	60 (4.44)	60 (4.44)	100 (5.67)
20	NL-44	100 (5.67)	60 (4.44)	60 (4.44)	60 (4.44)	100 (5.67)
	Mean	5.665	5.084	5.091	4.373	5.679
		C.D. (5%)	SE(m±)			
	G	0.078	0.028			
	T	0.039	0.014			
	G X T	0.175	0.062			

*values in ( ) angular transformed

4.1.7. Shoot length at different level of salinity stress

Shoot length at 100mM NaCl were found to significantly different from their corresponding control varieties. Highest shoot length was found in Vyttila-3 (8.3 cm) followed by PTB-7 (8.19 cm), their respective control plants were having shoot length of 8.99 cm and 8.63 cm respectively. The lowest shoot length was observed in Apo (5.32 cm). The seeds under 100mM NaCl stress showed a mean shoot length of 6.6 cm and 9.4 cm under control condition. Shoot length of rice varieties under 150mM NaCl recorded a reduction compared to that of shoot length at 100mM NaCl. MO–18 (6.7 cm) showed maximum shoot length at 150mM NaCl followed by PTB-7 (4.36 cm). The lowest shoot length was recorded in chomala (0.37 cm).

All the 20 varieties were germinated at 200mM NaCl. The maximum shoot length was observed in Vyttila-9 (4.95 cm) followed by MO-18 and PTB-7 (3.8 cm). MO-18 showed a maximum shoot length of 3.5 cm followed by Vyttila-3 (1.15 cm) at 250mM NaCl. The least shoot length was found in APO (0.11 cm). The plants under 250mM NaCl showed a mean shoot length of 0 .73cm. Shoot length at 100mM NaCl, 150mM NaCl, 200mM NaCl, 250mM NaCl and Control are represented in Table 11.

Table 11. Shoot length (cm) of seedlings under various salinity stress.

Sl. No.	Variety	100mM NaCl	150mM NaCl	200mM NaCl	250mM NaCl	Control
1	Chomala	5.79	0.37	0.31	0.00	6.60
2	MO – 18	6.93	6.07	3.80	3.50	7.73
3	PTB-35	5.94	1.19	0.78	0.68	9.21
4	PTB-60	6.75	1.93	1.30	0.64	7.64
5	PTB-39	5.98	1.35	1.13	0.14	10.0

6	PTB-55	6.17	2.25	1.95	0.67	8.51
7	PTB-30	7.76	2.02	1.82	0.76	8.63
8	PTB-7	8.19	4.36	3.80	0.37	9.18
9	CRdhan307	6.06	1.86	1.61	1.01	6.72
10	Apo	5.32	0.81	0.69	0.11	6.70
11	Vyttila-3	8.30	2.26	1.98	1.15	8.99
12	Vyttila-4	6.87	0.91	0.66	0.00	8.25
13	Vyttila-5	6.67	0.70	0.68	0.47	7.85
14	Vyttila-6	5.75	0.41	0.36	0.10	7.31
15	Vyttila-7	6.19	0.73	0.62	0.05	16.0
16	Vyttila-8	5.61	1.76	1.60	0.35	14.2
17	Vyttila-9	6.56	5.30	4.95	2.95	15.2
18	Vyttila-10	7.48	2.00	1.78	0.32	10.3
19	N-22	6.91	1.50	1.29	0.68	10.1
20	NL-44	6.77	0.69	0.58	0.78	8.94
	Mean	6.60	1.92	1.58	0.73	9.41
		C.D.(5%)	SE(m±)			
	G	0.342	0.122			
	T	0.171	0.061			
	G X T	0.764	0.272			

4.1.8. Root length at different level of salinity stress

There was a significant difference in root length between varieties under treatment condition and control condition. Maximum root length was shown by Vyttila-3 (22.2 cm) followed by N-22 (21.5 cm) and MO-18 (20.7 cm) at 100mM NaCl. Minimum root length was found in Apo (7.26 cm). The mean root length under salinity stress was found to be 18.5 cm and control plants shown a mean root length of 20.7 cm. Maximum root length at 150mM was observed in Vyttila-9 (17.4 cm) followed by MO–18 (11.3 cm). The minimum root length was shown by Apo (0.85 cm). Vyttila-9 (14.2 cm) recorded the maximum root length at 200mM NaCl. The lowest root length was recorded in Apo (0.68cm).

Out of 20 varieties 18 showed germination at the highest level of salinity 250mM, among which maximum root length was observed in MO-18 (5.20 cm) followed by Vyttila-9 (4.95 cm). The Minimum root length was observed in Vytitla-10 (0.26 cm). The data related to root length at 100mM NaCl, 150mM NaCl, 200mM NaCl, 250mM NaCl and Control are represented in Table 12.

Table 12. Root length (cm) of seedlings under various salinity stress

Sl. No.	Variety	100mM NaCl	150mM NaCl	200mM NaCl	250mM NaCl	Control
1	Chomala	15.6	3.11	2.93	0.00	20.8
2	MO-18	20.7	11.3	10.7	5.20	23.8
3	PTB-35	19.1	8.82	8.27	3.21	20.6
4	PTB-60	20.4	4.24	3.86	1.79	23.1
5	PTB-39	19.6	2.63	2.30	0.91	21.3
6	PTB-55	18.2	6.08	5.72	1.66	21.1

7	PTB-30	19.3	8.17	7.67	2.46	23.4
8	PTB-7	15.6	3.91	3.58	1.34	21.2
9	CRdhan307	19.5	5.95	5.58	2.59	19.7
10	Apo	7.26	0.85	0.68	2.56	14.3
11	Vyttila-3	22.2	9.07	9.00	7.07	21.4
12	Vyttila-4	17.0	0.91	0.66	0.00	18.6
13	Vyttila-5	21.0	0.70	0.68	0.47	22.6
14	Vyttila-6	19.8	0.41	0.36	0.10	20.2
15	Vyttila-7	18.6	0.73	0.62	0.05	19.1
16	Vyttila-8	20.1	1.76	1.60	0.35	21.3
17	Vyttila-9	17.5	5.30	4.95	2.95	18.1
18	Vyttila-10	17.0	2.00	1.78	0.32	20.7
19	N-22	21.5	1.50	1.29	0.68	22.2
20	NL-44	19.8	0.69	0.58	0.78	21.5
	Mean	17.0	1.92	1.58	0.73	20.7
		C.D.(5%)	SE(m±)			
	G	0.271	0.116			
	T	0.121	0.058			
	G X T	0.543	0.26			

4.1.9. Seedling vigour index at different levels of salinity stress

There was significant difference in seedling vigour index between treatment and control varieities at salinity stress condition. Maximum seedling vigour index was observed in Vyttila-3 (3,055) followed by N-22 (2,846) and MO-18 (2,769) and the minimum seedling vigour index was observed in Apo (1,258) at 100mM NaCl. The varieties under salinity stress of 100mM recorded a mean seedling vigour index of 2,503. Seedling vigour index of varieties were reduced at 150mM NaCl than that of 100mM NaCl. The maximum seedling vigour index was observed in Vyttila – 9 (2,276) followed by MO-18 (1,737) and Vyttila – 3 (1,133). Vyttila – 9 showed maximum seedling vigour index at salinity stress of 200mM NaCl. The varieties under salinity stress of 200mM NaCl showed a mean seedling vigour index of 508.8. Maximum seedling vigour index was observed in M0-18 (870) followed by Vyttila–3 (822) and Vyttila-9 (790) at salinity stress of 250mM NaCl. The results related to seedling vigour index at 100mM NaCl, 150mM NaCl, 200mM NaCl, 250mM NaCl and control are represented in Table 13.

Table 13. Seedling vigour index at different level of salinity stress.

Sl. No.	Variety	100mM NaCl	150mM NaCl	200mM NaCl	250mM NaCl	Control
1	Chomala	1,932	243.4	226.6	0.00	2,741
2	MO-18	2,769	1,737	1,272	870	3,111
3	PTB-35	2,509	1,001	693.6	311	2,985
4	PTB-60	2,723	493.6	364.0	145	3,077
5	PTB-39	2,564	238.8	230.4	63.0	3,134
6	PTB-55	2,440	833.0	670.0	139	2,961
7	PTB-30	2,706	1,019	858.0	322	3,211

8	PTB-7	2,380	827.0	549.0	171	3,040
9	CRdhan307	2,562	624.8	511.2	360	2,647
10	Apo	1,258	166.0	101.0	239	2,103
11	Vyttila-3	3,055	1,133	999.0	822	3,039
12	Vyttila-4	2,395	445.0	329.0	0.00	2,685
13	Vyttila-5	2,775	316.2	271.8	64.8	3,047
14	Vyttila-6	2,558	172.8	147.6	114	2,752
15	Vyttila-7	2,481	355.8	319.2	10.4	3,514
16	Vyttila-8	2,575	369.0	295.2	86.4	3,563
17	Vyttila-9	2,413	2,276	1,674	790	3,447
18	Vyttila-10	2,452	421.2	205.8	34.8	2,996
19	N-22	2,846	383.4	270.6	46.8	3,236
20	NL-44	2,663	322.8	189.6	53.4	3,052
	Mean	2,502	668.9	508.8	232	3,017
		C.D.(5%)	S.E.(m±)			
	G	75.89	27.007			
	T	37.945	13.503			
	G X T	169.696	60.389			

4.1.10. Na^+ - K^+ ratio at different levels of salinity stress

There was significant difference among varieties for Na^+- K^+ ratio at different level of salinity stress. Maximum amount of Na^+ - K^+ ratio was found in Vyttila-3 (1), Vyttila-7 (1), Vyttila-9 (1), Vyttila-10 (1), N-22 (1) and NL-44(1). The lowest Na^+ - K^+ ratio was observed in Chomala and MO-18 (0.33) under salinity stress of 100mM. The varieties under salinity stress recorded a mean value of 0.71. The

varieties under control condition recorded a mean Na^+ - K^+ ratio of 0.58. Na^+ - K^+ ratio was found to be increased at 150mM NaCl compared to that of 100mM NaCl. Maximum amount of Na^+ - K^+ ratio was found in Apo (5.75) followed by Vyttila–7 (4.25). The minimum amount was found in PTB-39 (1), PTB-7(1) and Vyttila–3 (1).The mean value under 150mM NaCl was found to be 2.377.

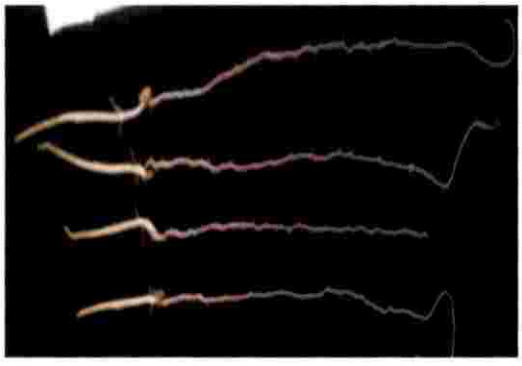
Maximum Na^+ - K^+ ratio was found in PTB-35 (6) at salinity stress of 200mM. The mean value at 200mM NaCl was found to be 3.17 for treatments and 0.58 for controls. Under salinity stress of 250mM NaCl maximum amount of Na^+ - K^+ ratio was observed in PTB – 55 (9.5) followed by PTB – 7 (9). The lowest amount of Na^+ - K^+ ratio was observed in Vyttila-6 (1.8) followed by N-22 and NL-44 (3). The mean value under 250mM NaCl was found to be 5.0. Na^+ - K^+ ratio at 100mM NaCl, 150mM NaCl, 200mM NaCl, 250mM NaCl and Control are represented in Table 14.

Table 14. Na^+ - K^+ ratio of seedlings under salinity stress.

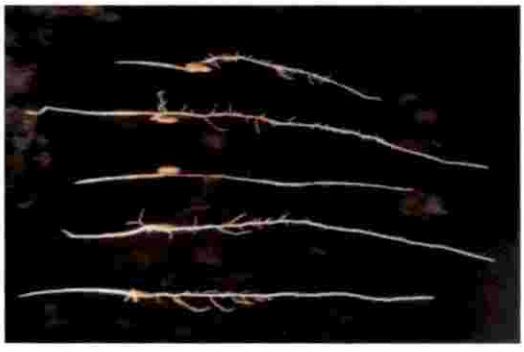
Sl. No.	Variety	100mM NaCl	150mM NaCl	200mM NaCl	200mM NaCl	Control
1	Chomala	0.33	2.00	3.50	No germination	0.16
2	MO-18	0.33	2.75	3.16	5.25	0.29
3	PTB-35	0.50	3.00	6.00	7.50	0.22
4	PTB-60	0.50	2.25	4.50	7.25	0.41
5	PTB-39	0.29	1.00	4.16	8.00	0.33
6	PTB-55	0.75	2.25	5.00	9.50	0.41
7	PTB-30	0.41	1.917	3.75	8.50	0.25
8	PTB-7	0.50	1.00	4.25	9.00	0.75
9	CRdhan307	1.00	4.00	4.16	4.50	0.50
10	Apo	0.75	5.75	4.00	6.50	0.50

11	Vyttila-3	1.00	1.00	2.75	3.25	0.50
12	Vyttila-4	0.75	3.50	2.00	No germination	1.00
13	Vyttila-5	0.75	2.50	5.50	6.50	1.00
14	Vyttila-6	0.75	3.50	2.50	2.83	1.00
15	Vyttila-7	1.00	4.25	1.66	3.50	1.00
16	Vyttila-8	0.75	1.25	1.25	3.50	1.00
17	Vyttila-9	1.00	1.25	1.75	3.50	1.00
18	Vyttila-10	1.00	0.87	1.33	4.50	1.00
19	N-22	1.00	1.25	1.00	3.00	0.11
20	NL-44	1.00	2.25	1.16	3.00	0.29
	Mean	0.71	2.37	3.171	5.00	0.588
		C.D.(5%)	SE(m±)			
	G	0.767	0.273			
	T	0.384	0.137			
	G X T	1.716	0.611			

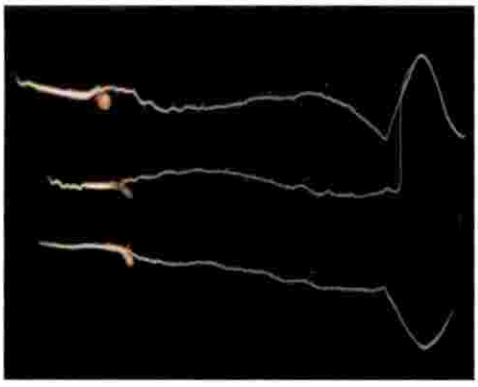
The varieties Vyttila–3, Vyttila–9 and MO-18 were slected as salinity tolerant varieties



Control



MO-18 Treatment (250mM NaCl)



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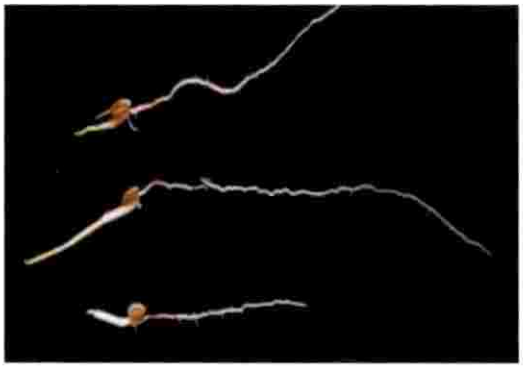
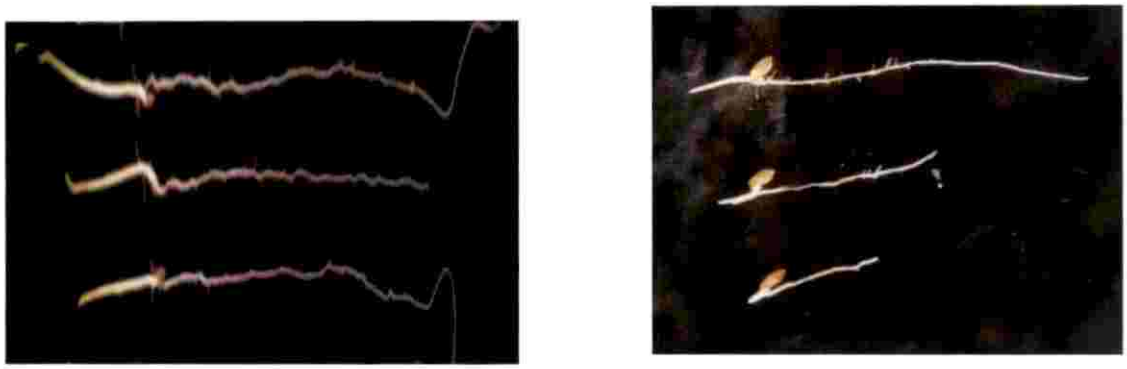


Vyttila -3 Treatment (250mM NaCl)



Control Vyttila -9 Treatment (250mM NaCl)

Plate 6. Growth pattern of selected varieties under 250mM NaCl

4.1.14. Germination percentage at different level of temperature stress

The results showed significant difference in germination percentage between treatment and control varieties. Among 20varieties, 100% germination was observed in 13 varieties. The least germination percentage was observed in Apo (40%) followed by Vyttila-10 (50%). The mean germination percentage was found to be 81.5%

The data related to germination percentage at $35^{O}C$ is represented in Table 15.

Table 15. Germination percentage (%) of seedlings under temperature stress of $35^{O}C$

Sl. No.	Variety	Treatment	Control	Mean
1	Chomala	100 (5.67)	100 (5.67)	100 (5.67)

2	MO-18	100 (5.67)	100 (5.67)	100 (5.67)
3	PTB-35	70 (4.65)	100 (5.67)	85.0 (5.06)
4	PTB-60	100 (5.67)	100 (5.67)	100 (5.67)
5	PTB-39	80 (5.06)	100 (5.67)	90 (5.40)
6	PTB-55	90 (5.40)	100 (5.67)	95 (5.54)
7	PTB-30	100 (5.67)	100 (5.67)	100 (5.67)
8	PTB-7	100 (5.67)	100 (5.67)	100 (5.67)
9	CR dhan 307	100 (5.67)	100(5.67)	100(5.67)
10	Apo	4 0(3.50)	100 (5.67)	70 (4.65)
11	Vyttila-3	0 (.573)	100 (5.67)	50 (3.12)
12	Vyttila-4	100 (5.67)	100 (5.67)	100 (5.67)

13	Vyttila-5	0 (.573)	100 (5.67)	50 (3.12)
14	Vyttila-6	100 (5.67)	100 (5.67)	100 (5.67)
15	Vyttila-7	100 (5.67)	100 5.67)	100 (5.67)
16	Vyttila-8	100 (5.67)	100 (5.67)	100 (5.67)
17	Vyttila-9	100 (5.67)	100 (5.67)	100 (5.67)
18	Vyttila-10	50 (3.126)	100 (5.67)	75 (4.40)
19	N-22	100 (5.67)	100 (5.67)	100 (5.67)
20	NL-44	100 (5.67)	100 (5.67)	100 (5.67)
	Mean	100 (5.67)	100 (5.67)	
		C.D.(5%)	SE(m±)	
	G	100 (5.67)	100 (5.67)	
	T	100 (5.67)	100 (5.67)	
	G X T	100	100	

		(5.67)	(5.67)	

*values in ( ) angular transformed values

4.1.16. Shoot length at different level of temperature stress

There was significant difference in shoot length between varieties under treatment and control condition. Among different temperature stress levels, all varieties except Vyttila-3 and Vyttila-10 were germinated at 35°C. None of the varieties were germinated at stress levels 40°C, 45°C and 50°C. Maximum shoot length was observed in N-22 (15.5 cm) followed by NL-44 (12.8 cm) and Vyttila-6 (11.1 cm) at 35°C. The mean shoot length at 35°C was found to be 5.09 cm for treatments and 8.33 cm for controls.

The data related to shoot length at 35°C is represented in Table 16.

Table 16. Shoot length (cm) of seedlings under temperature stress of 35°C

Sl. No.	Variety	Treatment	Control	Mean
1	Chomala	4.77	6.03	5.40
2	MO-18	2.75	5.13	3.94
3	PTB-35	2.00	4.27	3.13
4	PTB-60	4.38	5.80	5.09
5	PTB-39	3.66	6.93	5.29
6	PTB-55	1.84	5.27	3.55
7	PTB-30	4.91	6.58	5.74
8	PTB-7	6.42	11.15	8.78
9	CRdhan307	4.53	6.69	5.61
10	Apo	0.48	5.36	2.92

11	Vyttila-3	0.00	9.85	4.92
12	Vyttila-4	7.94	8.79	8.36
13	Vyttila-5	0.00	8.07	4.03
14	Vyttila-6	11.1	12.2	11.7
15	Vyttila-7	5.19	7.65	6.42
16	Vyttila-8	9.98	11.4	10.7
17	Vyttila-9	3.6	8.37	5.98
18	Vyttila-10	0.00	6.46	3.23
19	N-22	15.5	16.6	16.0
20	NL-44	12.8	13.9	13.3
	Mean	5.09	8.33	
		C.D.(5%)	SE(m±)	
	G	1.031	0.359	
	T	0.326	0.114	
	G X T	1.458	0.508	

4.1.17. Root length at different level of temperature stress

The results showed significant difference in root length between varieties under treatment and control condition. Maximum root length was observed in Vyttila – 6 (11.1 cm) followed by NL-44 (10.9 cm) and N-22 (10.33cm). The minimum root length was observed in Apo (1.87 cm).The mean root length at this stress level was found to be 5.88 cm for treatments and 9.63 cm for controls. The data related to root length at 35°C is represented in Table 17.

Table 17. Root length (cm) of seedlings under temperature stress of 35°C

Sl. No.	Variety	Treatment	Control	Mean
1	Chomala	9.53	11.25	10.39
2	MO-18	6.52	9.33	7.925
3	PTB-35	4.36	7.18	5.770
4	PTB-60	4.22	6.84	5.530
5	PTB-39	5.06	10.47	7.765
6	PTB-55	3.41	7.64	5.525
7	PTB-30	3.66	8.9	6.28
8	PTB-7	7.81	10.69	9.25
9	CRdhan307	4.52	8.36	6.44
10	Apo	1.87	6.09	3.98
11	Vyttila-3	0	12.8	6.435
12	Vyttila-4	10.21	11.2	10.705
13	Vyttila-5	1.99	9.96	5.975
14	Vyttila-6	11.1	10.5	10.81
15	Vyttila-7	5.14	8.44	6.79
16	Vyttila-8	9.97	10.6	10.295
17	Vyttila-9	3.63	9.74	6.685
18	Vyttila-10	3.31	9.62	6.465
19	N-22	10.3	11.4	10.87
20	NL-44	10.9	11.5	11.27
	Mean	5.880	9.63	
		C.D.(5%)	SE(m±)	

	G	1.699	0.592	
	T	0.537	0.187	
	G X T	2.403	0.838	

4.1.18. Seedling vigour index at different level of temperature stress

Among 20 varieties maximum seedling vigour index was observed in N-22 (2,584) followed by NL-44 (2,376) and Vyttila-6 (2,224). The least seedling vigour index was found in Vyttila-10 (91.00). The varieties under treatment condition recorded a mean seedling vigour index of 1,049. The data related to germination percentage at 35°C is represented in Table 18.

Table 18. Seedling vigour index under temperature stress of 35°C

Sl. No.	Variety	Treatment	Control	Mean
1	Chomala	1,430	1,728	1,579
2	MO-18	927.0	1,446	1,186
3	PTB-35	469.2	1,145	807.1
4	PTB-60	860.0	1,264	1,062
5	PTB-39	712.8	1,740	1,226
6	PTB-55	464.0	1,291	877.5
7	PTB-30	857.0	1,548	1,202
8	PTB-7	1,423	2,184	1,803
9	CRdhan307	905.0	1,505	1,205
10	Apo	94.20	1,145	619.0
11	Vyttila-3	0.000	2,272	1,136

12	Vyttila-4	1,815	1,999	1,907
13	Vyttila-5	0.000	1,803	901.5
14	Vyttila-6	2,224	2,278	2,251
15	Vyttila-7	1,033	1,609	1,321
16	Vyttila-8	1,995	2,211	2,103
17	Vyttila-9	723.0	1,811	1,267
18	Vyttila-10	91.00	1,608	849.5
19	N-22	2,584	2,807	2,695
20	NL-44	2,376	2,555	2,465
	Mean	1,049	1,797	
		C.D.(5%)	S.E.(m±)	
	G	209.641	73.077	
	T	66.294	23.109	
	G X T	296.477	103.34	

4.1.19. Cell membrane stability index at different level of temperature stress

The maximum cell membrane stability index was observed in N-22 (99.02%) and minimum cell membrane stability index was shown by MO-18 (4.72%).

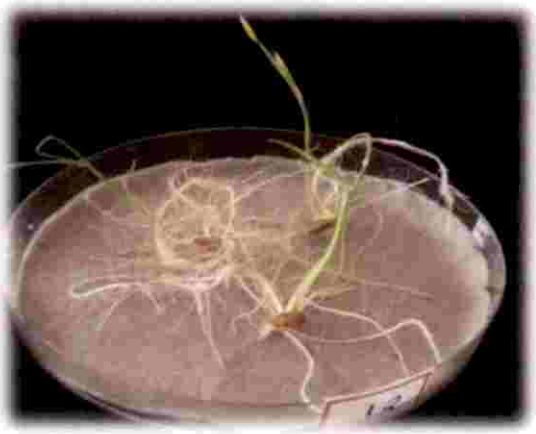
Cell membrane stability index at 35°C is represented in Table 19.

