VALIDATION OF TEMPERATURE INDUCTION RESPONSE (TIR) TECHNIQUE FOR INDUCING DROUGHT AND HEAT STRESS TOLERANCE IN RICE (*Oryza sativa* L.)

by

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THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"Validation of Temperature Induction Response (TIR) technique for inducing drought and heat stress tolerance 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, associateship, fellowship or other similar title, of any other university or society.

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Date: 19-06-2018

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CERTIFICATE

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LIST OF ABBREVIATIONS

TIR	Temperature Induction response
IMD	Indian Meteorological Department
DNA	Deoxyribo Nucleic Acid
FAO	Food and Agricultural Organization
U. S. A	United States of America
IRRI	International Rice Reseach Institute
g/m^2	grams/meter^2
RARS	Regional Agricultural Research Station
PCR	Polymerase Chain Reaction
SDS	Sodium Dodecyl Sulphate
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
^0C	Degree Celsius
m H_2O moles m^{-2} s^{-1}	milli H_2O moles $meter^{-2}$ $second^{-1}$
μ CO_2 moles m^{-2} s^{-1}	micro CO_2 moles $meter^{-2}$ $second^{-1}$
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
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
FYM	Farm Yard Manure
POX	Peroxidase
SOD	Superoxide dismutase
PAGE	Polyacrylamide gel electrophoresis
APX	Ascorbate peroxidase
CMT	Cell membrane thermo-stability
MSI	Membrane stability index
CSI	Chlorophyll stability index
gs	Stomatal conductance
ABA	Abscisic acid
EMF	Early morning flowering
DRO 1	Deep rooting 1
PSTOL 1	Phosphorous starvation tolerance 1
PS	Photosystem
RT	Reverse Transcriptase
WS	Water Stress
HSF	Heat Shock Factor

INTRODUCTION

INTRODUCTION

World population is increasing in an alarming rate. It is estimated that by 2050, the population will reach around 9 billion based on International Data Base, U. S, 2016. It is predicted that world population will reach 10 billion by 2050. The major challenge faced by this growing population will be to meet their daily nutritional requirements (FAO, 2017). Kumar and Gautham (2014) reported that in order to feed another two billion people in 2050, farmers need to produce 50% more food grains by 2020.

Rice (*Oryza sativa* L.) is a starch-rich food that provides 35–75% of caloric intake for over 3 billion people in the world, with approximately 90% of the rice produced and consumed in Asia (Fitzgerald *et al.*, 2009). Rice is the staple food for more than 60 % of the world population. Worldwide, rice is cultivated in an area of 164 million hectares with an annual production of 772.8 million tons (FAO, 2013). Govt. of India Directorate of Economics and Statistics, Ministry of Agriculture, 2017 reported that rice is cultivated in an area of 43.385 million ha with the production of 104.317 million tonnes.

Even though the rice production is increasing, the rate of increase is not sufficient for the present demand. So we need to produce 116 million tonnes of additional rice by 2035 (Kumar and Gautham, 2014). Farmers are adopting better cultural and management practices for rice production, but the productivity of rice is not increasing as expected. This fluctuation in rice yield in various agricultural regions is due to various environmental stresses (Jagadish *et al.*, 2007), which include both biotic and abiotic factors. Among abiotic factors, drought and high temperature are considered as key stress factors with high potential impact on crop yield (Barnabas *et al.*, 2008). Global climate change has caused detrimental effects on yield physiology of rice because of the rise in atmospheric temperatures (Jagadish *et al.*, 2007).

According to IPCC (2014) the expected rise in global air temperature by 2100 is 0.2°C–0.4°C per decade and it will lead to 1.8°C–4.3°C higher temperatures than the current level. Frequent episodes of heat stress enhance the current vulnerability of rice

productivity due to the future climate change and global warming (IPCC, 2014; Matsui *et al.*, 2001). It was reported that 0.51°C per 100 year is the warming trend for India during the period of 1901-2007. In tropical and subtropical areas, rice productivity is affecting mainly by high temperature (HT) (Shah *et al.*, 2011), and the elevated temperature results in grain sterility injurious to rice yield (Prasad *et al.*, 2006). According to India Meteorological Department (IMD), during the last 43 years, the mean maximum temperature over Kerala has risen by 0.8°C, the minimum by 0.2°C and the average by 0.5°C, indicating that the temperature trends in Kerala followed the trends of West Coast. February and March are the hot months of Kerala with a mean maximum of 33°C.

Coming to precipitation, by 2050 India, Southern America and Southern Africa would witness reduced rainfall of less than 0.6 mm per day. Worldwide food production is highly affected by drought. IMD (2012) reported that Kerala is facing rainfall deficit of 39% from its long period average for the period of 1st June to 25th July. Expected average rainfall from 1 June to 25 July for Kerala was 1255.1 mm while actual rainfall was only 760.9 mm, expected rainfall from 1 June to 2 September was 1812.2 mm while actual rainfall was 1330.6 mm As the world population continues to grow and water resources for crop production decline, the development of drought-tolerant cultivars and water-use-efficient crops is a global concern.

In order to withstand in this extreme weather, plants have evolved many adaptive and tolerant mechanisms by bringing about short and long-term physiological and biochemical mechanisms like mechanisms such as excess heat dissipation through evaporative cooling, maintaining membrane integrity and synthesis of HSPs (Wahid *et al.*, 2007). Thermo-tolerance is a multigenic trait and one of the approaches to improve thermotolerance is the transfer of superior alleles from intrinsically thermo-tolerant wild relatives, which require precise screening methods to measure the variability in thermo-tolerance (Harihar *et al.*, 2014). To screen thermotolerance at field level specific physiological parameters such as single leaf photosynthetic capacity,

quantification of chlorophyll fluorescence under stress are being used (Selmani and Wasson, 1993), but the limitation is that these measurements are highly influenced by environmental factors. An efficient screening technique to identify the thermo-tolerant lines from the segregating population have been developed, which is referred as Temperature Induction Response (TIR) technique. Induction of thermo- tolerance can be done by gradual increase in temperature to lethal temperature as would be experienced in natural environment (Larkindale *et al.*, 2005). Plants develop the ability to withstand under lethal temperatures by acclimation through both prevention of heat damage and repair of heat-sensitive components (Kheir *et al.*, 2012). Under future climatic scenarios, rice will be exposed to frequent episodes of drought and high temperature. To alleviate this threat, it is essential to improve the adaptability of rice. So in this present study, an attempt was made to standardize the temperature induction response technique for rice genotypes which is used for screening the temperature tolerance and the study was carried out with the following objective

- To validate the effect of TIR technique for combined drought and heat stress tolerance.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Rice (*Oryza sativa* L.) is an important cereal crop grown in about 1/3rd of the world's total cereal crop area, providing staple food and 35–60% of the calories for more than 2.7 billion people. Seventeen countries in Asia and the Pacific, 9 countries in North and South America and 8 countries in Africa predominantly depend on rice as their dietary energy source. According to 2012 FAOSTAT data, rice is the agricultural commodity with the third highest worldwide production, after sugarcane and maize. Rice coming under the family Poaceae and subfamily Oryzoidae. The two cultivated species of rice are *O. sativa*, the Asian rice, and *O. glaberrima*, the African rice. Rice, accounts for 35 to 60% of the calories consumed by 3 billion Asians and it is originated at least 130 million years ago and spread as a wild grass in Gondwanaland. India ranks first in cultivable area (43.92 million hectares) under rice and second in production (91.61 million tons) after China (Pandey *et al.*, 2010). Recent FAO reports shown that area harvested under rice in India is around 42.96 million hectares and the production is 158.75 million tonnes in the year 2016.

In order to meet the demand of ever growing population, rice production has been intensified in lowland and upland cropping systems, which are highly prone to weather fluctuations (Cassman *et al.*, 2005). Zhang (2007) reported that increasing rice productivity can significantly address the global food security challenge and the production levels need to be increased by 2 mt every year (Thankapandian *et al.*, 2010). But under field conditions crops are continuously exposed to a number of different abiotic stress factors. Among this, drought and heat stress are the two most important environmental factors influencing crop growth, development and yield. Simultaneous occurrence of multiple stresses affect the crop production and the effect considerably exceeds the simple additive effects of the action alone.

High-temperature stress is the rise in temperature beyond a critical threshold for a period of time, sufficient to cause irreversible damage to plant growth and

development (Wahid *et al.*, 2007). Current IPCC (2014) projections indicate that the mean global temperature will rise 0.2°C per decade in coming years and the global surface temperature change is projected to exceed 2°C by the end of 21st century. For every 1°C increase in temperature there will be 10% decrease in grain yield (Peng *et al.*, 2004). Prasad *et al.* (2017) observed that high day-time temperatures (30–38°C) coinciding with reproductive stage can cause significant damage to reproductive processes in cereals, for rice damage will occur when exposed to 30°/35°C (Satake and Yoshida, 1978). High temperature affects several physiological processes like germination, respiration, photosynthesis seedling survival etc. and the extent of damage depends on the crop species. One of the most sensitive apparatus to heat stress is PS II. Increased fluidity of thylakoid membranes and the dependence of PS II integrity on electron dynamics were the reason for this sensitivity (Havaux, 1992).

Indian Meteorological Department (IMD) has reported that Kerala as a whole has a rainfall deficit of 39% during the year 2012. During 2016, 64% rainfall deficit is reported in north east monsoon and 34% during south west monsoon and temperature may go up to 39-41°C during second/ third crop season. The unpredictability of drought affects various physiological process like photosynthesis in plants. Regeneration of ribulose bisphosphate (RuBP) and ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) are affected (Bota *et al.*, 2004). Decreased Rubisco activity (Parry *et al.*, 2002), impairment of ATP synthesis, photophosphorylation or decreased inorganic phosphorus and oxidative damage to chloroplast (Zhou *et al.*, 2007) are the main metabolic changes affected by drought and resulted in decline in photosynthesis.

Combined stress occurrence will produce greater detrimental effect on growth and productivity than exposure to a single stress in *Poa pratensis* (Wang and Huang, 2004), *Hordeum vulgare* (Savin and Nicolas, 1996), *Arabidopsis thaliana* (Rizhsky *et al.*, 2004), *Triticum aestivum* (Shah and Paulsen, 2003) and *Nicotiana tabacum* (Rizhsky *et al.*, 2002). Mittler (2006) also observed that plants under combined drought and heat stress exhibits high respiration with low photosynthesis, closed stomata, and

high canopy temperatures. Both these stresses enhances activity of oxygenase, leading to photorespiration and reduced photosynthesis.

Thermo-tolerance is a multi genic trait and it can be induced by gradual increase in temperature to normally lethal temperature as would be experienced in natural environment (Larkindale *et al.*, 2005). An efficient screening method to identify the thermo-tolerant lines have been developed, which is referred as Temperature Induction Response (TIR) technique. The reason behind observed genetic variability can be attributed to expression of stress responsive genes during the induction stress and differences in the gene expression. Rice will be exposed to frequent episodes of drought and high temperature in future. So it is important to focus on the development of techniques which will improve the adaptability of rice to cope with the changing climates.

2.1. RICE PRODUCTION vs DROUGHT

Blum (2011) reported that drought is the insufficiency of soil moisture content to meet plant water requirements resulting in reduced growth and development of the plant and hence low yield. Rice is more vulnerable to drought due to its semi aquatic phylogenetic origin. Bartels and Souer (2004) reported that the response of plants to water stress depends on the duration and severity of the stress and the developmental stage (Zhu *et al.*, 2005). Sokoto and Muhammad (2014) observed that at cell level, drought results in impaired in cell division and cell elongation due to decrease in turgor pressure. In the case of rice, the sensitive period is flowering stage, resulting in severe yield losses (Liu *et al.*, 2006). The physiological processes during flowering stage will negatively affected by water stress and it will lead to decreased spikelet fertility. There are studies which reported effect of drought on anther dehiscence (Ekanayake *et al.*, 1989) and germination of pollen (Saini and Westgate, 2000) and the responses were just like high-temperature stress (Jagadish *et al.*, 2010).

2.1.1. ADAPTATION MECHANISM TO DROUGHT TOLERANCE IN RICE

Plants, as sessile organisms, evolved certain mechanisms to cope with temporary water scarcity for their survival and reproduction. Chaves *et al.* (2003) reported that plant resistance to drought can be subdivided into escape, avoidance and tolerance strategies. Escape mechanism means the short life cycle, that is, plants successfully complete their reproduction before the onset of extreme stress conditions. Maintenance of an optimum water status during stress resulted in dehydration avoidance, mainly due to minimized water loss or through maximized water uptake by enhanced root growth. Tolerance strategies includes the maintenance of plant function at limited water availability and/or the recovery of plant water status and plant function after stress, which may involve osmotic adjustments, rigid cell walls or small cells and efficient scavenging of reactive oxygen species (ROS) (Sairam and Saxena, 2000).

Yoshida and Hasegawa (1982) observed that plants extract water in the deeper soil layers in order to maintain a high leaf water potential. The ability of rice to carry out key physiological processes, such as anther dehiscence, pollination, pollen germination, and fertilization, under stress but still maintain high seed-set is the indication of absolute tolerance (Jagadish *et al.*, 2010).

2.2. RICE PRODUCTION vs HEAT STRESS

High-temperature stress cause irreversible damage to plant growth and development. The optimum temperature for the normal development of rice ranges from 27 to 32°C (Satake and Yoshida, 1978). Rice responses to high temperature differ according to the developmental stage, with the highest sensitivity recorded at the reproductive stage. High temperature at the vegetative stage resulted in chlorosis and reduced tillering (Yoshida *et al.*, 1981). Jagadish *et al.* (2007) observed that temperatures >35°C at anthesis and lasting for more than 1 hour can lead to high sterility in rice. The anticipated global warming of the future would decrease the stability of rice yield, mainly through high temperature-induced spikelet sterility

(Horie, 1993) and this induced spikelet sterility was attributed to abnormal anther dehiscence (Matsui and Omasa, 2002), impaired pollination (Matsui *et al.*, 2005), and pollen germination (Jagadish *et al.*, 2010). Among the physiological processes in plants photosynthesis is more susceptible to heat stress (Yin *et al.*, 2010). It is reported that photosynthesis of rice leaves are considerably reduced at temperatures higher than 35°C (Taniyama *et al.*, 1988). Other processes affected by heat stress includes rubisco activation state, maximal efficiency of PSII photochemistry (Fv/Fm), the actual PSII efficiency in the light-adapted state and non-photochemical quenching in rice leaves (Yin *et al.*, 2010).

2.2.1. ADAPTATION MECHANISM TO HEAT STRESS TOLERANCE IN RICE

The mechanisms identified to minimize heat stress damage during flowering in rice, including heat escape by early morning flowering (Julia and Dingkuhn, 2012), heat avoidance through transpirational cooling (Julia and Dingkuhn, 2013) and heat tolerance through resilient reproductive processes (Jagadish *et al.*, 2010). Tolerant genotypes of rice exhibit many traits in order to cope with the extreme temperatures. Some of the mechanisms are listed below.

- Wassmann *et al.* (2009) reported that in tolerant cultivars panicle is surrounded
  with many leaves which will increase the transpirational cooling which may lead to reduction in spikelet sterility.
- Matsui and Omasa (2002) identified that length of the anther is more in tolerant genotypes, hence the number of pollen grains present per anther will be more.
- Large basal pore of anthers in genotypes also increase the chance of pollination (Matsui and Kagata, 2003). Pollen grains in the anther with large basal pore would readily drop out of the theca on to the stigmata.

- Paraheliotropism (plants will orient themselves to avoid incident light), increased trichomatous and stomatal densities, leaf rolling are another characters exhibited by tolerant cultivars.
- Tolerant genotypes also have the ability to produce certain substances under heat stress such as HSPs (Heat Shock Proteins), compatible osmolytes, anti-oxidants etc.

2.3. TEMPERATURE INDUCTION RESPONSE (TIR) TECHNIQUE

Temperature induction response (TIR) technique can be used as an efficient tool to identify and select the genotypes which are temperature tolerant at the seedling level from a large population. It has been standardized to identify thermo-tolerant genotypes in rice. This technique involves exposing rice seedlings to gradual induction temperature immediately followed by lethal temperature and measuring growth of the surviving seedlings at the end of the recovery period of 72 h. Plants are exposed to a gradual increase in temperature in the field and not directly to heat shock. The success of TIR technique depends on optimum induction cycle and lethal temperature. The standardization of induction temperature and lethal temperature is based on per cent growth reduction and survival percentage at the end of recovery period (Harihar *et al.*, 2014). Kheir *et al.* (2012) reported that TIR technique is the best method for screening cotton seedlings for heat tolerance. Using this technique they demonstrated that there is sufficient genetic variability present among cotton lines for high temperature tolerance. Cotton lines selected as tolerant to high temperature should be useful in breeding programs to overcome yield limitation. Gomathi *et al.* (2014) conducted an experiment for analyzing the responses of sugarcane variety CO 86032 for high temperature stress through TIR technique in settlings and callus, they observed that acclimated settlings recorded higher soluble protein, proline, glycine betaine, total phenols, POX, APX and SOD activities than non- acclimated settlings and callus. Srikanthbabu *et al.* (2002)

studied enhanced thermotolerance using temperature induction response technique in pea plants. They observed that induced pea seedlings showed higher recovery growth compared to seedlings which were directly exposed to lethal temperature. Moreover, in the induced seedlings accumulation of higher levels of hsp18.1 and hsp70 transcripts as well as HSP104 and HSP90 proteins were observed. The genotypes developed through TIR approach not only showed tolerance to high temperature, but also to other abiotic stresses like salinity and desiccation stress.

2.4. IMPACT OF DROUGHT AND HEAT STRESS ON PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS.

2.4.1. Cell Membrane Stability Index

Sustained function of cellular membranes is essential for processes such as photosynthesis and respiration under stress. Heat stress results in accelerated kinetic energy and movement of molecules across membranes thereby loosening chemical bonds within molecules of biological membranes. So by denaturation of proteins or an increase in unsaturated fatty acids, the lipid bilayer of biological membranes become more fluid (Savchenko *et al.*, 2002). This will increase solute leakage and we can use this physiological index as an indication of decreased cell membrane thermo-stability (CMT), has long been used as an indirect measure of drought and temperature tolerance. (Blum and Ebercon, 1981).

Leibler *et al.* (1986) suggested that lower membrane stability or higher injury reflects the extent of membrane lipid peroxidation, which in turn is a consequence of higher susceptibility to oxidative stress due to various environmental stresses including drought.

The relative injury due to heat stress as a result of electrolyte leakage from flag leaf for different cultivars of rice ranged between 44 and 56%. (Prasad *et al.*, 2006).

Tyagi *et al.* (1999) reported that the value of MSI was higher in tolerant genotypes under water stress. In his study he observed that the tolerant genotypes of rice CR 143-2-2 and N 22 under water stress showed a higher membrane stability index than susceptible genotypes PR 110 and PR 169.

2.4.2. Chlorophyll stability index

Kaloyereas (1958) reported that chlorophyll stability index appears to be a more reliable test of drought resistance than bound water or extractable sulfhydryl groups. The high CSI value helps the plant to withstand stress through enhanced availability of chlorophyll in the plant. This leads to increased photosynthetic rate, more dry matter production and higher productivity. The high CSI showed that the plant has the ability to convert the glutamate into proline and this proline seems to have diverse roles under different abiotic stresses.

High accumulation of proline and chlorophyll in plants indicate that the plant is able to survive in abiotic stress environment (Verma, 1999). Mahla *et al.* (2011) found that there is decline in chlorophyll stability (%) in terms of loss of chlorophyll content in all genotypes of wheat under high temperature stress conditions. This decrease in chlorophyll stability index was least in tolerant genotype and the highest in susceptible genotype.

Rodriguez *et al.* (2012) reported that chlorophyll degradation in a sensitive tomato cultivar was measurable and he suggested that this change might be considered as a drought response mechanism for minimizing light harvesting by chloroplasts.

2.4.3. Stomatal conductance

Medrano *et al.* (2002) reported that under mild to moderate drought stress decreased photosynthesis is due to stomatal closure. The ability to sustain leaf gas exchange under heat stress has a direct relationship with heat tolerance (Hall, 1992).

Stomatal conductance (gs) and net photosynthesis (Pn) are affected by heat stress in many plant species due to decreases in the activation state of rubisco (Crafts-Brander and Salvucci, 2002; Morales *et al.*, 2003).

Under drought stress stomatal conductance and the amount of water transpired in indica rice decreased substantially (Farooq *et al.*, 2010).

Ji *et al.* (2012) reported that stomatal conductance (gs) decreased in drought susceptible and drought tolerant genotypes with respect to control after drought stress treatment. However, the drought-stressed plants of tolerant genotypes showed lower rates of decrease in gs than those of drought susceptible.

Beena *et al.* (2014) identified that heat stress (33°C for 5 days) leads to increase in stomatal conductance and transpiration in rice.

Urban *et al.* (2017) found that the stomatal conductance increased with increasing leaf temperature and air temperature. Unlimited soil water availability and an increase in leaf temperature from 30- 40 °C led to an increase in stomatal conductance of 42 % in poplar but soil water deficit and increased CO_2 concentration significantly reduced stomatal conductance in poplar. Even though gs was reduced in these conditions, general trends of increasing gs with increasing leaf temperature remained similar.

Heat causes increases in stomatal conductance as the plant attempts to reduce the temperature of leaves by transpiration, whereas drought decreases the stomatal conductance to prevent water loss (Mittler and Blumwald, 2010). Tolerance to a combination of drought and heat mainly depends on to the maintenance of leaf temperature.

2.3.4. Photosynthetic rate

Photosynthesis is the key metabolic process affected directly by high temperature and drought. Scafaro *et al.* (2009) analysed the effect of high temperature on *O. sativa and O. meridionalis* and they found that there exist a

significant difference in the net photosynthetic rate between *O. sativa and O. meridionalis* at 27 °C but not at 45°C, the impact of heat on net photosynthesis was greater for *O. sativa* (53% fall) than for *O. meridionalis* (42% fall). Net photosynthetic rate for *O. sativa* was 26.6 μmol CO_2 m^{-2} s^{-1} at 27°C and it reduced to 12.6 μmol CO_2 m^{-2} s^{-1} at 45°C. In *O. meridionalis* 22.4 μmol CO_2 m^{-2} s^{-1} reduced to 13 μmol CO_2 m^{-2} s^{-1} at high temperature.

Depending on plant species the effects of drought, heat stress and their combination on photosynthesis will vary. According to Wang *et al.* (2010), simultaneous occurrence of drought and heat stress have a negative impact on wheat photosynthetic rates, to a more severe level than each of the different stresses applied individually.

Experimental results shown that the photosynthetic rate of flag leaves decreased 47% in rice varieties Zhenshan97B and in IRAT109 under the drought stress compared to control (Ji *et al.*, 2012).

2.4.5. Proline

Proline is a proteinogenic amino acid with an exceptional conformational rigidity, and is essential for primary metabolism. Under drought and heat stress, water potential of tissue decreases and it will lead to increased osmotic potential, hence turgidity of cells will be lost and collapse, resulted in wilting of plants. Tolerant varieties have the capacity to accumulate compatible osmolytes like proline, they suddenly reduces the osmotic potential and maintain the turgidity (Kishore *et al.*, 2005).

According to Wahid and Close (2007), proline synthesis may buffer cellular redox potential under heat and other environmental stresses. Compatible solutes protect plants from oxidative stress by scavenging of reactive oxygen species, and also their chaperone-like activities helps to maintain protein structures and functions.

Proline act as a molecular chaperone, able to protect protein integrity and enhance the activities of different enzymes. Proline involved in the prevention of protein aggregation and stabilization of M4 lactate dehydrogenase during extreme temperatures (Rajendrakumar, 1994). It has ROS scavenging activity and proline acting as a singlet oxygen quencher (Matysik, 2002).

Beena *et al.* (2012) observed that water deficit during panicle initiation stage cause an increase in proline content (89.6%) in selected recombinant inbred lines (RIL's) as compared to control plants.

2.4.6. Anti-oxidants

Environmental changes and developmental transitions in plant produces oxidative stress and it accumulates ROS in higher concentrations. This will resulted in disruption of membrane proteins, enzymes and cellular homeostasis, finally increases the membrane fluidity. In order to detoxify the ROS plant cells are equipped with anti-oxidative machinery comprised of both enzymatic and non-enzymatic compounds of low molecular weight. While Superoxide dismutase (SOD), Catalase (CAT), Peroxidase (POX), Glutathione peroxidase (GPX), Glutathione-S- Transferase (GST), Mono de-hydro ascorbate reductase (MDHAR) and De-hydro ascorbate reductase (DHAR) forms the enzymatic part, Ascorbate, phenolic compounds, carotenoids and tocopherols contribute in the non-enzymatic arm of the anti-oxidative defense of the cell (Asthir, 2015).

Recent studies confirmed that the participation of ROS generation in programmed cell death (PCD) is related with the pollen sterility of some cytoplasmic male sterile plants of rice (Li *et al.*, 2004) and wheat (Wan *et al.*, 2007).

2.4.6.1. Peroxidase

Increased activity of peroxidase in stressed seedlings can be correlated to oxidative reactions corresponding to accumulation of peroxides and free radicals in the plant cells (Radotic *et al.*, 2000).

Accumulation of excess H_2O_2 in cells was prevented by Ascorbate peroxidase (APX) through ascorbate-glutathione pathway (Foyer and Halliwell, 1976).

Stressful conditions induces enhanced expression of APX in cytosol as well as in cellular organelles (Yoshimura, 2000).

In drought stressed seedlings an increased cytosolic APX activity led to decrease in H_2O_2 concentration (Madhusudhan *et al.*, 2003).

Sharma and Dubey (2005) observed that the guaiacol peroxidase (GPX) activity in the seedlings increased significantly due to mild drought stress but declined significantly at higher drought stress level. In 20 days old mildly drought stressed rice seedlings GPX activity was increased between 33 and 56% but the activity was reduced by 13 to 61% under high drought stress levels.

Zhao *et al.* (2017) analysed the effect of HT exposure on POD activity in rice and they observed that activity was reduced on exposure to high temperature similar to that on SOD and CAT, but less pronounced, with the statically insignificant difference being observed between high temperature (35°C day/27°C night) and extremely high temperature (38°C day/30°C night) for HT tolerant cultivar Qianjiang 3, and also between control (28°C day/22°C night), and high temperature (38°C day/30°C night) for susceptible cultivar is Xieqingzao.

2.4.6.2. Super Oxide Dismutase (SOD)

High temperature exposure of plants leads to decrease in SOD and CAT activity and this reduction in SOD was closely related to the severity of heat stress and heat tolerance of rice cultivars. HT-sensitive cultivars decreased more

profoundly than those of HT-tolerant cultivars under the same HT regimes. (Karuppanapandian *et al.*, 2011).

Zhao *et al.* (2017) conducted an experiment to access SOD activity different rice genotypes under different temperature regimes. They observed that under high temperature (35°C day/27°C night) SOD activity of HT tolerant cultivar Qianjiang 3 decreased by 29.8% as compared to control (28°C day/22°C night), while the reduction in susceptible cultivar is Xieqingzao was 35.9 %. In extreme high temperature (38°C day/30°C night), reduction of Qianjiang 3 was 36.3% and that of Xieqingzao was 50.7%.

Sharma and Dubey (2005) studied the effect of mild and high drought stress on superoxide dismutase (SOD) activity and they observed that total SOD activity increased significantly in roots as well as shoots of both the rice cultivars (Malviya-36 and Pant12). The level of total SOD activity was higher in shoots than in roots. Twenty-day-old mild drought stressed ((PEG-6000 of 17%) seedlings showed about 71 to 78% increase in total SOD activity in roots and 56 to 90% increased activity in shoots compared to control seedlings. High drought stress (PEG-6000 of 41.2%) led to an increase between 15 and 105% in Cu/Zn-SOD, 56 to 93% in Fe-SOD and 53 to 63% in Mn-SOD activity in 20 days old seedlings.

2.5. IMPACT OF DROUGHT AND HEAT STRESS ON MORPHOLOGICAL AND YIELD PARAMETERS

2.5.1. Plant height

Lanceras *et al.* (2004) observed that the plant height of rice varieties IR62266 and CT9993 was not significantly different under well-watered (9.4mm average water per day) and very mild stress (5.4 mm per day). But there is considerable effect under severe drought stress (0.0 mm per day). Reduction in plant height was 21.2 cm for IR62266 and 2 cm for CT9993 under severe stress.

In mung bean and wheat increased plant height helps the plants from high temperature through increase in transpiration cooling effect (Kumar *et al.*, 2011, Hasanuzzaman *et al.*, 2013).

Beena *et al.* (2012) observed that plant height reduced 10.4% across the RIL's as compared to irrigated control during drought stress.

Under heat stress, plant height was increased in tolerant rice variety N22 (4.59%) and mutant NH219 (12.82%) (Poli *et al.*, 2013).

Simultaneous occurrence of drought and heat stress decreased stem growth and plant height. Changes in internal water status under drought affect stem growth and stem diameter shrinks (Simonneau *et al.*, 1993).

Severe heat stress decreases stem growth resulting in decreased plant height (Prasad *et al.*, 2006a).

According to Prasad *et al.* (2006), drought and heat stress often decrease stem growth and plant height. Severe heat stress decreases stem growth resulting in decreased plant height.

2.5.2. Leaf area

Leaf expansion is among the most sensitive growth processes to drought such that the expansion and development of the transpiration surface is drastically decreased. Reduction in leaf numbers, rate of expansion, and final leaf size are the general effects of mild drought on leaves. This loss of leaf area will serve as a drought-avoidance mechanism as reduction in leaf area can limit further water loss.

de Souza *et al.* (1997) observed that continued drought stress can accelerate leaf senescence and lead to death of leaf tissue, resulting in leaf drop, particularly old and mature leaves. Drought stress can also influence total leaf area through its effect on initiation of new leaves, which is usually decreased under drought stress.

Heat stress resulted in significant increases in leaf numbers, particularly when reproductive development was arrested without any decrease in leaf photosynthetic rates (Prasad *et al.*, 2006a).

Drought and heat stress can reduce leaf area production and also green leaf area duration, thus negatively affecting the available photosynthates to seeds, ultimately influencing grain size and yield (Prasad *et al.*, 2006).

2.5.3. Shoot dry weight

Boyer (1985) reported that increased root to shoot ratio was observed in plants during soil moisture deficit as a result of reduced shoot dry weight.

Sharp *et al.* (1994) observed that abscisic acid influences the relative growth rates of plant parts such as an increase in the root to shoot dry weight ratio, inhibition of leaf area development and production of prolific and deeper roots.

Mild drought stress changes pattern of resource allocation, generally root growth will be more than shoot growth. (Prasad *et al.*, 2006).

High temperatures reduce plant growth and the total dry weight of the plant by affecting the shoot net assimilation rates (Wahid *et al.*, 2007). Exposure of reproductive stage of wheat to high temperature hastened the decline in photosynthesis and leaf area, decreased shoot and grain mass as well as weight (Shah and Paulsen, 2003).

Wahid (2007) reported that high temperatures caused significant declines in shoot dry mass, relative growth rate and net assimilation rate in maize, pearl millet and sugarcane.

2.5.4. Root traits (Root dry weight, Root length, Root volume)

Growth of root was affected by water and heat stresses. Porter and Gawith (1999) observed that heat stress decreases root growth, and this reduction is due to very narrow optimum temperature range for root growth as compared with

other growth processes. Reduction in root number, root length as well as root diameter were observed under heat stress.

Heat stress occurrence during reproductive development reduces root growth due to decreased carbon partitioning to roots (Batts *et al.*, 1998).

Response of root growth to drought can be variable; root growth can be greater under moderate moisture stress, because of increased partitioning of carbohydrates to roots, whereas, reduction in root growth were observed in severe drought. Drought stress increases the concentrations of ABA in the root, which in turn maintain root growth and increase root hydraulic conductivity, which can postpone development of water stress by increase in water uptake (Gowda *et al.*, 2011).

Secondary traits such as deep, thick, coarse and highly branched roots as well as higher root to shoot ratio are reported in rice as drought adaptation (Blum, 2011).

Niones *et al.* (2015) reported that lateral root production in response to varying soil water content has been demonstrated as an important trait in maintaining dry matter production and grain yield.

Chang *et al.* (1986) found that deep rooted rice cultivars tolerate drought better than shallow rooted cultivars because of their ability to extract moisture from the deeper layers of soil.

Nag (2008) observed a reduction of 17.1 % in root volume due to less moisture availability.

Rejeth (2017) conducted a study in rice to analyse the effect of water stress on root traits, he observed that some genotypes showed significant increase in root length. The average increase in root length among these rice genotypes was 7.23% under water stress condition. Varieties like Ptb-15 with root length 58.8 cm was the highest, which can be considered as drought tolerant. He also analysed the effect on root volume, an overall decrease in root volume from 38.9cm^3 (irrigated condition) to 29.6 cm^3 (water stress condition) occurred among the genotypes under stress. Similar trend was observed for root dry weight, there is an overall decrease

of 32.6% in rice genotypes due to water stress condition compared to control condition. But tolerant varieties like Ptb-10, Ptb-7 and Ptb-55 showed an increase in root volume as well as root dry weight under stress condition.

2.5.5. Canopy Temperature

Canopy temperature can be used as a sensitive indicator of plant stress level, which is associated with stomatal conductance at the leaf level. Wanjura and Upchurch (1997) suggested canopy-temperature-based irrigation scheduling for rice may contribute to the reduction of heat damage.

Batts *et al.* (1998) observed that use of nitrogen fertilizers in heat-tolerant variety is a highly effective means of decreasing heat damage through lowering of the canopy temperature by enhancing evapotranspiration.

It is possible to screen rice varieties for reproductive-stage drought-avoidance traits, using canopy temperature (Garrity and O'Toole, 1995). Tolerant lines remained the coolest under stress.

There is an overall increase in leaf temperature of 3.95% among rice genotypes under water stress as compared to control condition. Decline in leaf water content and decreased transpiration rate can be the reasons for this increment (Rejeth, 2017).

2.5.6. Time of anthesis

Occurrence of flowering early in the morning was found to be a useful phenomenon for stress tolerance especially heat stress. In most of the cultivated accessions of rice the peak anthesis occurs between 10.00 am and 12.00 pm (Sheehy *et al.*, 2005).

Satake and Yoshida (1978) reported that high temperature during or soon after anthesis (1–3 h after anthesis in rice) induces spikelet sterility. Early morning flowering trait can be effectively used to escape from heat stress induced spikelet

sterility at anthesis by shedding viable pollen during the cooler hours in the morning on to a receptive stigma.

Ishimaru *et al.* (2009) transferred a promising EMF trait or allele from wild rice (*O. officinalis*) to mitigate heat stress damage during anthesis. Drought stress or heat stress during flowering and anthesis can lead to failure of fertilization because of decreasing pollen or ovule function.

2.5.7. Spikelet fertility

Prasad *et al.* (2006) observed a significant decrease in spikelet fertility and grain yield at high temperature of 5°C above the ambient air temperatures in various rice cultivars like Gainesville and Florida.

Bheemanahalli *et al.* (2017) recorded a shift in flowering pattern (early flowering) in dry seasons with higher spikelet sterility in rice compared to wet season.

Ekanayake *et al.* (1989) showed that water availability plays an important role in the occurrence of spikelet sterility in rice.

Serraj *et al.* (2009) observed that there is reduction in spikelet fertility and panicle exertion during drought which accounts for decline in rice grain yield.

High percentages of sterility and partially filled grains were observed when the daily mean temperature was 31.5°C (daily maximum was 36°C and daily minimum 27"C) at flowering stages (Moriya and Nara, 1971).

Prasad *et al.* (2006) observed a strong positive correlations between spikelet fertility, pollen production and pollen reception in rice. Spikelet fertility for heat tolerant cultivar N22 at ambient temperature were 89.4% and for high temperature (34/27°C) it is 81.1%.

2.5.8. Pollen viability

Prasad *et al.* (2006) concluded from his study that high temperature decreased pollen production by 51% and number of pollen grains on stigma by 43%. High temperature decreased pollen viability from 91 to 75%, when averaged across all rice cultivars.

Liu *et al.* (2006) observed significant reduction in pollen viability, spikelet fertility and grain yield under water deficit during reproductive stage.

Matsui and Omasa (2002) reported that HT stress given a day prior to anthesis affected the normal functioning of the pollen sac dehiscence and pollen viability in rice.

Bahuguna *et al.* (2015) studied the effect of temperature on pollen viability and found that rice varieties NL-44 and N22 both had about 87% fertility under extremely hot field conditions.

2.5.9. Days to 50% flowering

Rang *et al.* (2011) conducted an experiment on rice for studying the effect of high temperature and water stress on pollen characters, they observed that flowering period of all the five genotypes was significantly extended when exposed to HT, WS and HT + WS compared with control.

Sailaja *et al.* (2015) observed that there was considerable reduction in number of days to 50 % flowering in all the cultivars. Temperature tolerant variety N22 shows a value of 67 days under control while at elevated temperature the value decreased to 63 days.

Rejeth (2017) reported that early flowering was observed in most of the rice genotypes under water stress condition. This can be attributed to drought escape mechanism in rice.

Drought stress developed prior to flowering generally delayed the flowering of genotypes and such a delay was associated with drought susceptibility in rice (Pantuwan *et al.*, 2002).

2.5.10. Productive tiller number

Park *et al.* (1999) observed significant reduction in panicle numbers per hill plant height, leaf area, tiller number, and spikelet numbers per panicle under moisture stress in cultivars Japonica and Dongjinbyeo.

Mitra and Bhatia (2008) reported that plant height, number of tillers and total biomass were reduced in rice cultivar in response to HT.

Reduced number of tillers with promoted shoot elongation was observed in wheat plant when exposed to heat stress (Kumar *et al.*, 2011)

Djanaguiraman *et al.* (2010) discovered that green leaf area and productive tillers/plant were drastically reduced under HT (30/25 °C, day/night).

2.5.11. 1000 grain weight

There is a reduction in biomass production, seed number, individual seed weight and yield of all grain crops under high temperature which is reflected in the harvest index. (Prasad *et al.*, 2017).

Ishimaru *et al.* (2009) stated that one of the reason for the formation of chalky grains were high-temperature stress during grain ripening stage.

Drought directly shorten the seed-filling duration, resulting in smaller seed size and yield (Zhang *et al.*, 2015)

Water stress during the panicle initiation to flowering stages is especially critical in reducing grain yield (IRRI, 1980)

Sailaja *et al.* (2015) observed reduction in 1000 grain weight in rice cultivars under elevated temperature. Mean reduction of 29.4% in grain yield/hill

was observed. 5 % reduction observed in 1000 grain weight. Maximum reduction reported in the variety BPT5204 (72%) whereas minimum value for N22.

2.6. MOLECULAR STUDIES

Under natural conditions plants experience sub-lethal induction stress before being subjected to severe stress. This sub lethal stress triggers the expression of an array of stress responsive genes and resulted in alteration of different physiological and biochemical processes related to stress tolerance (Vierling, 1991; Bohnert *et al.*, 1995).

Presence of LEA protein (Babu *et al.*, 2004), and a water channel transporter aquaporin gene (Lian *et al.*, 2004) in rice led to increased drought resistance.

Masle *et al.* (2005) showed that expression of *ERECTA* gene led to reduction in stomatal frequency and conductance and greater photosynthetic rates, resulting in increased WUE under a wide range of water regimes.

Overexpression of *OsLEA3-1* gene under field conditions were reported by Xiao *et al.* (2007), which resulted in a significant enhancement of drought resistance without any yield penalty.

Kumar *et al.* (2003) reported that stress perception and transduction leading to the expression of transcription factors. He selected HT tolerant hybrids through TIR technique and identified that selected hybrids exhibited enhanced expression of HSP 90 and HSP 104. From this observation he concluded that several HSPs are up-regulated during stress that could be because of the efficiency of upstream regulatory mechanisms.

Klueva *et al.* (2001) reported that generally plants have two distinct mechanisms for heat tolerance; inherent and acquired heat tolerance. Pre-existing characteristics that promotes heat tolerance constitutes inherent heat tolerance whereas acquired thermo tolerance is a physiological response of heat exposure (Massui and Singh, 2003; Larkindale *et al.*, 2005).

Nollen and Morimoto (2002) observed that HSPs/chaperones have a role in stress signal transduction and gene activation and these responses interact with other stress-response mechanisms such as production of osmolytes (Diamant *et al.*, 2000) and antioxidants (Panchuk *et al.*, 2002).

Sailaja *et al.* (2015) selected one tolerant (N22) and susceptible (Vandana) rice cultivar based on the physiological, biochemical and yield studies in the previous experiment. They used these two cultivars for molecular study and observed upregulation and downregulation of many genes under heat stress. Heat shock transcription factors *OsHsfA2a, OsHsfA2e, OsHsfA7* were upregulated in N22, but in Vandana downregulation of *OsHsfA2a* occurred. Other highly upregulated genes in N22 are *Osfd* (13.7 fold), *Cyt-C-Oxi* (14.2 fold), *CWIP* (12.5 fold) and *FRH* (80 fold). In Vandana also *Osfd, Cyt-C-Oxi, CWIP* genes were upregulated, but the expression was less and Vandana exhibited downregulation of *FRH* under heat stress. Upregulation of heat shock protein genes and downregulation of SOD genes found in both the cultivars.

MATERIALS AND METHODS

MATERIALS AND METHODS

The present study entitled "Validation of Temperature Induction Response (TIR) technique for inducing drought and heat stress tolerance in rice (*Oryza sativa* L.)" was conducted in the field and rain out shelter maintained by Departmentt of Plant Physiology, College of Agriculture, Vellayani, Kerala Agricultural University during the years from 2016-2018. The main objective of the study was to validate the effect of Temperature induction response for combined drought and heat 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 IDENTIFICATION OF LETHAL AND INDUCTION TEMPERATURE FOR TIR TECHNIQUE.

3.1.1 Plant materials

Two rice varieties were used for this study Ptb 39 (Jyothi) and PTB 60 (Vaishak) collected from RARS, Pattambi.

3.1.2 Location

The study was conducted in the BOD Incubator (Equitron Incubator, Ecogain series 43L) maintained by Department of Agricultural Microbiology, College of Agriculture, Vellayani during 2017.

3.1.3 Experimental details

3.1.3.1. Standardization of lethal temperature

Lethal temperature is the temperature at which seedling mortality would be 100%. To standardize the lethal temperature, five days old rice seedlings kept in petri plates with wet filter paper (plate 1) were exposed to different temperatures for varying

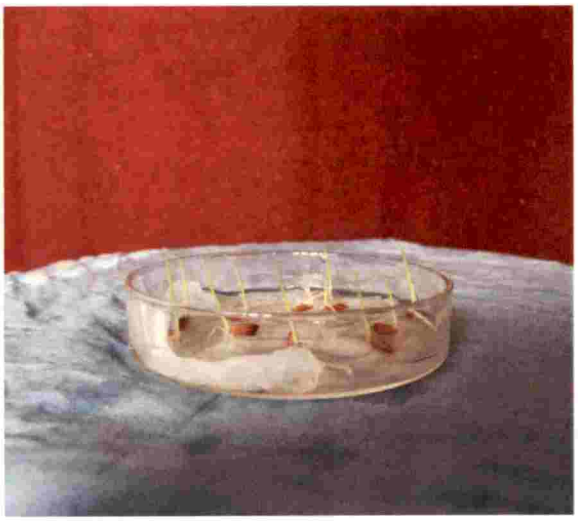


Plate 1. Germinated seeds of Jyothi and Vaishak before temperature treatments.

durations without prior induction in incubator. The seedlings were then allowed to recover at normal room temperature with 60% RH for 72 hrs. At the end of the recovery period, the percent survival of seedlings were taken to arrive at the challenging or lethal temperature. Here, the temperature at which 100% seedling mortality occurred was considered as challenging or lethal temperature. Temperatures used to standardize the lethal temperature is given below (Table.1).

Table 1: List of temperatures used to standardize lethal temperature.

Sl.No.	Treatments	Temperature	Duration
1	T1	49°C	2 hrs
2	T2	49°C	2 ½ hrs
3	T3	49°C	3 hrs
4	T4	50°C	2 hrs
5	T5	50°C	2 ½ hrs
6	T6	50°C	3 hrs
7	T7	51°C	2 hrs
8	T8	51°C	2 ½ hrs
9	T9	51°C	3 hrs
10	T10	52°C	2 hrs
11	T11	52°C	2 ½ hrs
12	T12	52°C	3 hrs
13	T13	Ambient Temperature	

3.1.3.2. Standardization of induction temperature

In order to develop certain degree of tolerance to lethal temperature, seedlings were exposed to a sub-lethal temperature, referred to as induction temperature. To standardize the induction protocol, five days old rice seedlings were taken in petri plates with wet filter paper. These plates with seedlings were exposed to a range of

gradual temperature for different durations in incubator. Then they were exposed to the standardized lethal temperature. The combination of induction and lethal temperature at which maximum recovery growth observed were selected for further study. At the end of lethal treatment, the seedlings were kept for recovery at room temperature (60% RH) for 72 hrs. Percent seedlings survival and recovery growth were measured. One more set of seedlings were kept at room temperature all through without exposing them to any kind of stress as control. Based on the extent of variation shown under different induction temperatures, the TIR protocol was standardized. Temperatures used to standardize the induction temperature is given below (Table 2).

Table 2: List of temperatures used to standardize induction temperature.

Sl.No.	Treatments	Temperature
1	T1	28-40 °C for 5 hrs & 40-52 °C for 30 min
2	T2	32-40 °C for 5 hrs & 40-52 °C for 30 min
3	T3	32-42 °C for 5 hrs & 42-52 °C for 30 min
4	T4	Control (Ambient temperature)

3.1.4 Parameters

3.1.4.1 Percentage Seedling Survival (%)

The number of seedlings survived in each treatment were assessed using the following formula

$$\% \text{ Seedling Survival} = \frac{\text{Number of seedlings survived}}{\text{Total number of seedlings}} \times 100$$

3.1.4.2 Recovery Growth (cm)

Before exposing the seedlings to various temperature treatments initial measurement (root and shoot length) of seedlings were made. Similarly, after treatments the final seedling growth was also measured and based on the initial and final growth of the seedlings, recovery growth of seedlings were determined.

Recovery Growth = Final growth – Initial growth

3.1.4.3 Percent Reduction in Recovery Growth (% RRG)

The recovery growth measured in the absolute control was used to calculate the % reduction in recovery growth of treatments.

$$\%\,RRG = \frac{\text{Recovery growth of control seedlings} - \text{Recovery growth of treated seedlings}}{\text{Recovery growth of control seedlings}} \times 100$$

3.2. TEMPERATURE INDUCTION RESPONSE (TIR) FOR COMBINED DROUGHT AND HEAT STRESS TOLERANCE IN RICE.

After standardizing the TIR protocol, ten rice varieties were exposed to standardized TIR protocol for validating TIR technique in inducing combined drought and heat stress tolerance.

3.2.1 Plant materials

Ten rice varieties were used for this study collected from RARS, Pattambi and NRRI, Cuttak. (Table 3), (plate 2).