Table 19. Cell membrane stability index (%) under temperature stress of 35°C

Sl. No.	Variety	Treatment
1	Chomala	10.65
2	MO -18	4.722

3	PTB-35	44.71
4	PTB-60	24.19
5	PTB-39	77.07
6	PTB-55	35.49
7	PTB-30	38.28
8	PTB-7	38.51
9	CRdhan307	56.69
10	Apo	7.234
11	Vyttila-3	No germination
12	Vyttila-4	15.41
13	Vyttila-5	No germination
14	Vyttila-6	64.11
15	Vyttila-7	45.17
16	Vyttila-8	63.78
17	Vyttila-9	37.47
18	Vyttila-10	66.66
19	N-22	99.02
20	NL-44	58.22
	C.D.	11.87
	SE(m±)	3.99

The varieties N-22, NL-44 and Vyttila – 6 were selected as temperature tolerant varieties

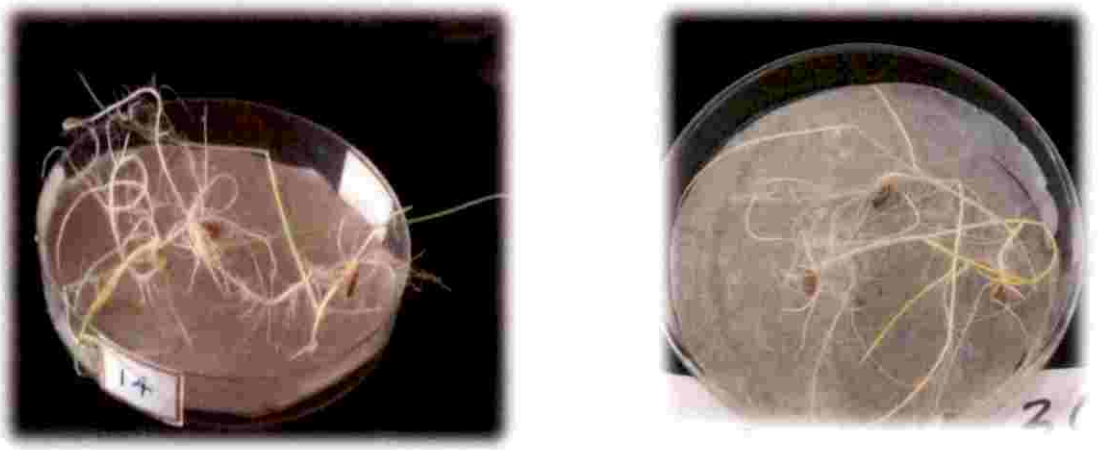


Control

N-22 Treatment (35^{O}C)

Control

NL-44 Treatment (35^{O}C)



Control **Vytilla – 6** **Treatment (35^{O}C)**

Plate 7. Growth pattern of selected varieties under 35^{0}C temperature stress

4.2. Varieties under combination of stresses

The selected varieties from highest tolerated level of individual stress were subjected to combination of stresses.

Among 20 varieties 9 tolerant varieties (PTB-7, PTB-60,PTB-35, Uma, Vyttila-3, Vytilla–9, N-22, NL-44 and Vytilla-6) are selected, 3 from highest tolerated level of salinity stress (Uma,Vyttila-3 and Vyttila-9), 3 from highest tolerated level of drought stress (PTB-7, PTB-60 and PTB-35) and 3 from highest tolerated temperature stress (N-22, NL-44 and Vytilla-6) were given combination of stress.

Highest tolerated level of drought stress was represented as D_h (-5bar PEG6000)

Highest tolerated level of salinity stress was represented as S_h (250mM NaCl)

Highest tolerated level of temperature stress was represented as T_h (35^oC)

4.2.1. Shoot length at highest tolerated level of drought and highest tolerated level of salinity

The results showed a significant difference in shoot length between varieties under treatment and control condition. Maximum shoot length at this stress level is recorded in PTB-7 (5.66 cm) followed by Vyttila-9 (0.73 cm) and PTB-35 (0.54 cm). Out of six varieties Only three varieties were found germinated at this stress level. The varieties under combined stress of highest tolerated level of salinity and highest tolerated level of drought recorded a mean shoot length of 1.15 cm and the control plants recorded mean shoot length of 9.72 cm. Shoot length at highest tolerated level of drought (D_h) and highest tolerated level of salinity stress (S_h) is represented in table 20.

Table 20. Shoot length (cm) of seedlings under combined stress of highest tolerated level of drought (D_h) (-5bar PEG6000) and highest tolerated level of salinity(S_h) (250Mm NaCl)

Sl. No.	Variety	Treatment	Control	Mean
1	MO-18	0.00	7.93	3.96
2	PTB-35	0.54	9.50	5.02
3	PTB-60	0.00	7.40	3.70
4	PTB-7	5.66	9.25	7.46
5	Vyttila-3	0.00	8.98	4.49
6	Vyttila-9	0.73	15.2	7.99

	Mean	1.15	9.72	
		C.D.(5%)	SE(m±)	
	G	0.065	0.022	
	T	0.038	0.013	
	G X T	0.092	0.031	

4.2.3. Root length at highest tolerated level of drought and highest tolerated level of salinity

The results showed a significant difference in root length in varieties under stress and control conditions. PTB-7 (13.64 cm) was recorded as the variety having maximum root length at combination highest tolerated drought stress and highest tolerated salinity stress. The varieties under combination of stress treatment recorded a mean root length of 3.52 cm and controls recorded a mean value of 21.1 cm. Root length at highest tolerated level of drought (D_h) and highest tolerated level of salinity stress (S_h) is represented in table 21.

Table 21. Root length (cm) of seedlings under combined stress of highest tolerated level of drought (D_h) (-5bar PEG6000) and highest tolerated level of salinity (S_h) (250mM)

Sl. No.	Variety	Treatment	Control	Mean
1	MO –18	0.00	22.9	11.4
2	PTB-35	3.11	20.8	11.9
3	PTB-60	0.00	23.0	11.5
4	PTB-7	13.6	21.0	17.3
5	Vyttila-3	0.00	21.0	10.5

6	Vyttila-9	4.40	17.9	11.1
	Mean	3.52	21.1	
		C.D.(5%)	SE(m±)	
	G	0.533	0.182	
	T	0.308	0.105	
	G X T	0.754	0.257	

4.2.4. Germination percentage at highest tolerated level of drought and highest tolerated level of salinity

All the varieties under control condition showed 100% germination. Out of six varieties only three varieties germinated under this combination stress treatment. Maximum germination was observed in PTB-7 (93%) followed by PTB–35 (60%) and Vyttila–9 (60%). The mean germination percentage at this stress level was recorded as 35 % for treatments and 100% for controls. The data related to germination percentage at highest tolerated level of drought (D_h) and highest tolerated level of salinity stress (S_h) is represented in Table 22.

Table 22. Germination percentage (%) of seedlings under combined stress of highest tolerated level of drought (D_h) (-5bar PEG6000) and highest tolerated level of salinity (S_h) (250Mm NaCl)

Sl. No.	Variety	Treatment	Control	Mean
1	MO-18	0 (0.04)	100 (0.95)	50 (.495)

2	PTB-35	60 (0.6)	100 (0.95)	80 (.775)
3	PTB-60	0 (0.04)	100 (0.95)	50 (.495)
4	PTB-7	93 (.9)	100 (0.95)	96.6 (.925)
5	Vyttila-3	0 (0.04)	100(0.95)	50 (.495)
6	Vyttila-9	60 (.6)	100 (0.95)	80 (.775)
	Mean	(.37)	(0.95)	
		C.D.(5%)	SE(m±)	
	G	0.03	0.01	
	T	0.017	0.006	
	G X T	0.042	0.014	

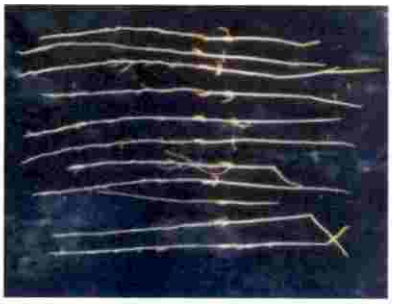
4.2.5. Seedling vigour index at highest tolerated level of drought and highest tolerated level of salinity

The results showed a significant difference in seedling vigour among varieties under stress and control condition. Maximum seedling vigour index was observed in PTB–7 (1615) followed by Vyttila–9 (293.6). The least seedling vigour index was observed in PTB-35 (209.2). The treatments recorded a mean vigour index of 353.1 and controls recorded a mean seedling vigour index of 2759. Seedling vigour index at highest tolerated level of drought (D_h) and highest tolerated level of salinity stress (S_h) is represented in Table 23.

Table 23. Seedling vigour index under combined stress of highest tolerated level of drought (D_h) (-5bar PEG6000) and highest tolerated level of salinity (S_h) (250mM NaCl)

Sl. No.	Variety	Treatment	Control	Mean
1	MO-18	0.000	2,818	1,409
2	PTB-35	209.2	2,712	1,460
3	PTB-60	0,000	2,799	1,399
4	PTB-7	1,615	2,716	2,166
5	Vyttila-3	0.000	2,699	1,349
6	Vyttila-9	293.6	2,810	1,551
	Mean	353.11	2,759	
		C.D.(5%)	SE(m±)	
	G	497.025	169.276	
	T	286.957	97.732	
	G X T	702.899	239.393	

PTB – 7 control



PTB – 7 treatment

Plate 8. 14th day germinated images of PTB -7 under the combined stress of D_h and S_h

4.2.6. Shoot length at highest tolerated level of temperature and highest tolerated level of salinity

Among six selected varieties from highest tolerated temperature (T_h) and highest tolerated salinity (S_h) none of them were found to have shoot germination at combination stress treatment. The control varieties recorded a mean shoot length of 9.98 cm. The data related to shoot length at highest tolerated level of temperature (T_h) and highest tolerated level of salinity stress (S_h) is represented in table 24.

Table 24. Shoot length (cm) of seedlings under combined stress of highest tolerated level of temperature (T_h) ($35^{O}C$) and highest tolerated level of salinity (S_h) (250Mm NaCl)

Sl. No.	Variety	Treatment	Control	Mean
1	MO-18	0.00	7.96	3.98
2	Vyttila-3	0.00	8.98	4.49
3	Vyttila-6	0.00	7.40	3.70
4	Vyttila-9	0.00	15.2	7.64
5	N-22	0.00	10.9	5.45
6	NL-44	0.00	9.38	4.69
	Mean	0.00	9.98	
		C.D.(5%)	SE(m±)	
	G	0.001	0.000	
	T	0.000	0.000	
	G X T	0.001	0.000	

4.2.7. Root length at highest tolerated level of temperature and highest tolerated level of salinity

Out of six varieties only three varieties were found to have root germination. The highest root length was observed in NL-44 (0.06 cm) followed by MO-18 (0.04cm) and the lowest was observed in N-22 (0.03 cm). Root length at highest tolerated level of temperature (T_h) and highest tolerated level of salinity stress (S_h) is represented in Table 25.

Table 25. Root length (cm) of seedlings under combined stress of highest tolerated level of temperature (T_h) (35°C) and highest tolerated level of salinity (S_h) (250mM NaCl)

Sl. No.	Variety	Treatment	Control	Mean
1	MO-18	0.04	23.2	11.6
2	Vyttila-3	0.00	21.0	10.5
3	Vyttila-6	0.00	19.9	9.95
4	Vyttila-9	0.00	18.0	9.03
5	N-22	0.03	22.2	11.1
6	NL-44	0.06	21.4	10.7
	Mean	0.02	20.9	
		C.D. (5%)	SE(m±)	
	G	0.016	0.005	
	T	0.009	0.003	
	G X T	0.022	0.008	

4.2.8. Germination percentage at highest tolerated level of temperature and highest tolerated level of salinity

Among six rice varieties three varieties were germinated and maximum germination percentage was observed in NL-44 (53.3%) followed by N-22 and MO-18. The mean germination percentage of varieties was 20%. The controls showed a germination of 100%. The data regarding to germination percentage at highest tolerated level of temperature (T_h) and highest tolerated level of salinity stress (S_h) is represented in Table 26.

Table 26. Germination percentage (%) of seedlings under combined stress of highest tolerated level of Temperature (T_h) (35°C) and highest tolerated level of salinity (S_h) (250mM)

Sl. No.	Variety	Treatment	Control	Mean
1	MO-18	33.3 (1.154)	100 (1.396)	66.6 (.495)
2	Vyttila-3	0 (1.02)	100 (1.396)	50.0 (.775)
3	Vyttila-6	0 (1.02)	100 (1.396)	50.0 (.775)
4	Vyttila-9	0 (1.02)	100 (1.396)	50.0 (.775)
5	N-22	33.3 (1.154)	100 (1.396)	66.6 (.495)
6	NL-44	53.3	100	76.6

		(1.238)	(1.396)	(.775)
	Mean	1.101	1.396	
		C.D.(5%)	SE(m±)	
	G	0.03	0.01	
	T	0.017	0.006	
	G X T	0.042	0.014	

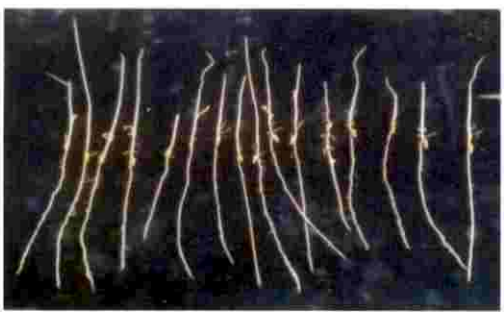
4.2.9. Seedling vigour index at highest tolerated level of temperature and highest tolerated level of salinity

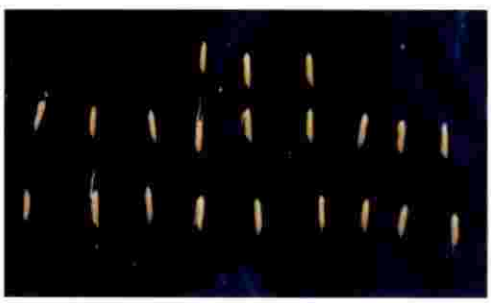
Maximum seedling vigour index at combined stress of highest tolerated level of temperature and highest tolerated level of salinity is recorded in NL-44 (3.46) followed by MO-18 (1.06). The least seedling vigour index was found in N-22 (1). The treatments recorded mean seedling vigor index of 0.92 and controls recorded mean vigour index of 2,098. The data related to seedling vigour index at highest tolerated level of temperature (T_h) and highest tolerated level of salinity stress (S_h) is represented in Table 27.

Table 27. Seedling vigour index under combined stress of highest tolerated level of Temperature (T_h) (35^{O}C) and highest tolerated level of salinity (S_h) (250mM NaCl)

Sl. No.	Variety	Treatment	Control	Mean
1	MO-18	1.067	2,322	1,161
2	Vyttila-3	0.000	2,100	1,050
3	Vyttila-6	0.000	1,990	995
4	Vyttila-9	0.000	1,806	903

5	N-22	1.000	2,222	1,111
6	NL-44	3.467	2,148	1,075
	Mean	0.922	2,098	
		C.D.(5%)	SE(m±)	
	G	0.844	0.287	
	T	0.487	0.166	
	G X T	1.193	0.406	





Control PTB-7 Treatment

Plate 9. 14th day germinated images of NL-44 under the combined stress of T_h and S_h

4.3 POT CULTURE EXPERIMENT OF SELECTED VARIETIES UNDER COMBINED STRESS OF HIGHEST TOLERATED DROUGHT X HIGHEST TOLERATED SALINITY AND HIGHEST TOLERATED TEMPERATURE X HIGHEST TOLERATED SALINITY

The selected 3 plants from each combination of stress treatment (D_h x S_h) (PTB-7, PTB-35, Vyttila-9) and (T_h x S_h) (N-22, NL-44, Uma) were subjected to field experiment using pot culture method. The highest tolerated levels of drought

and salinity stresses were induced by -5bar PEG6000 and 250mM NaCl by applying the solutions in to the pots with plant varieties for a period of 5 days during reproductive stages. Temperature and salinity stress were induced by transferring the plants in to temperature-controlled poly house (35^0C) and NaCl (250mM) during panicle initiation to maturity stage.

4.3.1. Plant height at D_h x S_h in pot culture experiment

Height of the plant was found reduced in plants under stress condition than that of their corresponding control plants. The mean plant height at combination stress of highest tolerated drought and temperature was found to be 92.0 cm and the control plants recorded a mean plant height of 105 cm. Maximum plant height was recorded in PTB-7 (111 cm) followed by Vyttila-9 (87.5 cm). The least plant height was found to be 77.5 cm in PTB – 35. The data related to plant height under the combined stress of highest tolerated level of drought (D_h) and highest tolerated level of salinity (S_h) are represented in Table 28.

Table 28. Height of the plant (cm) under combined stress of highest tolerated drought (D_h) and highest tolerated salinity (S_h).

Sl. No.	Variety	Treatment	Control	Mean
1	PTB-35	77.5	84	80.7
2	PTB-7	111	132	121
3	Vyttila-9	87.5	100	93.7
	Mean	92.0	105	
		C.D.(5%)	SE(m±)	
	G	10.423	2.955	
	T	8.510	2.412	
	G X T	N/S	4.178	

4.3.2. No. of productive tillers at D_h x S_h in pot culture experiment

Maximum No. of productive tillers was observed in PTB-7 (8.5) where as its well-irrigated control recorded 10.5 numbers of productive tillers. The plants recorded a mean number of six productive tillers under stress condition and 7.3 in control condition. The least no of productive tillers was observed in PTB-35 (4.0). No. of productive tillers under the combined stress of highest tolerated level of drought (D_h) and highest tolerated level of salinity (S_h) are represented in Table 29.

Table 29. No. of productive tillers under combined stress of highest tolerated drought (D_h) and highest tolerated salinity (S_h).

Sl. No.	Variety	Treatment	Control	Mean
1	PTB-35	4.0	5.0	4.5
2	PTB-7	8.5	10.5	9.5
3	Vyttila-9	5.5	6.5	6
	Mean	6.0	7.3	
		C.D.(5%)	SE(m±)	
	G	1.018	0.289	
	T	0.831	0.236	
	G X T	N/S	0.408	

4.3.3 Spikelet fertility percentage at D_h x S_h in pot culture experiment

Spikelet fertility percentage recorded a significant difference between control and treatment. The mean spikelet fertility of varieties under stress condition was recorded as 59.47% and for controls 80.95%. The maximum spikelet fertility was recorded as 64.93% for PTB-7 followed by 62.58% for PTB–35. The minimum spikelet fertility was found in Vyttila–9 (50.90%). The results related to spikelet fertility percentage under the combined stress of highest tolerated level of drought (D_h) and highest tolerated level of salinity (S_h) are represented in table 30.

Table 30. Spikelet fertility percentage (%) under combined stress of highest tolerated drought (D_h) and highest tolerated salinity (S_h).

Sl. No.	Variety	Treatment	Control	Mean
1	PTB-35	62.58	87.88	75.23
2	PTB-7	64.93	80.98	72.95
3	Vyttila-9	50.90	73.99	62.44
	Mean	59.47	80.95	
		C.D.(5%)	SE(m±)	
	G	3.808	1.079	
	T	3.109	0.881	
	G X T	N/S	1.526	

Control	Treatment (D_h x S_h)	Control	Treatment (D_h x S_h)	Control	Treatment (D_h x S_h)
PTB – 7		PTB -35		Vyttila - 9	

Plate 10. Variation in spikelet fertility percentage (%) of rice varieties at flowering stage under the combined stress of D_h x S_h and well irrigated condition.

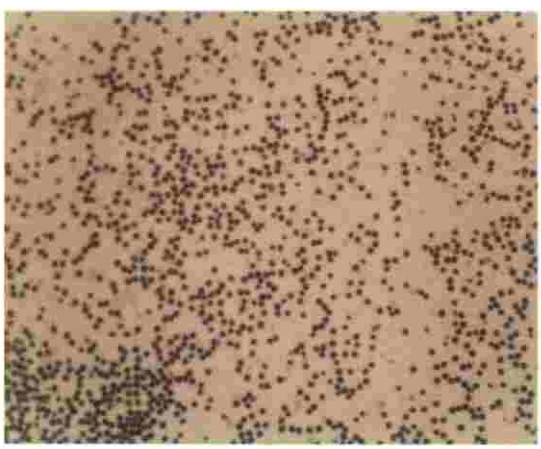
4.3.4. Pollen viability percentage at D_h x S_h in pot culture experiment

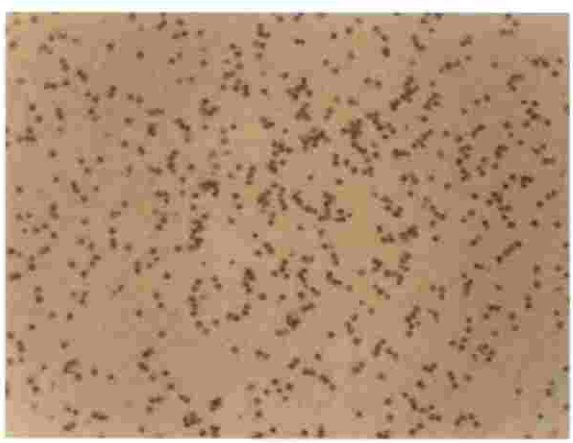
The results showed a significant diffference in pollen viability between control and treatment varieties. Maximum pollen viability percentage was observed in Vyttila–9 (80.19%) followed by PTB–35 (74.13%). The minimum pollen viability percentage was observed in PTB-7 (51.05%). The mean pollen viability percentage of varieties under treatment was recorded as 68.45%. The results related to pollen viability percentage under the combined stress of highest

tolerated level of drought (D_h) and highest tolerated level of salinity (S_h) are represented in table 31.

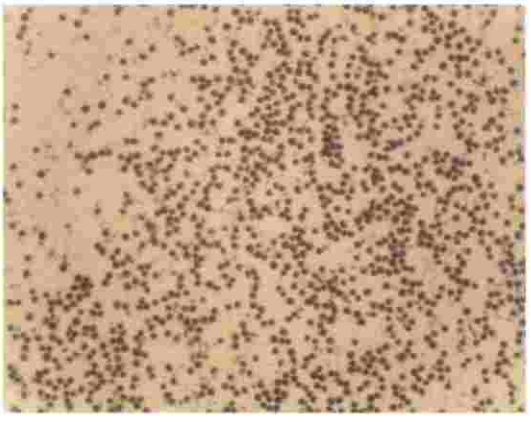
Table 31. Pollen viability percentage (%) under combined stress of highest tolerated drought (D_h) and highest tolerated salinity (S_h).

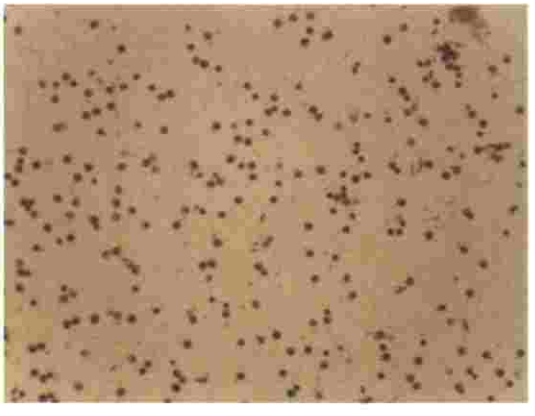
Sl. No.	Variety	Treatment	Control	Mean
1	PTB-35	74.13	88.12	81.12
2	PTB-7	51.05	93.28	72.16
3	Vyttila-9	80.19	90.43	85.31
	Mean	68.45	90.61	
		C.D.(5%)	SE(m±)	
	G	2.472	0.701	
	T	2.019	0.572	
	G X T	3.496	0.991	



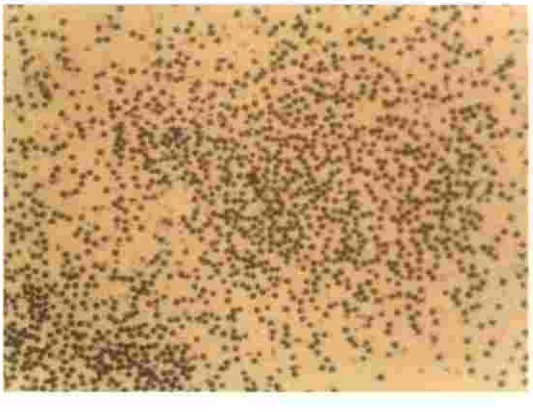


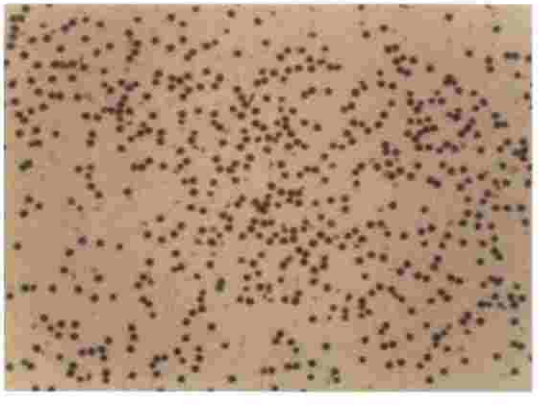
Control PTB – 7 Treatment (D_h x S_h)





Control PTB – 35 Treatment (D_h x S_h)





Control Vyttila – 9 Treatment (D_h x S_h)

Plate 11. Variation in pollen viability percentage (%) of rice varieties at flowering stage under the combined stress of D_h x S_h and well irrigated condition.

4.3.5. Yield per plant at D_h x S_h in pot culture experiment

The maximum yield among varieties under stress condition was observed in PTB-7 (9.0 g). The minimum yield was observed in Vyttila–9 (5.2 g). The results related to yield per plant under the combined stress of highest tolerated level of drought (D_h) and highest tolerated level of salinity (S_h) are represented in Table 32.

Table 32. Yield per plant (g) under combined stress of highest tolerated drought (D_h) and highest tolerated salinity (S_h).

Sl. No.	Variety	Treatment	Control	Mean
1	PTB-35	6.7	15.5	11.1
2	PTB-7	9.0	12.7	10.8
3	Vyttila-9	5.2	10.0	8.0
	Mean	7.0	13.0	
		C.D.(5%)	SE(m±)	
	G	0.882	0.25	
	T	0.72	0.204	
	G X T	1.247	0.354	

4.3.6. Proline content at D_h x S_h in pot culture experiment

There was a significant difference in proline content on varieties under stress and varieties under irrigated condition. The maximum proline content was observed in PTB-7(48.58 µg/g) followed by PTB-35 (38.69 µg/g). The minimum proline content was observed in Vyttila-9 (27.63 µg/g). The varieties under

control condition recorded a mean proline content of 22.51 µg/g. The results related to proline content under the combined stress of highest tolerated level of drought (D_h) and highest tolerated level of salinity (S_h) are represented in Table 33.

Table 33. Proline content (µg/g) under combined stress of highest tolerated drought (D_h) and highest tolerated salinity (S_h).

Sl. No.	Variety	Treatment	Control	Mean
1	PTB-35	38.69	24.13	31.41
2	PTB-7	48.85	22.58	35.72
3	Vyttila-9	27.63	20.82	24.23
	Mean	38.39	22.51	
		C.D.(5%)	SE(m±)	
	G	1.966	0.557	
	T	1.605	0.455	
	G X T	2.78	0.788	

4.3.7. Cell membrane stability index (%) at D_h x S_h in pot culture experiment

Among varieties maximum cell membrane stability index was observed in PTB–7 (90.07%) followed by Vyttila–9 (84.55%). The minimum cell membrane stability index was observed in PTB–35 (78.96%).