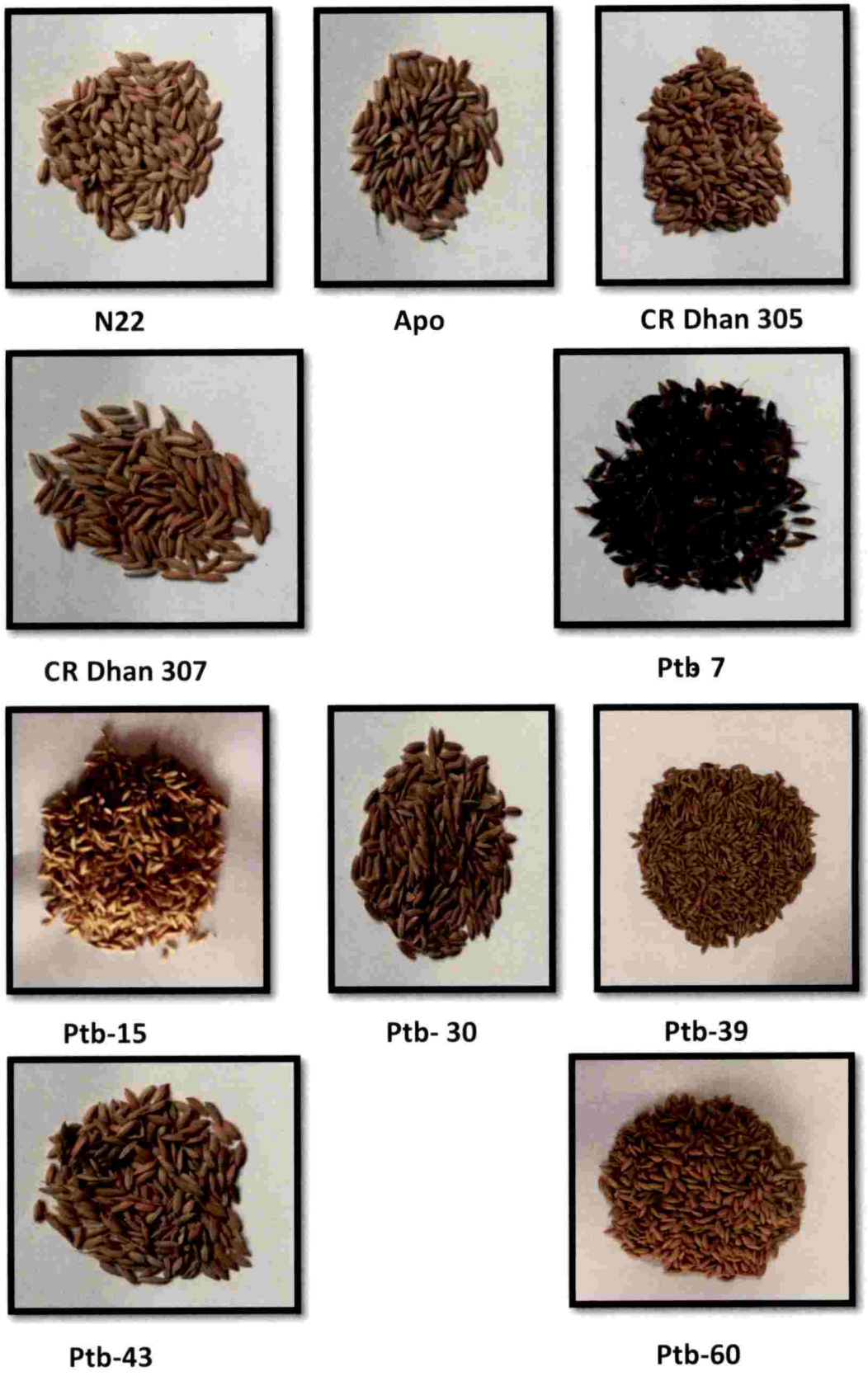


Plate 2. Seeds of different rice genotypes.

Table 3: List of rice accessions used in the study.

Sl.No.	Genotypes
V1	N22 (Nagina)
V2	Apo
V3	CR Dhan 305
V4	CR Dhan 307 (Maudamani)
V5	Ptb 7 (Parambuvattan)
V6	Ptb 15 (Kavunginpoothala)
V7	Ptb 30 (Chuvanna Modan)
V8	Ptb 39 (Jyothi)
V9	Ptb 43 (Swarnaprabha)
V10	Ptb 60 (Vaishak)

3.2.2. Location

The study was conducted in the field and rainout shelter of Department of Plant Physiology, College of Agriculture, Vellayani during 2016-18.

3.2.3. 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. Seedlings after standardized TIR technique (32-42 °C for 5 hrs & 42-52 °C for 30 min + 52°C for 3 hrs) were sown in plastic pro-trays (30cm x 15cm dimension) filled with soil and coir pith in the ratio 2:1 (plate 3). Twenty 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. The

Plate 3. Recovered seedlings after TIR treatment.

cultural operations including weeding and plant protection measures were carried out as per *ad hoc* recommendations of Kerala Agricultural University, Thrissur.

3.2.4. Methodology

In this study, plants were raised in earthen pots in field (plate 4, 5) and rainout shelter (plate 6,7). Separate set of plants with three replications were maintained for all eight treatments. Irrigation was given regularly for all the eight treatments up to panicle initiation stage according to their duration. Then irrigation was withhold to a period of 5 days to induce drought condition in two set of plants, one set was TIR treated plants and second set was plants grown under normal conditions. Next two set of plants, one was TIR treated plants and another was grown under normal conditions were exposed to high temperature (3-5°C more than ambient condition) from panicle initiation to maturity (plate 8). Both heat and drought stress were given to two set of plants, which were TIR treated and second set grown under normal condition. The control plants were well irrigated up to maturity. One set of TIR treated plants without any stress also maintained for comparison. Plants were kept upto maturity after stress. All the physiological parameters were taken ten days after stress. At the time of harvest, morphological and yield parameters were taken.

Plate 4. General view of control plants.

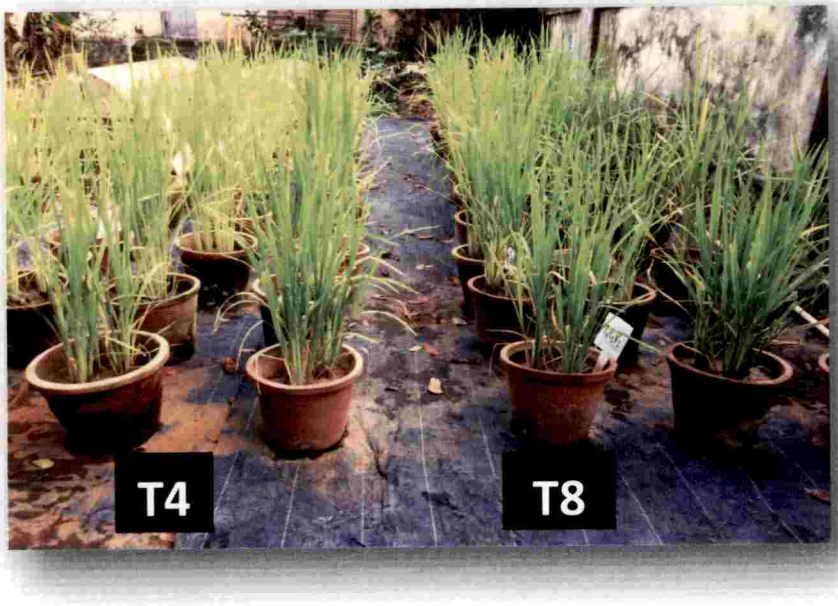


Plate 5. Plants under ambient conditions.

T4- TIR induced plants without stress

T8- Non-induced plants without stress (Control)

Plate 6. General view of experimental unit

Plate 7. Plants before stress.

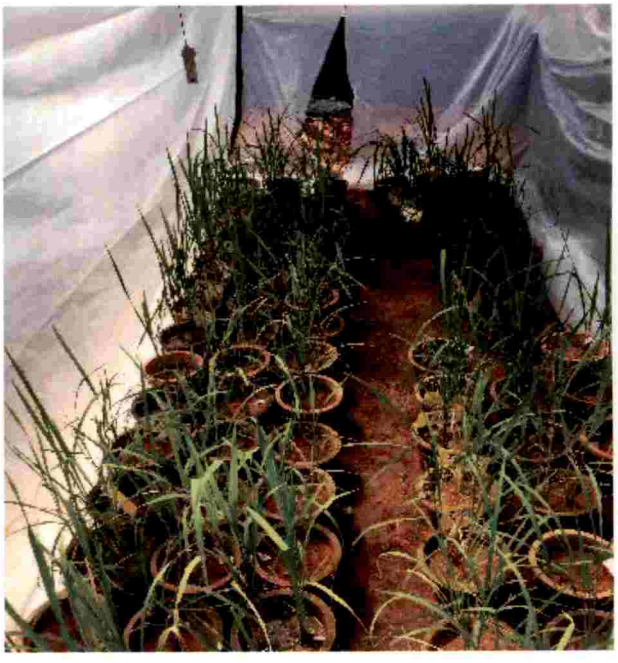


Plate 8. Plants under heat stress and combined drought and heat stress.

Table 4. Particulars of experiment 2.

1. Crop	Rice: 10 genotypes
2. Design	Completely Randomized Design (CRD)
3. Number of treatments	Six T1- TIR treated seeds + Drought + Heat T2- TIR treated seeds + Drought T3- TIR treated seeds + Heat T4- TIR control T5-Germinated seeds under ambient temperature + Drought + Heat T6-Germinated seeds under ambient temperature + Drought T7-Germinated seeds under ambient temperature + Heat T8- Control (Ambient temperature + Normal irrigation).
*TIR treatment: 32-42 °C for 5 hrs & 42-52 °C for 30 min + 52°C for 3 hrs	
4. Replication	Three

3.2.5. Observations

3.2.5.1. Morphological parameters

3.2.5.1.1. Plant height (cm)

Plant height was measured from the base of the plant to the tip of the primary panicle at the time of maturity and expressed in centimeters.

3.2.5.1.2. Leaf area (cm^2)

Leaf area was measured during maturity stage and calculated using the formula

Total leaf area= L x B x 0.75 x Total number of leaves.

where, L- Length of leaf blade, B- maximum width of leaf blade

3.2.5.1.3. Shoot dry weight (g)

Shoots collected after harvest were dried moisture free in a hot air oven at 80°C for 48 hours (till attaining constant weight). Then the dry weights were recorded in grams by using an electronic balance.

3.2.5.1.4. Root dry weight (g)

Roots collected were dried moisture free in a hot air oven at 80°C for 48 hours (till attaining constant weight). Then the dry weights were recorded in grams by using an electronic balance.

3.2.5.1.5. Root length (cm)

Root length was measured from the cut end to the tip of the longest rootlet by using a centimetre scale and expressed in cm.

3.2.5.1.6. Root volume (cm^3)

Root volume was determined in cubic centimetre by water displacement method. Roots were immersed in water in a 1000 ml measuring cylinder after removing from the soil and cleaning thoroughly. The displaced volume of the water was taken as the volume of the roots.

3.2.5.1.7. Canopy Temperature (°C)

Canopy temperature were measured using IR thermometer.

3.2.5.2. Physiological parameters

3.2.5.2.1. Cell membrane stability index

Cell membrane stability index was calculated as per the procedure described by Blum and Ebercon (1981). Samples collected from all the treatments were washed three times in deionised water to remove electrolytes adhered on the surface. Samples were kept in a capped vial (20ml) containing 10ml of deionised water and incubated in the dark for 24 hours at room temperature. The conductance was measured with a conductivity meter. Then these vials were autoclaved for 15 minutes to kill the leaf tissue and release the electrolytes. After cooling, the second conductivity reading was taken. These two measurements were carried out individually for all the treatments. Cell membrane stability index was calculated by using following formula and expressed as per cent.

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

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.

3.2.5.2.2. Chlorophyll stability index

Chlorophyll content of leaf samples were estimated as per the procedure by Arnon (1949). 100 mg of leaf sample was taken from fully expanded third leaf and were chopped inti pieces. 5 ml of DMSO (Dimethyl sulfoxide): Acetone (80%) (1:1) mixture was added to samples and incubated overnight. The supernatant was collected and absorbance was measured at 645 and 663 nm. Total chlorophyll content and chlorophyll stability index was calculated using the formula given below and expressed in mg g^{-1} of fresh leaf weight.

Total chlorophyll = {[20.2(OD at 645) + 8.01(OD at 663)] x V} / (Wx1000)

Where V = volume of the solution made up and W= fresh weight of leaves.

$$\text{Chlorophyll stability index} = \frac{\text{Total chlorophyll in stress}}{\text{Total chlorophyll in control}} \times 100$$

3.2.5.2.3. Stomatal conductance

Stomatal conductance was measured using Portable Photosynthetic System (CIRAS-3, PP systems U.S.A) at morning time between 9 am and 11 am and were expressed in m H_2O moles m^{-2} s^{-1}.

3.2.5.2.4. Photosynthetic rate

Photosynthetic rate was measured at morning time between 9 am and 11 am using Portable Photosynthetic System (CIRAS-3, PP systems U.S.A) and were expressed in μ CO_2 moles m^{-2} s^{-1}.

3.2.5.2.5. Proline content

Proline content was estimated as per the procedure described by Bates *et al.,* (1973). A known amount (0.5g) of mid-leaf portion was homogenized with 10ml of 3% aqueous sulphosalicylic acid and centrifuged at 3000 rpm for 15 minutes. 2ml of the supernatant was taken and mixed with an equal amount of glacial acetic acid and acid ninhydrin. The contents were allowed to react at 100^0C for one hour in water bath. The reaction was terminated by keeping it in ice bath for 10 min. 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

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

where, 115.5 is the molecular weight of proline.

3.2.5.2.6. Peroxidase

Peroxidase activity was measured according to the procedure described by Peru (1962). Leaf sample of 200 mg was homogenised in 1 ml of 0.1 M sodium phosphate buffer (pH 6.5) to which a pinch of polyvinyl pyrrolidone (PVP) was added. The supernatant was filtered through a muslin cloth and centrifuged at 5000 rpm for 15 minute at 4°C. The supernatant was used as the enzyme extract for the assay.

Reaction mixture containing 1 ml of 0.05 M pyrogallol and 50 µl of enzyme extract was taken in both reference and sample cuvettes, mixed and kept in spectrophotometer, reading was adjusted to zero at 420 nm. The enzyme reaction was started by adding 1 ml of 1 % hydrogen peroxide (H_2O_2) into sample cuvettes and change in absorbance was measured every 30 seconds upto 3 minutes. One unit of peroxidase is defined as the change in absorbance/minute at 420 nm.

3.2.5.2.7. Superoxide Dismutase

Superoxide dismutase activity was measured by the method described by Beauchamp and Fridovich (1971). Grind 1g of clean leaf tissue in 10 ml ice cold 50 mM potassium phosphate buffer, pH 7.8 in a pre-chilled pestle and mortar. Centrifuge the homogenate at 10000rpm for 10 min at 4°C and the supernatant was used for assay. Mix a 3 ml reaction mixture containing 50 mM potassium phosphate buffer, 13mM methionine, 2 µM riboflavin, 0.1 mM EDTA, 75 µM NBT and 50 µl of crude enzyme extract, in duplicate. Make up the volume equal by adding double distilled water. Set a blank without enzyme and NBT to calibrate the spectrophotometer. Set another control having NBT but no enzyme as reference control. Expose all the tubes to 400 W bulb (4 x 100 W bulbs) for 15 min. Read the absorbance immediately at 560 nm. Calculate the percentage inhibition. The 50 % inhibition of the reaction between riboflavin and NBT in the presence of methionine was taken as 1 unit of SOD activity.

3.2.5.3. Observations in Spikelet

3.2.5.3.1. Time of anthesis

Time of anthesis was observed from08.00 am to 12.00 pm. Visual observation was taken directly from the plot.

3.2.5.3.2. Spikelet fertility (%)

The total numbers of filled and unfilled spikelets of three randomly selected primary tillers of the target plants in each treatment were counted. Then,

Spikelet fertility (%) was calculated by using the formula

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

3.2.5.3.3. Pollen viability (%)

Pollen viability was measured using 1 % iodine- potassium iodide (IKI) solution which was prepared by dissolving 2.5 g of KI and 250 mg of iodine made up to 125 ml. Spikelets from each treatment were collected just before anthesis and it was crushed and stained using IKI solution in glass slides. The fully stained grains represent fertile pollen and unstained, shriveled, empty grains denote sterile grains. The fertile pollen grains were visually counted under compound microscope, Leica. The pollen viability was calculated using the formula given below and expressed as percentage.

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

3.2.5.4. Yield parameters

3.2.5.4.1. Days to 50% flowering

The number of days taken from sowing to exertion of 50% of panicles in each replication was taken as days to 50% flowering

3.2.5.4.2. Productive tiller number

In each replication, the number of panicle bearing tillers at the time of harvest was counted and recorded.

3.2.5.4.3. 1000 grain weight (g)

One thousand seeds were taken randomly from each replication, weighed and expressed in grams.

3.2.5.5. Molecular observations

3.2.5.5.1. SDS PAGE

Electrophoresis is widely used to separate and characterise proteins by applying electric current. Electrophoretic separation of proteins were done by the procedure described by Laemelli (1970).

1 g of root samples were homogenised in 1.5 ml of ice cold denaturing buffer. The extract was centrifuged at 5000 rpm for 15 minutes. The supernatant was collected and mixed with chilled acetone in the ratio 1:1 and the protein was allowed to precipitate by keeping the mixture at 4°C for 30 minutes. The sample was centrifuged at 3600 rpm for 10 minutes. The supernatant was removed and the pellet was re suspended in 50 µl of denaturing buffer and vortexed. The homogenate was centrifuged at 5000 rpm for 15 minutes. The supernatant was mixed with 10µl of

sample buffer and kept in a boiling water bath for 3 minutes. These samples were subjected to electrophoresis using SDS – PAGE.

Reagents

a) Electrode buffer pH – 8.3.

b) 0.5 M Tris- HCl pH- 6.8.

c) 10 % SDS.

d) 1.5 M Tris- HCl pH- 8.8.

e) Acrylamide stock- 30 %.

f) Sample buffer.

g) Polymerising agents.

h) Staining solution.

i) Destaining solution.

Procedure

After thorough cleaning and drying the plates and spacers, assembled them properly. Separating gel was casted first followed by stacking gel by mixing the various reagents prepared earlier.

After keeping the comb in position stacking gel was poured over the polymerized separating gel. Then the comb was removed and samples were loaded into the wells. Standard with known molecular weight was also loaded to one well. The electrophoresis was performed at 80 V till the dye reached the separating gel. Then the voltage increased to 100 V till the dye reached bottom of the gel. Immediately after electrophoresis gel was removed from glass plates and placed in the staining solution overnight. Then the gel was transferred to destaining solution. The protein appeared as bands and the gel was photographed.

3.2.5.5.2. **RT- PCR analysis**

Expression level of *PSTOL1* (Phosphorous Starvation Tolerance 1) and *DRO1* (Deeper Rooting 1) was studied in induced and non-induced plants of one tolerant genotype (N22) and one susceptible genotype (Ptb 39- Jyothi) under combined drought and heat stress by using RT (Reverse Transcriptase) PCR. RNA was isolated using TRIZOL™ reagent.

RNA isolation

100 mg of tissue were frozen in liquid nitrogen and grinded to a fine powder. 1 mL of Trizol reagent were added to the powdered tissue and mixed gently to homogenize the mixture and incubated at room temperature for 5 min for complete dissociation of nucleoprotein complexes. This homogenate was transferred to a 2 ml pre chilled microfuge tube. Then 0.2 mL chloroform were added to it and shaken vigorously for 15 seconds. Then it was incubated at RT for 5 mins and kept in ice for 10 mins. After that centrifugation was done at 12000 rpm for 15 mins at 4° C. The aqueous phase was transferred to a fresh tube and 0.5 mL of ice cold isopropanol (100%) were added to each tube and incubated at RT for 10 mins. After mixing centrifuged at 12000 rpm for 10 mins at 4° C. Then the supernatant were removed and pellet washed with 1 ml of 75% alcohol (in DEPC treated water). Sample briefly vortexed and spun at 7500 rpm for 5 mins at 4° C. After centrifugation, alcohol was aspirated and the RNA pellet was air dried for 30 - 40 mins. Then the pellet was dissolved in 30 µl RNase free water and incubate at 55 – 60° C for 10 mins. The quantity of RNA was checked in a 2% agarose gel by loading 1 µl sample along with loading dye.

Reverse Transcriptase PCR analysis

The cDNA synthesis was performed using Thermo scientific verso cDNA Synthesis kit Product code AB-1453/A. About 4µl of 5X cDNA synthesis buffer , 2µl

of dNTP mix, 1µl of anchored oligo dT, 1µl of RT Enhancer, 1µl of Verso Enzyme Mix and 5 µl of RNA template (1ng of total RNA) were added to an RNAse free tube. Then the total reaction volume was made up to 20 µl with the addition of sterile distilled water. The solution was mixed by pipetting gently up and down. The thermal cycler (Eppendorf Master Cycler) was programmed to undergo cDNA synthesis. The following cycling conditions were employed, 30minutes at 42°C and 2 minutes at 95°C.

The amplification was done using Thermoscientific amplification kit. The following components were added to a new PCR vial in a PCR work station. For each 50 µL reaction: 25 µL of PCR Master Mix (2X) , 2 µLof Forward primer (0.1-1.0 µM),2 µLof Reverse primer(0.1-1.0 µM), 5 µLof Template DNA (10 pg - 1 µg). The components were made upto 50 µL with sterile distilled Water (nuclease-free). Initial denaturation at 95°C for 3 minutes, followed by denaturation at 95°C for 30s , annealing at 58°C (PSTOL1) and 60 °C (DRO1) for 30 s and extension at 72°c for 1 minute which was repeated for 35 cycles and the final extension at 72°C for 5 minutes. After the amplification, the PCR product was separated by agarose gel electrophoresis.

Table 5. Details of primer used in RT PCR

OLIGO NAME	FORWARD	REVERSE
	SEQUENCE (5' ->3')	SEQUENCE (5' ->3')
Dro 1	ATATGGGCGTACGGTAGCTG	AGAGATTGGGGAGGGACAAA
Pstol 1	TGAGATAGCCGTCAAGATGCT	AAGGACCACCATTCCATAGC

1.5% agarose gel was prepared in 1X TE buffer and melted in hot water bath at 90°C. Then the melted agarose was cooled down to 45◦C. 6µl of 10 mg/ml of ethidium bromide was added and poured in to gel casting apparatus with the gel comb. After setting, the comb was removed from the gel. The electrophoresis buffer was poured in the gel tank and the platform with the gel was placed in it so as to immerse the gel. The gel was loaded with the samples and run at 50 V for 30 minutes. The stained gel was visualized using a gel documentation system (E gel imager, Invitrogen).

RESULTS

RESULTS

The present study "Validation of Temperature Induction Response (TIR) technique for inducing drought and heat stress tolerance in rice (*Oryza sativa* L.)" was implemented in two experiments in the Department of Plant Physiology, College of Agriculture, Vellayani. The objective of the first experiment was to identify the lethal and induction temperature for TIR technique in rice. Second experiment was conducted for understanding the effect of TIR technique in inducing stress tolerance in rice. The data obtained during the course of investigation were statistically analysed and the results are presented in this chapter.

4.1 STANDARDIZATION OF LETHAL TEMPERATURE

Seedlings of Jyothi (Ptb 39) and Vaishak (Ptb 60) were exposed to four different temperatures (49°C, 50°C, 51°C, 52°C) in three different durations (2 h, 2 ½ h, 3 h). After the recovery period of 72 hrs in 30°C, the temperature at which 100 % mortality of the seedlings occurred was selected as the lethal temperature. Percentage seedling survival after the treatment was presented in table 6 and the results showed that as the intensity of lethal temperature increased from 49 to 52°C, the percent survival of the seedlings reduced markedly (plate 9). 100% mortality was observed when the seedlings were exposed to 52°C for 3 hrs and this temperature was selected as the lethal temperature.

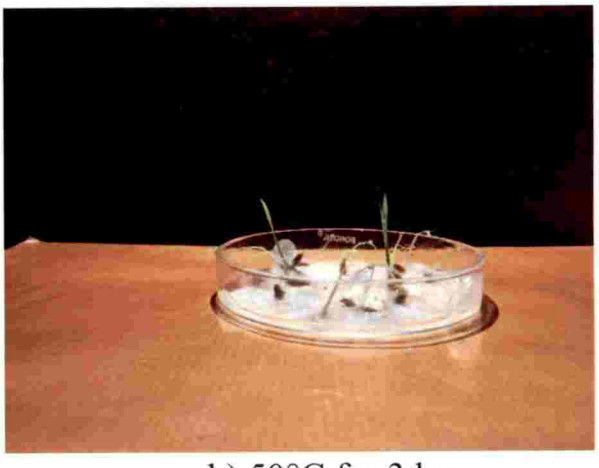
4.2 STANDARDIZATION OF INDUCTION TEMPERATURE

Germinated seedlings of Jyothi and Vaishak were exposed to a gradual induction temperature followed by standardized lethal temperature. Later, these seedlings were allowed for recovery at 30°C for 72 h. At the end of recovery period, recovery growth and percent reduction in recovery growth were measured. For comparison one set of seedlings were kept as absolute control in room temperature

Table 6. Percentage of seedling survival under of rice genotypes under different treatments

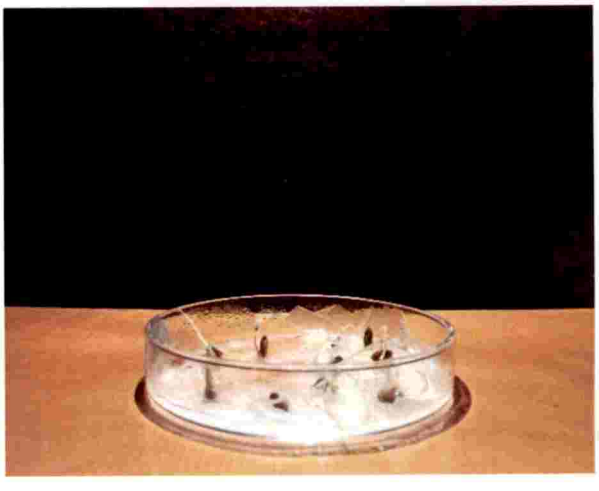
Sl.No.	Treatments	Jyothi	Vaishak	Mean
1	49°C for 2 hrs	96.7	98.4	97.5
2	49°C for 2 ½ hrs	95.0	95.0	95.0
3	49°C for 3 hrs	91.7	91.7	91.7
4	50°C for 2 hrs	73.3	88.3	80.8
5	50°C for 2 ½ hrs	66.7	78.3	72.5
6	50°C for 3 hrs	50.0	70.0	60.0
7	51°C for 2 hrs	48.3	46.7	47.5
8	51°C for 2 ½ hrs	41.7	41.7	41.7
9	51°C for 3 hrs	23.3	22.9	23.1
10	52°C for 2 hrs	11.7	13.3	12.5
11	52°C for 2 ½ hrs	9.8	10.0	9.9
12	52°C for 3 hrs	0	0	0
13	Control (Ambient temperature)	100.0	100.0	100.0
	Mean B	54.5	58.2	
		C.D(5%)	SE (m)	
	G	1.6	0.6	
	T	4.1	1.4	
	GxT	5.9	2.1	

a) 49°C for 3 hrs



b) 50°C for 3 hrs

c) 51°C for 3 hrs



d) 52°C for 3 hrs

Plate 9. Seedlings after different temperature treatments.