The results related to cell membrane stability index under the combined stress of highest tolerated level of drought (D_h) and highest tolerated level of salinity (S_h) are represented in Table 34.

Table 34. Cell membrane stability index (%) under combined stress of highest tolerated drought (D_h) and highest tolerated salinity (S_h).

Sl. No.	Variety	Treatment
1	PTB-35	78.96
2	PTB-7	90.07
3	Vyttila-9	84.55
	C.D.	6.631
	SE(m±)	1.423

4.3.8. Malondialdehyde content at D_h x S_h in pot culture experiment

Varieties under stress condition recorded a significant increase in malondialdehyde content than corresponding control. The maximum malondialdehyde content was observed in Vyttila-9 (2.97 m mol g^{-1}). The minimum malondialdehyde content was found in PTB-7 (2.21 m mol g^{-1}) . Varieties under control condition recorded mean malondialdehyde content of 0.96 m mol g-1. The results related to malondialdehyde content under the combined stress of highest tolerated level of drought (D_h) and highest tolerated level of salinity (S_h) are represented in Table 35.

Table 35. Malondialdehyde (m mol g^{-1}) content under combined stress of highest tolerated drought (D_h) and highest tolerated salinity (S_h) .

Sl. No.	Variety	Treatment	Control	Mean
1	PTB-35	2.36	0.89	1.62
2	PTB-7	1.29	0.93	1.11

3	Vyttila-9	2.97	1.07	2.02
	Mean	2.21	0.96	
		C.D.(5%)	SE(m±)	
	G	0.393	0.111	
	T	0.321	0.091	
	G X T	0.555	0.157	

4.3.9. Chlorophyll a/b ratio at D_h x S_h in pot culture experiment

The maximum chlorophyll a/b ratio was recorded in Vyttila–9 (1.89) followed by PTB–7 (1.80). The lowest chlorophyll a/b ratio was found in PTB–35 (1.57). The varieties under combined stress of drought and salinity recorded a mean value of 1.75 and controls recorded a mean value of 2.43. The results related to Chlorophyll a/b ratio under the combined stress of highest tolerated level of drought (D_h) and highest tolerated level of salinity (S_h) are represented in Table 36.

Table 36. Chlorophyll a/b ratio under combined stress of highest tolerated drought (D_h) and highest tolerated salinity (S_h).

Sl. No.	Variety	Treatment	Control	Mean
1	PTB-35	1.57	2.31	1.94
2	PTB-7	1.80	2.43	2.12
3	Vyttila-9	1.89	2.53	2.21
	Mean	1.75	2.43	
		C.D.(5%)	SE(m±)	

	G	0.206	0.058	
	T	0.168	0.048	
	G X T	N/S	0.083	

4.3.10. Na^+ - K^+ ratio at D_h x S_h in pot culture experiment

The results showed a significant difference in Na^+- K^+ ratio between control and treated varieties. Under combined stress of highest tolerated drought and highest tolerated salinity Na^+ - K^+ ratio of varieties under treatment was found to be increased compared to that of varieties under control condition. Maximum Na^+ - K^+ ratio was recorded in Vyttila–9 (0.739) followed by PTB–7 (0.494) and PTB–35 (0.403). The varieties under combined stress of drought and salinity recorded a mean value of 0.545 and varieties under control condition recorded a mean value of 0.498. Na^+-K^+ ratio under the combined stress of highest tolerated level of drought (D_h) and highest tolerated level of salinity (S_h) are represented in Table 37.

Table 37. Na^+- K^+ ratio under combined stress of highest tolerated drought (D_h) and highest tolerated salinity (S_h) in field condition

Sl. No.	Variety	Treatment	Control	Mean
1	PTB-35	0.403	0.356	0.379
2	PTB-7	0.494	0.495	0.495
3	Vyttila-9	0.739	0.642	0.691
	Mean	0.545	0.498	
		C.D.(5%)	SE(m±)	

	G	0.111	0.032	
	T	N/S	0.026	
	G X T	N/S	0.045	

4.3.11. Superoxide dismutase activity at D_h x S_h in Pot culture experiment

SOD activity was found to be increased in varieties under combined stress of drought and salinity than their corresponding controls. The maximum SOD activity was observed in genotype PTB–7 (0.428 g^{-1} min^{-1}) followed by Vyttila – 9 (0.360g^{-1} min^{-1}). The least SOD was observed in PTB – 35 (0.343 g^{-1} min^{-1}). Varieties under combined stress of drought and salinity recorded a mean SOD activity of 0.287 g^{-1} min^{-1}. The results related to super oxide dismutase activity (g^{-1} min^{-1}) under the combined stress of highest tolerated level of drought (D_h) and highest tolerated level of salinity (S_h) are represented in Table 38.

Table 38. Superoxide dismutase activity (g^{-1} min^{-1}) under combined stress of highest tolerated drought (D_h) and highest tolerated salinity (S_h) in field condition

Sl. No.	Variety	Treatment	Control	Mean
1	PTB-35	0.343	0.251	0.297
2	PTB-7	0.428	0.294	0.361
3	Vyttila-9	0.360	0.317	0.339
	Mean	0.377	0.287	
		C.D.(5%)	SE(m±)	

	G	0.044	0.012	
	T	0.036	0.01	
	G X T	N/S	0.018	

4.3.12. Plant height at T_h x S_h in pot culture experiment

The results showed significant difference in plant height between varieties under control and treatment condition. Under the combined stress of highest tolerated temperature and highest tolerated salinity mean plant height was found to be reduced in treatments compared to that of varieties under well irrigated condition. Maximum plant height was observed in N-22 (125 cm) followed by NL-44 (109 cm). The least plant height was found in MO-18 (79 cm). The varieties under treatment showed a mean height of 104 cm whereas controls showed a mean plant height of 111 cm. The results related to plant height under the combined stress of highest tolerated level of temperature (T_h) and highest tolerated level of salinity (S_h) are represented in Table 39.

Table 39. Plant height (cm) under combined stress of highest tolerated temperature (T_h) and highest tolerated salinity (S_h).

Sl. No.	Variety	Treatment	Control	Mean
1	MO-18	79	100	89
2	N-22	125	122	123
3	NL-44	109	112	110
	Mean	104	111	
		C.D.(5%)	SE(m±)	

	G	16.718	4.739	
	T	N/S	3.869	
	G X T	N/S	6.702	

4.3.13. No. of productive tillers at T_h x S_h in pot culture experiment

The mean no. of productive tillers in varieties under treatment was four and varieties under well irrigated condition was found to be 5.5. The maximum no. of productive tiller was observed in N-22 (5) where as the minimum no. of productive tiller was found in MO-18 (3). The results related to No. of productive tillers under the combined stress of highest tolerated level of temperature (T_h) and highest tolerated level of salinity (S_h) are represented in Table 40.Table 40. No. of productive tillers under combined stress of highest tolerated temperature (T_h) and highest tolerated salinity (S_h) in field condition

Sl. No.	Variety	Treatment	Control	Mean
1	MO-18	3	5.5	4.25
2	N-22	5	4.5	4.75
3	NL-44	4	6.5	5.25
	Mean	4	5.5	
		C.D.(5%)	SE(m±)	
	G	N/S	0.250	
	T	0.720	0.204	
	G X T	1.247	0.354	

4.3.14. Spikelet fertility percentage at T_h x S_h in pot culture experiment.

There was a significant difference in spikelet fertility percentage in varieties under stress condition and varieties under control condition. Maximum spikelet fertility percentage was observed in N-22 (62.65%) followed by NL-44 (38.05%). The least spikelet fertility percentage was observed in MO-18 (31.75 %). Varieties under stress condition recorded a mean spikelet fertility percentage of 44.15% and varieties under well irrigated condition recorded a mean spikelet fertility percentage of 78.51%. The results related to spikelet fertility percentage under the combined stress of highest tolerated level of temperature (T_h) and highest tolerated level of salinity (S_h) are represented in table 41.

Table 41. Spikelet fertility percentage (%) under combined stress of highest tolerated temperature (T_h) and highest tolerated salinity (S_h) in field condition

Sl. No.	Variety	Treatment	Control	Mean
1	MO-18	31.75	76.15	53.95
2	N-22	62.65	82.23	72.44
3	NL-44	38.05	77.16	57.60
	mean	44.10	78.51	
		C.D.(5%)	SE(m±)	
	G	4.709	1.335	
	T	3.845	1.090	
	G X T	6.660	1.888	

Treatment control treatment control treatment control

(T_h x S_h) (T_h x S_h) (T_h x S_h)

N-22 NL-44 MO-18

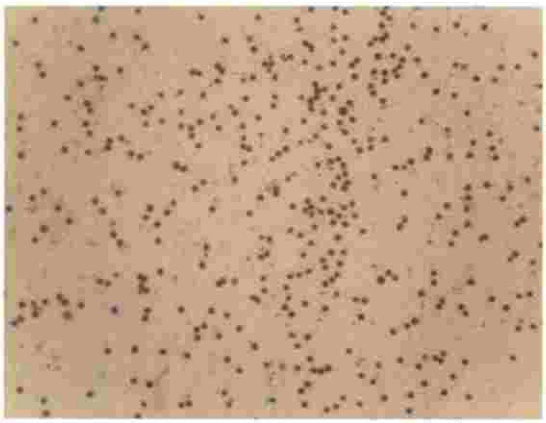
Plate 12. Variation in spikelet fertility percentage (%) of rice varieties at flowering stage under the combined stress of T_h x S_h and well irrigated condition.

4.3.15. Pollen viability percentage at T_h x S_h in pot culture experiment

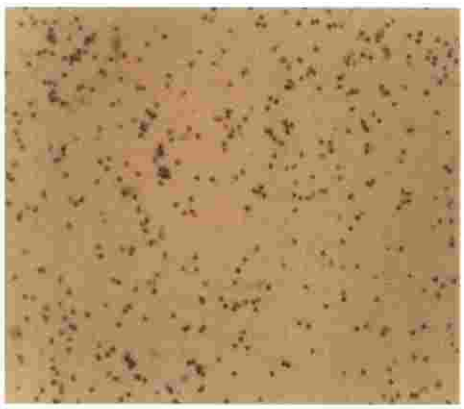
Among three selected varieties maximum pollen viability under combined stress of highest tolerated level of temperature and highest tolerated level of salinity was observed in NL-44 (79.24%) followed by N-22 (74.04%). The minimum pollen viability percentage was observed in MO-18 (64.95%). Varieties under stress condition recorded a mean pollen viability percentage of 72.74% and the varieties under control condition recorded a mean pollen viability percentage of 89.37%. The results related to pollen viability percentage under the combined stress of highest tolerated level of temperature (T_h) and highest tolerated level of salinity (S_h) are represented in Table 42.

Table 42. Pollen viability percentage (%) under combined stress of highest tolerated temperature (T_h) and highest tolerated salinity (S_h).

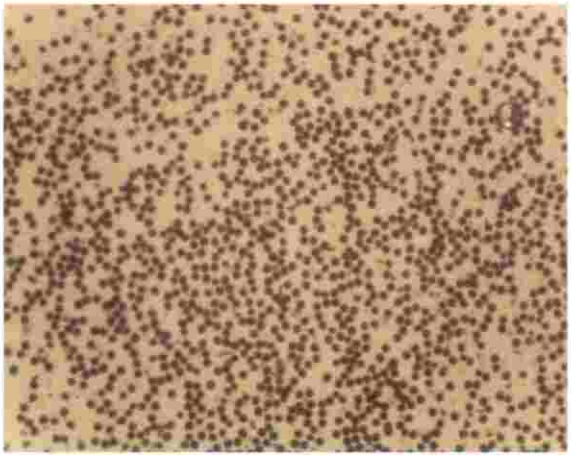
Sl. No.	Variety	Treatment	Control	Mean
1	MO-18	64.95	80.82	72.88
2	N-22	74.04	93.32	83.68
3	NL-44	79.24	93.96	86.60
	mean	72.74	89.37	
		C.D.(5%)	SE(m±)	
	G	2.980	0.845	
	T	2.433	0.69	
	G X T	N/S	1.195	

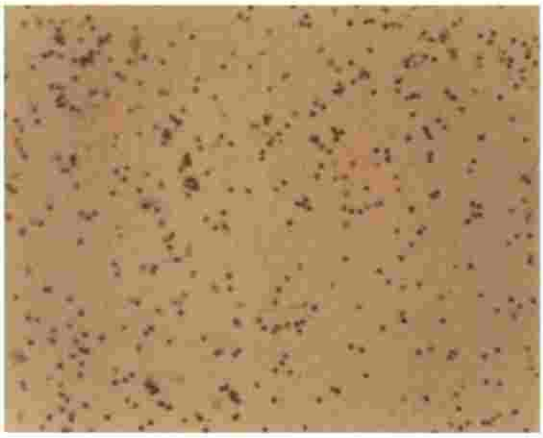


Control

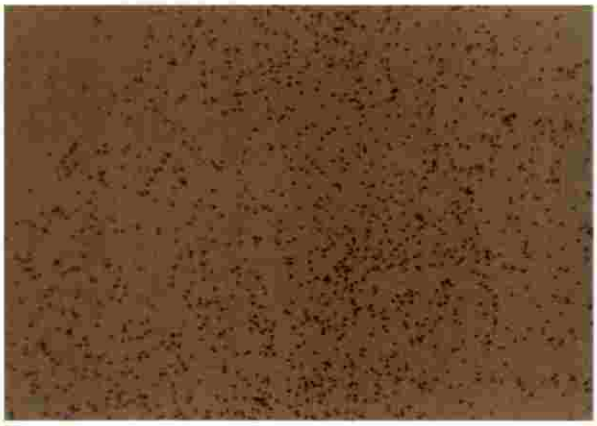


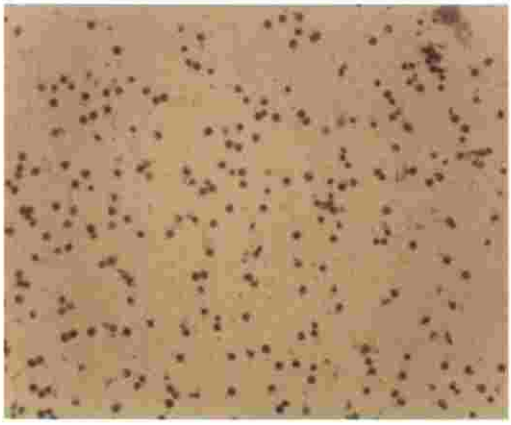
N-22 Treatment (T_h x S_h)





Control NL – 44 Treatment (T_h x S_h)





Control MO-18 Treatment (T_h x S_h)

Plate 13. Variation in pollen viability percentage (%) of rice varieties at flowering stage under the combined stress of T_h x S_h and well irrigated condition.

4.3.16. Yield per plant at T_h x S_h in pot culture experiment

Yield per plant was found to be significantly reduced in varieties under stress condition than that of varieties under well irrigated condition. Among three

varieties maximum yield per plant was observed in N-22 (6.5 g) followed by NL – 44 (5.0 g). The minimum yield per plant was observed in MO-18 (4.5 g). The varieties under stress condition recorded a mean yield of 5.3 g and varieties under well-watered recorded a mean yield of 10.9 g. The results related to yield per plant under the combined stress of highest tolerated level of temperature (T_h) and highest tolerated level of salinity (S_h) are represented in Table 43.

Table 43. Yield per plant (g) under combined stress of highest tolerated temperature (T_h) and highest tolerated salinity (S_h).

Sl. No.	Variety	Treatment	Control	Mean
1	MO-18	4.5	10	7.2
2	N-22	6.5	12	9.5
3	NL-44	5.0	10	7.6
	Mean	5.3	11	
		C.D.(5%)	SE(m±)	
	G	1.463	0.586	
	T	1.194	0.479	
	G X T	N/S	0.829	

4.3.17. Proline content at T_h x S_h in pot culture experiment

Proline content in varieties under stress condition were found to be increased than that of varieities under control condition the mean proline content of varieties under stress condition was recorded as 26.07µg/g tissue and the plants under well irrigated condition was found to be 22.79µg/g tissue. The maximum proline content was observed in N-22 (27.33 µg/g tissue) followed by NL-44 (26.12µg/g tissue) The minimum proline content was found in MO-18 (24.76µg/g

tissue). The results related to proline content under the combined stress of highest tolerated level of temperature (T_h) and highest tolerated level of salinity (S_h) are represented in Table 44.

Table 44. Proline content (µg/g) under combined stress of highest tolerated temperature (T_h) and highest tolerated salinity (S_h).

Sl. No.	Variety	Treatment	Control	Mean
1	MO-18	24.76	19.97	22.36
2	N-22	27.33	24.90	26.12
3	NL-44	26.12	23.48	24.80
	Mean	26.07	22.79	
		C.D.(5%)	SE(m±)	
	G	0.970	0.275	
	T	0.792	0.225	
	G X T	1.372	0.389	

4.3.18. Cell membrane stability index at T_h x S_h in pot culture experiment

The maximum cell membrane stability index was observed in N-22(69.17%) followed by NL-44(60.19%). The minimum cell membrane stability index was observed in MO-18 (49.52%). The results related to cell membrane stability index under the combined stress of highest tolerated level of temperature (T_h) and highest tolerated level of salinity (S_h) are represented in Table 45.

Table 45. Cell membrane stability index (%) under combined stress of highest tolerated temperature (T_h) and highest tolerated salinity (S_h)

Sl. No.	Variety	Treatment
1	MO-18	49.52
2	N-22	69.17
3	NL-44	60.197
	C.D.	10.556
	SE(m±)	2.264

4.3.19. Malondialdehyde content at T_h x S_h in pot culture experiment

The results showed significant difference in MDA content between varieties under control and treatment condition. Maximum malondialdehyde content was observed in MO-18 (2.63 m $molg^{-1}$) followed by NL-44 (2.42 m $molg^{-1}$). The minimum malondialdehyde was found in N-22. The varieties under stress recorded a mean malondialdehyde content of 2.39 m $molg^{-1}$ and varieities under well-watered condition recorded a mean malondialdehyde content of 0.98 m $molg^{-1}$. The results related to malondialdehyde content under the combined stress of highest tolerated level of temperature (T_h) and highest tolerated level of salinity (S_h) are represented in Table 46.

Table 46. Malondialdehyde content (m mol g^{-1}) under combined stress of highest tolerated temperature (T_h) and highest tolerated salinity (S_h).

Sl. No.	Variety	Treatment	Control	Mean
1	MO-18	2.63	0.96	1.80

2	N-22	2.13	0.94	1.53
3	NL-44	2.42	1.04	1.73
	Mean	2.39	0.98	
		C.D.(5%)	SE(m±)	
	G	0.136	0.038	
	T	0.111	0.031	
	G X T	0.192	0.054	

4.3.20. Chlorophyll a/b ratio at T_h x S_h in pot culture experiment

Among the varieties under the combination stress maximum chlorophyll a/b ratio was observed in MO-18 (1.23) followed by N-22 (1.07). The minimum amount of chlorophyll a/b was found in NL-44 (1.01). The results related to Chlorophyll a/b ratio under the combined stress of highest tolerated level of temperature (T_h) and highest tolerated level of salinity (S_h) are represented in Table 47.

Table 47. Chlorophyll a/b ratio under combined stress of highest tolerated temperature (T_h) and highest tolerated salinity (S_h).

Sl. No.	Variety	Treatment	Control	Mean
1	MO-18	1.23	2.07	1.65
2	N-22	1.07	2.08	1.58
3	NL-44	1.01	2.01	1.51
	mean	1.10	2.05	
		C.D.(5%)	SE(m±)	
	G	N/S	0.07	

	T	0.201	0.057	
	G X T	N/S	0.099	

4.3.21. Na^+ - K^+ ratio at T_h x S_h in pot culture experiment

The maximum Na^+ - K^+ ratio under stress condition was observed in MO-18 (0.72) followed by NL-44 (0.54). The minimum Na^+ - K^+ ratio under stress condition was found in N-22 (0.46). Varieties under combined stress of highest tolerated temperature and highest tolerated salinity recorded a mean Na^+ - K^+ ratio of 0.57 and well-watered control varieties recorded a mean value of 0.42.

The results related to Na^+ - K^+ ratio under the combined stress of highest tolerated level of temperature (T_h) and highest tolerated level of salinity (S_h) are represented in Table 48.

Table 48. Na^+ - K^+ ratio under combined stress of highest tolerated temperature (T_h) and highest tolerated salinity (S_h) in pot culture experiment

Sl. No.	Variety	Treatment	Control	Mean
1	MO-18	0.72	0.69	0.70
2	N-22	0.46	0.30	0.38
3	NL-44	0.54	0.29	0.41
	mean	0.57	0.42	
		C.D.(5%)	SE(m±)	
	G	0.07	0.020	
	T	0.057	0.016	
	G X T	0.099	0.028	

4.3.22. SOD activity at T_h x S_h in Pot culture experiment

Among the selected three varieties maximum SOD was found in N-22(0.42 g^{-1} min^{-1}) followed by MO-18 (0.37 g^{-1} min^{-1}). The minimum SOD activity was observed in NL-44 (0.34 g^{-1} min^{-1}) under stress condition. The mean SOD value of varieties under stress condition was found to be 0.381g^{-1} min^{-1} and 0.262 g^{-1} min^{-1} for varieties under control condition.

Superoxide dismutase activity under the combined stress of highest tolerated level of temperature (T_h) and highest tolerated level of salinity (S_h) are represented in table 49.

Table 49. Super oxide dismutase activity (g^{-1} min^{-1}) under combined stress of highest tolerated temperature (T_h) and highest tolerated salinity (S_h).

Sl. No.	Variety	Treatment	Control	Mean
1	MO-18	0.37	0.23	0.30
2	N-22	0.42	0.24	0.33
3	NL-44	0.34	0.29	0.32
	mean	0.38	0.26	
		C.D.(5%)	SE(m±)	
	G	N/S	0.013	
	T	0.036	0.010	
	G X T	0.063	0.018	

4.4 DNA EXTRACTION

The genomic DNA isolated from 20 rice varieties were analyzed and confirmed by agarose gel electrophoresis. All the DNA isolated were appeared on agarose gel ensuring good quality.

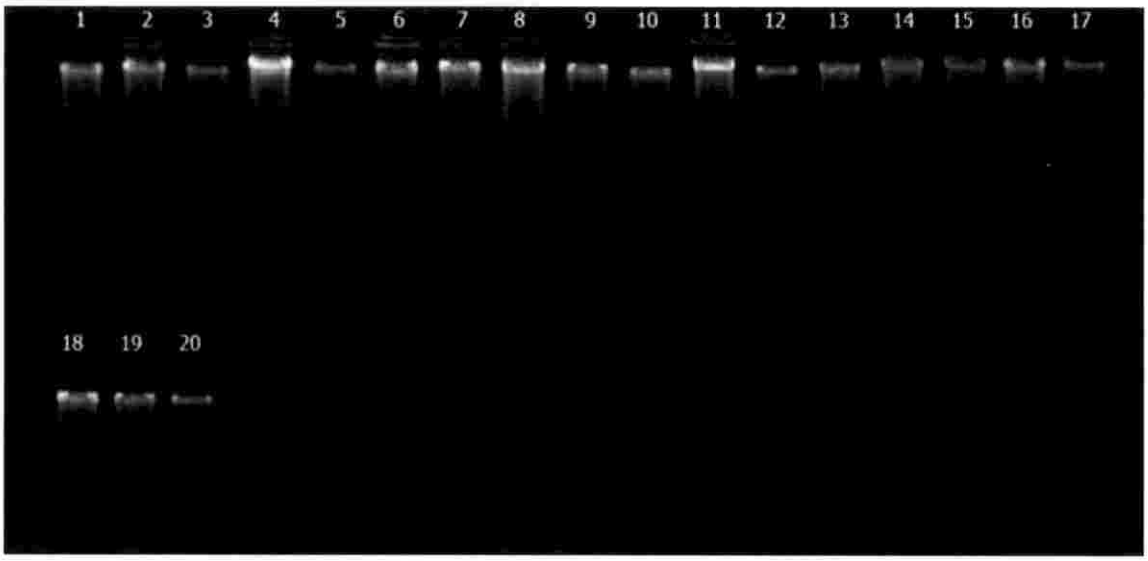


Plate 14. Gel profile with DNA bands of rice (Lane 1 – chomala, Lane 2 - MO-18, Lane 3 – PTB 35, Lane 4 -PTB 60, Lane 5 – PTB 39, Lane 6 – PTB 55, Lane 7 – PTB 30, Lane 8 – PTB 7, Lane 9 – CR dhan 307, Lane 10 – Apo, Lane 11 – Vyttila -3, Lane 12 – Vyttila -4, Lane 13 – Vyttila -5, Lane 14 – Vyttila -6, Lane 15 – Vyttila -7, Lane 16 – Vyttila -8, Lane 17 – Vyttila -9, Lane 18 – N-22, Lane 19 – NL -44, Lane 20 – Vyttila -10)

4.4.1 quality and quantity of dna samples of rice varieties selected for polymorphism analysis using ssr microsatellite markers.

Sl. No	Variety	A_{260}/A_{280} value	DNA concentration (ng/µl)
1	Chomala	1.66	2940.0
2	MO-18	1.89	2319.0
3	PTB 35	1.82	2070.0
4	PTB 60	1.88	1563.0
5	PTB 39	1.85	2610.0
6	PTB 55	1.82	3270.0
7	PTB 30	1.79	2880.0
8	PTB 7	1.73	3060.0
9	CR dhan 307	1.76	2403.0
10	APO	1.98	1812.0
11	Vyttila–3	1.87	2568.0
12	Vyttila-4	1.83	2431.0
13	Vyttila-5	1.88	2009.0
14	Vyttila-6	1.84	2379.0
15	Vyttila-7	1.85	2198.0
16	Vyttila-8	1.82	2029.0
17	Vyttila-9	1.99	1280.0
18	Vyttila-10	1.98	1319.0
19	N-22	1.86	2219.0
20	NL-44	1.85	2198.0

4.5 SCREENING OF PRIMERS BY PCR

PCR reaction was carried out using selected primers for drought, salinity and temperature providing appropriate conditions. Out of 30 primers 9 showed polymorphism in 3% agarose gel electrophoresis and all other primers were monomorphic. The polymorphic markers for temperature tolerance were RM6100, showed polymorphic bands with size ~ 150bp, RM7076 with polymorphic band of size ~ 180bp, RM5749 with polymorphic band of size ~ 170bp and RM26212 is linked to grain yield and located in chromosome 4showed a polymorphic band of size ~ 180bp. The polymorphic markers for salinity tolerance were RM1287, RM8094, and RM10843 they showed polymorphic bands with size between150 – 220bp, 80 - 250bp, 151 - 161bp respectively. RM490 showed polymorphism for drought tolerance with a band size of ~ 98bp. Banding patterns of amplified product with RM6100, RM7076, RM5749, RM26212, RM1287, RM8094, RM10843 and RM490 in 3% agarose gel is represented in plates.

Among 9 SSR markers distinct polymorphic bands for temperature tolerance was shown by markers RM 6100 in varieties N-22 and NL-44, RM 7076 in N-22 and PTB–7.

Distinct polymorphism for salinity tolerance between salinity tolerant and susceptible varieties was shown by RM 1287.

Distinct bands for drought tolerance was shown by RM 490 in PTB-7

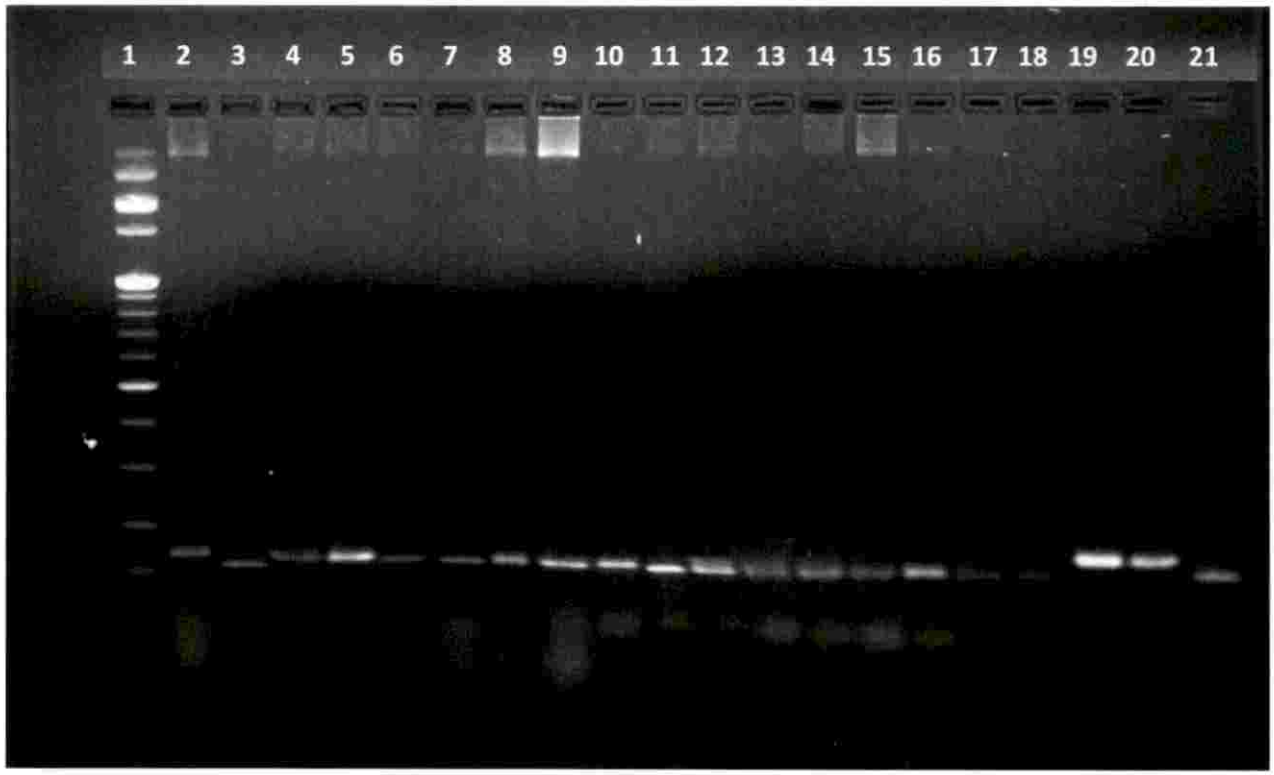


Plate 15. Amplification pattern of 20 rice varieties obtained by SSR marker RM 6100(Lane 1 – 100bp ladder, Lane 2 – chomala, Lane 3 - M0-18, Lane 4 – PTB 35, Lane 5 -PTB 60, Lane 6 – PTB 39, Lane 7 – PTB 55, Lane 8 – PTB 30, Lane 9 – PTB 7, Lane 10 – CR dhan 307, Lane 11 – Apo, Lane 12 – Vyttila -3, Lane 13 – Vyttila -4, Lane 14 – Vyttila -5, Lane 15 – Vyttila -6, Lane 16 – Vyttila -7, Lane 17 – Vyttila -8, Lane 18 – Vyttila -9, Lane 19 – N-22, Lane 20 – NL -44, Lane 21 – Vyttila -10)

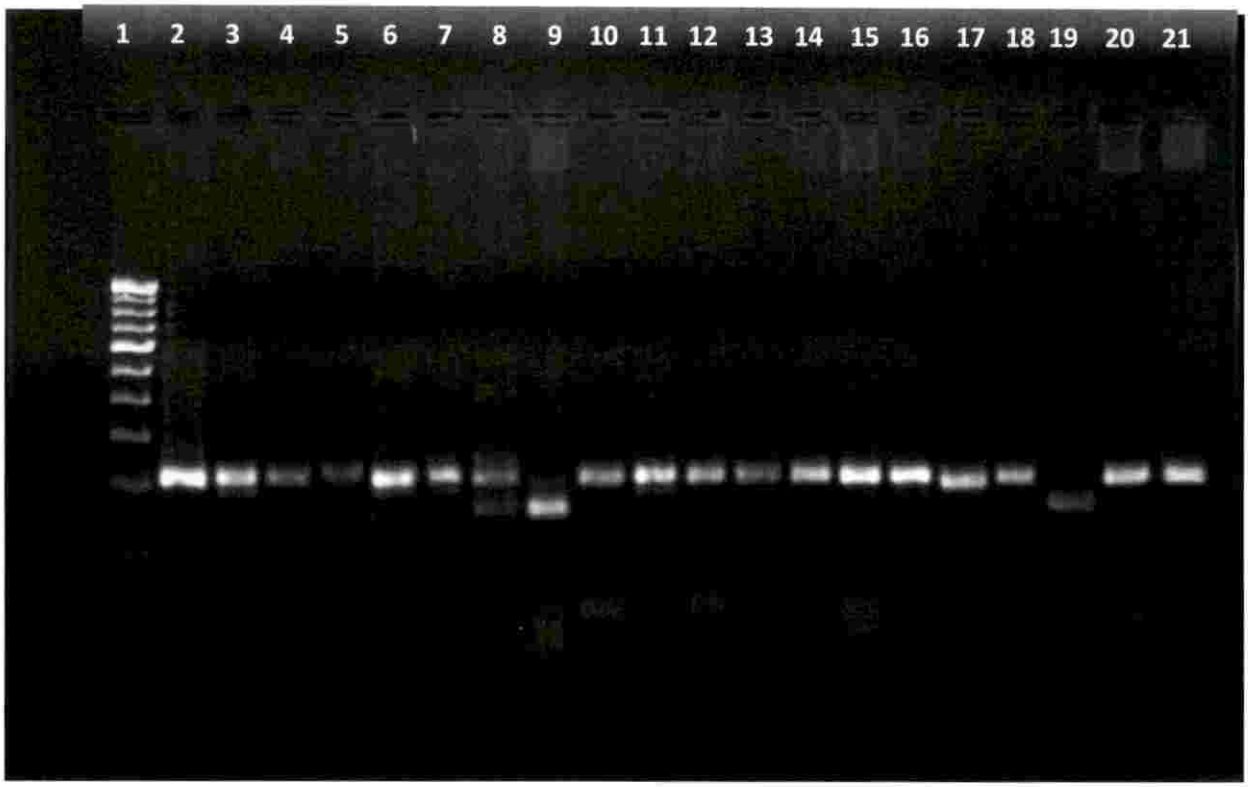


Plate 16. Amplification pattern of 20 rice varieties obtained by SSR marker RM 7076(Lane 1 – 100bp ladder, Lane 2 – chomala, Lane 3 - MO-18, Lane 4 – PTB 35, Lane 5 -PTB 60, Lane 6 – PTB 39, Lane 7 – PTB 55, Lane 8 – PTB 30, Lane 9 – PTB 7, Lane 10 – CR dhan 307, Lane 11 – Apo, Lane 12 – Vyttila -3, Lane 13 – Vyttila -4, Lane 14 – Vyttila -5, Lane 15 – Vyttila -6, Lane 16 – Vyttila -7, Lane 17 – Vyttila -8, Lane 18 – Vyttila -9, Lane 19 – N-22, Lane 20 – NL -44, Lane 21 – Vyttila -10)

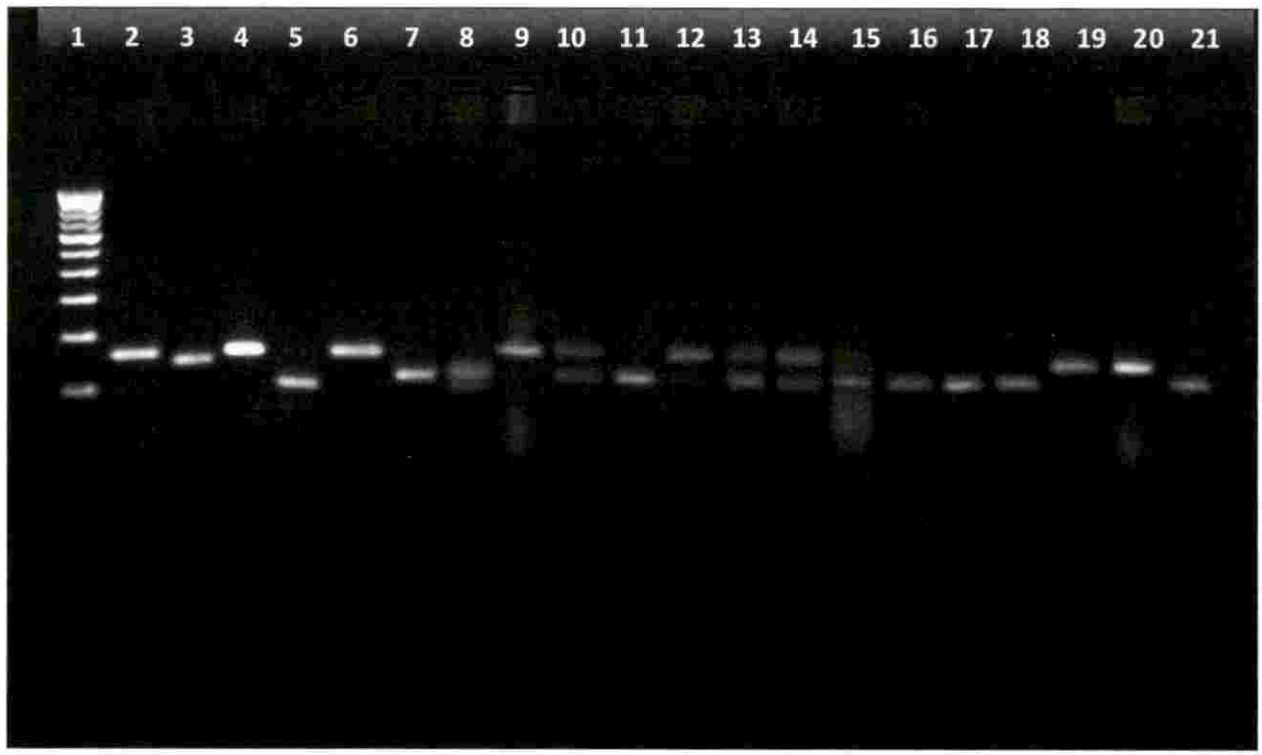


Plate 17. Amplification pattern of 20 rice varieties obtained by SSR marker RM 5749(Lane 1 – 100bp ladder, Lane 2 – chomala, Lane 3 - M0-18, Lane 4 – PTB 35, Lane 5 -PTB 60, Lane 6 – PTB 39, Lane 7 – PTB 55, Lane 8 – PTB 30, Lane 9 – PTB 7, Lane 10 – CR dhan 307, Lane 11 – Apo, Lane 12 – Vyttila -3, Lane 13 – Vyttila -4, Lane 14 – Vyttila -5, Lane 15 – Vyttila -6, Lane 16 – Vyttila -7, Lane 17 – Vyttila -8, Lane 18 – Vyttila -9, Lane 19 – N-22, Lane 20 – NL -44, Lane 21 – Vyttila -10)

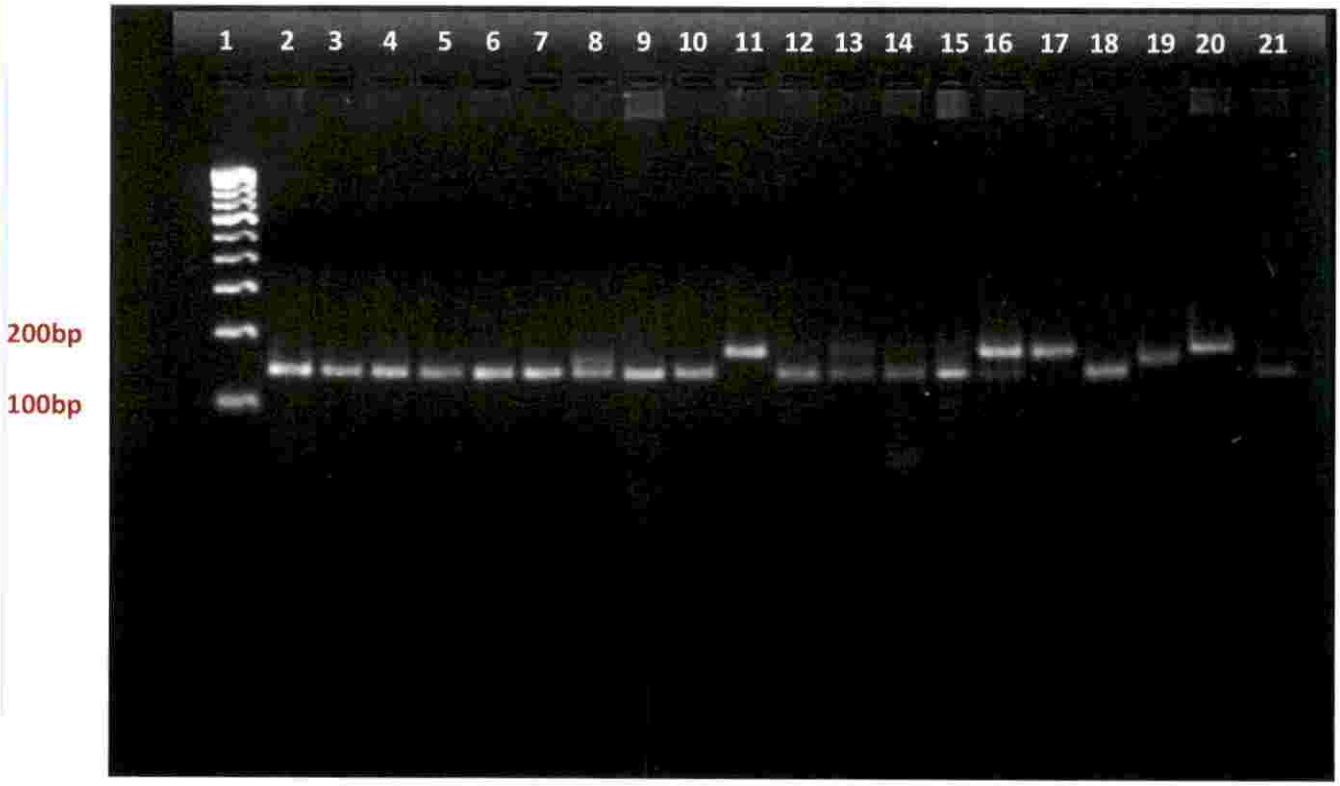


Plate 18. Amplification pattern of 20 rice varieties obtained by SSR marker RM 26212 (Lane 1 – 100bp ladder, Lane 2 – chomala, Lane 3 - MO-18, Lane 4 – PTB 35, Lane 5 -PTB 60, Lane 6 – PTB 39, Lane 7 – PTB 55, Lane 8 – PTB 30, Lane 9 – PTB 7, Lane 10 – CR dhan 307, Lane 11 – Apo, Lane 12 – Vyttila -3, Lane 13 – Vyttila -4, Lane 14 – Vyttila -5, Lane 15 – Vyttila -6, Lane 16 – Vyttila -7, Lane 17 – Vyttila -8, Lane 18 – Vyttila -9, Lane 19 – N-22, Lane 20 – NL -44, Lane 21 – Vyttila -10)

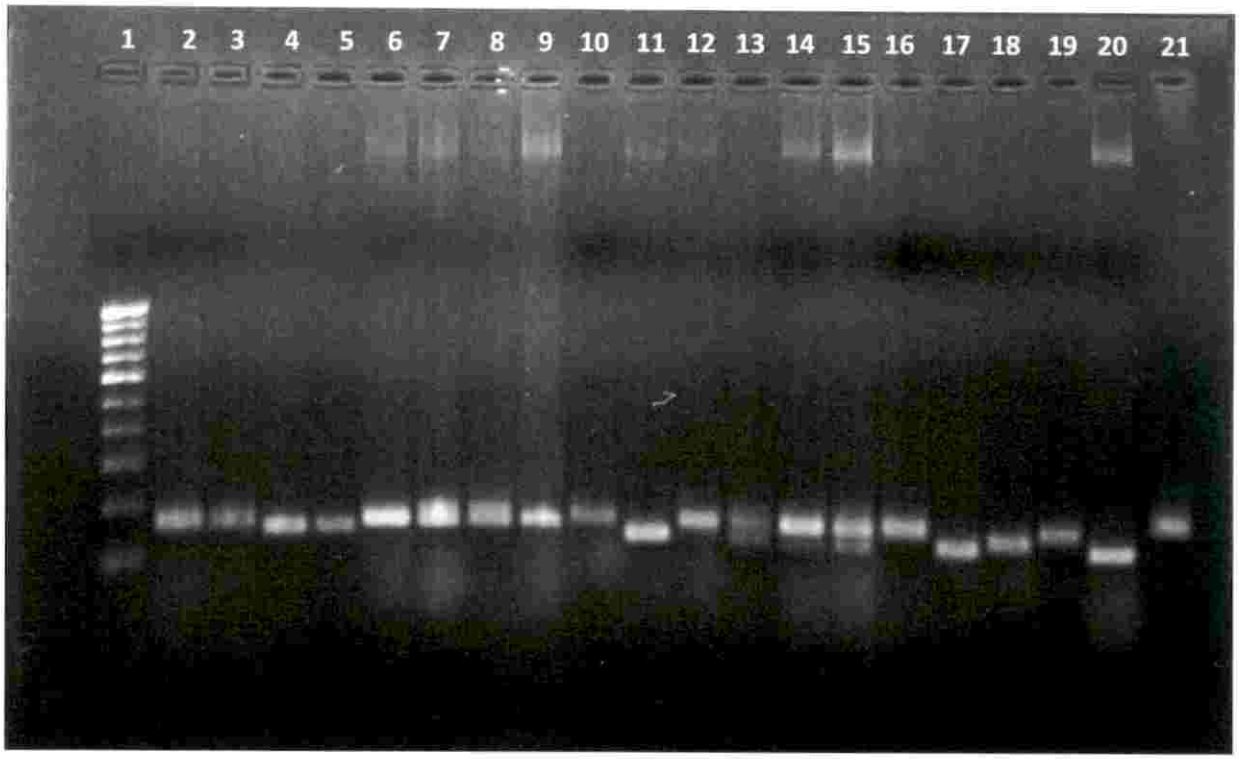


Plate 19. Amplification pattern of 20 rice varieties obtained by SSR marker RM 1287 (Lane 1 – 100bp ladder, Lane 2 – chomala, Lane 3 - MO-18, Lane 4 – PTB 35, Lane 5 -PTB 60, Lane 6 – PTB 39, Lane 7 – PTB 55, Lane 8 – PTB 30, Lane 9 – PTB 7, Lane 10 – CR dhan 307, Lane 11 – Apo, Lane 12 – Vyttila -3, Lane 13 – Vyttila -4, Lane 14 – Vyttila -5, Lane 15 – Vyttila -6, Lane 16 – Vyttila -7, Lane 17 – Vyttila -8, Lane 18 – Vyttila -9, Lane 19 – N-22, Lane 20 – NL -44, Lane 21 – Vyttila -10)

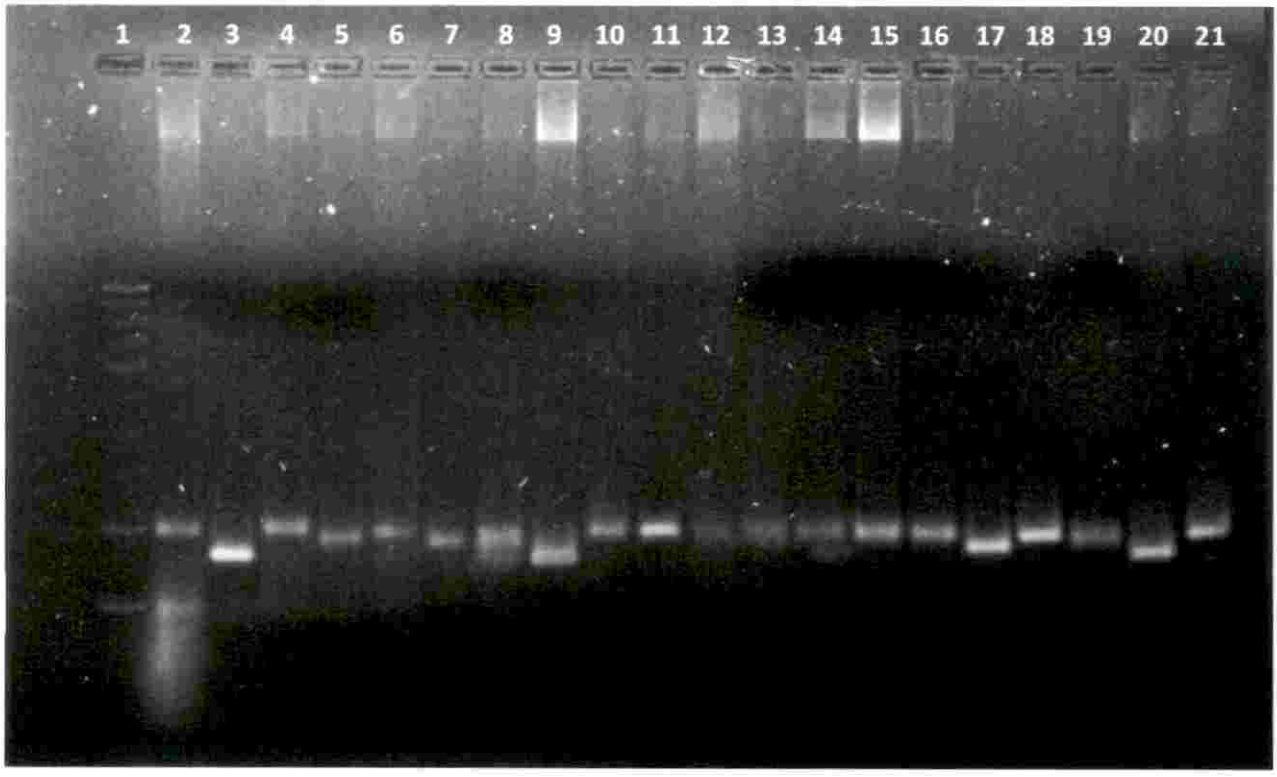


Plate 20. Amplification pattern of 20 rice varieties obtained by SSR marker RM 8094 (Lane 1 – 100bp ladder, Lane 2 – chomala, Lane 3 - MO-18, Lane 4 – PTB 35, Lane 5 -PTB 60, Lane 6 – PTB 39, Lane 7 – PTB 55, Lane 8 – PTB 30, Lane 9 – PTB 7, Lane 10 – CR dhan 307, Lane 11 – Apo, Lane 12 – Vyttila -3, Lane 13 – Vyttila -4, Lane 14 – Vyttila -5, Lane 15 – Vyttila -6, Lane 16 – Vyttila -7, Lane 17 – Vyttila -8, Lane 18 – Vyttila -9, Lane 19 – N-22, Lane 20 – NL -44, Lane 21 – Vyttila -10)

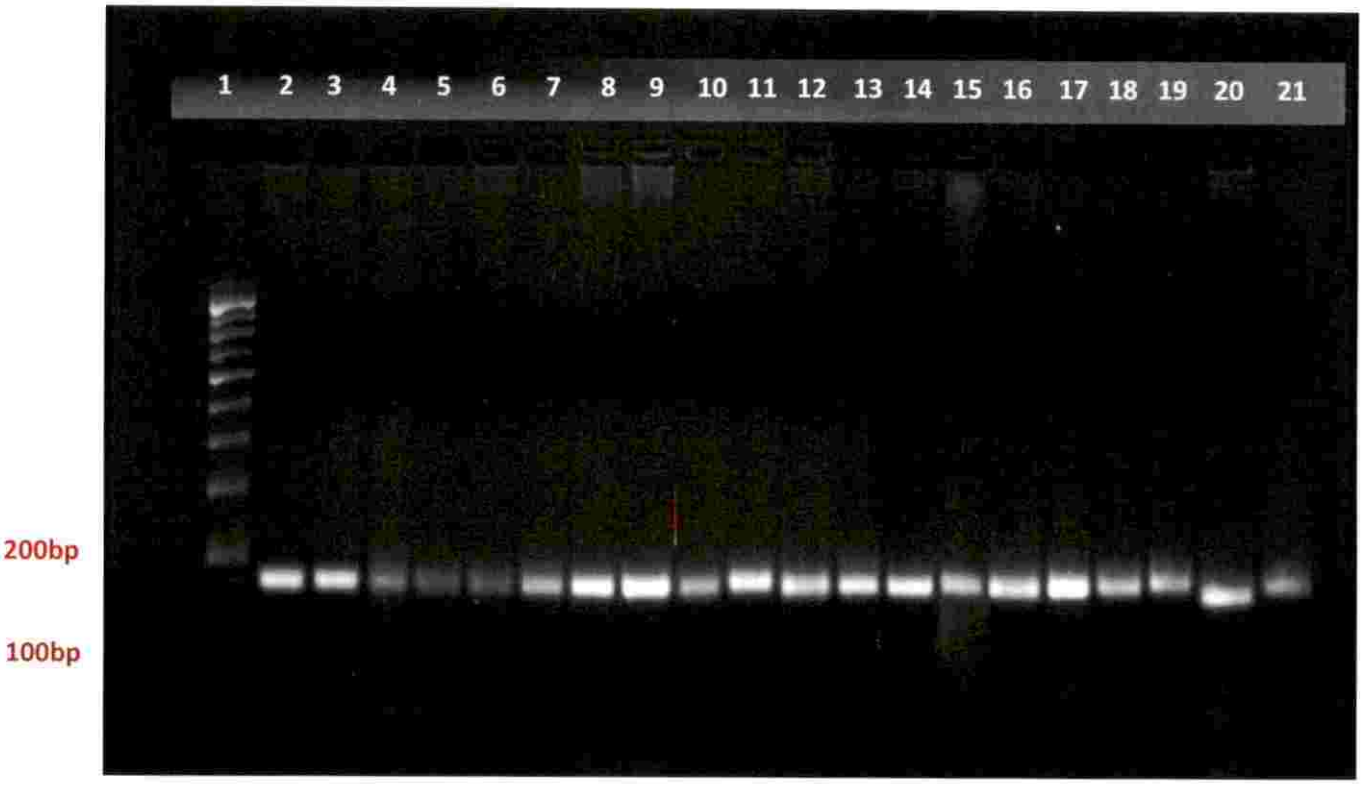


Plate 21. Amplification pattern of 20 rice varieties obtained by SSR marker RM 10843 (Lane 1 – 100bp ladder, Lane 2 – chomala, Lane 3 - MO-18, Lane 4 – PTB 35, Lane 5 -PTB 60, Lane 6 – PTB 39, Lane 7 – PTB 55, Lane 8 – PTB 30, Lane 9 – PTB 7, Lane 10 – CR dhan 307, Lane 11 – Apo, Lane 12 – Vyttila -3, Lane 13 – Vyttila -4, Lane 14 – Vyttila -5, Lane 15 – Vyttila -6, Lane 16 – Vyttila -7, Lane 17 – Vyttila -8, Lane 18 – Vyttila -9, Lane 19 – N-22, Lane 20 – NL -44, Lane 21 – Vyttila -10)