The treatment which showed maximum recovery growth and minimum percent reduction in recovery growth were selected as induction temperature.

4.2.1. Recovery growth

The results of recovery growth are presented in table 7. The results showed that among the treatments mean recovery growth is maximum (7.8 cm) for the third treatment (32-42°C for 5 h & 42-52°C for 30 min followed by 52°C for 3 h). Both the genotypes have higher recovery growth in this treatment, 8.8 cm for Jyothi and 6.8 cm for Vaishak.

4.2.2. Percent reduction in recovery growth

The percent reduction in recovery growth of genotypes are shown in table 8. The least reduction in recovery growth (22.2%) is recorded in the third treatment (32-42°C for 5 h & 42-52°C for 30 min followed by 52°C for 3 h). Between the genotypes Vaisakh showed least reduction (33.3 %) than Jyothi (38.9%).

4.3. EFFECT OF DROUGHT AND HEAT STRESS ON MORPHOLOGICAL PARAMETERS.

4.3.1. Plant height

Plant height was measured from the base of the shoot to tip of the top most panicle at maturity. Table 9 shows the variation in plant height of ten rice genotypes under different treatments. Differences in the plant height was significant with respect to genotype and treatment. As compared to control (T8), all the genotypes except N22, showed a reduction in plant height when exposed to different stress treatments. The plants exposed to combined heat and water stress showed highest percentage reduction in plant height. Temperature stress has relatively more deleterious effect on plant height in all the genotypes than moisture stress. The induced plants without any stress (T4- TIR control) have higher plant height as compared to absolute control plants. Induced plants under stress have more plant height as compared to non-induced plants.

Table 7. Recovery growth of seedlings after different induction temperature treatments

Treatments	Jyothi	Vaishak	Mean
T1-28-40 °C for 5 hrs & 40-52 °C for 30 min	5.3	5.1	5.2
T2-32-40 °C for 5 hrs & 40-52 °C for 30 min	6.6	5.7	6.1
T3-32-42 °C for 5 hrs & 42-52 °C for 30 min	8.8	6.8	7.8
T4-Control (Ambient temperature)	11.3	8.8	10.0
Mean	8.0	6.6	
		C.D.(5%)	SE(m)
	G	0.4	0.1
	T	0.5	0.2
	GxT	0.7	0.2

Table 8. Percentage reduction in recovery growth of seedlings after different induction temperature treatments

Treatments	Jyothi	Vaishak	Mean
T1-28-40 °C for 5 hrs & 40-52 °C for 30 min	53.0	42.4	47.7
T2-32-40 °C for 5 hrs & 40-52 °C for 30 min	41.8	35.0	38.4
T3-32-42 °C for 5 hrs & 42-52 °C for 30 min	21.8	22.5	22.2
Mean	38.9	33.3	
		S.D(5%)	SE(m)
	G	5.3	1.7
	T	4.3	1.4
	GxT	NS	2.4

Among the genotypes Ptb-15 (147.1 cm) recorded the highest mean value, followed by Ptb-43(142.3 cm) and the genotypes Ptb-39 (97.3 cm) recorded the minimum plant height followed by Apo (108.3 cm). Induced plants without any stress showed higher mean value (134.6 cm) among the treatments and non- induced plants under combined drought and heat stress possess lowest plant height (110.5 cm).

4.3.2. Leaf area

A significant difference was observed with respect to genotype, treatment and their interactions for leaf area which is shown in table 10. Maximum leaf area was observed for Ptb-60 (7079.7 cm^2) followed by Ptb-15 (6822.8 cm^2) and Ptb-43 (6426.1 cm^2) under control but the mean leaf area value is higher for Ptb-43 (5707.1 cm^2) followed by Ptb-60 (5657.1 cm^2) and Ptb-15 (4496.9 cm^2). This indicated that reduction in leaf area was less in Ptb-43 as compared to Ptb-60 and Ptb-15. Among the treatments, induced plants without any stress (3934.8 cm^2) showed 7.7 % more leaf area than control plants (3654.7 cm^2). Mean value of leaf area was less in Ptb-39 (1076.1cm^2) followed by Ptb-30 (1190.6 cm^2). Percentage reduction in leaf area was highest in non-induced plants exposed to combined drought and heat stress (42.1%), followed by non-induced plants exposed to heat stress (37.9%) and non-induced plants exposed to moisture stress (34.9%). Corresponding values for induced plants are 30.3%, 25.4% and 20.8 % respectively.

4.3.3. Shoot dry weight

The results related to shoot dry weight after harvest in ten genotypes in different treatments are presented in table 11. There was significant difference exist between treatments, genotypes and interactions. Mean shoot dry weight was highest for Ptb-15 (25.1 g) and Ptb-7 (23.7 g) among the genotypes. Minimum value recorded in Ptb-39 (15.4 g) and CR Dhan 305 (18.4 g). 33.8% reduction in shoot dry weight was recorded in Ptb-15 in non-induced plants exposed to combined stress whereas in induced plants, the recorded reduction was 29.4 %. In Ptb-39 reduction was 53.8 % for

Table 9. Plant height (cm) of rice genotypes at maturity under stress conditions.

Sl.No.	Genotypes	T1	T2	T3	T4	T5	T6	T7	T8	Mean
1	N22 (Nagina)	115.3	115.0	127.5	104.3	112.3	111.3	110.7	103.3	112.5
2	APO	103.7	112.7	104.0	122.7	98.3	105.3	98.7	121.7	108.3
3	CR Dhan 305	117.3	118.7	120.0	133.3	96.0	100.3	109.7	126.7	115.2
4	CR Dhan 307 (Maudamani)	113.8	120.3	117.0	127.7	102.3	117.0	111.7	123.0	116.6
5	Parambuvattan (Ptb 7)	140.7	137.5	136.3	150.0	111.3	127.7	131.3	149.7	135.6
6	Kavunginpoothala(Ptb15)	142.0	154.3	144.7	162.0	132.0	141.0	140.7	160.3	147.1
7	Chuvannamodan (Ptb 30)	116.0	124.3	121.0	127.7	110.3	122.7	112.2	125.3	119.9
8	Jyothi (Ptb 39)	93.0	99.7	98.7	107.7	91.2	92.0	92.0	104.0	97.3
9	Swarna prabha (Ptb 43)	140.0	146.0	140.0	155.7	129.0	135.3	138.3	154.3	142.3
10	Vaishak (Ptb 60)	126.7	145.3	136.3	155.3	122.3	125.3	130.0	151.7	136.6
	Mean	120.7	127.4	124.5	134.6	110.5	117.8	117.5	132.0	
			C.D (5%)	SE (m)						
		G	6.7	2.4						
		T	6.0	2.1						
		GxT	NS	6.8						

Table 10. Leaf area (cm^2) of rice genotypes at maturity under stress conditions.

Sl.No.	Genotypes	T1	T2	T3	T4	T5	T6	T7	T8	Mean
1	N22 (Nagina)	1412.1	1792.5	1864.9	2488.1	1167.1	1468.3	1502.8	2059.1	1719.3
2	APO	2179.7	2439.9	2130.6	2979.5	1691.5	2036.8	1950.2	3010.7	2302.4
3	CR Dhan 305	1797.0	2353.8	1992.8	2852.0	1419.6	1746.1	1641.4	2638.6	2055.2
4	CR Dhan 307 (Maudamani)	1454.5	1650.5	1694.6	2731.1	1086.3	1541.5	1259.9	2267.2	1710.7
5	Parambuvattan (Ptb 7)	2143.8	2170.6	2062.2	3746.9	1535.8	1664.8	1587.1	2935.5	2230.8
6	Kavunginpoothala(Ptb15)	3784.4	4549.7	4337.0	7032.3	2963.7	3350.7	3134.9	6822.8	4496.9
7	Chuvannamodan (Ptb 30)	1074.1	1175.1	1148.0	1761.0	859.9	889.6	858.6	1758.8	1190.6
8	Jyothi (Ptb 39)	936.2	1213.2	995.2	1641.6	704.5	786.3	782.9	1548.6	1076.1
9	Swarna prabha (Ptb 43)	5354.2	5879.1	5672.2	6780.7	5038.9	5324.0	5181.2	6426.1	5707.1
10	Vaishak (Ptb 60)	5329.2	5734.5	5348.8	7334.9	4701.2	5001.6	4806.7	7079.7	5667.1
	Mean	2546.5	2895.9	2724.6	3934.8	2116.9	2381.0	2270.6	3654.7	
			C.D (5%)	SE (m)						
		G	86.8	31.1						
		T	77.7	27.8						
		GxT	245.7	87.9						

Table 11. Shoot dry weight (g) of rice genotypes at maturity under stress conditions.

Sl.No.	Genotypes	T1	T2	T3	T4	T5	T6	T7	T8	Mean
1	N22 (Nagina)	23.4	23.2	22.4	24.5	20.6	22.6	21.1	22.6	22.6
2	APO	23.3	23.5	22.7	21.2	21.6	21.1	20.5	20.1	21.7
3	CR Dhan 305	16.8	18.9	18.1	26.8	14.4	13.6	14.4	24.2	18.4
4	CR Dhan 307 (Maudamani)	20.4	21.6	19.7	23.5	16.3	18.6	18.3	24.4	20.4
5	Parambuvattan (Ptb 7)	24.0	25.6	21.8	25.9	21.5	23.9	22.8	24.4	23.7
6	Kavunginpoothala(Ptb15)	22.8	24.3	23.5	32.5	21.4	22.1	21.9	32.3	25.1
7	Chuvannamodan (Ptb 30)	18.9	20.3	19.5	24.5	18.4	20.4	20.3	23.4	20.7
8	Jyothi (Ptb 39)	13.2	15.0	14.5	27.3	11.0	9.0	9.3	23.8	15.4
9	Swarna prabha (Ptb 43)	18.9	19.2	19.1	32.9	18.6	19.3	19.8	33.2	22.6
10	Vaishak (Ptb 60)	21.5	22.5	21.4	29.7	18.3	20.3	19.5	28.1	22.7
	Mean	20.3	21.4	20.3	26.9	18.2	19.1	18.8	25.6	
			C.D (5%)	SE (m)						
		G	1.1	0.4						
		T	1.0	0.3						
		GxT	3.0	1.1						

non-induced plants and 44.5 % for induced plant under combined stress.

4.3.4. Root dry weight

Data regarding root dry weight under different treatments in ten genotypes are presented in table 12. Differences were significant with respect to genotype, treatment and their interactions. Root dry weight in all the genotypes decreased under stress except for N22 and Apo. In general, the highest reduction in root dry weight was observed under combination of heat and moisture stress in all the genotypes. Effect of different stresses in non-induced plants was more as compared to those plants which are thermally induced. Maximum root dry weight was recorded in Apo (10.9 g) followed by CR Dhan 305 (9.5 g), CR Dhan 307 (9.5 g) and Ptb-30 (9.5 g).

4.3.5. Root length

Data pertaining to root length of ten genotypes under different treatments after maturity is presented in table 13. Root length is maximum in Ptb-15 (50.5 cm) and Apo (50.0 cm). Minimum root length was in Ptb-39 (32.7 cm) and Ptb-60 (39.1 cm). Among the treatments, induced plants under drought shows maximum root length (47.8 cm) followed by non-induced plants under drought (45.1 cm). Minimum root length was recorded in control plants (39.5 cm) and non-induced plants under combined heat and drought stress (40.1 cm). 21 % increase in root length was recorded in induced plants under drought than control plants. In all the varieties root length was increased under drought but the increase was more in induced plants than non-induced plants (plate 10).

4.3.6. Root volume

Data regarding root volume of ten genotypes under different treatments after maturity is presented in table 14. In all the treatments Ptb-15 showed maximum root volume followed by Ptb-30. The mean value for Ptb-15 was 40.2 cm^3 and for Ptb-30

Table 12. Root dry weight (g) of rice genotypes after harvest under stress conditions.

Sl.No.	Genotypes	T1	T2	T3	T4	T5	T6	T7	T8	Mean
1	N22 (Nagina)	9.5	9.6	9.4	8.7	8.1	8.9	8.5	8.1	8.9
2	APO	11.3	11.6	10.9	10.8	10.5	10.8	10.5	10.4	10.9
3	CR Dhan 305	8.2	9.7	9.4	12.4	7.9	8.9	8.1	11.4	9.5
4	CR Dhan 307 (Maudamani)	8.1	9.6	9.5	12.4	7.7	9.2	8.2	11.2	9.5
5	Parambuvattan (Ptb 7)	8.3	8.8	8.2	9.9	7.9	8.5	8.0	9.4	8.6
6	Kavunginpoothala(Ptb15)	8.6	9.6	8.7	10.8	8.4	9.5	8.4	10.3	9.3
7	Chuvannamodan (Ptb 30)	8.4	9.1	8.6	11.8	8.2	9.4	8.7	11.5	9.5
8	Jyothi (Ptb 39)	7.6	8.0	7.5	10.2	6.8	7.4	7.1	9.4	8.0
9	Swarna prabha (Ptb 43)	8.4	8.7	8.4	9.2	8.1	8.6	8.1	8.9	8.5
10	Vaishak (Ptb 60)	8.2	9.0	8.3	10.3	7.7	8.7	7.9	9.8	8.7
	Mean	8.7	9.4	8.9	10.7	8.1	9.0	8.4	10.0	
			C.D (5%)	SE (m)						
		G	0.2	0.1						
		T	0.2	0.1						
		GxT	0.6	0.2						

Table 13. Root length (cm) of rice genotypes after harvest under stress conditions.

Sl.No.	Genotypes	T1	T2	T3	T4	T5	T6	T7	T8	Mean
1	N22 (Nagina)	50.6	45.7	42.1	39.7	42.4	43.9	42.2	37.6	43.0
2	APO	50.2	55.3	52.6	46.0	48.4	51.6	50.6	45.0	50.0
3	CR Dhan 305	41.5	42.6	41.6	40.9	37.0	40.5	38.9	38.3	40.2
4	CR Dhan 307 (Maudamani)	41.5	43.9	42.7	38.5	38.9	41.7	42.1	36.7	40.8
5	Parambuvattan (Ptb 7)	47.2	48.9	45.7	47.3	42.9	47.7	43.2	39.7	45.3
6	Kavunginpoothala(Ptb15)	49.6	56.3	52.8	50.2	47.2	54.9	46.0	46.6	50.5
7	Chuvannamodan (Ptb 30)	44.2	52.9	44.3	41.9	40.3	49.9	40.6	40.8	44.4
8	Jyothi (Ptb 39)	30.6	33.8	32.9	38.2	28.4	34.8	28.1	34.3	32.7
9	Swarna prabha (Ptb 43)	42.0	48.8	43.0	43.2	41.1	42.8	40.4	40.6	42.7
10	Vaishak (Ptb 60)	36.5	50.2	37.3	39.1	35.0	43.7	35.3	35.6	39.1
	Mean	43.4	47.8	43.5	42.5	40.1	45.1	40.8	39.5	
			C.D (5%)	SE (m)						
		G	1.8	0.7						
		T	1.6	0.6						
		GxT	5.2	1.8						

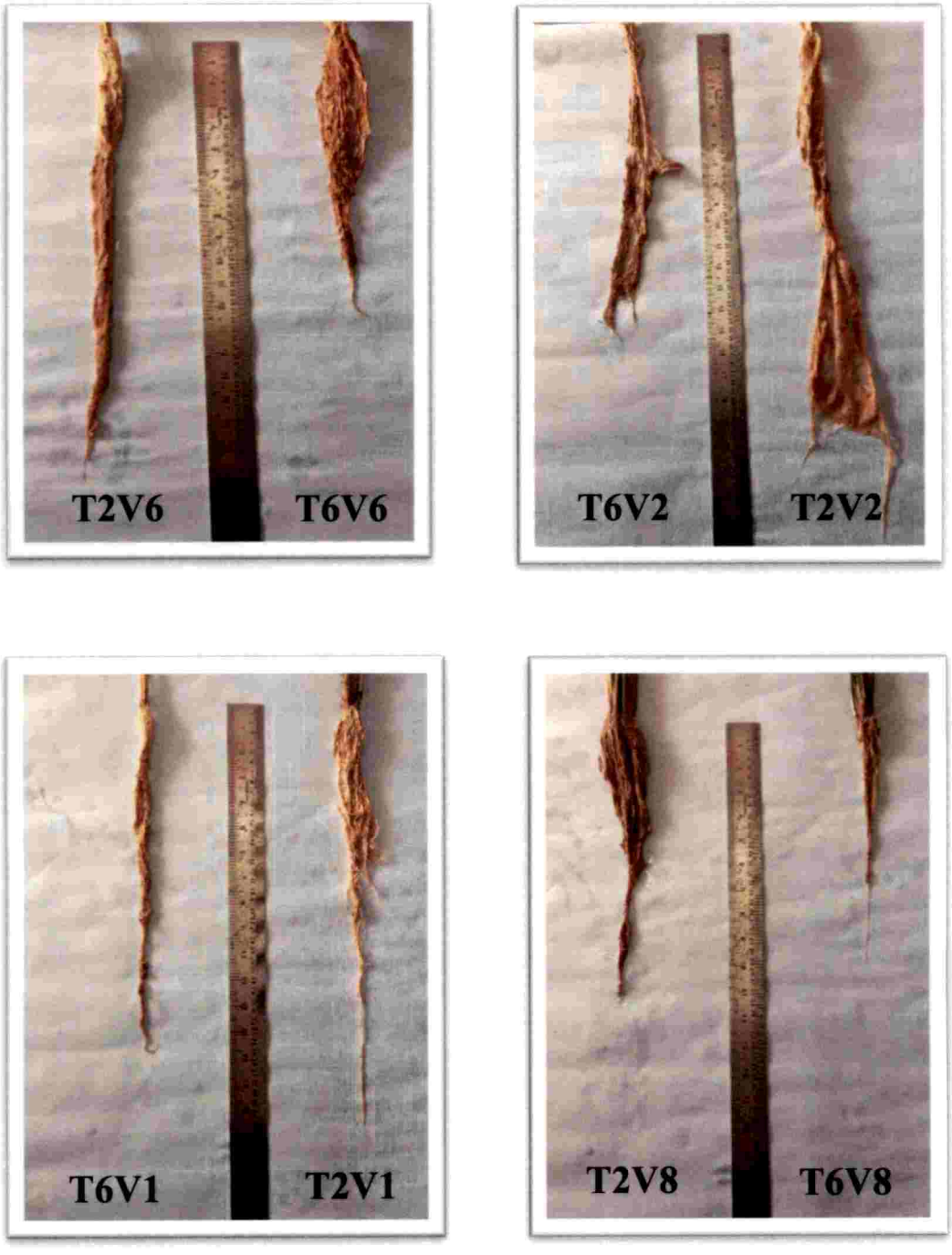


Plate 10. Variation in root length of induced and non-induced plants of different genotypes under drought.

(35.4 cm^3). Minimum root volume shown by Ptb-39 (19.9 cm^3). Among the treatments induced plants without stress showed maximum root volume (34.1 cm^3) and non-induced plants under combined drought and heat stress showed minimum root volume (24.4 cm^3). In all the genotypes induced plants exhibited higher value of root volume than non-induced plants. The percentage increase in root volume of induced plants under combined drought and heat stress and non-induced plants ranged from 1.3 % to 20.0 %.

4.3.7. Canopy temperature

The canopy temperature of rice genotypes was measured and the data is presented in table 15. Maintenance of minimum canopy temperature through evaporative cooling system is an adaptive mechanism shown by tolerant genotypes. N22 recorded minimum canopy temperature among the genotypes in all the treatments (26.3°C) followed by Ptb-7 (27.8°C). Ptb-39 (31.0°C) recorded maximum canopy temperature followed by CR Dhan 305 (30.3°C). Plants that were exposed to stress without induction resulted in higher value of canopy temperature than induced plants. Among the treatments, induced plants without stress showed minimum value and non-induced plants under combined drought and heat stress showed maximum value.

4.4. EFFECT OF DROUGHT AND HEAT STRESS ON PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS.

4.4.1 Cell membrane stability index

Cell membrane stability of rice genotypes were studied ten days after stress treatment and the data is presented in table 16. It was observed that among the genotypes N22 (96.3%) showed the highest cell membrane stability followed by Ptb 30 (93.5%), Ptb 15 (93.0%) and the least membrane stability was recorded in Ptb 39 (74.8%) and CR Dhan 305 (76.5%). Among the treatments, TIR induced plants without any stress showed maximum membrane stability (106.0%). Plants under water

Table 14. Root volume (cm^3) of rice genotypes after harvest under stress conditions.

Sl.No.	Genotypes	T1	T2	T3	T4	T5	T6	T7	T8	Mean
1	N22 (Nagina)	31.0	32.3	31.3	29.8	29.3	31.7	31.1	28.2	30.6
2	APO	30.7	33.5	31.4	32.1	30.3	32.4	30.8	29.5	31.3
3	CR Dhan 305	19.8	21.6	21.2	33.5	18.5	19.1	19.5	32.8	23.3
4	CR Dhan 307 (Maudamani)	21.6	22.1	21.6	33.5	20.4	20.2	20.6	32.7	24.1
5	Parambuvattan (Ptb 7)	26.1	34.2	24.7	33.6	22.1	33.1	21.4	32.6	28.5
6	Kavunginpoothala(Ptb15)	38.3	42.5	40.4	46.7	32.0	41.7	35.0	44.6	40.2
7	Chuvannamodan (Ptb 30)	33.2	37.6	35.3	40.5	31.5	35.6	32.4	37.0	35.4
8	Jyothi (Ptb 39)	19.3	20.1	18.9	25.3	16.6	18.4	16.6	23.7	19.9
9	Swarna prabha (Ptb 43)	19.8	21.9	20.7	30.3	17.7	18.8	17.9	27.8	21.9
10	Vaishak (Ptb 60)	29.4	32.3	30.8	35.4	25.9	29.2	27.7	33.8	30.6
	Mean	26.9	29.8	27.6	34.1	24.4	28.0	25.3	32.3	
			C.D (5%)	SE (m)						
		G	1.0	0.4						
		T	1.0	0.3						
		GxT	2.8	1.0						

Table 15. Canopy temperature (°C) of rice genotypes at maturity under stress conditions.

Sl.No.	Genotypes	T1	T2	T3	T4	T5	T6	T7	T8	Mean
1	N22 (Nagina)	26.4	26.0	26.3	25.9	26.8	26.3	26.7	26.3	26.3
2	APO	29.2	28.8	29.1	27.1	29.8	29.1	29.5	27.4	28.7
3	CR Dhan 305	30.6	29.8	30.3	29.1	31.5	30.3	31.5	29.1	30.3
4	CR Dhan 307 (Maudamani)	30.4	29.4	30.3	29.2	31.5	29.9	31.4	28.6	30.1
5	Parambuvattan (Ptb 7)	28.4	27.2	27.7	27.1	28.8	27.7	28.4	27.2	27.8
6	Kavunginpoothala(Ptb15)	29.1	28.2	28.1	27.8	29.5	28.7	29.0	28.1	28.6
7	Chuvannamodan (Ptb 30)	28.7	28.3	28.3	28.2	29.0	28.6	28.9	28.3	28.5
8	Jyothi (Ptb 39)	31.3	30.7	31.1	29.1	32.7	31.3	32.4	29.6	31.0
9	Swarna prabha (Ptb 43)	30.5	29.7	30.1	28.3	30.8	29.7	30.7	28.9	29.8
10	Vaishak (Ptb 60)	29.1	28.3	29.2	28.1	29.4	28.7	29.0	28.2	28.8
	Mean	29.4	28.6	29.1	28.0	30.0	29.0	29.8	28.2	
			C.D (5%)	SE (m)						
		G	0.2	0.1						
		T	0.2	0.1						
		GxT	0.5	0.2						

Table 16. Cell membrane stability index (%) of rice genotypes at flowering under stress conditions.