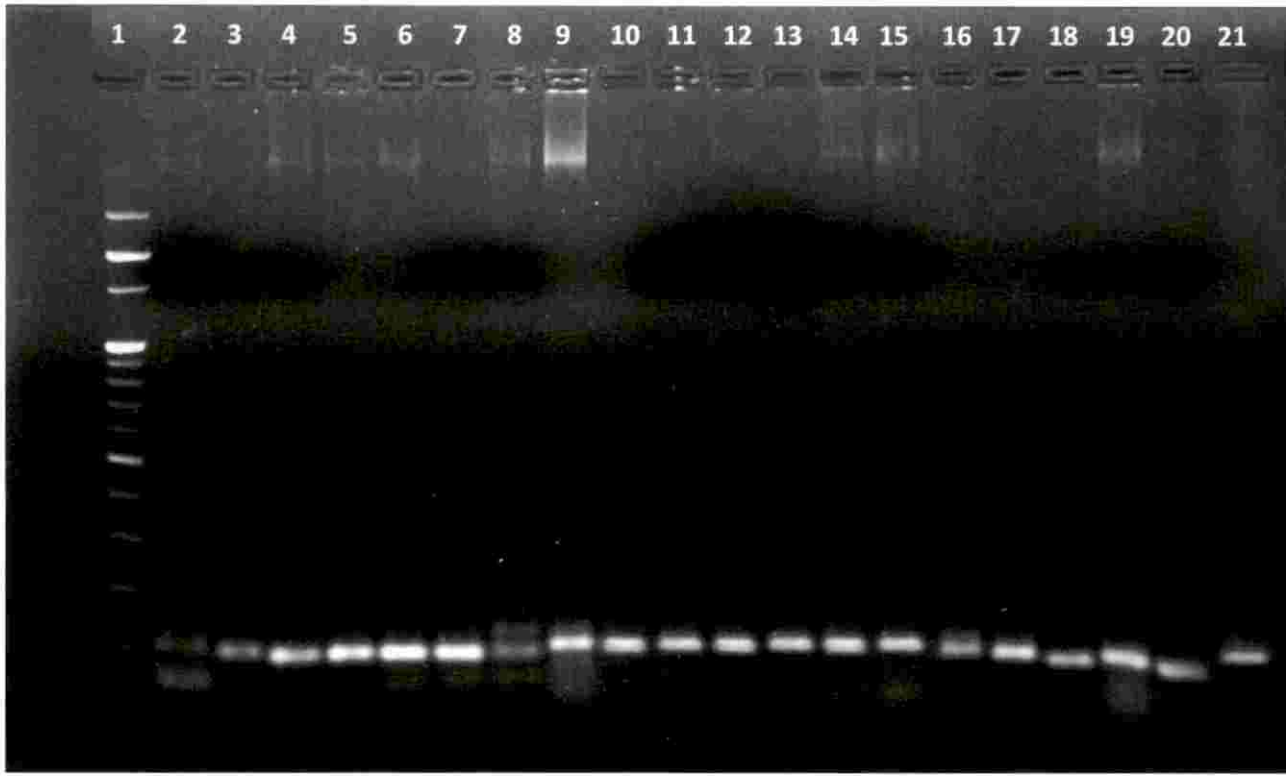


Plate 22. Amplification pattern of 20 rice varieties obtained by SSR marker RM 490 (Lane 1 – 100bp ladder, Lane 2 – chomala, Lane 3 - MO-18, Lane 4 – PTB 35, Lane 5 -PTB 60, Lane 6 – PTB 39, Lane 7 – PTB 55, Lane 8 – PTB 30, Lane 9 – PTB 7, Lane 10 – CR dhan 307, Lane 11 – Apo, Lane 12 – Vyttila -3, Lane 13 – Vyttila -4, Lane 14 – Vyttila -5, Lane 15 – Vyttila -6, Lane 16 – Vyttila -7, Lane 17 – Vyttila -8, Lane 18 – Vyttila -9, Lane 19 – N-22, Lane 20 – NL -44, Lane 21 – Vyttila -10)

Discussion

5. DISCUSSION

Global climate change is a major threat that affects crop production. Climate change leads to drought, flood, acidification of soil, salinization, variation in temperature and other adverse environmental conditions. Crop yield and quality are the principal factors which get affected directly or indirectly due to these stress factors. The world's population is also estimated to be 9 billion by 2050 and therefore in order to feed another two billion individuals in the next 40 years, there is a need to boost crop output (Ashikari and Ma, 2015).

Plants are simultaneously subjected to a mixture of biotic and abiotic stresses, which reduces crop output. In relation to several common reactions as part of its stress tolerance approach, the multiple abiotic stress on crops display tailor-made physiological and molecular response. These customized responses are only proposed in crops subjected to concurrent stress. Individual stress surveys cannot infer this data. Plants have developed complicated processes to perceive outside signals to survive under stress circumstances, which enable optimal response to environmental circumstances (Fujita *et al.*, 2006).

Plants have stress tolerance or stress prevention *via* mechanisms of acclimatization and adaptation developed by natural selection. Following recognition of stress, regulatory response eventually restores homeostasis or reduces the impacts of episodic shock. When plants are adapted to similar environments functional conservation of traits that enable higher yields in abiotic stress was identified across species (Mickelbar *et al.*, 2015).

The direct selection of tolerant genotype under abiotic stress condition is somewhat hindered by the influence of environmental factors which are having a significant role in the response of plants and it is also a time-consuming process.

DNA markers can be used for the identification of QTLs by the advances in molecular biology methods. The application of QTLs has enhanced the selection effectiveness, especially in those characteristics regulated by multiple genes and extremely affected by environment (Flowers, 2004). QTLs and markers are of advantages over direct phenotypic screening, particularly as a PCR-based methodology, markers reduce the time needed to screen individuals and reduce the environmental impact of the study feature (Yamaguchi and Blumwald, 2005).

In the present study, 20 rice varieties collected from RARS, Pattambi. RARS, Vyttila. NRRI, Cuttack, Orissa were evaluated for physio-morphological and yield parameters for single abiotc stress (drought, salinity and temperature) and the varieties having better tolerance capacity were subjected to combination of stress treatments. The selected varieties from combination stress were analyzed in pot culture study for various physiological and yield parameters. Then DNA polymorphism analysis was carried out by using reported microsatellite markers linked to drought, salinity and temperature. Significant variations were observed for all the parameters studied and the results obtained are discussed in this chapter with appropriate support from previous studies.

5.1 EFFECT OF ABIOTIC STRESS ON PHYSIOLOGICAL, BIOCHEMICAL AND YIELD PARAMETERS

In this study, various physiological and biochemical parameters were studied for identifying multiple abiotic stress tolerant varieties and this section explains the basis of results obtained.

5.1.1 Variation in seedling vigour index at different drought stress levels.

Increased stress severity resulted in drastic decrease in the seedling vigor index (Bina and Bostani, 2017). Nautiyal (2009) also reported that germination and seedling vigour was found to be affected under simulated drought stress in different PEG

solutions. This may be due to the decreased availability of water required for early seed germination and development. In the present study, seedling vigour index was reduced under stress condition. In accordance with increase in drought stress level seedling vigour index was also reduced. Among 20 varieties maximum seedling vigour index at -5bar PEG 6000 was observed in PTB-7, PTB-60 and PTB-35. Similar results were also reported by Kaya *et al.* (2006). Variation of seedling vigour index at different drought stress level are depicted in Fig.1.

5.1.2. Variation in proline content (µg/g tissue) at different drought stress levels.

Proline accumulation in plant tissues is a clear indicator of environmental stress, especially in crops under drought stress (Routley, 1966). This may be due to the ability of proline to maintain osmotic balance and help the plants to adapt under stress condition. In the present study drought stress recorded maximum proline content in PTB-7 at -5bar PEG6000 followed by PTB-35. A negative correlation was observed between seedling vigour index and increase in drought stress levels and these findings are in accordance with Vendruscolo *et al.* (2007) who reported a severe accumulation of proline in wheat plants subjected to drought stress for 15 days. Similar observations were also reported in rice by Beena *et al.* (2012). Variation in proline content at different drought stress level is depicted in Fig 2.

5.1.3. Variation in seedling vigour index at different salinity stress levels.

Salinity decreases the rate of germination and seedling vigour index, leading to lower plant development and final crop yield (Janmohammadi *et al.*, 2008). This may be due to decreased shoot length, root length and germination percentage under stress conditions. Among the different varieties studied at 250mM NaCl maximum seedling vigour index was observed in MO-18 followed by Vyttila-3 and Vyttila-9. The seedling vigour index was reduced according to the increase in stress levels. Similar

findings were also reported by Zhang *et al.* (2007). Variation in seedling vigour index at different salinity stress level is expressed in Fig 3.

5.1.4. Variation in Na^+ - K^+ ratio at different salinity stress levels.

High levels of Na adversely influence the acquisition of K (Munns *et al.*, 2010). Sodium competes with K for absorption through common transport scheme and does this efficiently since the concentration of Na in saline environment is generally significantly higher than that of K. The sensitivity of the plants to salinity is due to the failure to prevent Na and Cl from transpiration streams (Gorham *et al.*, 1990). In the present study Na^+- K^+ ratio was found to be increased in accordance with the increase in concentration of NaCl. Minimum Na^+- K^+ ratio was observed in N-22 and Maximum Na^+- K^+ ratio was observed in PTB-55 at 250 mM NaCl (9.5). Similar results were reported by Bohra and Doerffling, (1993). Variation in Na^+ - K^+ ratio at different salinity stress level is depicted in Fig. 4.

5.1.5. Variation in seedling vigour index at different temperature stress levels.

Increase in temperature stress significantly affects seed germination. Most varieties lack the ability to withstand temperature stress, and a significant germination differences and related characteristics were observed under temperature stress condition (Ashraf and Abu-Shakra, 1978). This may be due to the decreased water potential and wet condition required for early seed development and establishment under stress conditions (Babu and Rosaiah, 2017). In the present study, Seedling vigour index was reduced under temperature stress condition. The maximum seedling vigour index was observed in N-22. Similar results were also reported by Yu and Tuinstra, (2001). Variation in seedling vigour index at 35^0C temperature stress is depicted in Fig. 5.

5.1.6. Variation in cell membrane stability index (%) under temperature stress levels.

The cell membrane stability index in crops is correlated with water and elevated temperature tolerance (Blum and Ebercon, 1981). This may be due to the production of high concentration of malondialdehyde under stress condition, which leads to lipid peroxidation and oxidation of fatty acids in cell membrane and ultimately lead to cell damage and decreased cell membrane stability index (Dacosta and Hoang, 2007). Among varieties CMS index was found maximum in N-22 under temperature stress condition. Similar results were also reported by Prasad *et al.* (2006). Variation in cell membrane stability index at $35^{O}C$ temperature stress is depicted in Fig. 6.

5.1.7. Variation in Seedling vigour index under the combined stress of drought and salinity.

Seedling vigour index was reduced under all single abiotic stress treatments such as drought, temperature and salinity. A drastic decrease in seedling vigour index was observed under the combination stress of highest tolerated level of drought and highest tolerated level of salinity. This is due to the decreased water availability needed for early seed development and establishment (Babu and Rosaiah, 2017). Among varieties the maximum seedling vigour index at this stress level was observed in PTB – 7. Variation in seedling vigour index under drought and salinity are shown in Fig. 7.

5.1.8 Variation in Seedling vigour index under the combined stress of temperature and salinity condition

Under the combination stress of highest tolerated level of salinity and temperature resulted a severe reduction in seedling vigour index compared to that of

individual stress treatments. This may be due to poor water availability required for germination and seed development under stress conditions (Zhang *et al.*, 2007). The maximum seedling vigour index at this stress level was observed in NL-44. . Variation in seedling vigour index under temperature and salinity are shown in Fig. 8.

5.1.7 Variation in plant height (cm) under the combined stress of drought and salinity.

The plant height was reduced under drought stress, especially under severe drought stress (Anosheh *et al.*, 2012). Munns *et al.* (2006) reported a reduction in plant height under salinity stress, This may be due to changes in internal water conditions affecting stem growth and shrinkage of stem diameter. In the present study plant height was reduced under the combination of drought and salinity stress. The decreased plant height under stress condition may be due to either inhibiton of cell growth or cell division by water deficits (Ahmed *et al.*, 2013). Among varieties the maximum plant height under drought and salinity stress was observed in PTB-7. Variation in plant height at drought and salinity is expressed in Fig. 9.

5.1.8 Variation in Number of productive tillers under combined stress of drought and salinity.

Number of productive tillers reduces under combined stress of drought and salinity this directly affect the grain yield (Bhutta, 2006). This may be due to decreased translocation of assimilates under water stress condition. In the present study also, Under the combination stress of drought and salinity, maximum number of productive tillers was observed in PTB-7 rice variety. Similar results were also reported by Blum *et al.* (1990) and Castillo *et al.* (2007) in rice. Variation in no. of productive tillers at drought and salinity is expressed in Fig. 10.

5.1.9. Variation in spikelet fertility percentage (%) under combined stress of drought and salinity.

Jagadish *et al.* (2008) reported a 50% reduction in all spikelets under combined stress of drought and salinity. This may be due to the effect of abiotic stress on behavior of tapetum, anther dehiscence, pollen release, pollen germination and fertilization (Zhou *et al.*, 2017). In the present study spikelet fertility decreased under the combination stress of drought and salinity. Among the varieities the maximum spikelet fertility was observed in PTB-7. Variation in spikelet fertility percentage (%) at drought and salinity stress is expressed in Fig. 12.

5.1.10. Variation in Pollen viability percentage (%) under combined stress of drought and salinity.

Abdullah *et al.* (2001) reported a considerable reduction in pollen viability in salinized plants. In the present study pollen viability was reduced in plants under various treatment condition. High salinity can lead to physiological drought-like osmotic stress, and the high soil salt deposition makes it increasingly difficult for plants to acquire water and nutrients and thus affecting pollen germination and viability (Verslues *et al.*, 2006). The highest pollen viability percentage was observed in Vyttila–9. Variation in pollen viability percentage (%) at drought and salinity stress is expressed in Fig. 11.

5.1.11. Variation in yield per plant (g) under the combined stress of drought and salinity.

Water deficits massively reduce crop yields by decreasing the amount of spikelets at the flowering stage (Ekanayake *et al.*, 1989). Soil salinity adversely affect crop growth, Germination and yield (Sairam *et al.*, 2002). This may be due to, reduction in pollen viability under severe salt stress condition and which in turn reduces the crop yield. Under the combined stress of drought and salinity yield per

plant was reduced. Among the varieties maximum yield was observed in PTB-7. Similar results were also reported by Daei *et al.* (2009). Variation in yield per plant at drought and salinity stress is expressed in Fig. 13.

5.1.12. Variation in proline content (µg/g) under the combined stress of drought and salinity.

Osmoprotectants, such as proline, glycine betaine, mannitol, and sugars are the common metabolites which are produced when plants are exposed to different stresses viz, drought, salinity and temperature, it confer stress tolerance to plants (Yamada *et al.*, 2005). This may be due to the role of proline in osmotic adjustment and conferring ability in plants to withstand cellular dehydration due to salinity, drought or extreme temperatures (Szabados and Savoure, 2010). In the present study, Proline content increased in varieties under stress treatment. Among the varieties maximum proline content was observed in PTB-7. Variation in proline content at drought and salinity stress is expressed in Fig. 14.

5.1.13. Variation in cell membrane stability index (%) under the combined stress of drought and salinity.

The cell membrane stability index in crops is correlated with water and elevated temperature tolerance (Blum and Ebercon, 1981). The decrease in cell membrane stability is due to the increased permeability and leakage of ions out of the cell during drought and salinity stress conditions (Elbasyoni *et al.*, 2017). Among the varieties, CMS index was found maximum in PTB -7 (99.02%). In all varieties CMS was found to be reduced under stress condition. Similar findings were observed by Sairam *et al.* (1997). Variation in cell membrane stability index at drought and salinity stress is expressed in Fig. 15.

5.1.14 Variation in malondialdehyde content (m mol g^{-1}) under the combined stress of drought and salinity.

Malondialdehyde is an oxidative damage indicator. In the present study malondialdehyde content was found increasing in all varieties under stress than that of their corresponding control plants. Among the varieties the maximum malondialdehyde content was observed in Vyttila-9. The results obtained are similar with the findings of Jiang and Huang, (2001) where in they reported that the lipid peroxidation was more in combined stresses of drought and temperature than either stress alone in kentucky bluegrass. Fazeli *et al.* (2007) also reported that NaCl treatment led to a gradual increase in the levels of malondialdehyde in *Phaseolus vulgaris* L. High salinity induced an increase of 44% and 56% of malondialdehyde levels in Tema and Djadida varieties respectively. Variation in malondialdehyde content at drought and salinity stress is expressed in Fig. 16.

5.1.15. Variation in chlorophyll a/b ratio under the combined stress of drought and salinity

Jaleel *et al.* (2008) reported a slight decrease in chlorophyll a and b and total chlorophyll content in mild salinity stress, but severe decrease of these pigments was observed under elevated salinity levels. The proportion of chlorophyll a, chlorophyll b also differed considerably under salinity stress. Mafakheri *et al.* (2010) also reported a reduction in chlorophyll a/b in drought stress condition. In the present study, chlorophyll a/b ratio was found decreased in varieties in both combination of stress treatments. A reduction of 27.7% was recorded in highest levels of drought and salinity stress. Among the different varieties studied maximum chlorophyll a/b was observed in Vyttila–9. Variation in chlorophyll a/b ratio at drought and salinity stress is expressed in Fig. 17.

5.1.16. Variation in Na^+ - K^+ ratio under the combined stress of drought and salinity

Plants must retain elevated levels of K^+ and low levels of Na^+ in cytosol, and elevated levels of K^+/Na^+ are essential for salt tolerance (Gao *et al.*, 2007). Osmotic balance breakdown results in turgidity loss, cell dehydration and subsequently cell death (Cicek and Cakirlar, 2008). The minimum amount of Na^+ - K^+ ratio under the combined stress of drought and salinity was observed in PTB-7. The varieties under salinity stress observed increase in Na^+ - K^+ ratio than their corresponding controls. Similar results were also reported by Al-karaki (2000). The minimum Na+- K+ ratio was observed in PTB-7. Variation in Na^+ - K^+ ratio at drought and salinity stress is expressed in Fig. 18.

5.1.17. Variation in SOD activity under combined stress of drought and salinity

Salt, drought, heat and oxidative stress results in the formation of ROS (such as O_2, H_2O_2, and OH^-) that harm the membranes and macromolecules (Mittler, 2002). The increased SOD activity helps the plants to scavenge free radicals and reactive oxygen species and thus impart tolerance in plants. In the present study also, SOD was found increasing in plants under stress than the control plants. The maximum amount of SOD was observed in PTB–7, under the combined effect of highest tolerated levels of drought and salinity. The plants under combined stress of drought and salinity recorded an increase in SOD production than that of control plants. Similar results were reported by Panda and Khan (2004) and Jiang and Huang (2001). Variation in SOD activity at drought and salinity stress is expressed in Fig. 19.

5.1.18. Variation in plant height under combined stress of temperature and salinity.

Plant height was reduced under temperature stress condition. The mean plant height of varieties under temperature and salinity stress was found to reduce. The reduction in plant height under temperature stress is due to decreased cell growth especially cell elongation (Went, 1953). The highest plant height was observed in N-22. The mean plant height under stress condition was reduced in treatments than control. Similar results were reported by Shao *et al.* (2008) and chu *et al.* (1974) in rice. Variation in plant height at temperature and salinity stress is expressed in Fig. 20.

5.1.19. Variation in Number of productive tillers under combined stress of temperature and salinity

Salinity adversely affect a number of yield elements including the panicles, tillers and spikelets per plant, floret sterility, individual size of grain, and even delayed heading (Grattan *et al.*, 2002). Increasing temperatures from 24 to 32°C resulted in reductions in tiller number (Harsant *et al.*, 2013). The mean number of productive tillers was reduced in rice varieties compared to their corresponding control plants. The highest number of productive tillers was observed in N-22. Variation in number of productive tillers at temperature and salinity stress is expressed in Fig. 21.

5.1.20. Variation in pollen viability under the combined stress of temperature and salinity

Pollen viability and germination are generally adversely affected under saline stress conditions. Pollen viability was reduced in all varieties under treatment condition than that of their corresponding controls. This may be due to the tapetum, the innermost cell layer of the anther wall which plays a crucial role in supplying

nutrients to these microspores and in regulating their release. Temperature and salinity stress during tapetal development (such as early degeneration, hypertrophy, or mutations in the archesporial cell) lead to aborted micro gametogenesis and male sterility (Chaudhury *et al.*, 1993). The maximum pollen viability under temperature and salinity stress was observed in NL-44. Variation in pollen viability at temperature and salinity stress is expressed in Fig. 22.

5.1.21. Variation in spikelet fertility under the combined stress of temperature and salinity

High temperature stress reduces the spikelet fertility. Spikelet sterility was greatly decreased at temperatures higher than 35°C (Shah *et al.*, 2011). The sterility under high temperature is due to inadequate anther dehiscence and poor pollen development, and thus limited numbers of pollen grains germinating on the stigma (Jagadish *et al.*, 2007). In the present study, spikelet fertility was reduced in all varieties under treatment than control. The highest spikelet fertility was observed in N-22. Variation in spikelet fertility percentage at temperature and salinity stress is expressed in Fig. 23.

5.1.22. Variation in yield per plant under the combined stress of temperature and salinity

Yield per plant was reduced in all experimented varieties under stress conditions. This is mainly attributed to the decline in pollen viability, retention of pollen in anthers, and pollen germination under stress conditions (Harsant *et al.*, 2013). The maximum yield per plant was observed in N-22. Similar results were also reported by Gregoria *et al.* (1997). Variation in yield per plant at temperature and salinity stress is expressed in Fig. 24.

5.1.23. Variation in Proline content under the combined stress of temperature and salinity

Proline has various roles, such as stabilizing proteins, membranes and sub cellular structures, and protecting cellular functions by scavenging reactive oxygen species under abiotic stress conditions (Kishor *et al.*, 2005). Proline act as an osmoprotectant and helps plants to maintain cellular homeostasis under saline stress condition (Huang *et al.*, 2013). The maximum proline content under this stress level was observed in N-22. Varieties under stress condition were found to have increased proline content than control. Variation in yield per plant at temperature and salinity stress is expressed in Fig. 25.

5.1.24. Variation in cell membrane stability index (%) under the combined stress of temperature and salinity.

Antioxidants minimize the toxic impacts of ROS under ordinary physiological circumstances. However, under temperature and salinity stress conditions antioxidant concentration is decrease leading to cell harm (Kreiner *et al.*, 2002). Increased cell damage due to ROS can reduce the thermal stability of the membrane, thereby disrupting the motion of water, ion and organic-solutes. Under the combined temperature and salinity stress, maximum cell membrane stability index was observed in N-22. Variation in cell membrane stability index at temperature and salinity stress is expressed in Fig. 26.

5.1.25. Variation in malondialdehyde content (m $molg^{-1}$) under combined stress of temperature and salinity.

Change in MDA content is a reliable measure of structural integrity of membrane under temperature stress condition. This may be due to high temperature

stress affecting membrane stability through lipid peroxidation and leading to the production of peroxide ions and malondialdehyde (MDA). Maximum MDA content in all the treatments at this stress level was observed in MO-18. MDA content in treatments were higher than that of their corresponding controls. Variation in malondialdehyde content at temperature and salinity stress is expressed in Fig. 27.

5.1.26. Variation in Chlorophyll a/b ratio under combined stress of temperature and salinity.

As the most significant photosynthetic pigment, chlorophyll plays an significant role in controlling crop yields. Chlorophyll, however, is quite fragile, not very stable and readily influenced by abiotic stress. The decrease in chlorophyll content under stress condition may be due to stress-induced disruption in biosynthetic pathways or in pigment degradation, loss of the chloroplast membrane, and increased lipid peroxidation thus resulting in production of reactive oxygen species (ROS) (Reddy *et al.*, 2004). The maximum chlorophyll a/b ratio at this stress level was observed in MO-18. The varieties under treatment resulted a decrease in chlorophyll a/b ratio than control. Similar findings were also reported by Mohan *et al.* (2000). Variation in chlorophyll a/b ratio at temperature and salinity stress is expressed in Fig. 28.

5.1.27. Variation in Na^+ - K^+ ratio under combined stress of temperature and salinity.

Salinity harms all factors related plant physiology and biochemistry and significantly reduces yield. This is mainly attributed to the higher ionic imbalances resulting in passive accumulation of Na in root and shoot during salinity stress (Bohra and Doerffling, 1993). As rice plants are salinity susceptible, maintenance of low Na^+-K^+ ratio is beneficial for rice plants. The lowest Na^+-K^+ ratio was observed in N-22 and highest was observed in MO-18. Variation in Na^+ - K^+ ratio at temperature and salinity stress is expressed in Fig. 29.