Sl.No.	Genotypes	T1	T2	T3	T4	T5	T6	T7	Mean
1	N22 (Nagina)	93.0	96.7	97.9	109.8	89.9	95.7	91.1	96.3
2	APO	90.0	93.5	91.9	108.1	79.6	85.1	83.7	90.3
3	CR Dhan 305	79.3	82.0	82.4	103.0	60.8	66.3	61.6	76.5
4	CR Dhan 307 (Maudamani)	81.3	85.7	85.2	102.3	78.8	79.8	80.0	84.7
5	Parambuvattan (Ptb 7)	85.1	94.0	87.3	107.1	81.0	92.7	84.7	90.3
6	Kavunginpoothala(Ptb15)	92.4	96.7	93.2	107.0	85.8	87.0	88.9	93.0
7	Chuvannamodan (Ptb 30)	88.4	97.7	91.6	108.7	86.6	92.0	89.2	93.5
8	Jyothi (Ptb 39)	80.3	80.1	80.8	100.9	59.5	61.2	61.1	74.8
9	Swarna prabha (Ptb 43)	87.2	89.5	87.7	104.8	73.4	81.9	81.9	86.6
10	Vaishak (Ptb 60)	92.0	96.1	92.1	108.2	85.2	87.0	85.6	92.3
	Mean	86.9	91.2	89.0	106.0	78.1	82.9	80.8	
			C.D (5%)	SE (m)					
		G	0.7	0.3					
		T	0.6	0.2					
		GxT	1.9	0.7					

stress showed comparatively higher membrane stability than heat stress and combined heat and drought stress. Membrane stability index of induced plants increased by 11.3%, 10.04% and 10.15% under combined stress, moisture stress and heat stress respectively than non-induced plants.

4.4.2. Chlorophyll stability index

Data on chlorophyll stability index of rice genotypes under different treatments are depicted in table 17. In all genotypes chlorophyll stability was increased under the treatment with thermal induction than without induction. Among the genotypes, N22 recorded the highest chlorophyll stability (108.3%) followed by Apo (100.5%) and Ptb-7 (97.1%). Ptb-39 recorded the lowest value (87.4 %) followed by Ptb-43 (90.6%) and CR Dhan 305 (92.0 %). Induced plants without stress (111.0%) showed the maximum chlorophyll stability among the treatment. Combined drought and heat stress reduced the chlorophyll stability index in both induced and non-induced plants, but the reduction was more in non-induced plants.

4.4.3. Stomatal conductance

Data pertaining to stomatal conductance of rice genotypes under different treatments is presented in table 18. Mean stomatal conductance under different treatments was highest in N22 (878.2m moles m^{-2} s^{-1}) and minimum for Ptb 39 (464.4 m moles m^{-2} s^{-1}). Plants under drought stress recorded minimum conductance than heat stress and combined drought and heat stress. Among the treatments induced plants without stress (828.1 m moles m^{-2} s^{-1}) showed the highest value for conductance and the value was minimum for non-induced plants under drought. This result indicates that stomatal closure was more in drought stressed plants. Eventhough, Jyothi recorded the lowest mean stomatal conductance under different treatments, under absolute control the highest stomatal conductance was recorded by Vaishak (Ptb60) followed by Jyothi (Ptb39).

Table 17. Chlorophyll stability index (%) of rice genotypes at flowering under stress conditions.

Sl.No.	Genotypes	T1	T2	T3	T4	T5	T6	T7	Mean
1	N22 (Nagina)	100.8	103.5	103.0	157.5	97.8	98.1	97.3	108.3
2	APO	98.3	98.7	100.6	119.9	94.1	96.7	95.4	100.5
3	CR Dhan 305	91.5	94.1	92.8	101.8	85.5	88.7	89.2	92.0
4	CR Dhan307 (Maudamani)	90.8	93.3	92.7	101.7	89.7	90.2	90.0	92.6
5	Parambuvattan (Ptb 7)	97.0	100.3	99.7	112.4	87.5	89.7	92.7	97.1
6	Kavunginpoothala(Ptb15)	94.6	96.2	96.8	103.2	88.4	88.2	89.5	93.8
7	Chuvannamodan (Ptb 30)	94.6	97.3	95.7	104.0	87.6	90.4	90.9	94.4
8	Jyothi (Ptb 39)	82.9	89.5	89.2	101.4	77.6	84.0	87.5	87.4
9	Swarna prabha (Ptb 43)	89.2	91.3	91.6	103.0	86.3	86.2	86.7	90.6
10	Vaishak (Ptb 60)	90.6	91.5	93.8	105.4	88.4	91.1	89.2	92.9
	Mean	93.0	95.6	95.6	111.0	88.3	90.3	90.8	
			C.D (5%)	SE (m)					
		G	1.7	0.6					
		T	1.5	0.5					
		GxT	4.6	1.7					

Table 18. Stomatal conductance (m moles m^{-2} s^{-1}) of rice genotypes at flowering under stress conditions.

Sl.No.	Genotypes	T1	T2	T3	T4	T5	T6	T7	T8	Mean
1	N22 (Nagina)	888.3	855.3	898.0	899.7	929.7	812.0	926.7	816.0	878.2
2	APO	834.3	659.3	810.3	759.3	818.3	626.7	761.3	753.3	752.9
3	CR Dhan 305	632.0	251.7	626.3	828.0	608.0	223.0	603.7	766.0	567.3
4	CR Dhan 307 (Maudamani)	631.0	459.7	593.3	872.7	616.3	468.0	595.7	807.7	630.5
5	Parambuvattan (Ptb 7)	740.0	531.7	730.7	713.0	695.7	414.0	646.3	684.3	644.5
6	Kavunginpoothala(Ptb15)	827.3	646.7	815.7	789.7	796.7	557.3	782.0	766.7	747.8
7	Chuvannamodan (Ptb 30)	852.7	667.3	804.0	760.0	836.0	625.0	784.0	736.7	758.2
8	Jyothi (Ptb 39)	434.0	239.3	357.7	887.3	386.0	211.0	349.3	850.7	464.4
9	Swarna prabha (Ptb 43)	583.3	242.7	601.7	877.3	574.0	237.3	569.7	853.3	567.4
10	Vaishak (Ptb 60)	662.7	398.0	644.7	894.3	586.7	337.0	656.3	876.0	632.0
	Mean	708.6	495.2	688.2	828.1	684.7	451.1	667.5	791.1	
			C.D (5%)	SE (m)						
		G	17.4	6.2						
		T	15.6	5.6						
		GxT	49.3	17.6						

4.4.4 Photosynthetic rate

Data recorded for photosynthetic rate of rice genotypes under different treatments is presented in table 19. Observed mean value was maximum for Apo (23.2 μ mol CO_2 m^{-2} s^{-1}) and minimum for Ptb-39 (12.6μ mol CO_2 m^{-2} s^{-1}). In control set Ptb-15 recorded maximum photosynthetic rate (25.4 μ mol CO_2 m^{-2} s^{-1}) followed by Ptb-7 (25.2 μ mol CO_2 m^{-2} s^{-1}). Heat stress treatment alone and combined drought and heat stress recorded more reduction in photosynthetic rate than drought stress. Also it is observed that TIR induced plants under stress showed higher photosynthetic rate than non-induced plants. Among the treatments, induced plants without stress recorded maximum value (24.2 μmol CO_2 m^{-2} s^{-1}) followed by control (23.3 μ mol CO_2 m^{-2} s^{-1}) and minimum photosynthetic rate was observed in non-induced plants under heat stress.

4.4.5 Proline

The results related to proline content of rice genotypes under different treatments is presented in table 20. Proline content was increased in all the treatments for the ten genotypes. There is significant difference exist between treatments, genotypes and their interactions. Among the genotypes N22 recorded the maximum proline content (115.3 μg /g fresh tissue) followed by Ptb-15(106.5 μg /g fresh tissue) and the minimum for Ptb-39 (19.0 μg /g fresh tissue) followed by CR Dhan 305 (20.1 μg /g fresh tissue). Among the treatments, induced plants under combined drought and heat stress recorded high proline content (65.2 μg /g fresh tissue) and the minimum for control plants (45.3 μg /g fresh tissue).

4.4.6 Peroxidase

Peroxidase content recorded under all the treatments for ten genotypes is presented in table 21. Results revealed that peroxidase activity was recorded more under drought stress and induced plants under drought showed the maximum activity ($0.57g^{-1}min^{-1}$) than non-induced plants ($0.34\ g^{-1}\ min^{-1}$). Non-induced plants under

Table 19. Photosynthetic rate (μ mol CO_2 m^{-2} s^{-1}) of rice genotypes after stress under stress conditions.

Sl.No.	Genotypes	T1	T2	T3	T4	T5	T6	T7	T8	Mean
1	N22 (Nagina)	19.3	19.7	19.2	20.8	18.5	19.0	18.8	20.2	19.4
2	APO	21.7	23.8	22.6	26.1	21.2	23.5	21.7	24.9	23.2
3	CR Dhan 305	14.6	16.4	16.0	24.3	14.7	14.8	11.0	24.3	17.0
4	CR Dhan307 (Maudamani)	19.3	20.1	18.5	22.5	22.2	18.1	16.9	22.1	20.0
5	Parambuvattan (Ptb 7)	19.6	20.5	20.5	25.8	17.6	19.3	19.2	25.2	21.0
6	Kavunginpoothala(Ptb15)	18.0	19.6	18.8	26.2	19.4	22.8	21.9	25.4	21.5
7	Chuvannamodan (Ptb 30)	20.6	21.8	21.7	25.8	18.7	19.5	19.2	23.7	21.4
8	Jyothi (Ptb 39)	10.7	10.8	10.5	22.2	8.6	8.4	8.0	21.5	12.6
9	Swarna prabha (Ptb 43)	12.2	12.9	12.4	22.3	11.6	12.4	11.6	21.2	14.6
10	Vaishak (Ptb 60)	20.8	21.0	21.2	26.2	19.2	20.1	20.0	24.4	21.6
	Mean	17.7	18.7	18.1	24.2	17.2	17.8	16.8	23.3	
			C.D (5%)	SE (m)						
		G	0.7	0.3						
		T	0.7	0.2						
		GxT	2.1	0.8						

Table 20. Proline (µg /g fresh tissue) of rice genotypes at flowering under stress conditions.

Sl.No.	Genotypes	T1	T2	T3	T4	T5	T6	T7	T8	Mean
1	N22 (Nagina)	128.2	126.3	125.2	101.1	119.1	116.5	117.3	88.4	115.3
2	APO	96.9	95.6	94.9	67.8	85.3	83.9	83.9	67.7	84.5
3	CR Dhan 305	21.7	20.4	20.1	19.8	19.9	19.6	19.7	19.9	20.1
4	CR Dhan 307 (Maudamani)	38.1	37.3	36.2	36.8	36.2	35.3	35.9	34.9	36.3
5	Parambuvattan (Ptb 7)	59.0	57.6	58.8	44.6	49.5	53.2	53.6	43.7	52.5
6	Kavunginpoothala(Ptb15)	120.6	118.8	119.6	77.2	119.1	117.4	105.8	73.2	106.5
7	Chuvannamodan (Ptb 30)	87.4	85.3	85.7	47.1	81.5	75.7	76.3	45.6	73.1
8	Jyothi (Ptb 39)	20.5	20.4	19.2	18.1	19.2	18.3	18.6	17.5	19.0
9	Swarna prabha (Ptb 43)	27.9	25.6	26.2	20.9	24.6	23.9	24.6	20.3	24.2
10	Vaishak (Ptb 60)	51.5	51.5	51.0	46.4	48.0	42.1	43.4	41.3	46.9
	Mean	65.2	63.9	63.7	48.0	60.3	58.6	57.9	45.3	
			C.D (5%)	SE (m)						
		G	3.4	1.2						
		T	3.0	1.1						
		GxT	9.6	3.4						

Table 21. Peroxidase (activity g^{-1} min^{-1}) of rice genotypes after stress under stress conditions

Sl.No.	Genotypes	T1	T2	T3	T4	T5	T6	T7	T8	Mean
1	N22 (Nagina)	0.21	0.90	0.27	0.70	0.09	0.52	0.14	0.39	0.40
2	APO	0.13	1.03	0.20	0.20	0.04	0.22	0.06	0.24	0.27
3	CR Dhan 305	0.11	0.44	0.10	0.23	0.04	0.21	0.06	0.27	0.18
4	CR Dhan 307 (Maudamani)	0.13	0.30	0.17	0.28	0.01	0.17	0.05	0.15	0.16
5	Parambuvattan (Ptb 7)	0.13	0.73	0.11	0.42	0.03	0.48	0.06	0.31	0.28
6	Kavunginpoothala(Ptb15)	0.11	0.45	0.15	0.29	0.07	0.32	0.13	0.27	0.22
7	Chuvannamodan (Ptb 30)	0.11	0.71	0.13	0.30	0.05	0.43	0.12	0.29	0.27
8	Jyothi (Ptb 39)	0.09	0.48	0.13	0.20	0.01	0.44	0.05	0.18	0.20
9	Swarna prabha (Ptb 43)	0.11	0.33	0.15	0.25	0.04	0.24	0.02	0.15	0.16
10	Vaishak (Ptb 60)	0.14	0.33	0.14	0.22	0.03	0.40	0.09	0.18	0.19
	Mean	0.13	0.57	0.16	0.31	0.04	0.34	0.08	0.24	
			C.D (5%)	SE (m)						
		G	0.04	0.01						
		T	0.04	0.01						
		GxT	0.11	0.04						

combined drought and heat stress exhibited minimum peroxidase activity (0.04 g^{-1} min^{-1}). N22 recorded the highest activity (0.4 g^{-1} min^{-1}) among the genotypes followed by Ptb-7 (0.28 g^{-1} min^{-1}) and the minimum activity showed by Ptb-43 and CR Dhan 307 (0.16 g^{-1} min^{-1}). In general, peroxidase content was decreased under heat stress and combined drought and heat stress.

4.4.7. Superoxide dismutase

Data recorded for superoxide dismutase activity of rice genotypes under different treatments is presented in table 22. Among the genotypes, Ptb-15 recorded the highest SOD activity (0.314 g^{-1} min^{-1}) followed by N22 (0.313 g^{-1} min^{-1}) and the minimum activity was shown by CR Dhan305 (0.22 g^{-1} min^{-1}) and CR Dhan307 (0.233 $g^{-1}min^{-1}$). The results revealed that SOD activity was increased under drought and decreased activity were shown by plants under heat stress treatment and combined drought and heat stress. Plants under heat stress recorded lowest SOD activity than combined drought and heat stress. Maximum activity was observed in induced plants under drought (0.325 g^{-1} min^{-1}) and the minimum activity was shown by non-induced plants under heat stress. TIR induced plants recorded highest activity and it ranged between 2.1 % to 27.1 %.

4.5. EFFECT OF DROUGHT AND HEAT STRESS ON YIELD PARAMETERS.

4.5.1. Time of anthesis

Flower opening time of ten genotypes under different treatments shown in table 23. Differences were significant with respect to genotype and treatment, but not with respect to their interactions. Among the ten genotypes, N22 (9.27 am) showed early flowering character. Flowers of Ptb-39 opened in late hours (11.44 am) with respect to other genotypes.

Table 22. Superoxide dismutase (activity g^{-1} min^{-1}) of rice genotypes at flowering under stress conditions

Sl.No.	Genotypes	T1	T2	T3	T4	T5	T6	T7	T8	Mean
1	N22 (Nagina)	0.311	0.353	0.292	0.336	0.293	0.331	0.280	0.309	0.313
2	APO	0.302	0.343	0.292	0.324	0.292	0.327	0.286	0.309	0.309
3	CR Dhan 305	0.231	0.241	0.199	0.238	0.224	0.237	0.154	0.236	0.220
4	CR Dhan307 (Maudamani)	0.222	0.271	0.220	0.254	0.219	0.235	0.210	0.230	0.233
5	Parambuvattan (Ptb 7)	0.311	0.345	0.294	0.334	0.287	0.329	0.270	0.305	0.310
6	Kavunginpoothala(Ptb15)	0.311	0.351	0.295	0.331	0.305	0.321	0.285	0.316	0.314
7	Chuvannamodan (Ptb 30)	0.298	0.350	0.295	0.341	0.272	0.346	0.271	0.317	0.311
8	Jyothi (Ptb 39)	0.248	0.328	0.223	0.308	0.216	0.286	0.191	0.258	0.257
9	Swarna prabha (Ptb 43)	0.240	0.331	0.235	0.290	0.216	0.289	0.206	0.276	0.260
10	Vaishak (Ptb 60)	0.247	0.333	0.239	0.304	0.210	0.314	0.207	0.302	0.269
	Mean	0.272	0.325	0.258	0.306	0.253	0.301	0.236	0.286	
			C.D (5%)	SE (m)						
		G	0.02	0.01						
		T	0.01	0.01						
		GxT	NS	0.02						

Table 23. Time of anthesis (am) of rice genotypes at flowering under stress conditions.

Sl.No.	Genotypes	T1	T2	T3	T4	T5	T6.	T7	T8	Mean
1	N22 (Nagina)	9.27	9.19	9.16	9.28	9.28	9.33	9.32	9.35	9.27
2	APO	10.22	10.23	10.08	10.28	10.23	11.29	10.22	10.32	10.23
3	CR Dhan 305	11.25	10.53	11.25	10.38	11.29	11.27	11.13	10.47	11.10
4	CR Dhan307 (Maudamani)	10.48	10.41	10.51	10.40	11.25	10.50	11.30	10.51	10.57
5	Parambuvattan (Ptb 7)	10.12	10.18	10.09	10.13	10.17	10.17	10.18	10.20	10.16
6	Kavunginpoothala(Ptb15)	10.23	10.15	10.17	10.22	10.27	10.17	10.24	10.22	10.21
7	Chuvannamodan (Ptb 30)	10.15	10.10	10.06	10.13	10.11	10.16	10.13	10.22	10.13
8	Jyothi (Ptb 39)	11.08	11.26	11.27	10.50	11.27	11.10	11.12	11.09	11.44
9	Swarna prabha (Ptb 43)	10.47	10.03	11.06	10.42	10.52	11.05	11.02	10.48	10.56
10	Vaishak (Ptb 60)	11.25	11.12	11.14	10.47	11.07	11.27	11.12	10.53	11.10
	Mean	10.33	10.30	10.32	10.22	10.39	10.37	10.38	10.30	

4.5.2. Spikelet fertility

Data recorded for spikelet fertility of ten genotypes under different treatments is recorded in table 24. There is significant difference exist with respect to treatments, genotypes and their interactions. Highest spikelet fertility was recorded in N22 (90.5 %) and Apo (83.4 %). Ptb-39 recorded the minimum spikelet fertility (54.9 %), followed by Ptb-43 (59.7%). Results revealed that heat stress has more deleterious effects on spikelet than drought. Among the treatments, induced plants without stress recorded maximum fertility (87.2 %) followed by control plants (85.1%). Minimum value for fertility was observed in non-induced plants under heat stress (62.8 %). TIR induced plants showed more fertile spikelets than non-induced plants (Plate 11).

4.5.3 Pollen viability

Data related to pollen viability of ten genotypes under different treatments is recorded in table 25. There is significant difference exist with respect to treatments, genotypes and their interactions. TIR induced plants showed higher percent of pollen viability than non-induced plants (Plate 12).Highest pollen viability was recorded in N22 (91.8 %) and Apo (84.8 %). Ptb-39 recorded the minimum pollen viability (56.2 %), followed by Ptb-43 (61.1%). Like spikelet fertility, pollen viability was also affected by heat stress than drought. Among the treatments, induced plants without stress recorded the maximum fertility (88.5 %) followed by control plants (86.4%). Minimum value for fertility was observed in non-induced plants under heat stress (64.2 %).

4.6 YIELD PARAMETERS

4.6.1. Days to 50 % flowering

Data regarding days to 50 % flowering of genotypes under different treatments is recorded in table 26. In general among the treatments, early days to 50% flowering was recorded in plants under drought in both induced and non-induced set of plants

Table 24. Spikelet fertility (%) of rice genotypes at maturity under stress conditions.

Sl.No.	Genotypes	T1	T2	T3	T4	T5	T6	T7	T8	Mean
1	N22 (Nagina)	91.3	90.7	90.5	93.6	87.5	88.3	89.0	92.7	90.5
2	APO	87.0	89.0	88.3	91.0	82.0	82.8	57.5	90.1	83.4
3	CR Dhan 305	59.7	60.8	61.4	87.1	51.6	54.3	52.2	83.2	63.8
4	CR Dhan 307 (Maudamani)	61.8	66.4	64.8	86.2	48.4	52.1	52.5	84.1	64.5
5	Parambuvattan (Ptb 7)	71.5	73.0	73.0	77.9	69.3	70.8	70.9	71.9	72.3
6	Kavunginpoothala(Ptb15)	72.4	73.6	73.3	85.7	71.5	72.2	71.4	84.4	75.6
7	Chuvannamodan (Ptb 30)	78.3	79.8	80.6	87.6	72.4	73.5	75.3	85.1	79.1
8	Jyothi (Ptb 39)	52.0	50.5	49.8	90.1	36.1	36.7	36.7	87.1	54.9
9	Swarna prabha (Ptb 43)	51.7	56.8	54.8	88.7	45.9	43.8	48.1	88.1	59.7
10	Vaishak (Ptb 60)	71.4	78.3	72.7	83.8	70.9	76.6	74.6	84.0	76.5
	Mean	69.7	71.9	70.9	87.2	63.6	65.1	62.8	85.1	
			C.D (5%)	SE (m)						
		G	3.4	1.2						
		T	3.0	1.1						
		GxT	9.6	3.4						

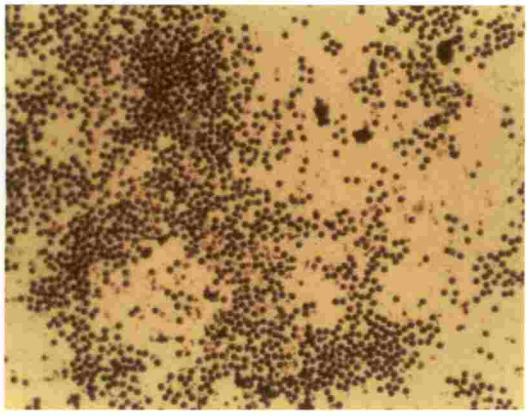
T1V7 T5V7

T1V3 T5V3

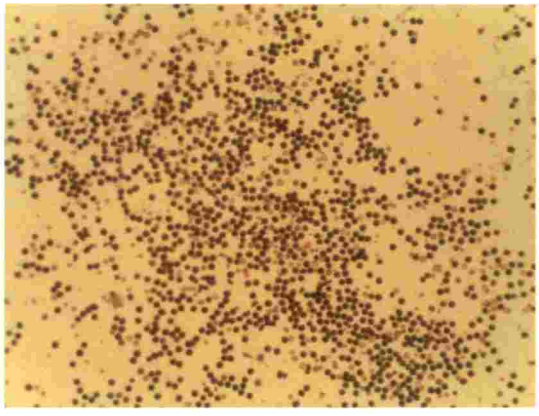
Plate 11. Variation in spikelet fertility of induced and non-induced plants of different genotypes under combined drought and heat stress.

Table 25. Pollen viability (%) of rice genotypes at flowering under stress conditions.

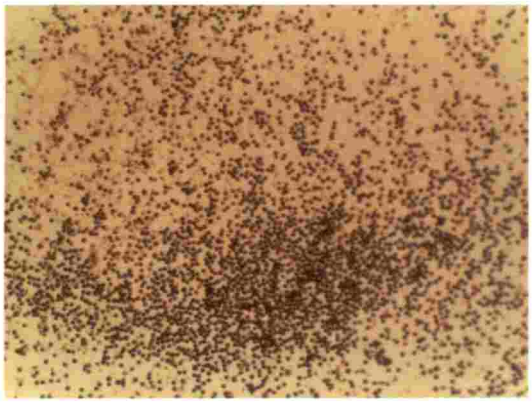
Sl.No.	Genotypes	T1	T2	T3	T4	T5	T6	T7	T8	Mean
1	N22 (Nagina)	92.7	92.1	91.9	94.9	88.9	89.6	90.4	94.0	91.8
2	APO	88.3	90.3	89.6	92.4	83.3	84.1	58.8	91.5	84.8
3	CR Dhan 305	61.1	62.2	62.8	88.4	52.9	55.7	53.6	84.5	65.2
4	CR Dhan 307 (Maudamani)	63.2	67.7	66.2	87.6	49.8	53.4	53.9	85.4	65.9
5	Parambuvattan (Ptb 7)	72.9	74.4	74.3	79.2	70.7	72.2	72.3	73.3	73.6
6	Kavunginpoothala(Ptb15)	73.8	75.0	74.7	87.1	72.9	73.6	72.8	85.8	77.0
7	Chuvannamodan (Ptb 30)	79.7	81.1	82.0	89.0	73.8	74.9	76.7	86.5	80.5
8	Jyothi (Ptb 39)	53.4	51.9	51.2	91.5	37.4	38.1	38.1	88.5	56.2
9	Swarna prabha (Ptb 43)	53.1	58.2	56.2	90.1	47.3	45.2	49.4	89.5	61.1
10	Vaishak (Ptb 60)	72.8	79.6	74.0	85.2	72.3	78.0	76.0	85.3	77.9
	Mean	71.1	73.3	72.3	88.5	64.9	66.5	64.2	86.4	
			C.D (5%)	SE (m)						
		G	3.4	1.2						
		T	3.1	1.1						
		GxT	9.7	3.5						



T1V1



T5V1



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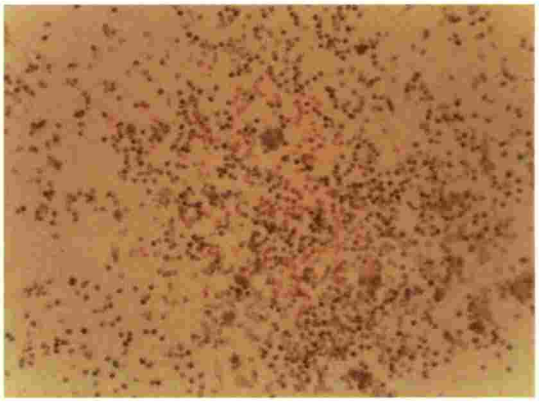

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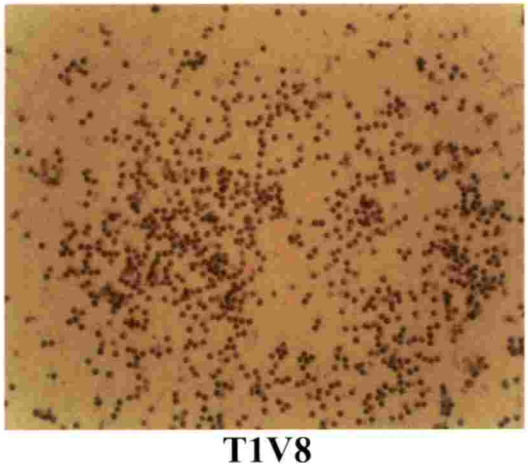
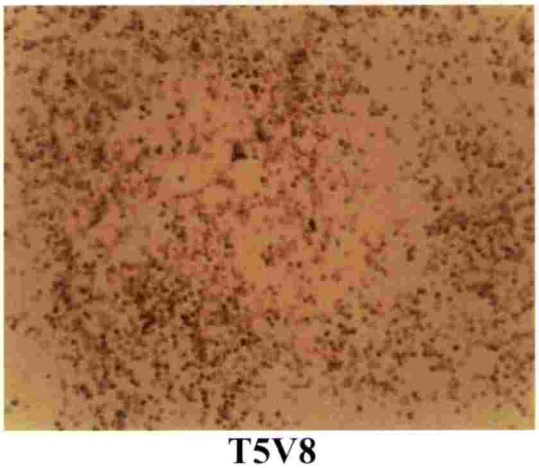


T1V8



T5V8

Plate 12. Variation in pollen viability of induced and non-induced plants of different genotypes under combined drought and heat stress.

Table 26. Days to 50 % flowering of rice genotypes at flowering under stress conditions.

Sl.No.	Genotypes	T1	T2	T3	T4	T5	T6	T7	T8	Mean
1	N22 (Nagina)	63	64	63	65	66	64	64	66	64
2	APO	98	98	98	98	96	97	98	99	98
3	CR Dhan 305	95	93	96	93	101	96	100	98	97
4	CR Dhan307 (Maudamani)	98	97	98	98	106	98	103	98	99
5	Parambuvattan (Ptb 7)	84	81	84	84	86	84	85	88	85
6	Kavunginpoothala(Ptb15)	105	96	102	111	111	101	105	114	106
7	Chuvannamodan (Ptb 30)	87	80	90	84	97	82	94	92	88
8	Jyothi (Ptb 39)	110	99	94	94	113	102	108	95	102
9	Swarna prabha (Ptb 43)	92	84	91	82	95	87	97	85	89
10	Vaishak (Ptb 60)	83	77	81	79	85	78	83	81	81
	Mean	92	87	90	89	95	89	94	92	
			C.D (5%)	SE (m)						
		G	1.8	0.7						
		T	1.7	0.6						
		GxT	5.2	1.9						

compared control. Among the genotypes, early flowering was showed by N22, Apo, Ptb-7 and Ptb-15. In Ptb-39 and Ptb-43 flowering period was extended under various stress treatments. Delay in flowering was observed in CR Dhan 305, CR Dhan 307, Ptb-30 and Ptb-60 under heat stress and combined drought and heat stress. But under drought these genotypes showed early flowering. Delayed flowering was shown by non-induced plants under stress as compared with induced plants under stress.

4.6.2. Productive tiller number

Data pertaining to productive tiller number of genotypes under different treatments is recorded in table 27. There is significant difference exist with respect to treatments, genotypes but not with their interactions. Stress conditions reduced number of productive tillers in all the genotypes and the reduction was more under combined drought and heat stress. Among the genotypes, Ptb-7 produced more number of productive tillers (10) and N22 produced least number (5). Induced plants without stress produced more productive tillers (10) than control plants (9). Least number was observed in non-induced plants under combined drought and heat stress (5). Results showed that heat stress has more deleterious effects on tiller number than drought and TIR induction helps the plants to produce more number of productive tillers than stressed plants without induction.

4.5.6. 1000 grain weight

1000 grain weight recorded in ten genotypes under different treatments is presented in table 28. The results showed that significant reduction was observed in 1000 grain weight under all the stress conditions. Combined drought and heat stress recorded lowest 1000 grain weight than other two stress treatments. The mean 1000 grain weight of genotypes was maximum for induced plants without stress (25.8 g), whereas the minimum value (21.4 g) recorded in non-induced plants under combined drought and heat stress. Among the genotypes, Ptb-43 showed highest mean value

Table 27. Productive tiller number of rice genotypes at maturity under stress conditions.

Sl.No.	Genotypes	T1	T2	T3	T4	T5	T6	T7	T8	Mean
1	N22 (Nagina)	4	5	5	6	4	5	4	5	5
2	APO	5	6	6	7	5	6	5	7	6
3	CR Dhan 305	6	6	6	10	4	5	5	9	6
4	CR Dhan 307 (Maudamani)	6	7	7	10	6	6	6	9	7
5	Parambuvattan (Ptb 7)	9	9	11	14	7	9	8	12	10
6	Kavunginpoothala(Ptb15)	9	9	7	11	6	8	7	9	8
7	Chuvannamodan (Ptb 30)	8	9	8	10	6	6	8	9	8
8	Jyothi (Ptb 39)	7	9	9	11	5	7	8	9	8
9	Swarna prabha (Ptb 43)	8	7	7	10	5	7	6	9	7
10	Vaishak (Ptb 60)	6	7	6	8	5	6	5	7	6
	Mean	7	8	7	10	5	6	6	9	
			C.D (5%)	SE (m)						
		G	1.0	0.4						
		T	1.0	0.3						
		GxT	NS	1.1						

Table 28. 1000 grain weight (g) of rice genotypes after harvest under stress conditions

Sl.No.	Genotypes	T1	T2	T3	T4	T5	T6	T7	T8	Mean
1	N22 (Nagina)	21.4	21.6	22.3	24.0	20.6	21.2	21.8	23.6	22.1
2	APO	21.6	22.0	21.8	22.7	20.5	20.9	21.1	21.9	21.6
3	CR Dhan 305	23.4	23.2	22.9	26.8	20.4	21.2	20.9	26.7	23.2
4	CR Dhan 307 (Maudamani)	21.7	23.1	24.1	27.7	21.0	22.5	21.8	27.7	23.7
5	Parambuvattan (Ptb 7)	21.6	23.1	23.2	26.5	20.7	22.1	21.5	25.3	23.0
6	Kavunginpoothala(Ptb15)	19.3	19.9	19.8	21.3	19.9	19.9	20.0	20.2	20.0
7	Chuvannamodan (Ptb 30)	24.4	24.8	24.9	25.7	24.0	24.0	23.8	27.4	24.9
8	Jyothi (Ptb 39)	22.3	22.6	22.9	27.8	20.9	21.3	22.2	27.2	23.4
9	Swarna prabha (Ptb 43)	24.6	24.6	24.7	27.8	24.1	24.3	24.0	26.8	25.1
10	Vaishak (Ptb 60)	22.7	22.8	23.6	27.6	21.7	22.6	22.7	26.9	23.8
	Mean	22.3	22.8	23.0	25.8	21.4	22.0	22.0	25.4	
			C.D (5%)	SE (m)						
		G	0.5	0.2						
		T	0.4	0.2						
		GxT	1.4	0.5						

(25.1 g) followed by Ptb-30 (24.9 g) and lowest for Ptb-15 (20.0 g). Reduction in 1000 grain weight was minimum for induced plants under stress than non-induced plants.

4.7. MOLECULAR ANALYSIS

4.7.1. SDS PAGE

The root protein profile by SDS-PAGE in samples of induced and non-induced plants of two selected genotypes (tolerant- N22 and susceptible- Ptb-39), is given in plate13. The differences were observed in terms of band intensities. The samples were loaded in the following order, Lane 1- non-induced plants of susceptible genotype, Lane 2- non-induced plants of tolerant genotype, Lane 3- induced plants of susceptible genotype and Lane 4- induced plants of tolerant genotype and lane 5- protein ladder. The protein expression was comparatively higher in induced plants of susceptible and tolerant genotypes as compared to non-induced plants. Protein bands of sizes around 20 and 75 kDa were observed in all the samples. The intensity of band was more in induced plants of tolerant genotype, N22.

4.7.2 RT PCR analysis

Expression level of *PSTOL 1*was analysed in root samples of induced and non-induced plants of two selected genotypes (tolerant- N22 and susceptible- Ptb-39), and the result is given in plate 14. An amplicon of 523 bp was obtained in all samples except non-induced plants of susceptible genotype. Expression of the gene was observed in induced plants of both the genotypes and non-induced plants of tolerant genotype. But the intensity was less in non-induced tolerant genotype compared to induced plants.

Expression level of *DRO1* gene was analysed in root samples of induced and non-induced plants of two selected genotypes (tolerant- N22 and susceptible- Ptb-39), and the result is given in plate 15. The relative intensity of bands were analysed using

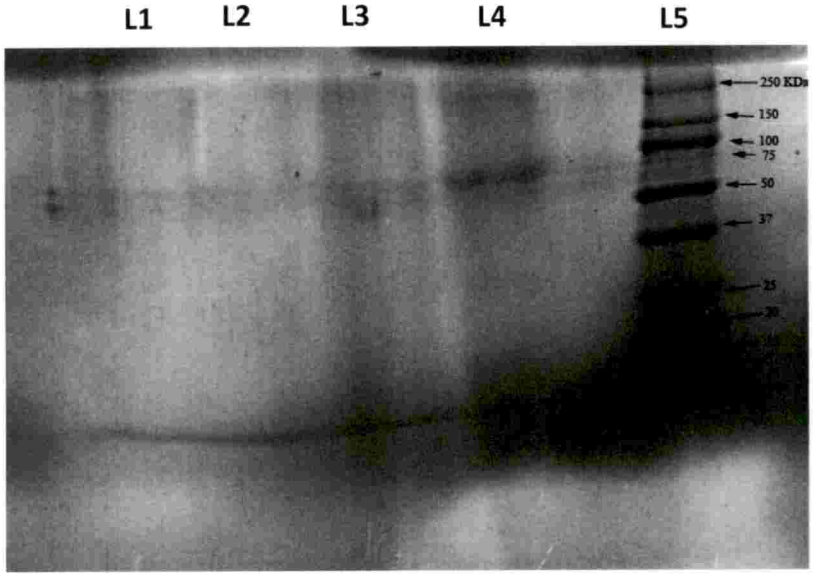


Plate 13. Protein Profiling (SDS PAGE) of induced and non-induced plants of Ptb-39 and N22 under combined drought and heat stress.

Lane 1. Non-induced plant of Ptb-39.
Lane 2. Induced plant of Ptb-39.
Lane 3. Non-induced plant of N22.
Lane 4. Induced plant of N22.
Lane 5. Protein ladder

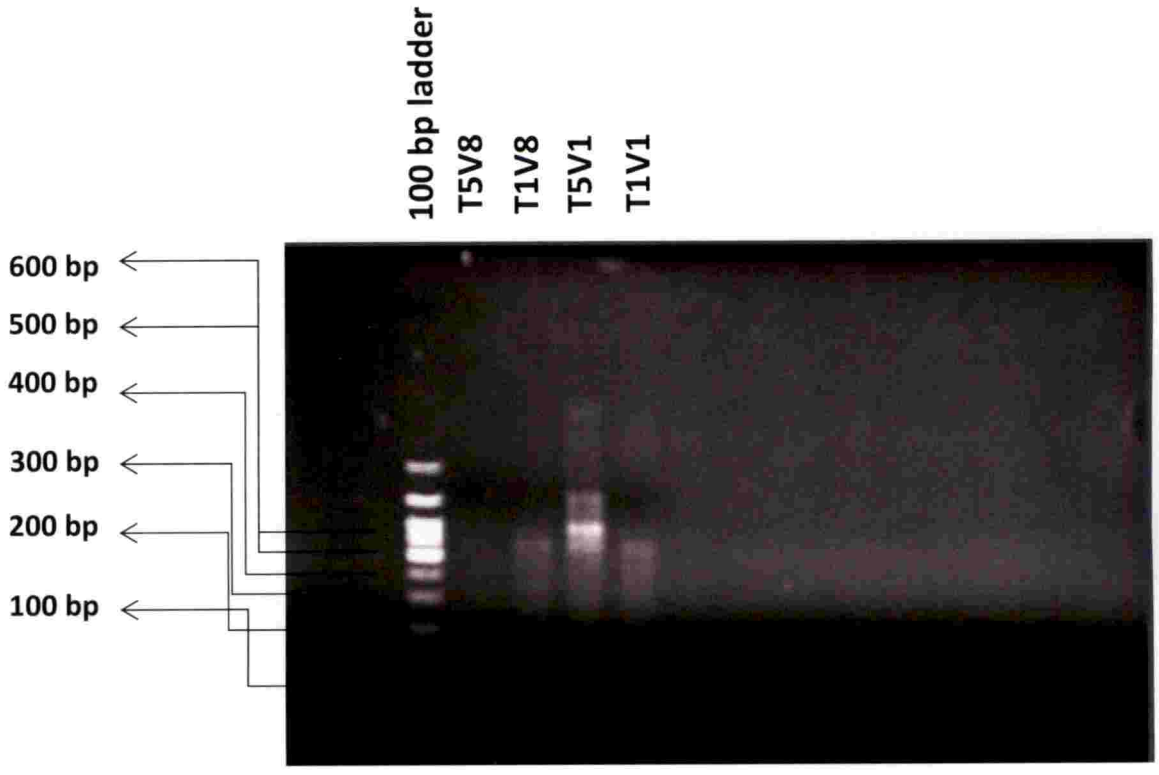


Plate 14. Expression Analysis of gene *PSTOL1* using Reverse Transcriptase PCR in induced and non-induced plants of Ptb-39 and N22 under combined drought and heat stress.

Lane 1. 100 bp DNA adder
Lane 2. Non-induced plant of Ptb-39.
Lane 3. Induced plant of Ptb-39.
Lane 4. Non-induced plant of N22.
Lane 5. Induced plant of N22.

Image J Software (figure 1). Expression of *DRO1* gene was recorded highest in induced plants of tolerant genotype followed by induced plants of susceptible genotype. Relative intensity was comparatively less in non-induced plants of both the genotypes.

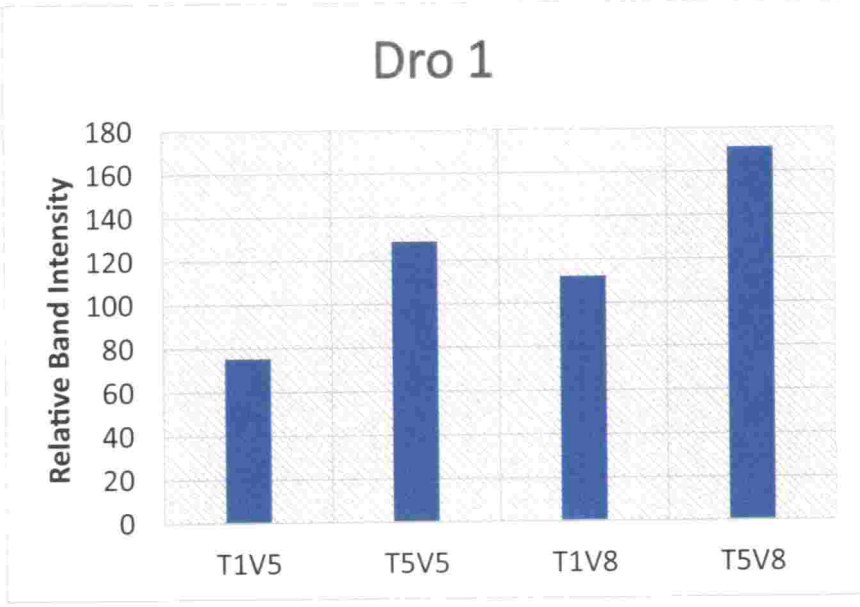


Figure 1. Expression analysis of gene *Dro 1* in induced and non-induced plants of Ptb-39 and N22 under combined drought and heat stress.

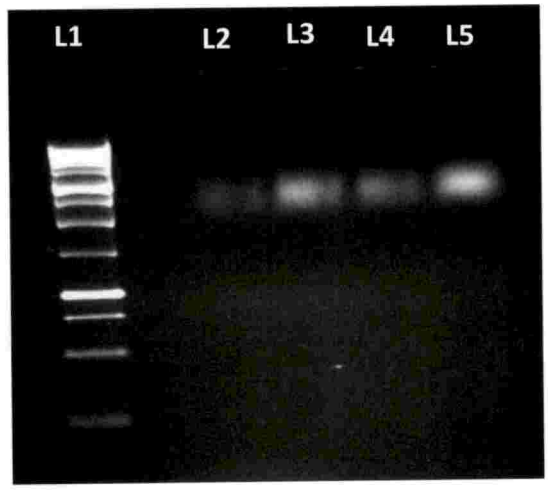


Plate 15. Expression Analysis of gene *DRO1* using Reverse Transcriptase PCR in induced and non-induced plants of Ptb-39 and N22 under combined drought and heat stress.

Lane 1. 100 bp DNA ladder
Lane 2. Non-induced plant of Ptb-39.
Lane 3. Induced plant of Ptb-39.
Lane 4. Non-induced plant of N22.
Lane 5. Induced plant of N22.

DISCUSSION

DISCUSSION

Rice is among the most widely consumed cereal crop in the world, and one of the most vulnerable crop to global climate change. In the changing climatic scenario, rice could more frequently be subjected to simultaneous stress conditions during sensitive developmental stages. Drought and heat stress represent two general abiotic stress conditions, which in practice often occur simultaneously in field condition and in combination have a significantly greater detrimental effect on the growth and productivity of rice compared with its effect individually. There exists a strong relationship between the plant water status and temperature, thus making it difficult to separate the effects of heat and drought stress under field conditions. These two stresses induce many biochemical, molecular, and physiological changes that influence crop yield and quality. Crop growth, development, biomass accumulation, and yield depends on the ability of the crop to withstand, acclimate, or recover from the stress. This involves a complex network of molecular and biochemical processes that integrate together to achieve a specific response of the whole crop.

Plants exposed to mild stress prior to a lethal stress survive better than those encountered chronic lethal stress at the first instance. This phenomenon of acquired tolerance for various abiotic stresses have been shown to operate for a range of stresses like high temperature, drought, salinity, chilling etc But many plants exhibit basal tolerance to stress conditions, due to their inherent ability to survive under extreme weather parameters above the optimal for growth. Acquired stress tolerance is a multigenic trait dependent on many attributes

One of the way to improve thermo-tolerance in plants is to transfer superior alleles from intrinsically thermo-tolerant wild relatives but it requires precise screening methods. Screening of thermo-tolerance at field level can be done based on specific physiological parameters (Selmani and Wasson, 1993), but these measurements are highly influenced by environmental factors. So the best alternative

for screening acquired thermo-tolerance would be to develop suitable laboratory protocol. From this perspective, a protocol called temperature induction response (TIR) technique has been developed. TIR was standardized for many crops including rice and it is reported as an efficient tool for improving stress tolerance in terms of physiological, biochemical and molecular characteristics, but the field level study was rare in this area.

In the present programme, TIR protocol for rice was standardized and used to analyse the effect of TIR technique for combined drought and heat stress tolerance. Both TIR induced plants and non-induced plants were exposed to heat stress, drought stress and combined drought and heat stress. Observations on morphological, physiological and biochemical parameters were taken and molecular studies were carried out in TIR induced and non-induced plants of one tolerant and one susceptible genotype under combined drought and heat stress.

In the first experiment, lethal and induction temperature were identified using two rice genotypes, Jyothi and Vaishak. Results showed that as the temperature increased from 49 to 52°C, the percent survival of the seedlings reduced markedly. Seedlings exposed to 52°C for 3 h showed 100 % mortality. Hence, the exposure to 52°C for 3 h was identified as lethal temperature condition. A similar study was conducted in banana, where 55°C for 2h was selected as lethal temperature with 11% survivability (Vidya *et al.*, 2017). Harihar *et al.* (2014) reported lethal temperature of rice as 52°C for 3 h with 98 % mortality. In pea, the threshold for challenging temperature identified was 48°C for 1h with 10% survivability (Srikanthbabu *et al.*, 2002), where as in ragi a higher level temperature of 57°C for 2 hours duration with 96 % mortality was observed (Babu *et al.*, 2013). Chandola (2015) reported 48°C for 2 h as the lethal temperature in tomato using thirty genotypes at which more than 95% of genotypes showed reduction in recovery growth. A lethal temperature of 54°C for 3 h was selected as best challenging temperatures for screening of rice seedlings for intrinsic heat tolerance at cellular level (Vijayalakshmi *et al.*, 2015).

In the present study induction temperature was standardized as 32-42°C for 5 hrs & 42-52 °C for 30 min with 52°C for 3 h at which maximum recovery growth and least percentage reduction in recovery growth were observed (figure 2 and 3). According to earlier studies 28°C to 42°C for 2.5h selected as induction temperature in sunflower (Kumar *et al.*, 2003). Vidya *et al.* (2017) standardized 42°C for 2½ h as induction temperature in banana. 38°C (1h), 42°C (1h), 46°C (1h) with 48°C (2 h) was reported as the induction temperature in tomato at which at least one genotype showed 100% survival (Chandola, 2015). In ragi 38-54°C for 5h selected as the induction temperature (Babu *et al.*, 2013). 36-44° C for 5h was standardized as the induction temperature in rice (Harihar *et al.*, 2014). Vijayalakshmi *et al.* (2015) reported that gradual induction temperature from 38 to 48°C for 3 h in rice.

Lindquist and Craig (1988) reported that the induction stress helps in preparing plants for higher temperatures stress. During induction stress, the stress tolerant genes like HSP's, HSF's were expressed, which helpes the plants to withstand higher temperatures. Arondel *et al.* (1990) reported that during induction stress HSP's were substantially synthesized and facilitated in promoting physiological and biochemical processes essential for the adaptation to lethal stress. Thus, the accumulation of HSPs during induction stress might confer tolerance to the seedlings when subsequently exposed to lethal stress.