5.1.28. Variation in SOD activity under combined stress of temperature and salinity.

The severe effects of heat stress are oxidative damage to cells by reactive oxygen species, as found with low temperature, drought, and salinity stress. Plants have developed enzymatic and nonenzymatic scavenging systems to quench active oxygen, and to eliminate the detrimental effects of active oxygen (Bowler *et al.*, 1992). The tolerant variety will have higher SOD activity. The maximum SOD activity under this stress condition was observed in N-22 and minimum was observed in NL-44. Variation in SOD activity at temperature and salinity stress is expressed in Fig. 30.

5.2 VALIDATION QTLs LINKED TO DROUGHT, SALINITY AND TEMPERATURE USING REPORTED SSR MARKERS.

SSR markers offer many benefits over other markers for genetic research. They are extremely polymorphic and abundantly reliable markers with locus specification. In addition, they are distributed over the genome and require little genomic DNA in order to be analyzed. In many plant species, such as soybeans, wheat, maize, barley, rice and potatoes, microsatellite markers have been reported. However, owing to its elevated polymorphism analysis and ease of use, microsatellites (di-or tri-nucleotide repeat sequences) are increasingly used for the identification of varieties as well as for marker assisted breeding (Maniruzzaman *et al.*, 2014).

In the present study 30 reported microsatellites for drought, temperature and salinity were used to screen 20 rice varieties for the identification of multiple stress tolerant varieties. Among the 30 SSR markers only 9 SSR markers, RM 6100, RM 7076, RM 5749, RM 26212 showed polymorphism for heat tolerance. RM 1287,

RM 10843, RM 8094 showed polymorphism for salinity tolerance. RM 490 showed polymorphism for drought tolerance.

RM 6100 is associated with heat tolerance at flowering stage (Bharathkumar *et al.*, 2014) and is located in chromosome 10. Buu *et al.* (2014) reported markers RM 7076, RM 3586, 26212 and RM 5749 were polymorphic for heat tolerance. Vu *et al.* 2012 reported SSR markers RM 1287, RM 8094, RM 3412, RM 493 and RM 140 were linked to the Saltol QTL on chromosome 1. The microsatellite marker, RM 8094 found in Saltol is considered as the superior marker for genetic diversity analysis for analysis of genetic diversity. Rice genotypes with the Pokkali band type for locus RM 8094 marker were either highly tolerant or tolerant to salinity stress at the seedling stage (Nejad *et al.*, 2008).

A major QTL (Saltol) derived from the salt-tolerant cultivar Pokkali has been located on chromosome 1. This QTL confers salinity tolerance at the vegetative stage (Bonilla *et al.*, 2002). RM8085 was mapped on chromosome 1 and is linked to leaf rolling and leaf drying under drought stress (Salunkhe *et al.*, 2011). The QTL on chromosome 9 is associated with spikelet fertility under stress and root and shoot traits (Yue *et al.*, 2006). Major QTL on chromosomes 4 (Ctb1) and 8 (qCTB8) for cold tolerance at the booting stage were identified in a tropical japonica cultivar, Silewah, and markers have been used for introducing the tolerance gene (Ctb1) into japonica cultivars (Kuroki *et al.*, 2007).

Hence in the present study, the varieties N-22, NL-44 and MO-18 are adjuncted as the best tolerant varieties for the combined stress salinity and temperature. But for the drought and salinity condition PTB-7, PTB-35 and Vyttila-9 are adjuncted to be the best tolerant varieties.

Identification of multiple stress tolerant varieties is an essential requirement for developing multiple stress tolerant varieties and the markers linked to different

abiotic stress were found to be usefull tools for the immediate selection of tolerant varieties than considering the phenotypic characters for the selection.

However, the genetic diversity analysis with SSR markers will contribute for the selection of tolerant parents for the future breeding programs. In addition, it will help in identifying future strategies for the sustainable management of genetic resources of rice.

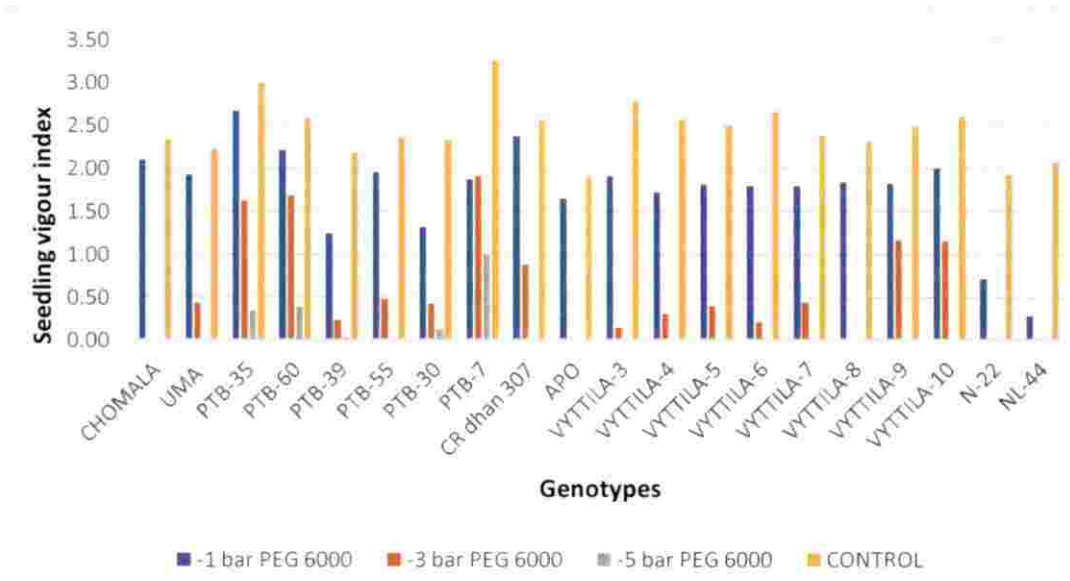


Fig 1. Variation in seedling vigour index at different drought stress levels

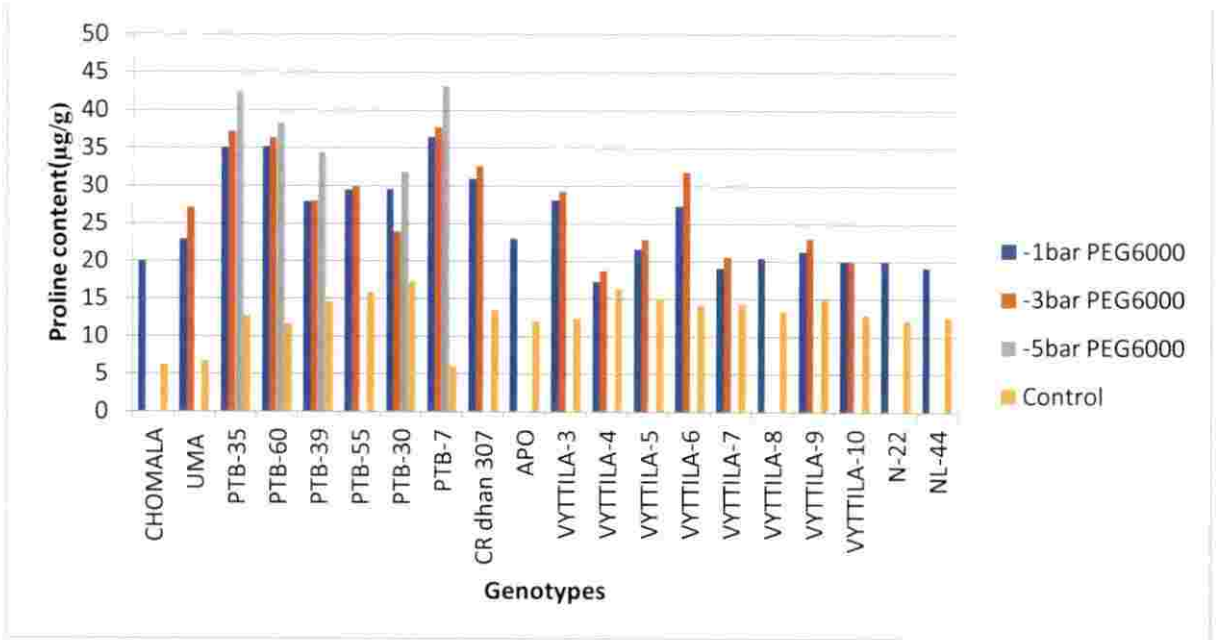


Fig 2. Variation in proline content at different drought stress levels

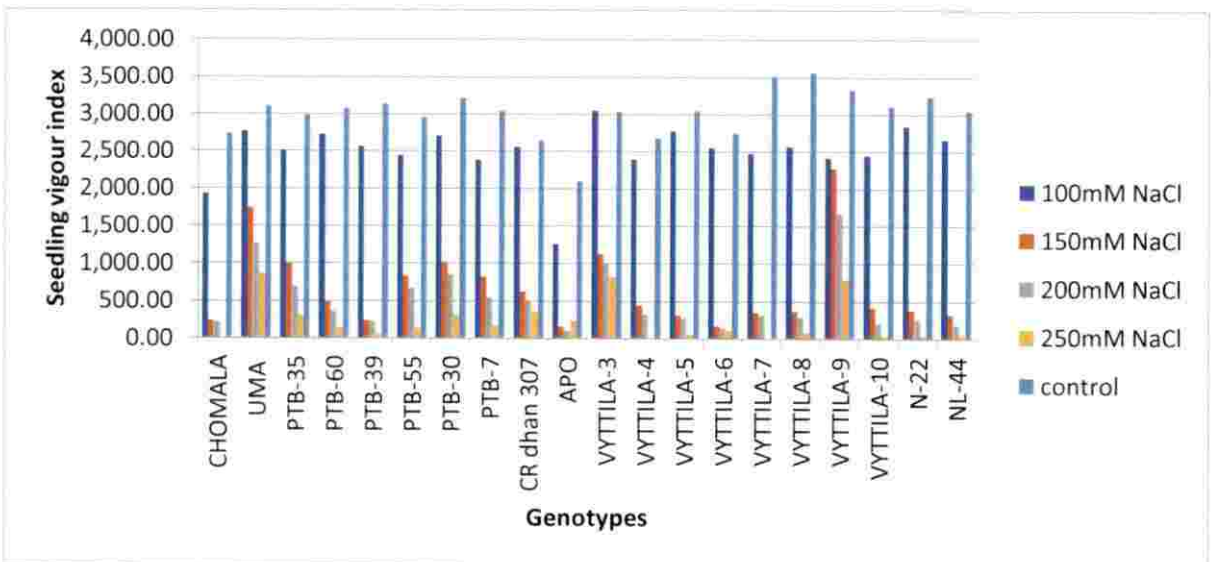


Fig 3. Seedling vigour index at different salinity stress levels

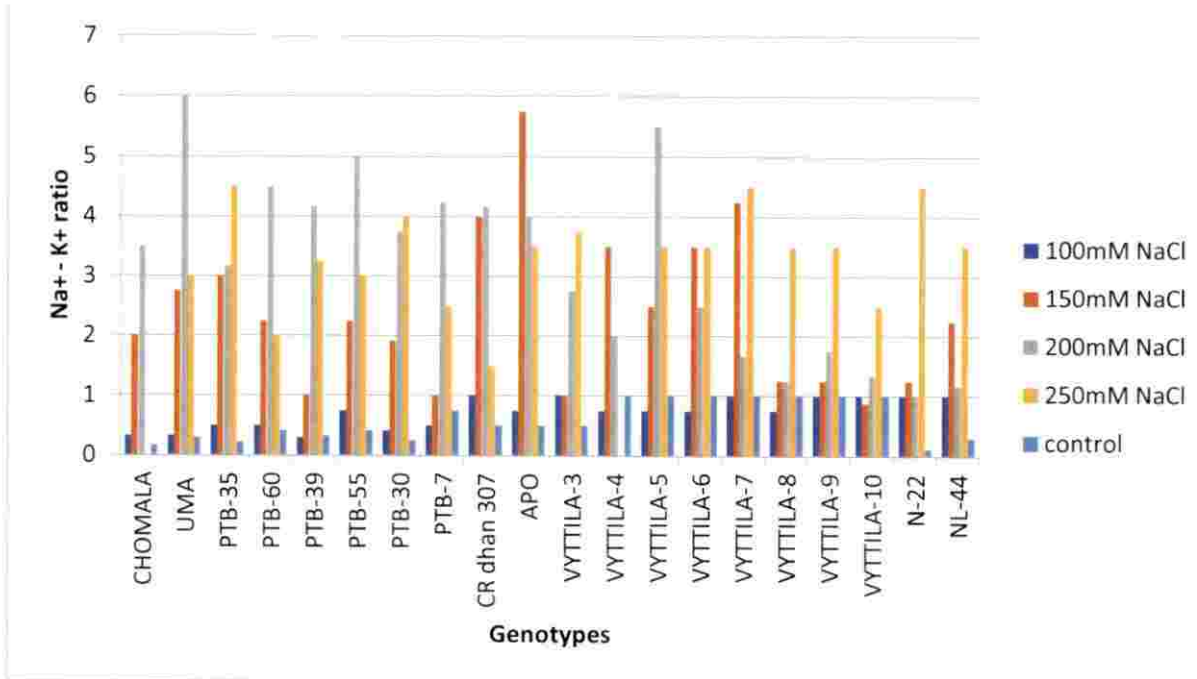


Fig 4. Variation in Na^+ - K+ ratio at different salinity stress levels

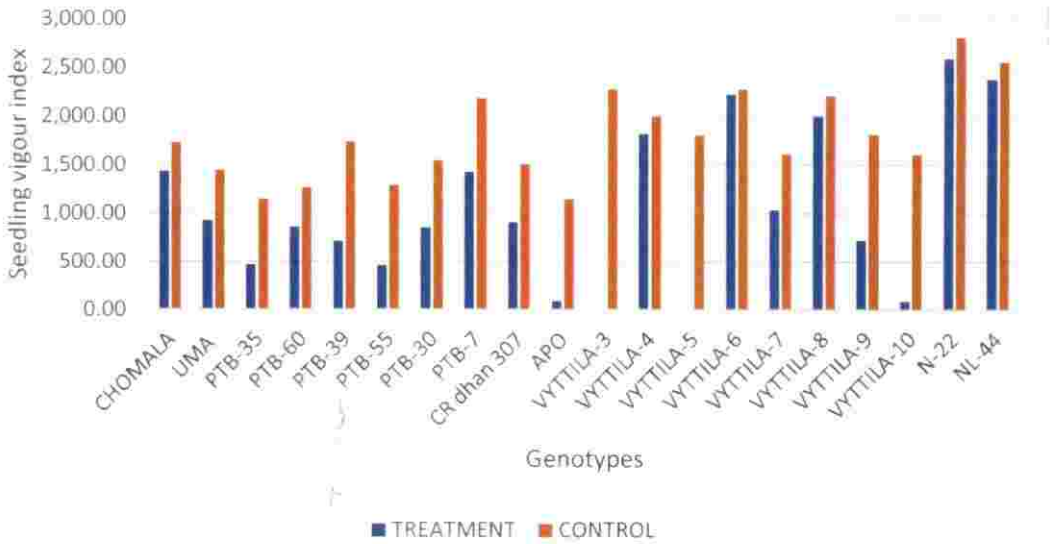


Fig 5. Variation in seedling vigour index at temperature stress of 35^{O}C

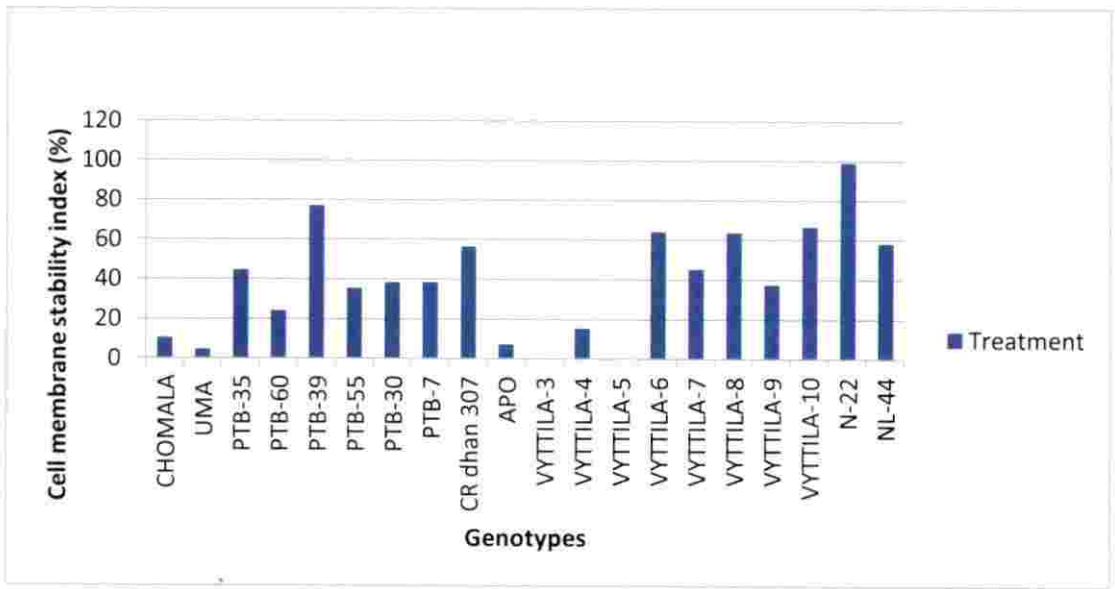


Fig 6. Variation in cell membrane stability index (%) at temperature stress of 35°C

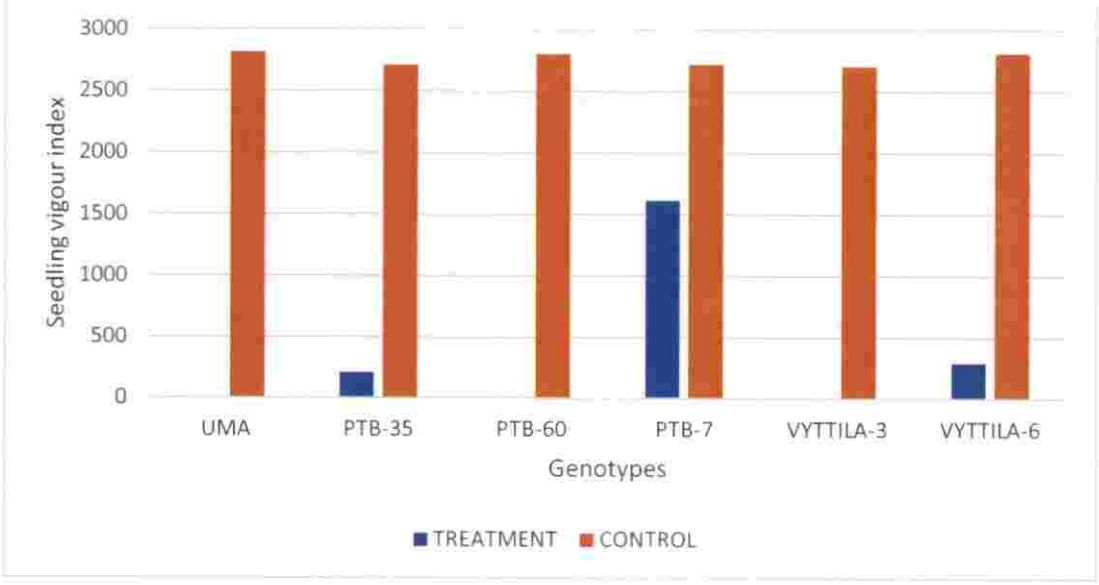


Fig 7. Variation in seedling vigour index under the combined stress of drought and salinity

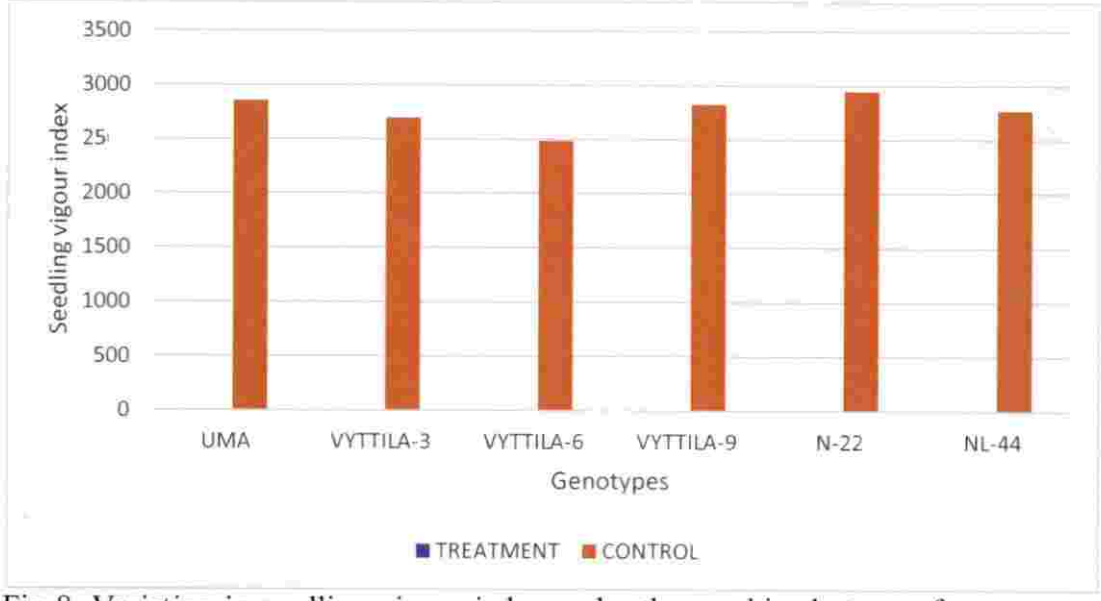


Fig 8. Variation in seedling vigour index under the combined stress of temperature and salinity

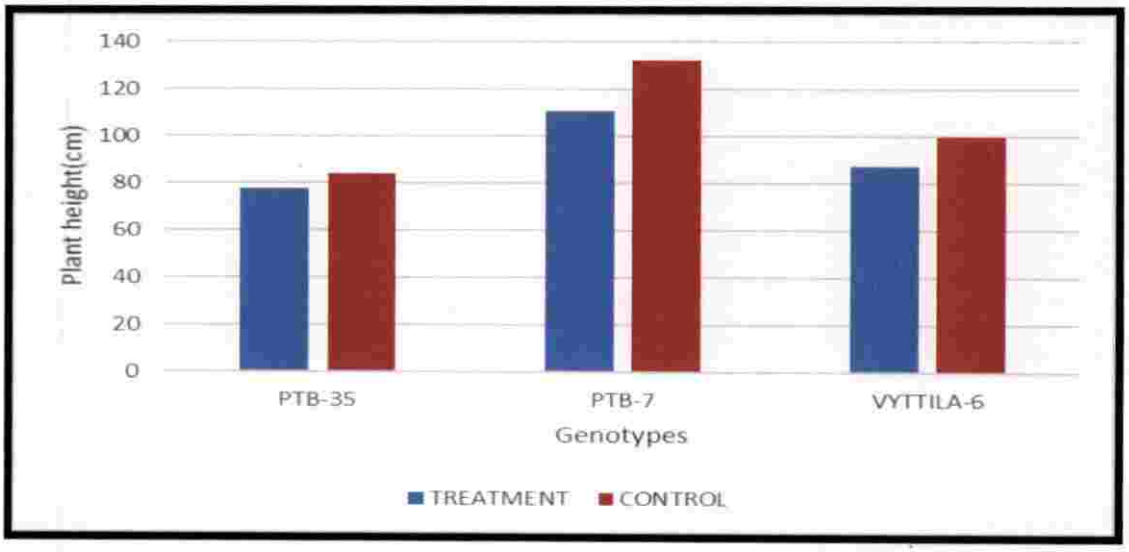


Fig 9. Variation in plant height (cm) under the combined stress of drought and salinity

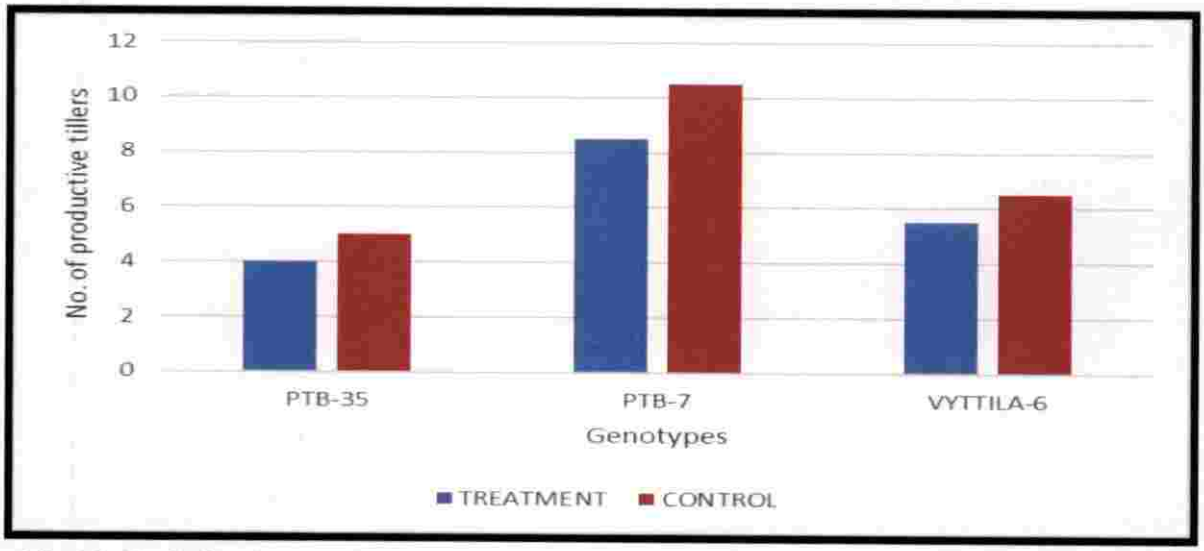


Fig 10. Variation in no. of productive tillers under the combined stress of drought and salinity

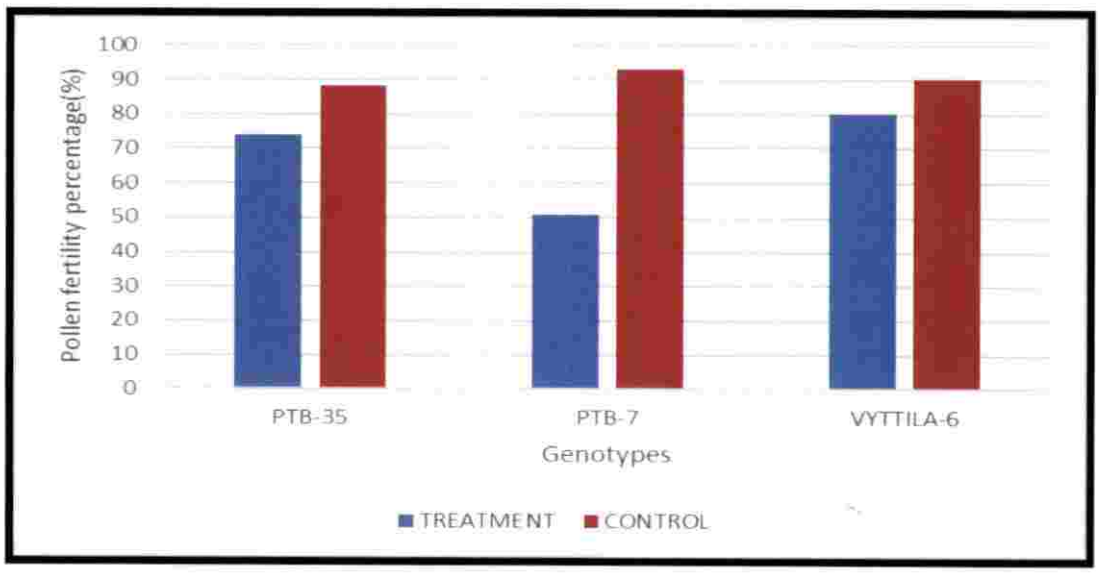


Fig. 12. Variation in pollen viability percentage (%) under combined stress of drought and salinity.