5.1. EFFECT OF DROUGHT AND HEAT STRESS ON MORPHOLOGICAL PARAMETERS.

Drought and heat stress affect stem growth and plant height. Mitra and Bhatia (2008) observed that plant height, number of tillers and total biomass were reduced in rice cultivar when exposed to heat stress. Under drought stress, stem diameter shrinks in response to changes in internal water status (Simonneau *et al.*, 1993). In the present study, an overall reduction of 16.3% in plant height was observed among the non-induced plants of different genotypes under combined drought and heat stress,

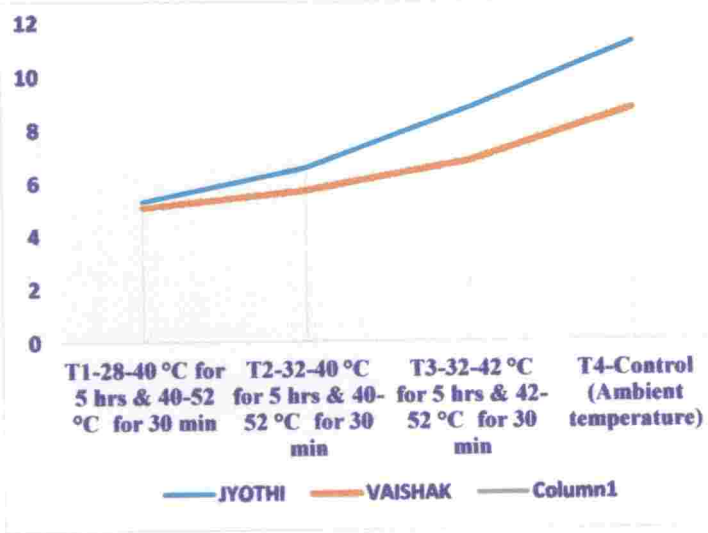


Figure 2. Recovery growth of seedlings under different induction temperatures.

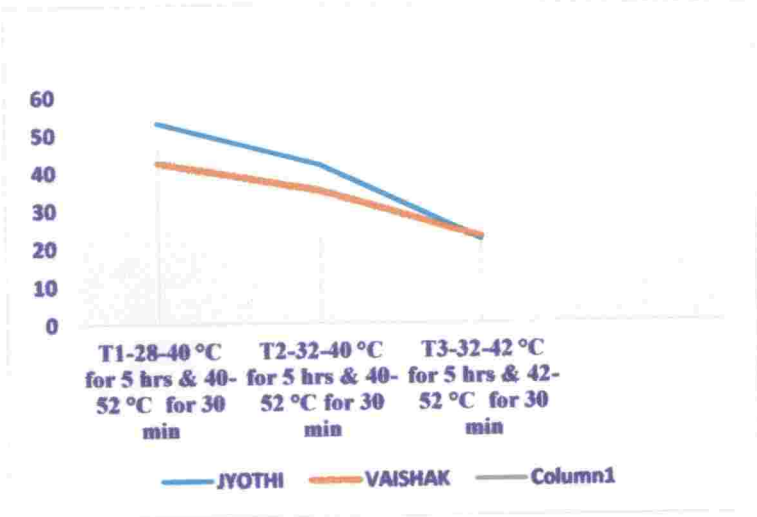


Figure 3. Percent reduction in Recovery growth of seedlings under different induction temperatures

whereas the reduction in induced plants were 8.5% (figure 4) Similar results were reported by Chandola, 2015 in tomato, where plant height was found to increase in induced plants as compared to control and non-induced treatments. Observed reduction in plant height due to heat stress was 10.9 % in non-induced plants and 5.7 % in induced plants. Prasad *et al.* (2006) observed similar results and reported that severe heat stress decreases stem growth resulting in decreased plant height.

Drought stress limits leaf area expansion, such that the expansion and development of the transpiration surface is drastically decreased. Drought sensitivity is expressed in terms of reduction in cell size and in the number of cells produced by leaf meristems (Tardieu *et al.*, 2000). Greer and Weedon, (2012) observed that heat stress results in reduced leaf area and pre-mature leaf senescence which have negative impacts on total photosynthesis performance of plant. In wheat green leaf area and productive tillers/plant were drastically reduced under HT (30/25 °C, day/night). In the present study, all the stress treatments produced significant reduction in leaf area (34.8% under stress, 42.1% under combined stress and 37.9 % under heat stress) but the reduction was less in induced plants. Variation among the treatments were depicted in figure 5. This results was in line with Chandola, (2015) that tomato plants exposed to induction treatment of 33°C for 1h + 36°C for 1h + 39°C for 1h and then subsequently exposed to 45°C 2hr had only 50% reduction in leaf area as against 85% when directly challenged with high temperature.

Reduction in the shoot dry weight have been reported in wheat under heat stress by Shah and Paulsen, (2003) and in maize by Wahid (2007).Similar results under drought was reported by Boyer (1985). Considerable reduction in shoot dry weight was observed in the present study under drought, heat stress and combined drought and heat stress (figure 6). Prasad *et al.* (2008) opinioned that under drought stress, pattern of resource allocation generally favors root growth rather than shoot growth. High temperatures caused significant reductions in shoot dry mass, relative

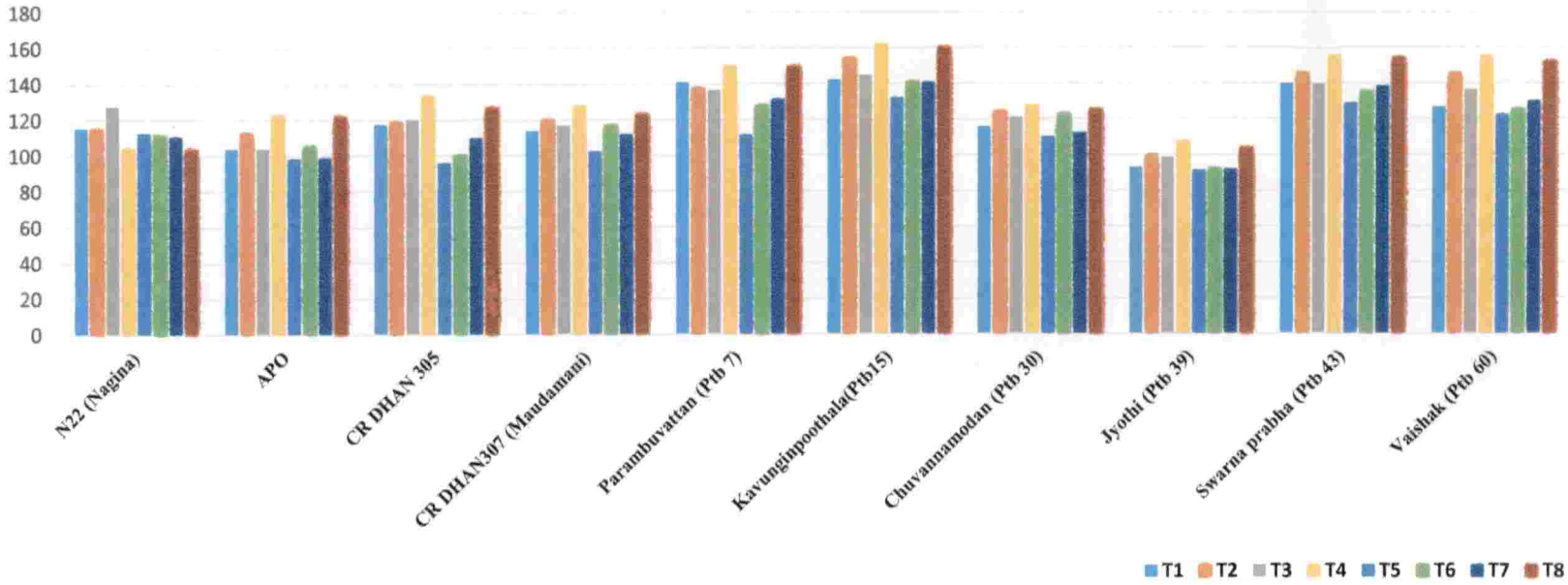


Figure 4. Variation in plant height (cm) of different genotypes under stress conditions.

T1- TIR treated seeds + Drought + Heat; **T2**- TIR treated seeds + Drought; **T3**- TIR treated seeds + Heat; **T4**- TIR control; **T5**-Germinated seeds under ambient temperature + Drought + Heat; **T6**-Germinated seeds under ambient temperature+ Drought; **T7**-Germinated seeds under ambient temperature + Heat; **T8**- Control (Ambient temperature + Normal irrigation).

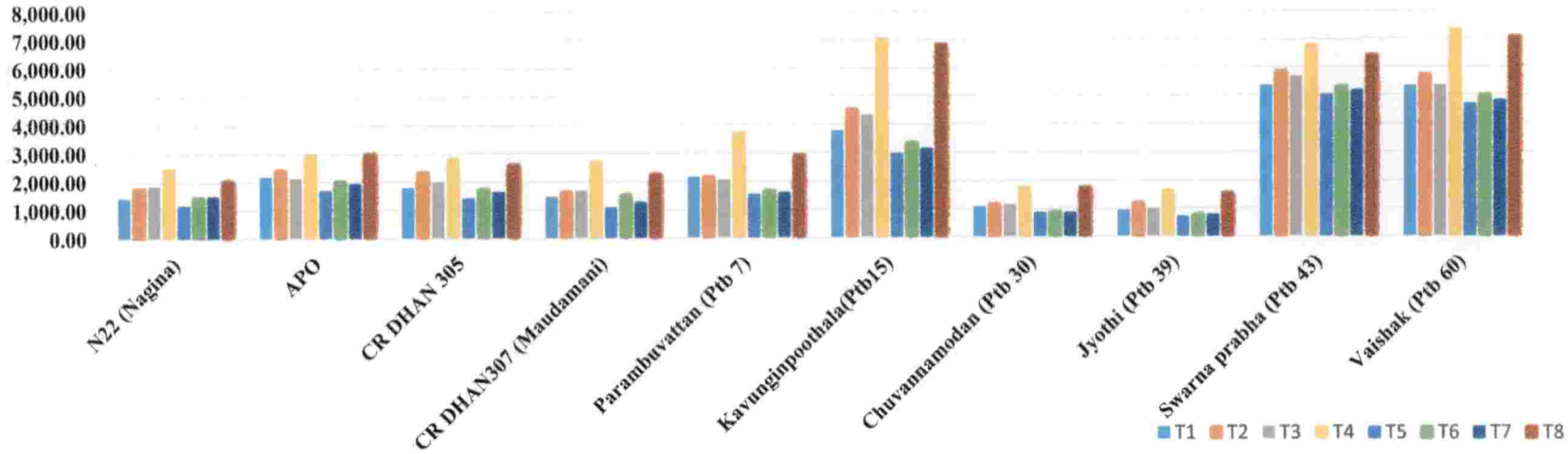


Figure 5. Variation in leaf area (cm^2) of different genotypes under stress conditions.

T1- TIR treated seeds + Drought + Heat; **T2**- TIR treated seeds + Drought; **T3**- TIR treated seeds + Heat; **T4**- TIR control; **T5**-Germinated seeds under ambient temperature + Drought + Heat; **T6**-Germinated seeds under ambient temperature+ Drought; **T7**-Germinated seeds under ambient temperature + Heat; **T8**- Control (Ambient temperature + Normal irrigation).

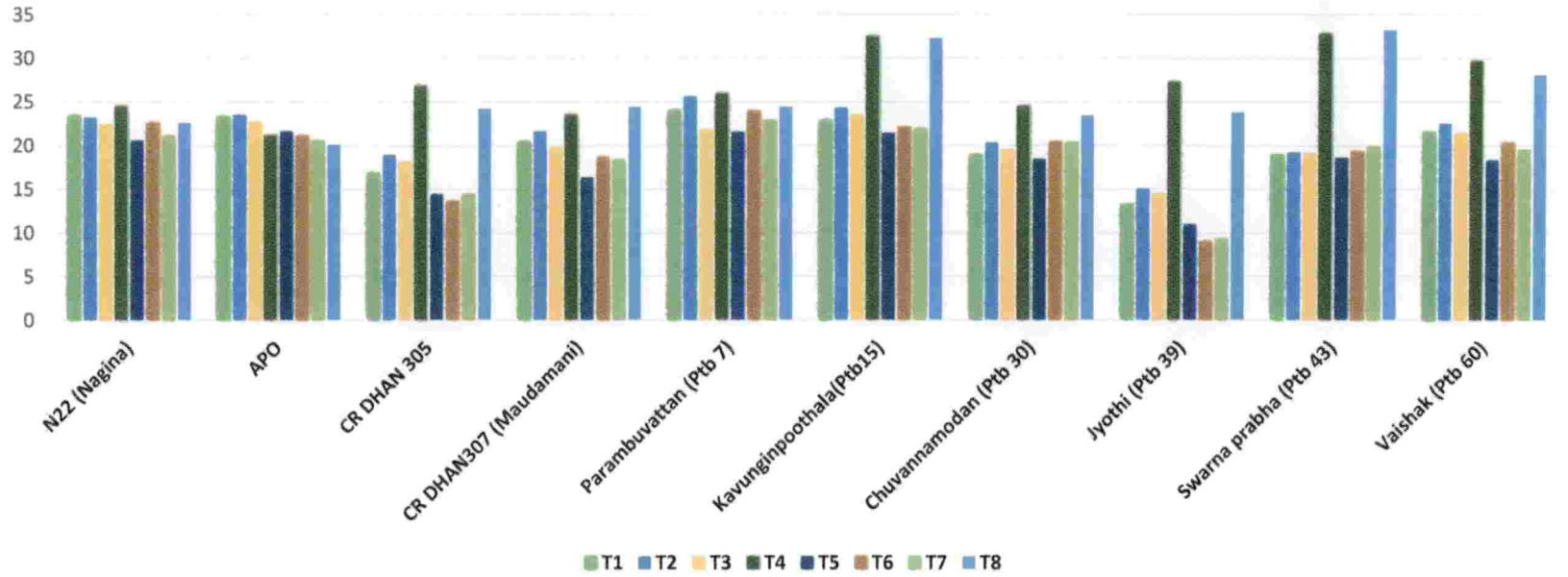


Figure 6. Variation in shoot dry weight (g) of different genotypes under stress conditions.

T1- TIR treated seeds + Drought + Heat; **T2**- TIR treated seeds + Drought; **T3**- TIR treated seeds + Heat; **T4**- TIR control; **T5**-Germinated seeds under ambient temperature + Drought + Heat; **T6**-Germinated seeds under ambient temperature+ Drought; **T7**-Germinated seeds under ambient temperature + Heat; **T8**- Control (Ambient temperature + Normal irrigation).

growth rate, and net assimilation rate in maize and pearl millet (Ashraf and Hafeez, 2004) and sugarcane (Wahid, 2007).

Root dry weight all the genotypes except N22 and Apo were reduced in the present study (figure 7). This decline in root dry weight was attributed to decreased supply of photosynthates to roots due to decrease in leaf water potential under drought stress (Cruz and O'Toole, 1985) and due to decreased carbon partitioning to roots under heat stress (Batts *et al.*, 1998). This decrease in root dry weight can be supported with the findings of Rejeth (2017), where an overall reduction of 32.6% was observed in rice genotypes under drought stress. Among the genotypes N22 and Apo showed increase in root dry weight, these findings were in line with the findings of Ganapathy *et al.* (2010) and Cruz *et al.* (1986) where they reported that increase in root dry weight under stress condition is a function of the ability to tolerate stress in rice.

Root growth decreases under heat stress because it has been shown that root growth has a very narrow optimum temperature range when compared with other growth processes (Porter and Gawith, 1999). Heat stress reduced root number as well as root length and root diameter. In the present study, mean root length of genotypes were increased under stress and induced plants under drought showed an increase of 6.8 % than non-induced plants (figure 8). Rejeth (2017) reported an average increase of 7.23 % in root length under drought stress. Greater root length density at depth has also been reported in drought-tolerant genotypes (Henry *et al.*, 2011). Greater root length will minimize the occurrence of stress through development of a healthy root system, which in the case of drought permits to absorb water from deeper soil layer (Lopes and Reynolds, 2010) and in the case of heat stress permits transpiration rates that better match evaporative demand (Amani *et al.*, 1996), thereby increasing carbon fixation as a result of canopy cooling.

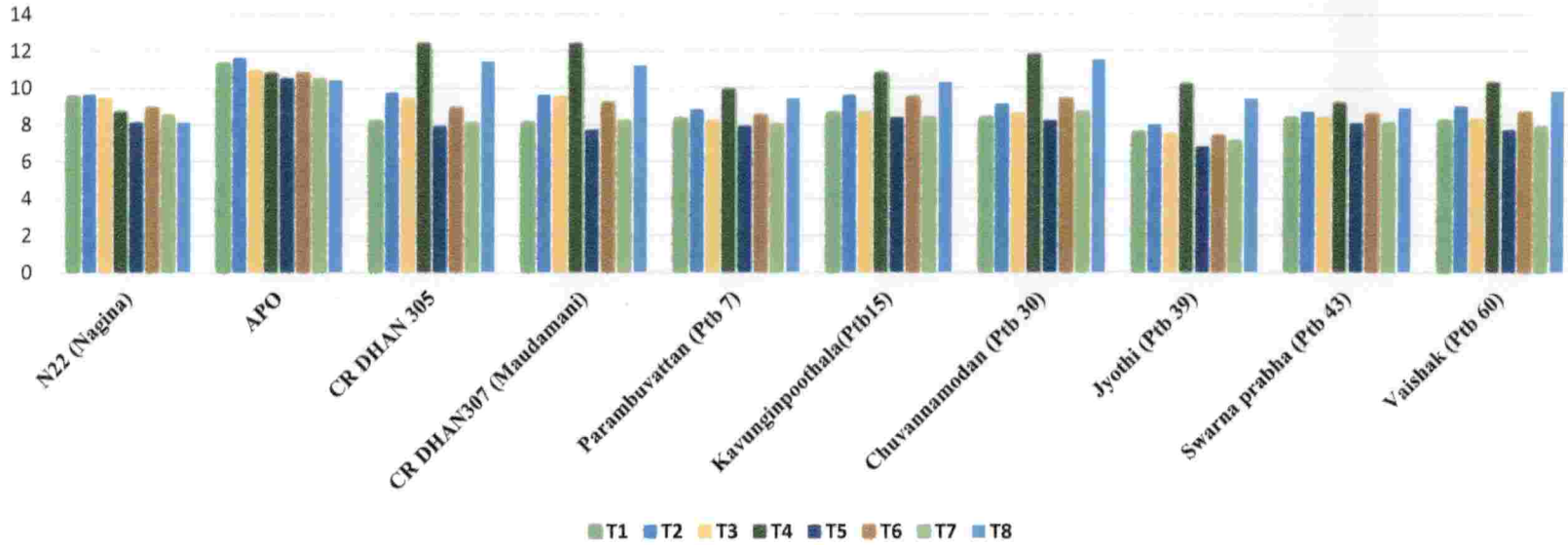


Figure 7. Variation in root dry weight (g) of different genotypes under stress conditions.

T1- TIR treated seeds + Drought + Heat; **T2**- TIR treated seeds + Drought; **T3**- TIR treated seeds + Heat; **T4**- TIR control; **T5**-Germinated seeds under ambient temperature + Drought + Heat; **T6**-Germinated seeds under ambient temperature+ Drought; **T7**-Germinated seeds under ambient temperature + Heat; **T8**- Control (Ambient temperature + Normal irrigation).

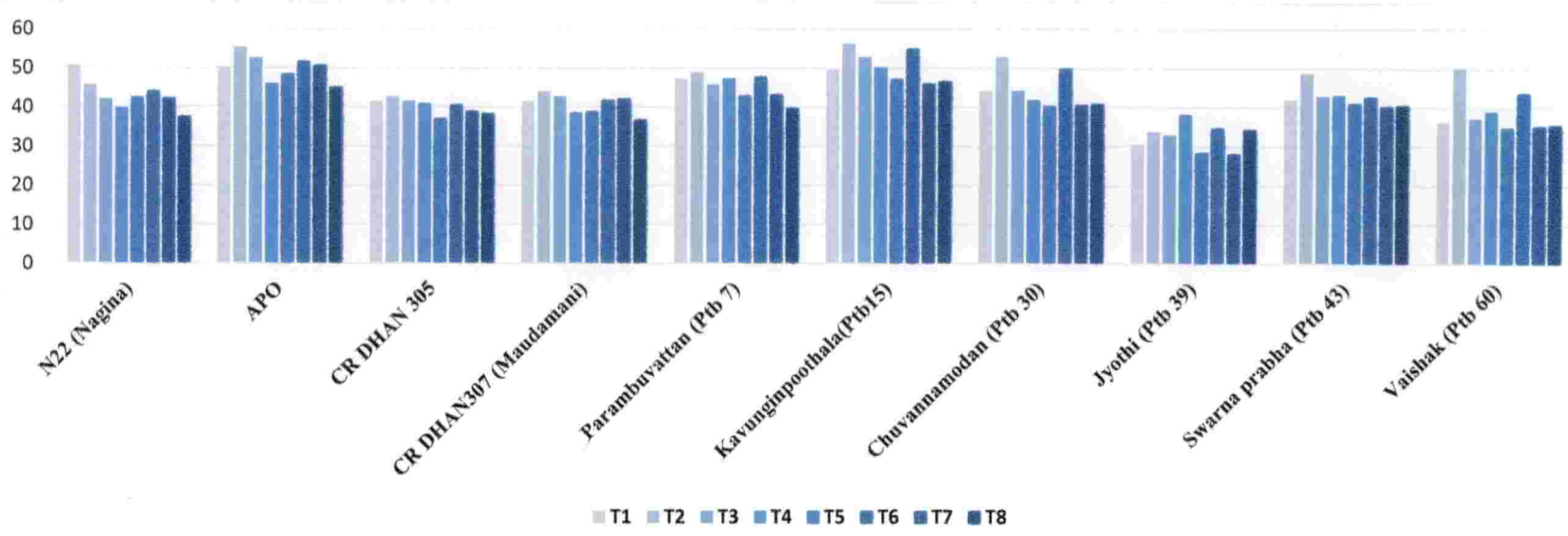


Figure 8. Variation in root length (cm) of different genotypes under stress conditions.

T1- TIR treated seeds + Drought + Heat; **T2**- TIR treated seeds + Drought; **T3**- TIR treated seeds + Heat; **T4**- TIR control; **T5**-Germinated seeds under ambient temperature + Drought + Heat; **T6**-Germinated seeds under ambient temperature+ Drought; **T7**-Germinated seeds under ambient temperature + Heat; **T8**- Control (Ambient temperature + Normal irrigation).

There exist a positive association between root length and root volume in rice (Zuno *et al.*, 1990), this trend may not be always exist in susceptible genotypes. Reduction in root number, root length as well as root diameter will lead to decreased reduction in root volume under heat stress. In the present study, the percentage reduction in root volume was 13.3, 21.7 and 24.5 % under drought, heat stress and combined drought and heat stress respectively in non-induced plants, whereas in induced plats the corresponding values were 7.7, 14.5 and 16.7%. The variation in the root length among the genotypes are shown in figure 9. There exist a considerable increase in root volume in induced plants under stress as compared to non-induced plants. Even though most of the genotypes showed reduction in root volume under stress, genotypes such as N22 and Apo showed increase in root volume under stress. This increase was attributed to their ability to withstand under drought by increasing root biomass and absorb moisture from deeper layers of soil. This result was in line with findings of Ekanayaka *et al.* (1985), he opinioned that deep and thick root system allows access to water in deeper layer and considered as an important trait determining drought tolerance in rice.

TIR induced plants showed cooler canopy as compared to non-induced plants under stress. In all the varieties canopy temperature was increased under stress treatments and the highest increase of 6.4% was shown by non-induced plants under combined drought and heat stress (figure 10). Decline in leaf water content and decreased transpiration rate can be the reasons for this increment (Rejeth, 2017). Rizhsky *et al.* (2002) reported that plants subjected to a combination of drought and heat stress has several unique aspects such as high respiration rate combined with low photosynthesis, closed stomata, and high canopy temperatures. Maintenance of canopy temperature through the transpirational cooling system would be insufficient with the occurrence of combined water deficit conditions with heat stress (Chandola, 2015).