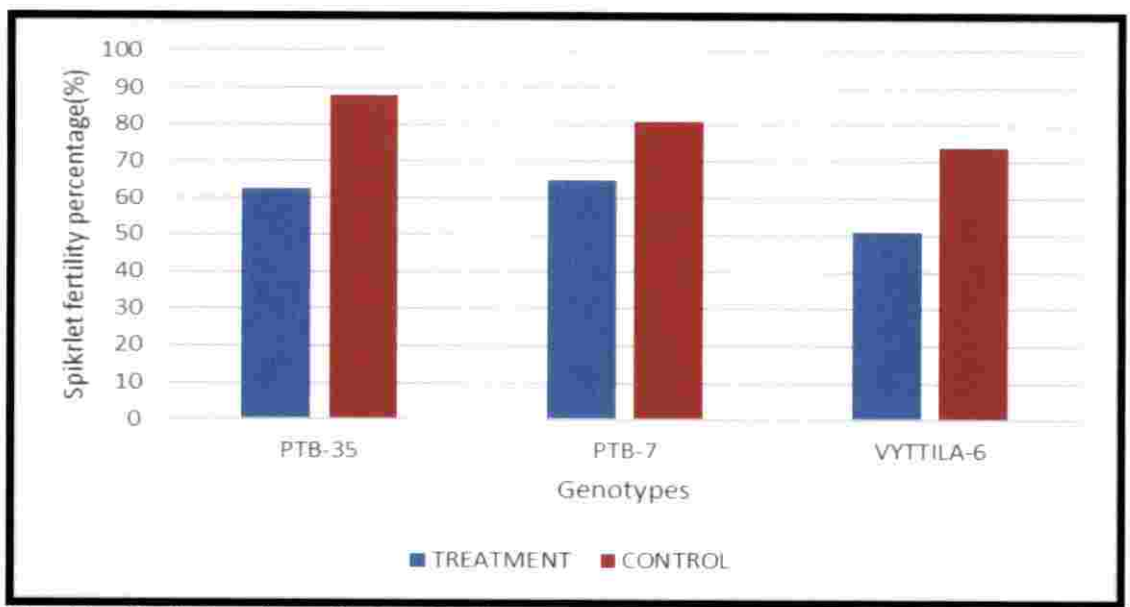


Fig 11. Variation in spikelet fertility percentage (%) under combined stress of drought and salinity

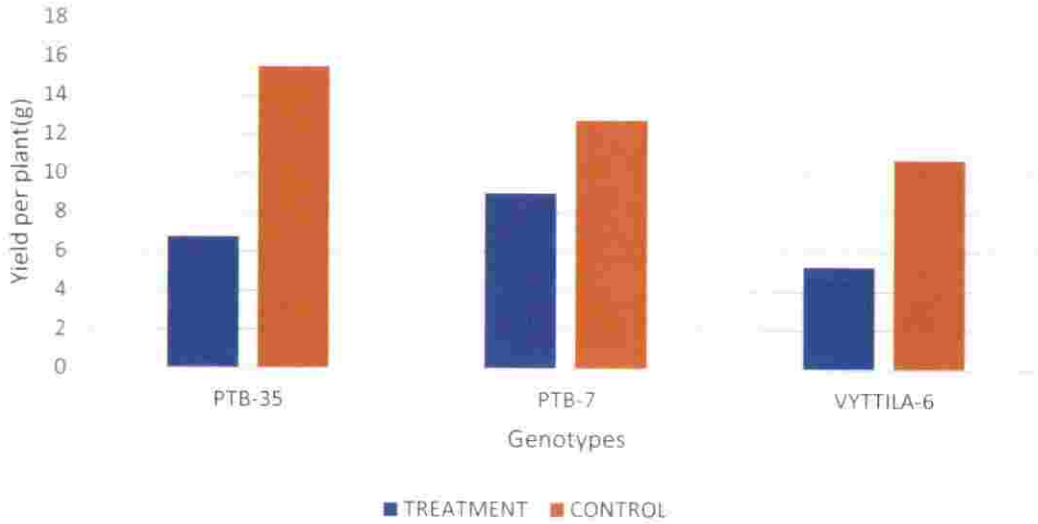


Fig. 13. Variation in yield per plant (g) under the combined stress of drought and salinity

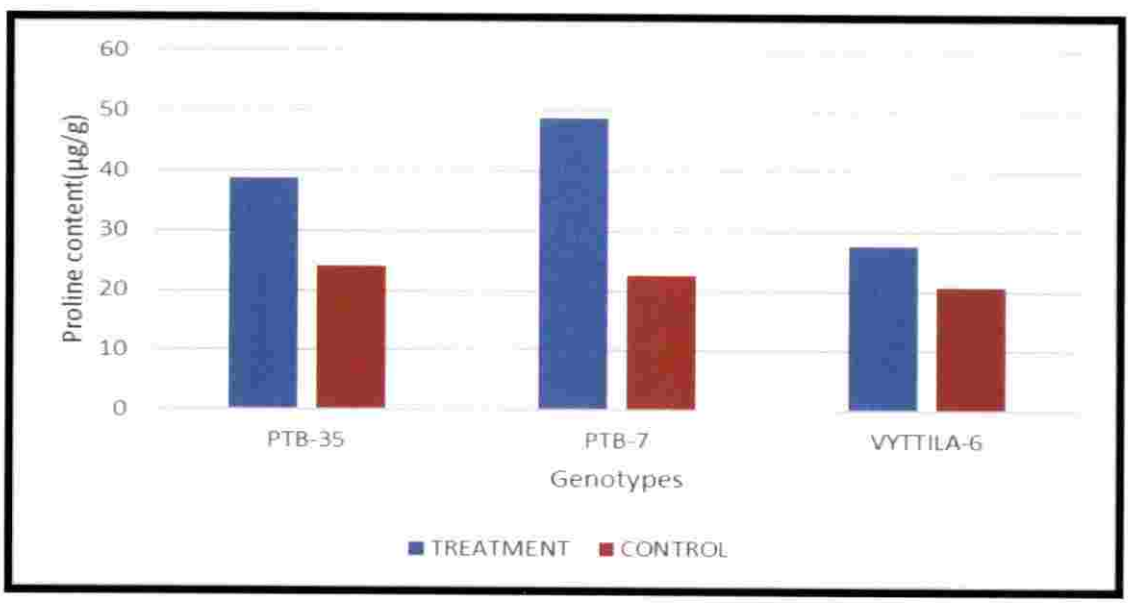


Fig 14. Variation in proline content (µg/g) under the combined stress of drought and salinity

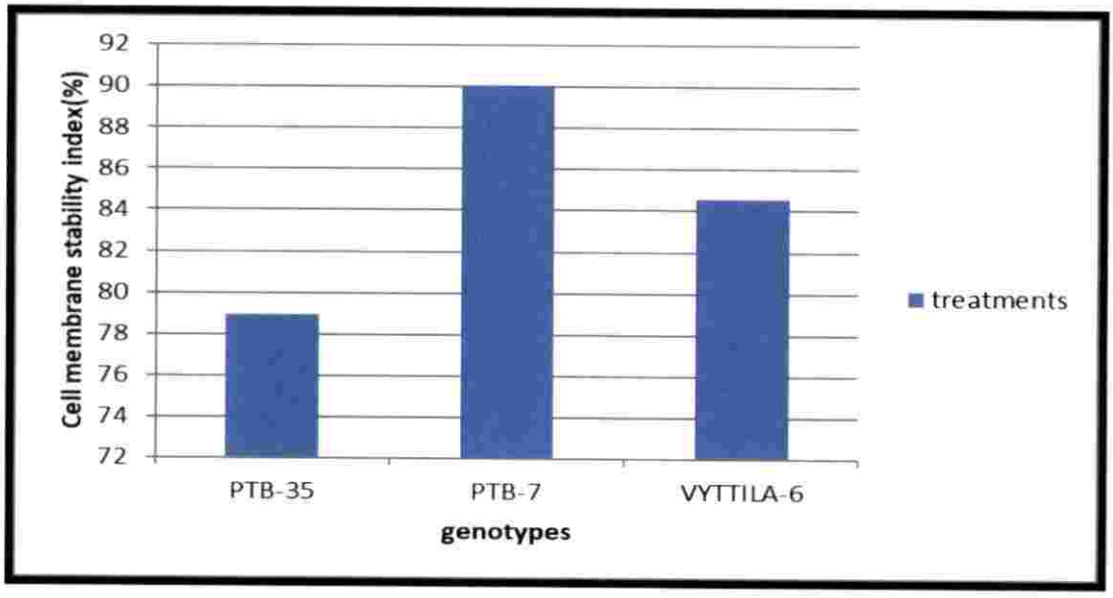


Fig 15. Variation in cell membrane stability index (%) under the combined stress of drought and salinity

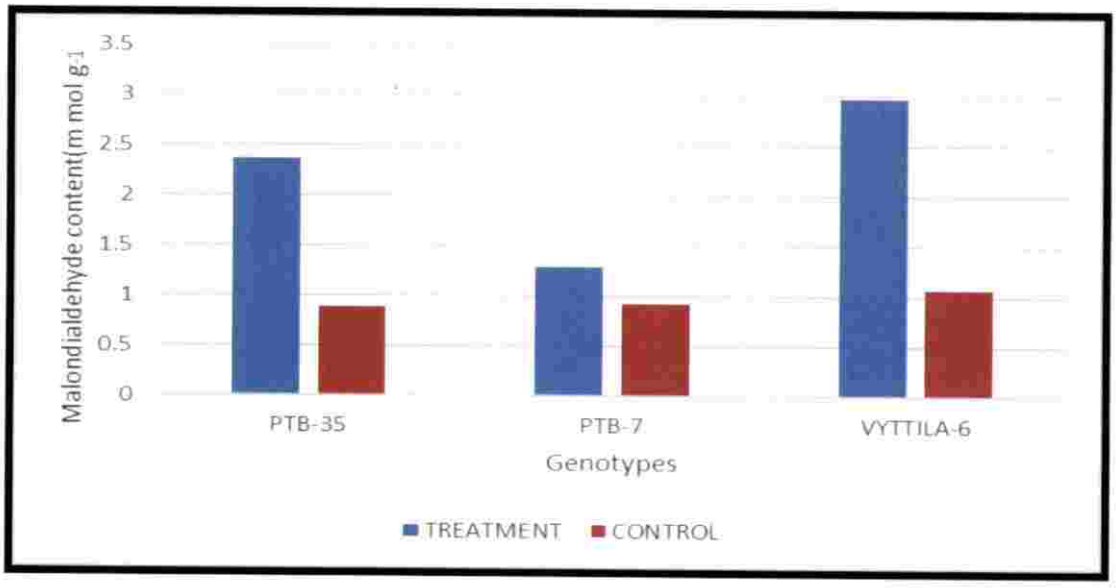


Fig 16. Variation in malondialdehyde content (m mol g^{-1}) under the combined stress of drought and salinity

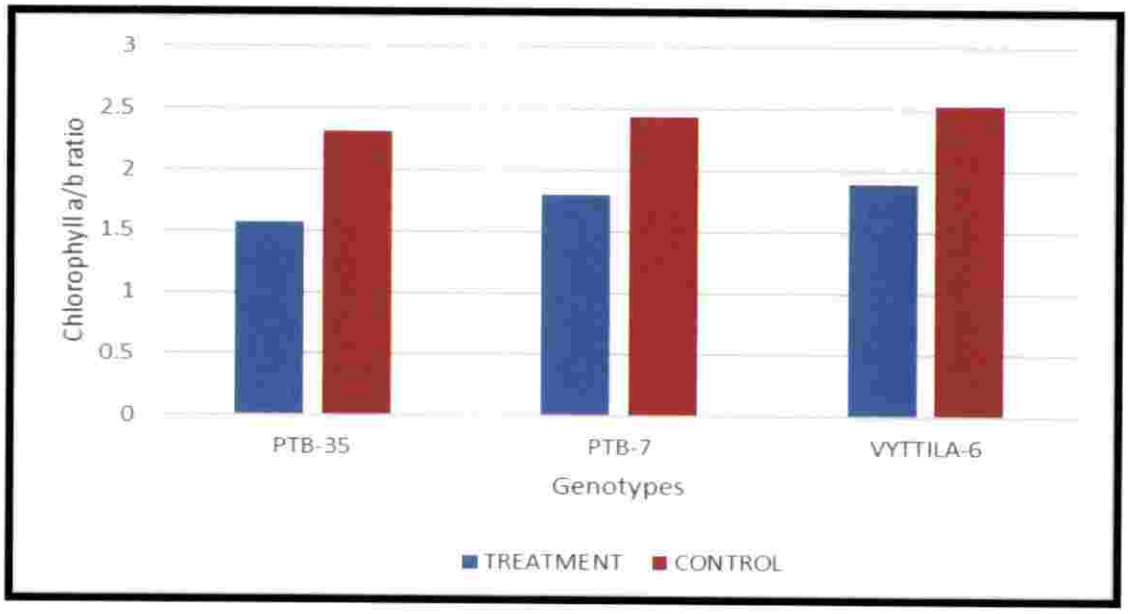


Fig. 17. Variation in chlorophyll a/b ratio under the combined stress of drought and salinity

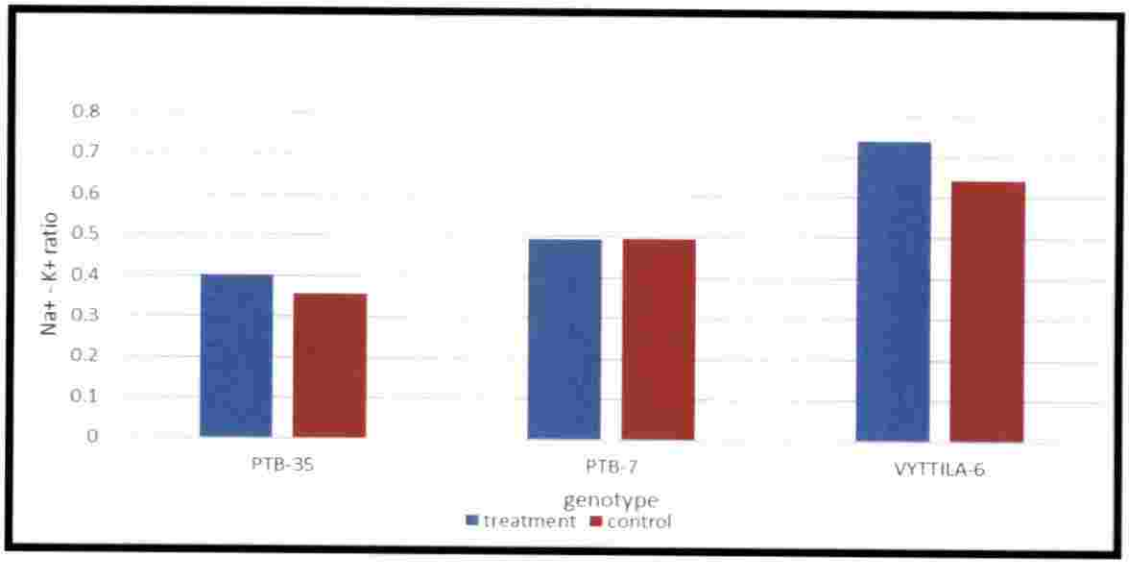


Fig. 18. Variation in Na^+ - K^+ ratio under combined stress of drought and salinity

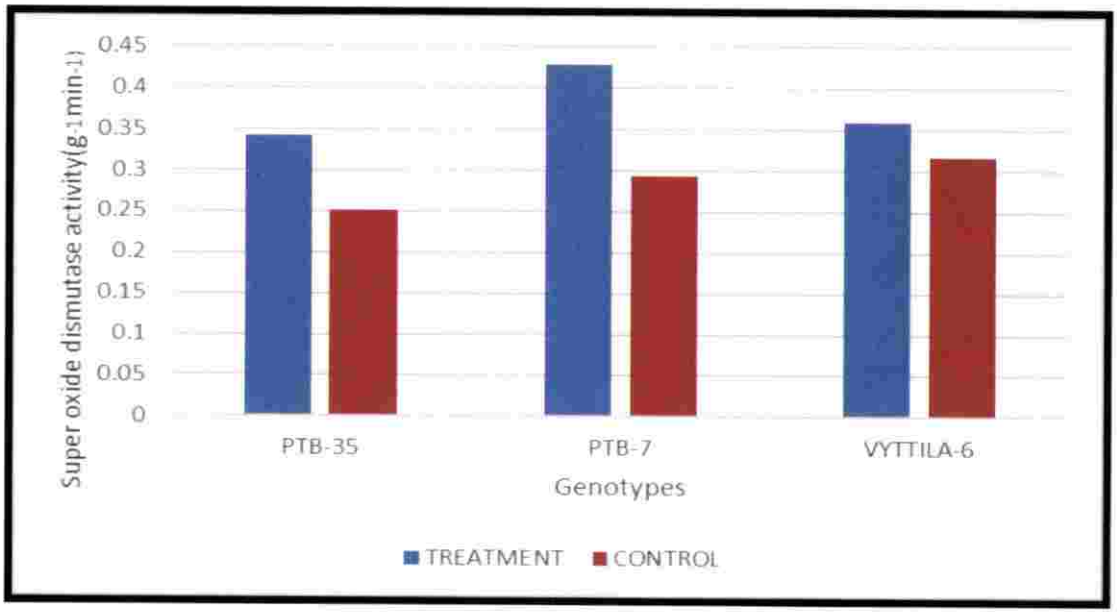


Fig. 19. Variation in superoxide dismutase (g^{-1} min^{-1}) under the combined stress of drought and salinity

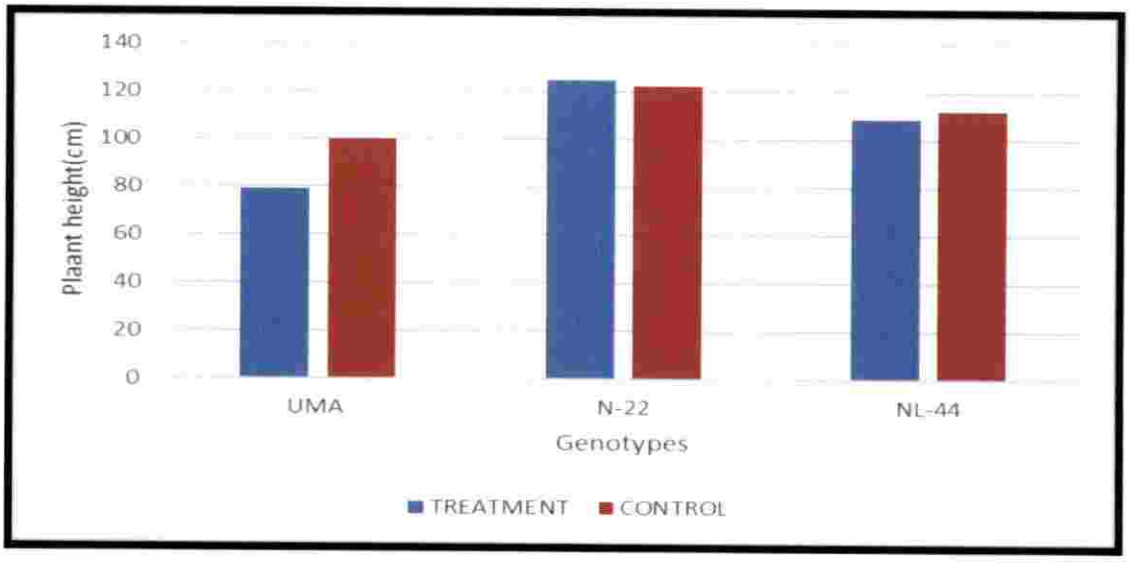


Fig. 20. Variation in plant height (cm) under the combined stress of temperature and salinity

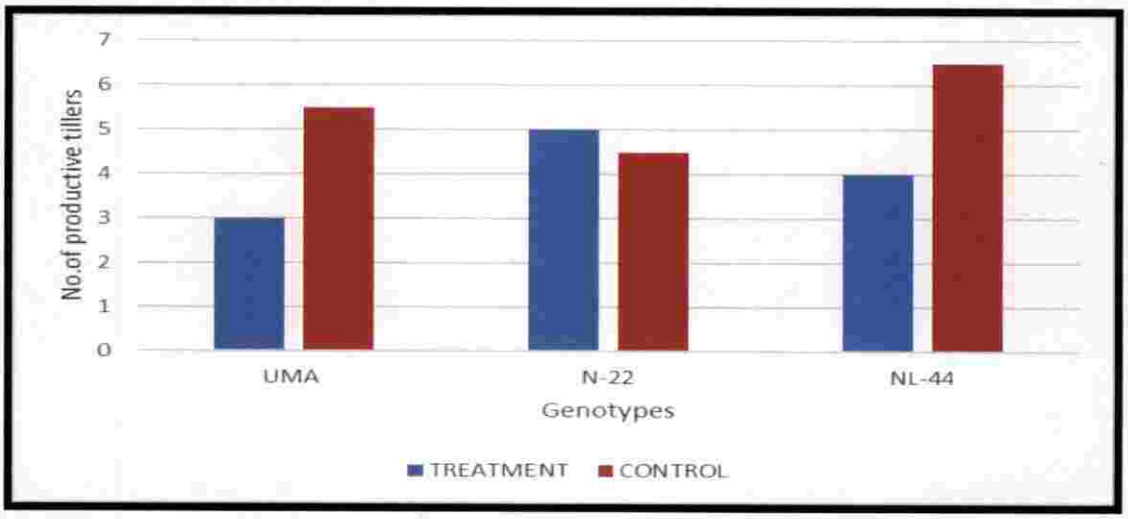


Fig. 21. Variation in no. of productive tillers under the combined stress of temperature and salinity

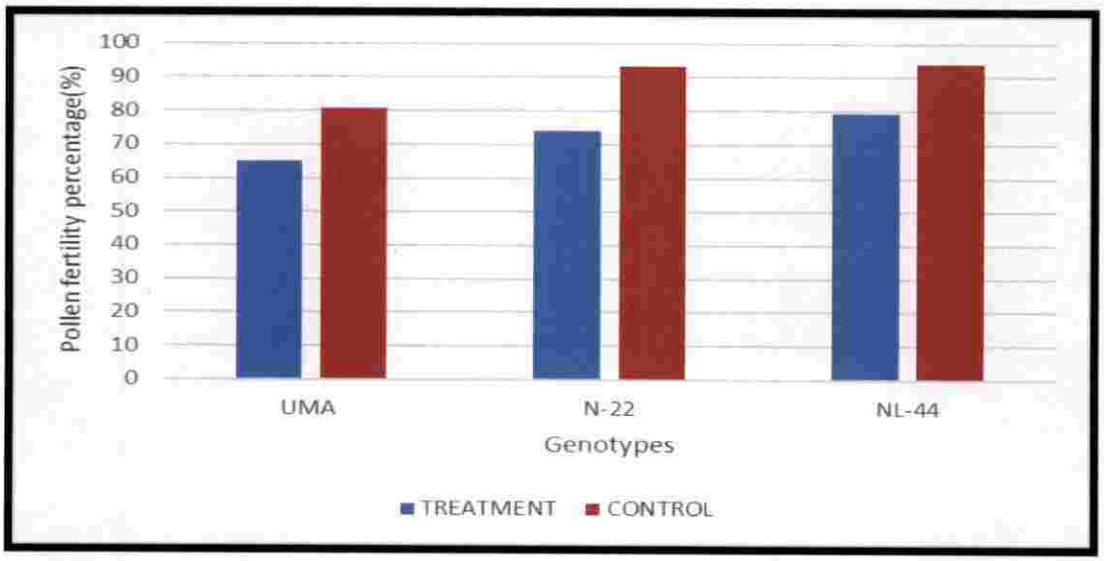


Fig 22. Variation in pollen viability percentage (%) under the combined stress of temperature and salinity

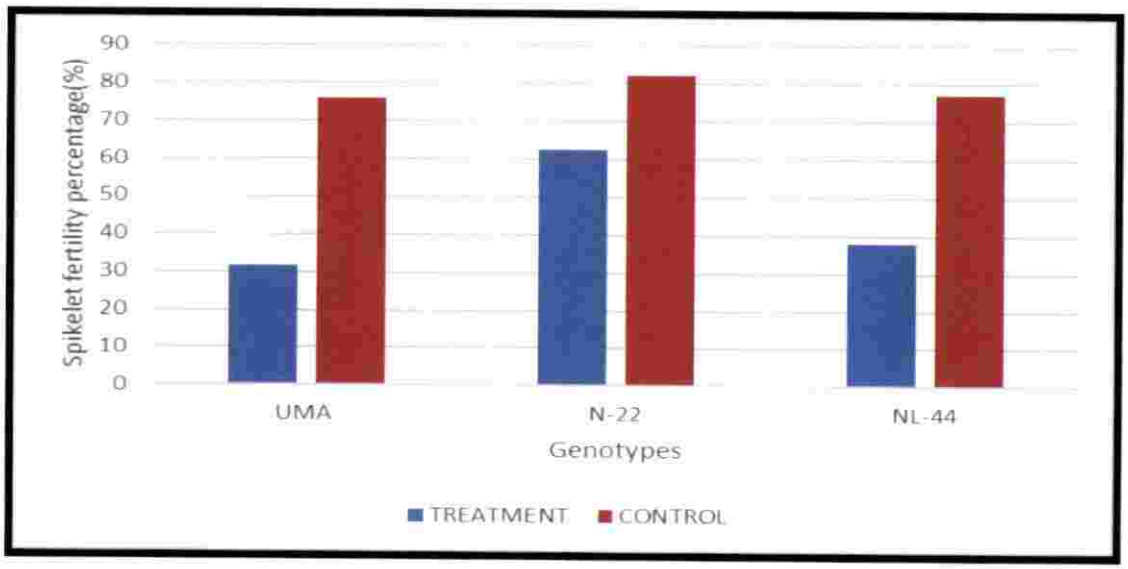


Fig. 23. Variation in Spikelet fertility percentage (%) under the combined stress of temperature and salinity

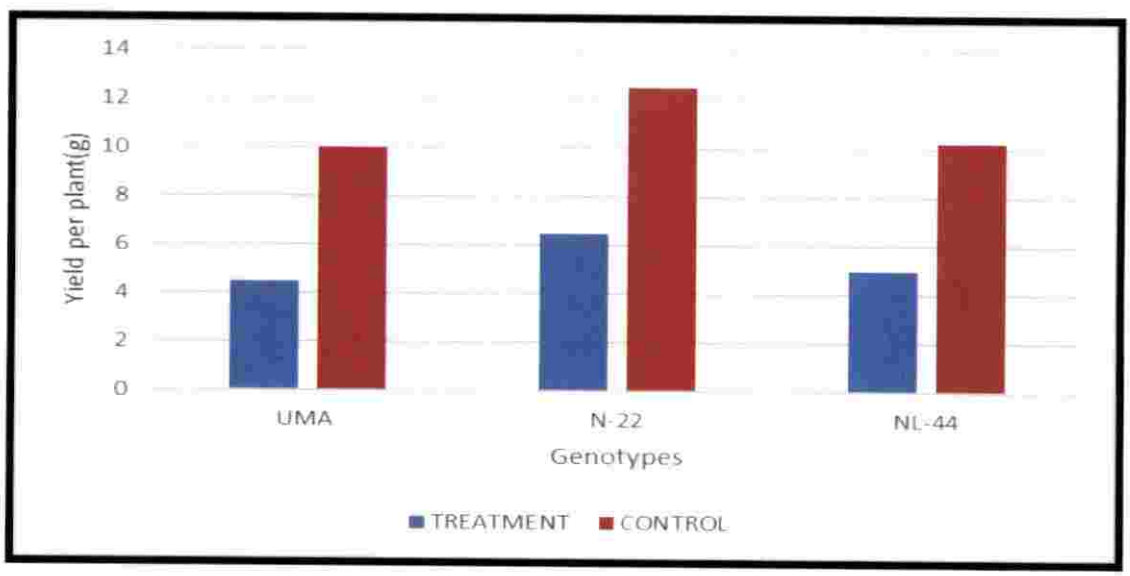


Fig 24. Variation in yield per plant (g) under the combined stress of temperature and salinity

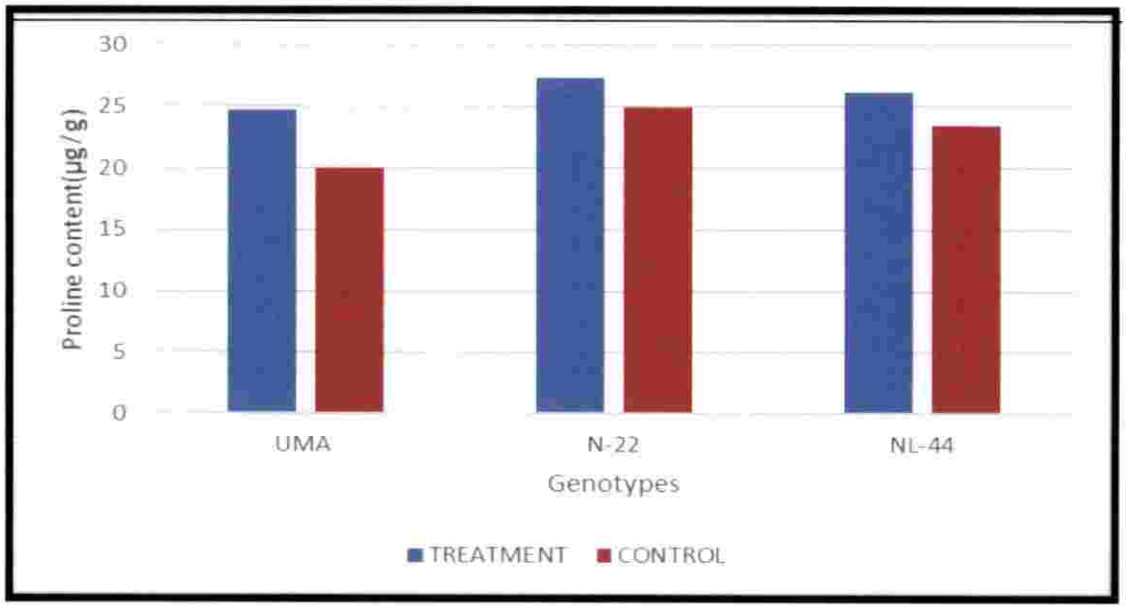


Fig 25. Variation in proline content (µg/g) under the combined stress of temperature and salinity

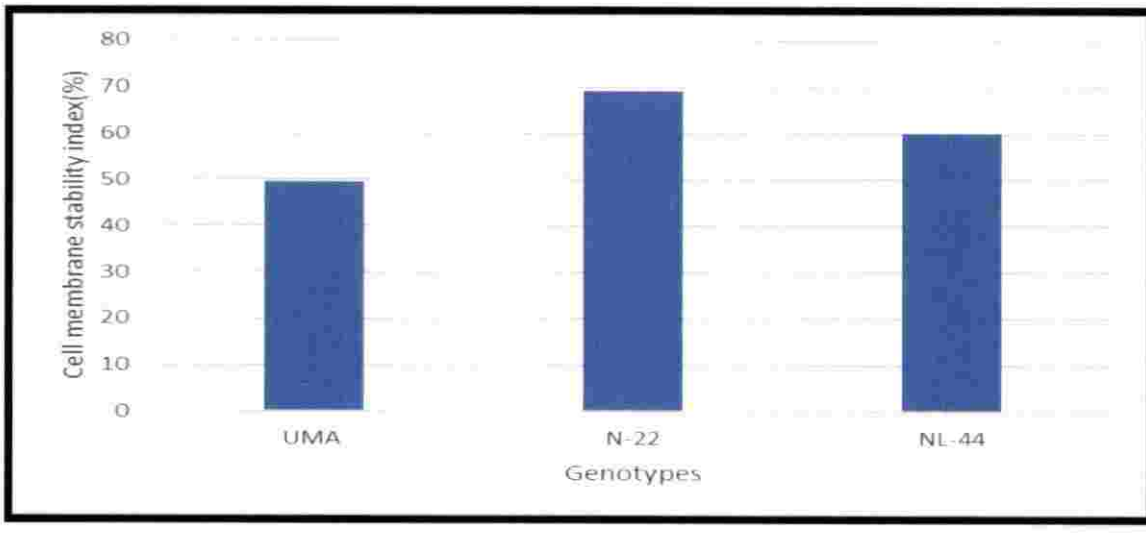


Fig 26. Variation in cell membrane stability index (%) under combined stress of temperature and salinity

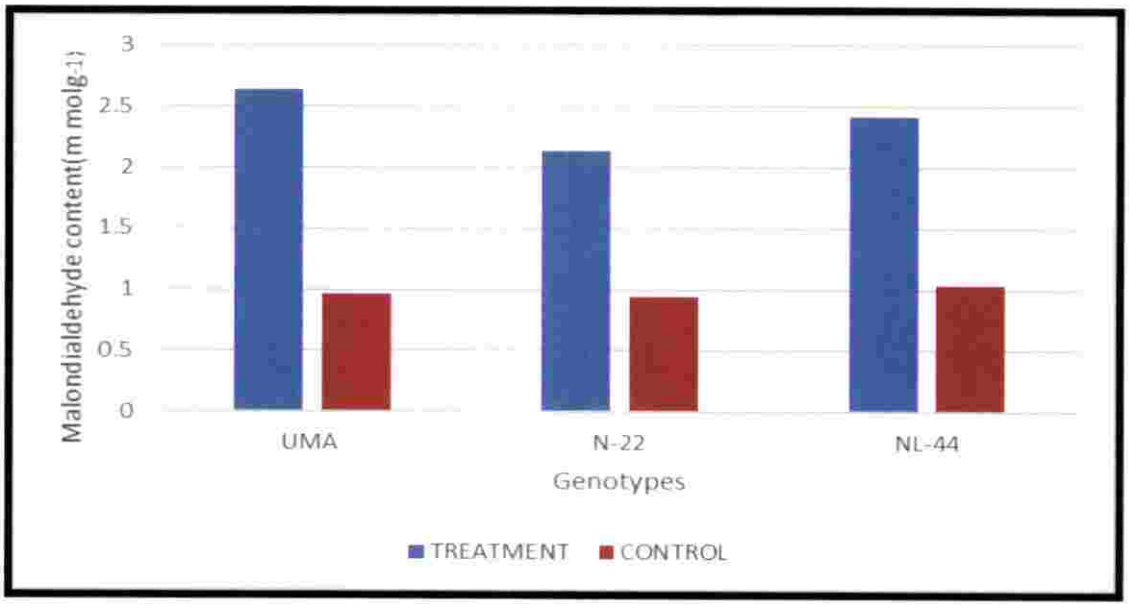


Fig 27. Variation in malondialdehyde content (m mol g^{-1}) under the combined stress of temperature and salinity

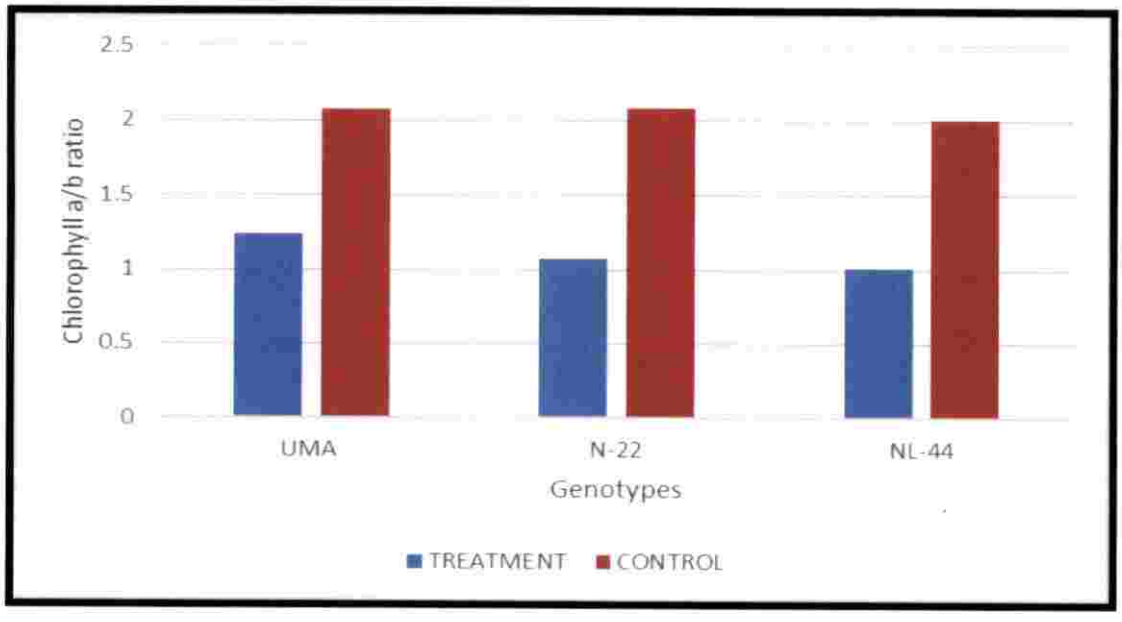


Fig 28. Variation in chlorophyll a/b ratio under the combined stress of temperature and salinity

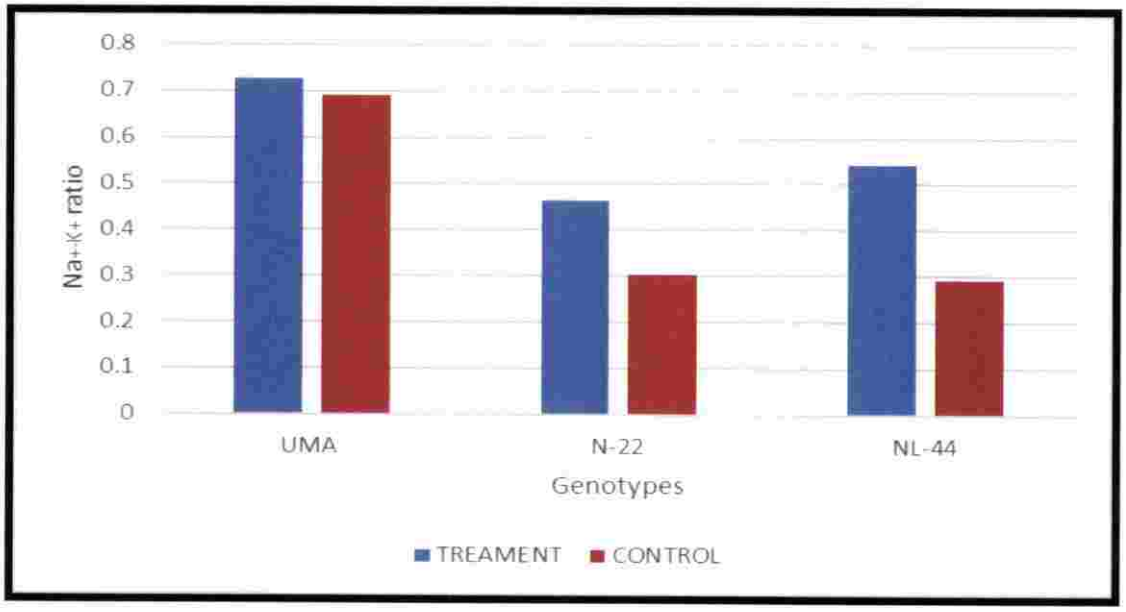


Fig 29. Variation in Na^{+} -K^{+} under the combined stress of temperature and salinity

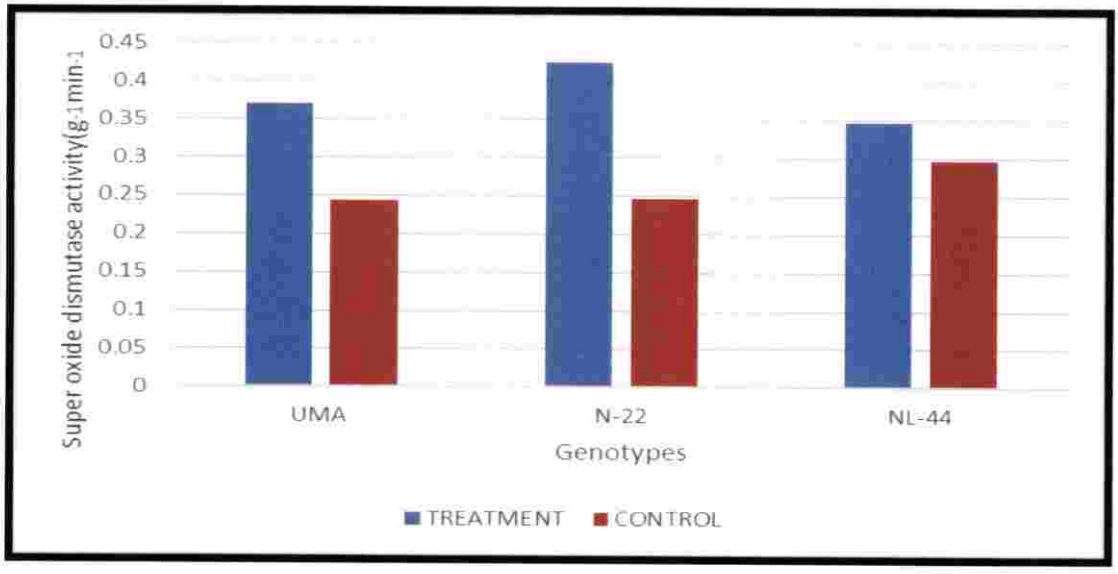


Fig 30. Variation in SOD activity (g^{-1} min^{-1}) under the combined stress of temperature and salinity

Summary

SUMMARY

The present programme was conducted to assess the multiple abiotic stress tolerance mechanism in rice (*Oryza sativa* L.). It was conducted in four experiments and the salient findings are given below.

In the first experiment preliminary screening for single stress tolerance study for drought (-1bar, -3bar, -5bar, -7bar PEG6000), salinity (100mM, 150mM, 200mM, 250mM NaCl) and temperature (35^0c, 40^0c, 45^0c, 50^0c) was carried out and measurements regarding to morphological, physiological and biochemical parameters were studied in all 20 genotypes on 14^{th} day of germination. The maximum stress levels on which germination occurred were -5bar water potential for drought, 250 mM NaCl for salinity and 35^0C for temperature. Morphological parameters like shoot length, root length, germination percentage were decreased in every 20 genotypes in every stress treatment compared to control plants. Among 20 genotypes PTB–7, PTB –60 and PTB–35 recorded maximum seedling vigour index under -5bar PEG 6000 drought stress condition.Vyttila-9, M0-18 and Vyttila – 3 presented maximum seedling vigour index at 250 mM NaCl. N-22, NL-44 and Vyttila-6 showed maximum seedling vigour index. These varieties were selected for combination stress treatment in experiment II.

The highest level of tolerance for each stress was selected (D_h (-5 bar), S_h (100 mM NaCl), T_h (35^0C)) from experiment I and best three genotypes selected from each stress were subjected to combination of stresses in experiment II. The combination of stresses given in experiment II were D_h x S_h, D_h x T_h, S_h x T_h and D_h x S_h x T_h. Germination were observed in D_h x S_h and T_h x S_h stress treatments. Morphological parameters such as shoot length, root length, germination percentage and seedling vigour index was measured. Under the combined stress of D_h x S_h, only three varieties showed germination and they were PTB – 7, Vyttila – 9 and PTB – 35 of which maximum seedling vigour index recorded in PTB–7. Under the combined

stress of T_h x S_h the genotypes showed germination were N-22, NL–44 and MO-18 of which NL -44 showed maximum seedling vigour index. The root length and shoot length were reduced in all stress treatments compared to control, the reduction was much more severe in T_h x S_h stress condition than that of D_h x S_h stress condition.

In experiment III Pot culture experiment was done for all the selected treatment combinations and genotypes from Experiment II. Drought and salt stress was imposed during reproductive stage for five days by adding highest tolerated level of PEG6000 (D_h)(-5 bar) and NaCl (Sh)(100mM) by applying the corresponding solutions in to the pots containing plants. Temperature stress treatment (T_h) (35^0C) was imposed from panicle initiation to maturity stage by keeping plants in a temperature controlled poly house. Morphological, Physiological, biochemical and yield parameters were studied in six genotypes under both selected combination of stress treatments. Physiological and yield parameters were reduced in varieties under treatment than that of their corresponding controls. All the biochemical parameters except chlorophyll a/b ratio and cell membrane stability were increased in all varieties under combined stress treatment than that of control.

Among 20 varieties, based on physiological, biochemical and yield parameters PTB-7 is selected as the rice variety tolerant to drought and salinity stress. N-22 is selected as the variety tolerant to temperature and salinity stress.

Validation of the QTLs controlling stress tolerance in rice was carried out using reported SSR markers linked to drought salinity and temperature. Markers RM 6100, RM 7076, RM 5749 and RM 26212 showed polymorphism for temperature tolerance. RM1287, RM8094, and RM 10843 showed polymorphism for salinity tolerance and RM490 showed polymorphism for drought tolerance. Among the markers distinct polymorphism for temperature tolerance between temperature tolerant (N-22 and NL-44) and susceptible varieties was shown by RM 6100. RM 7076 in tolerant varieties PTB-7 and NL-44. RM 1287 showed distinct

polymorphism for salinity tolerance in PTB-7 and N-22. Drought tolerance between drought tolerant (PTB-7) and susceptible varieties was shown by RM 490.

Genotypic and phenotypic identification of stress tolerance in rice was investigated.

Future line of work

This investigation was conducted in twenty rice genotypes in paper towel and pot culture method in protected conditions, so indeed a revalidation of the effect of tolerance mechanism is needed when cultivated in the field conditions. Abiotic stress tolerant genotypes identified in this study can be used as donors for developing new varieties which are high yielding and tolerant to multiple abiotic stresses.

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APPENDICES

APPENDIX-I

Acid Ninhydrin

Warm 1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml 6M phosphoric acid with agitation until dissolved. Store at 4°C and use within 24 hrs.

APPENDIX-II

Potassium phosphate buffer (50 mM)- pH. 7.8.

A: 50 mM solution of K_2HPO_4 - 4.35 g in 500 ml.

B: 50 mM solution of KH_2PO_4 - 3.40 g in 500 ml.

Solution A and solution B were added with constant stirring until pH 7.8 reached.

APPENDIX-III

1. CHEMICALS FOR PLANT GENOMIC DNA ISOLATION

CTAB extraction buffer

Cetyltrimethyl ammonium bromide (CTAB)	3.0 ml
5 M NaCl	2.8 ml
0.5 M EDTA (pH 8.0)	0.4 ml
1 M Tris-Cl (pH 8.0)	1.0 ml
Polyvinylpyrrolidone (PVP) (MW 40 kDa)	0.3g
β-Mercaptoethanol	0.02 mL
H_2O	2.48 mL

1X TE Buffer (100 ml)

1M Tris-Hcl (pH-8)	1 ml

0.25 EDTA (pH-8)	0.4 ml

Final volume was adjusted to 100 ml and autoclaved.

II. CHEMICALS FOR AGAROSE GEL ELECTROPHORESIS

Gel loading dye

Formamide	50 ml
Xylene cyanol	50 mg
Bromophenol blue	50 mg
0.5 M EDTA	1 ml

10 X TBE Buffer (Tris-Borate-EDTA): 1000 ml

Tris base	107 g
Boric acid	55 g
Na_2EDTA	9.8 g

ASSESMENT OF MULTIPLE ABIOTIC STRESS TOLERANCE MECHANISMS IN RICE (*Oryza sativa* L.)

by

ALIF ALI B. S.
(2014-09-109)

ABSTRACT

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ABSTRACT

The study entitled "Assessment of multiple abiotic stress tolerance mechanisms in rice (*Oryza sativa* L.)" undertaken at the Department of Plant Physiology, College of Agriculture, Vellayani during 2018-19. The objective was to study the multiple abiotic *viz.* drought, salinity and high temperature stress tolerance mechanisms in rice and to validate the identified QTLs for stress tolerance in rice.

The investigation comprises four experiments, In experiment I initial screening of 20 rice genotypes for single abiotic stress tolerance was studied. Stresses were induced using different concentrations of PEG6000, NaCl and temperature controlled incubator for providing drought, salinity and temperature stresses respectively. Germination study was carried out using paper towel method. In the first experiment drought stress were given at concentrations -1bar, -3bar, -5bar and -7bar water potentials of PEG6000, salinity stress was given at 100mM, 150mM, 200mM, 250mM NaCl and temperature stress were given at 35^0C, 40^0C, 45^0C and 50^0C for all 20 rice varieties with two replications. The physio-morphological and biochemical parameters were studied on 14^{th} day of germination.

The highest level drought, salinity and temperature stresses at which germination occurred was selected as D_h (-5 bar), S_h (250mM NaCl) and temperature (T_h) (35^0C) respectively. Among 20 rice varieties, PTB-7, PTB-60 and PTB-35 showed maximum seedling vigour at highest level of drought stress (D_h) stress condition Vyttila-9, MO-18 and Vyttila-3 recorded maximum seedling vigour index at highest tolerated level of salinity (S_h) stress condition and N-22, NL-44 and Vyttila -6 showed maximum seedling vigour index at highest tolerated level of temperature stress (T_h) stress condition. These nine genotypes were selected for the evaluation of combination stress treatment

In the second experiment, The combination stress treatments given were D_h x S_h, D_h x T_h, T_h x S_h and D_h x S_h x T_h. Rice varieties did not germinated at D_h x S_h and D_h x T_h. The maximum seedling vigour index at D_h x S_h and combination stress treatment was observed in PTB-7, Vyttila-9, PTB-35 and at T_h x S_h was observed in NL-44, MO-18 and N-22 respectively. These rice varieties were selected as tolerant varieties.

In experiment III six rice varieties selected from combination stress treatment were evaluated for yield parameters in pot culture experiment. The design of the experiment was CRD with two replications and one control. Drought and salt stress were imposed during reproductive stage for 5 days by applying -5bar PEG6000 and 250mM NaCl solutions respectively into the pots containing rice varieties, Temperature stress was induced using a temperature controlled polyhouse from panicle initiation to maturity stage. Physio-morphological, biochemical and yield parameters were studied under the combination stress treatments. Highest yield under the combination stress treatment of D_h x S_h was observed in PTB-7 and highest yield under the combination stress treatment T_h x S_h was observed in N-22. Based on morpho-physiological and yield parameters PTB-7 was selected as the tolerant variety under drought and saline condition and N-22 was selected as the tolerant variety under temperature and salinity condition.

In experiment IV all the 20 genotypes were analyzed for the identification of reported markers linked to stress tolerance such as drought salinity and temperature. Reported microsatellite markers linked to drought, salinity and temperature were used to screen 20 rice varieties. Among the markers distinct polymorphism for temperature tolerance between temperature tolerant (N-22 and NL-44) and susceptible varieties was shown by RM 6100. RM 7076 showed distinct polymorphism in tolerant varieties PTB-7 and NL-44 . RM 1287 showed distinct polymorphism for salinity tolerance in PTB-7 and N-22. Drought tolerance between drought tolerant (PTB-7) and susceptible varieties was Shown by RM 490.