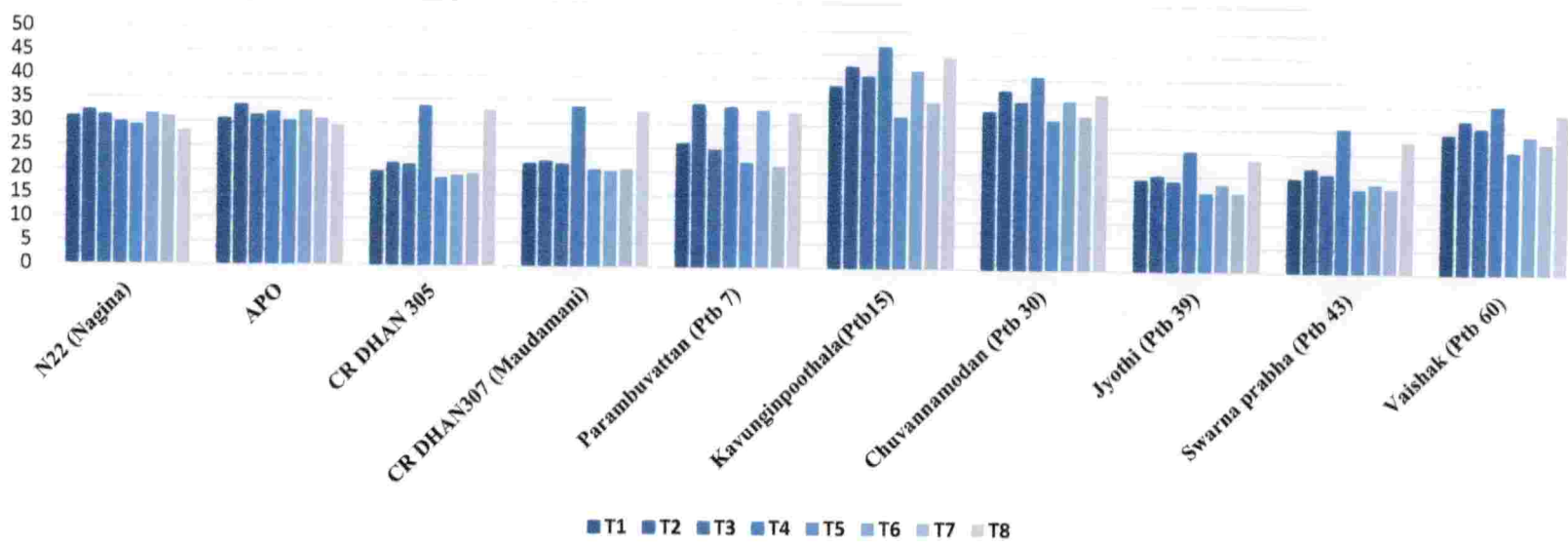


Figure 9. Variation in root volume (cm^3) of different genotypes under stress conditions.

T1- TIR treated seeds + Drought + Heat; **T2**- TIR treated seeds + Drought; **T3**- TIR treated seeds + Heat; **T4**- TIR control; **T5**-Germinated seeds under ambient temperature + Drought + Heat; **T6**-Germinated seeds under ambient temperature+ Drought; **T7**-Germinated seeds under ambient temperature + Heat; **T8**- Control (Ambient temperature + Normal irrigation).

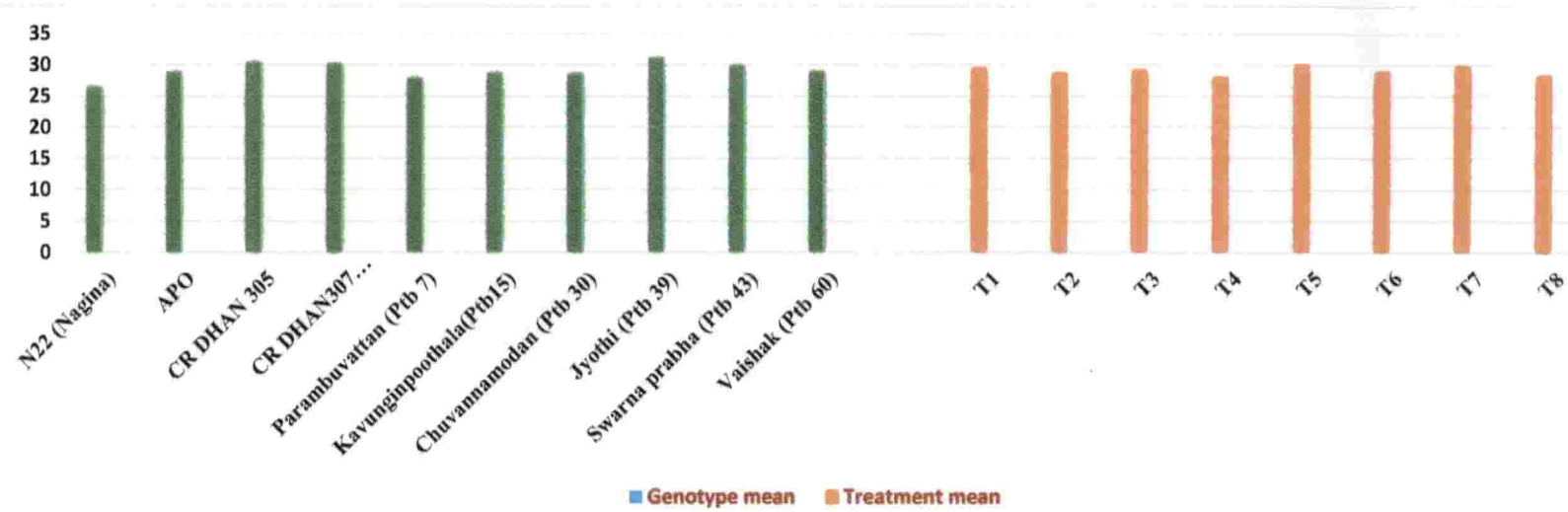


Figure 10. Variation in canopy temperature (°C) among the genotypes and the treatments.

T1- TIR treated seeds + Drought + Heat; **T2**- TIR treated seeds + Drought; **T3**- TIR treated seeds + Heat; **T4**- TIR control; **T5**-Germinated seeds under ambient temperature + Drought + Heat; **T6**-Germinated seeds under ambient temperature+ Drought; **T7**-Germinated seeds under ambient temperature + Heat; **T8**- Control (Ambient temperature + Normal irrigation).

5.2. EFFECT OF DROUGHT AND HEAT STRESS ON PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS

The physiological processes during the sensitive periods will negatively affected by stress conditions. The integrity and functions of biological membranes are sensitive to stress conditions, as the tertiary and quaternary structures of membrane proteins are highly sensitive to alterations in the environment especially heat stress. Such alterations enhance the permeability of membranes, as evident from increased loss of electrolytes. In the present study cell membrane stability of rice genotypes were decreased considerably under stress and depicted in figure 11. Reduction of membrane stability recorded for drought stress was 17.1 % in non-induced plants and 8.8 % in induced plants. Respective values for heat stress was 19.2 % and 11%. In the case of combined stress, induced plants showed 13.1 % reduction and non-induced plants recorded 21.9 %. These results are in line with findings of Chandola, (2015) he reported that thermally induced plants showed increase in stability as compared to the plants which were not induced at seedling stage. In genotype GT, heat stress with thermal induction inferred highest stability (221.2%) but plants without thermal induction reduced to 24.9%. In genotype NDTVR-60, it was also highest (83.8%) under heat stress with thermal induction. The minimum increase (29.9%) in thermally induced plants were observed under combination of heat and water stress (T8).Increase in cell membrane stability due to thermal induction was further proved by him, as these plants were having lower malondialdehyde (MDA) content, a product of membrane peroxidation. Falcone *et al.* (2004) reported that since PSI and PSII are integral thylakoid membrane protein complexes, any change in membrane fluidity will have adverse effect on membrane integrity associated PSI and PSII complexes and ultimately Fv/Fm.

High temperature affects chloroplast stroma and thylakoid membrane (Wang *et al.*, 2010) and water stress reported to reduce chlorophyll content in plants (Arjum *et al.*, 2011). In the present study variation in chlorophyll stability index of

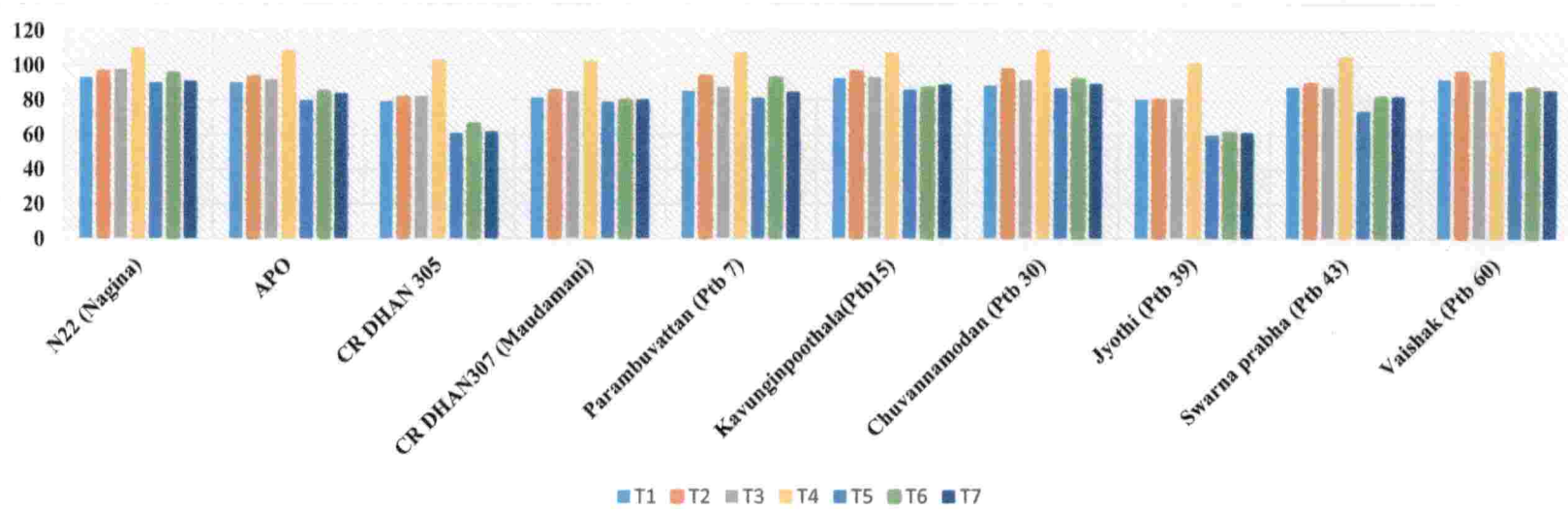


Figure 11. Variation in cell membrane stability index (%) of different genotypes under stress conditions.

T1- TIR treated seeds + Drought + Heat; **T2**- TIR treated seeds + Drought; **T3**- TIR treated seeds + Heat; **T4**- TIR control; **T5**-Germinated seeds under ambient temperature + Drought + Heat; **T6**-Germinated seeds under ambient temperature+ Drought; **T7**-Germinated seeds under ambient temperature + Heat; **T8**- Control (Ambient temperature + Normal irrigation).

rice genotypes under stress conditions were presented in figure 12. Stability of chlorophyll membrane was reduced significantly under stress and the highest reduction observed under combined stress condition. Stability of induced plants were higher than non-induced plants. Similar results were obtained by Chandola, (2015), where reduction in chlorophyll stability was recorded under all the treatment without induction. Maximum reduction of 25.7% reported in plants subjected to combination of heat and water stress without thermal induction. The maximum per cent increase (17.4%) was observed under heat stress with thermal induction. Decreased chlorophyll content and chlorophyll stability index under combined drought and heat stress was reported in wheat by Sairam *et al.* (1997). Decrease in chlorophyll content is due to increased chlorophyllase activity under high temperature (Todorov *et al.*, 2003). Burke (1998) reported that the chlorophyll stability was higher in acclimated wheat plants and Howarth *et al.* (1997) got similar results in sorghum.

Conductance through stomata will vary within the genotypes according to their ability to tolerate stress conditions. In this present study, stomatal conductance reduced under drought stress and the reduction was less in plants under heat stress and combined stress condition (figure 13). In induced plants, conductance through stomata was higher as compared to non-induced plants in order to maintain optimum CO_2 intake. Stomatal closure is one of the adaptation strategy to avoid tissue dehydration under moisture stress. This finding can be supported by the results obtained by Rejeth (2017), where all the genotypes showed significant reduction in stomatal conductance under drought. Farooq *et al.* (2010) also reported reduction in stomatal conductance under drought stress in *indica* rice. Increase in stomatal conductance under heat stress was responsible for the decrease in leaf temperature through transpiration. Tolerance of plants to combined drought and heat stress mainly depends on the maintenance of leaf temperature (Mittler and Blumwald, 2010). Morales *et al.* (2003) reported that high-temperature pre-conditioning in tomato plants showed good osmotic adjustment by maintaining the osmotic potential and stomatal conductance, and better growth than non-conditioned plants.

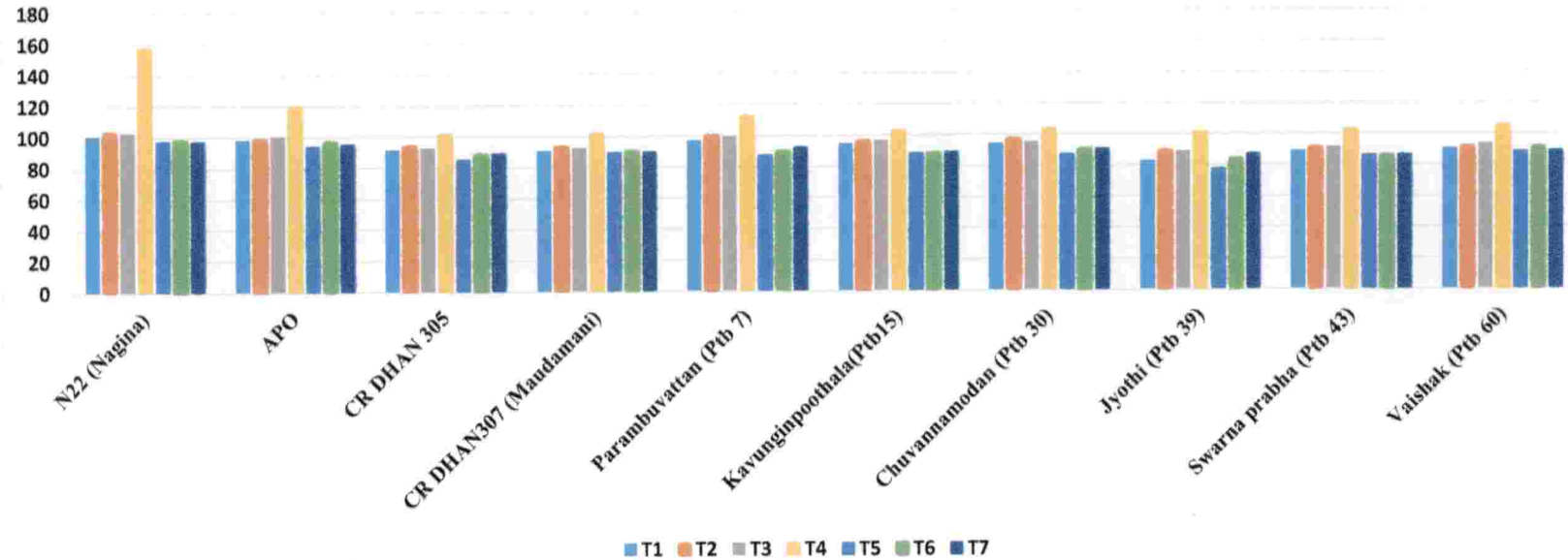


Figure 12. Variation in chlorophyll stability index (%) of different genotypes under stress conditions.

T1- TIR treated seeds + Drought + Heat; **T2-** TIR treated seeds + Drought; **T3-** TIR treated seeds + Heat; **T4-** TIR control; **T5**-Germinated seeds under ambient temperature + Drought + Heat; **T6**-Germinated seeds under ambient temperature+ Drought; **T7**-Germinated seeds under ambient temperature + Heat; **T8-** Control (Ambient temperature + Normal irrigation).

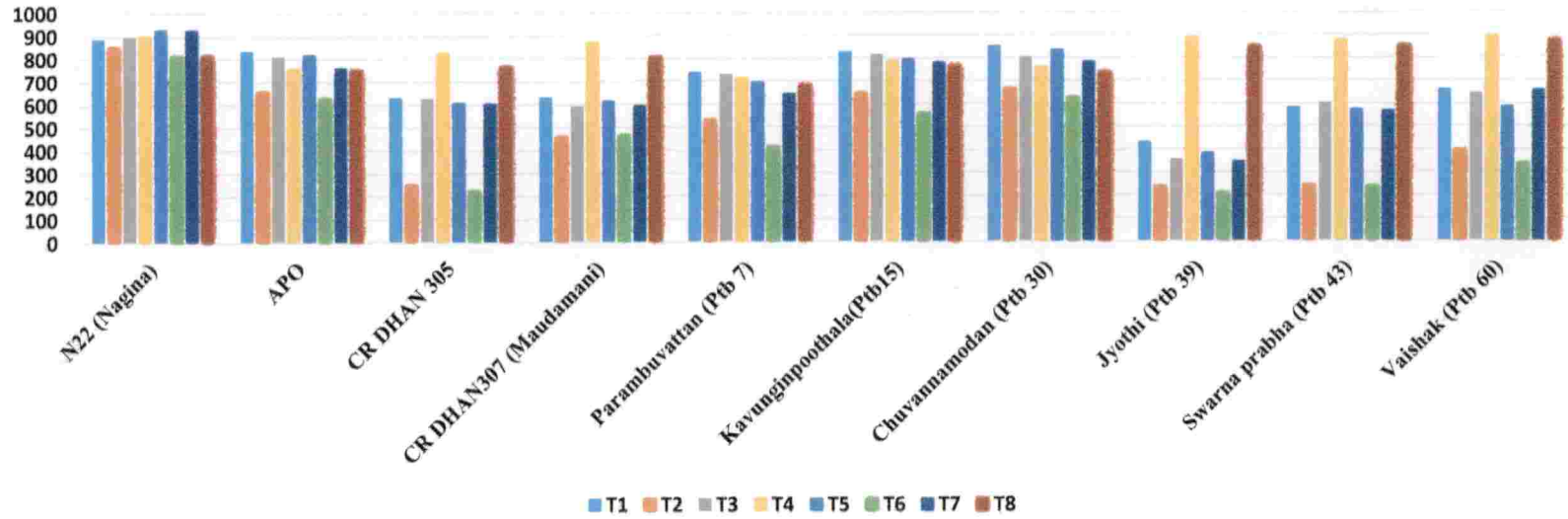


Figure 13. Variation in stomatal conductance (m moles m^{-2} s^{-1}) of different genotypes under stress conditions.

T1- TIR treated seeds + Drought + Heat; **T2**- TIR treated seeds + Drought; **T3**- TIR treated seeds + Heat; **T4**- TIR control; **T5**-Germinated seeds under ambient temperature + Drought + Heat; **T6**-Germinated seeds under ambient temperature+ Drought; **T7**-Germinated seeds under ambient temperature + Heat; **T8**- Control (Ambient temperature + Normal irrigation).

Reduction in photosynthetic rate under stress conditions was mainly due to various factors. Under drought, photosynthesis can be influenced either through pathway regulation by stomatal closure and decreasing flow of CO_2 into mesophyll tissue (Flexas *et al.*, 2004) or by directly impairing metabolic activities (Farquhar *et al.*, 1989). Decline in regeneration of ribulose bisphosphate (RuBP) and ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) protein content (Bota *et al.*,2004) was reported under drought and hence decreased Rubisco activity (Parry *et al.*, 2002). Cornic (2000) opinioned that decreased conductance through stomata is the primary cause of decline in photosynthesis. In the present study, an overall reduction of 28.5 % were observed in non-induced plants under stress conditions whereas in induced plants the recorded reduction was 24.8 % (Figure 14). These findings are in line with the findings of Lea and Leegood (1999), they reported that the solubility of oxygen is decreased to a lesser extent than CO_2 under heat stress resulting in increased photorespiration and lower photosynthesis. It is reported that high temperature can reduce photosynthetic rate by 40-60% at mid-ripening, leading to more rapid senescence of the flag leaf (Oh-e *et al.*, 2007).

Kishore *et al.* (2005) reported that, proline is known to occur in higher plants and the accumulation will increase in response to environmental stresses, which helps in maintaining turgidity of cells under stress. In this study, proline content increased during stress in all the genotypes under different stress treatments to an extent of 28.3 to 44.6 per cent compared to ambient conditions (Figure 15). Induced plants showed the highest increase under combined drought and heat stress. This result can be supported by findings of Beena *et al.* (2012) they reported an increase of 89.6 % in proline content under drought stress in selected recombinant inbred lines. Similar findings were also reported in rice under drought stress by Rejeth (2017). Boominathan (2013) confirmed role of proline content in high temperature tolerant rice genotypes by analysing the proline content in both tolerant and susceptible genotypes and it was observed that higher per cent increase in proline content over control in the tolerant genotypes N22 (32.1,

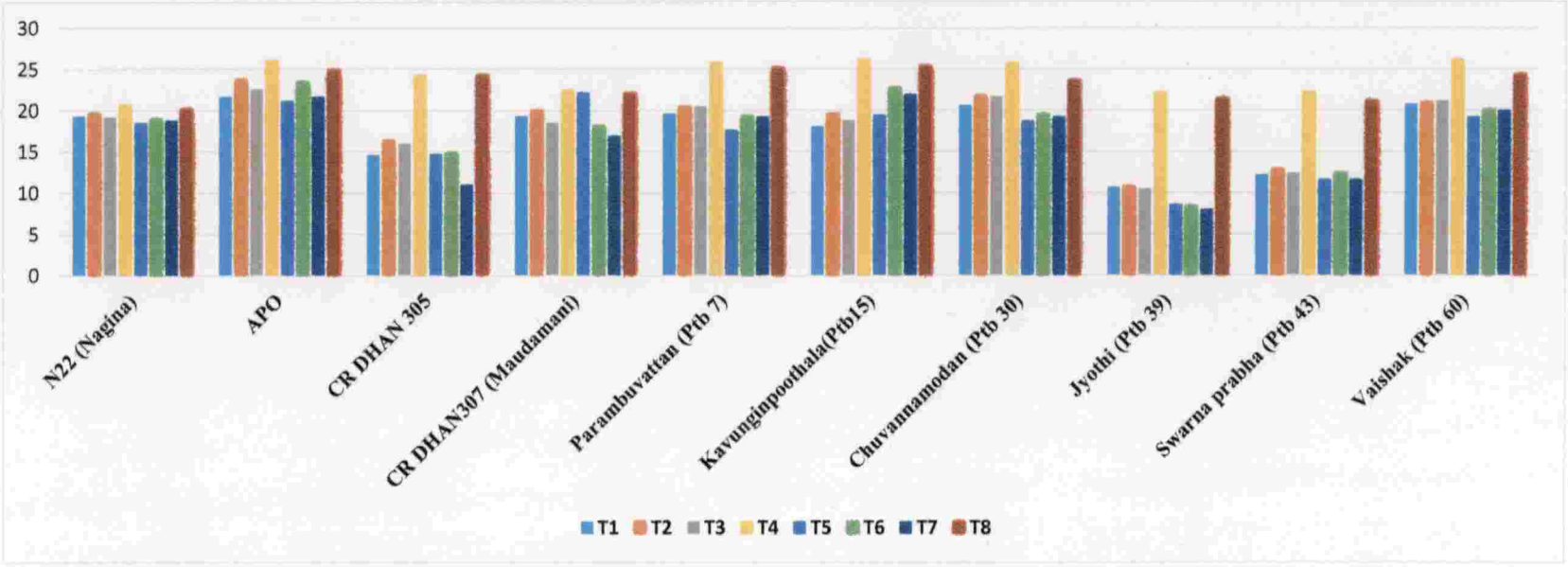


Figure 14. Variation in photosynthetic rate (μ moles m^{-2} s^{-1}) of different genotypes under stress conditions.

T1- TIR treated seeds + Drought + Heat; **T2**- TIR treated seeds + Drought; **T3**- TIR treated seeds + Heat; **T4**- TIR control; **T5**-Germinated seeds under ambient temperature + Drought + Heat; **T6**-Germinated seeds under ambient temperature+ Drought; **T7**-Germinated seeds under ambient temperature + Heat; **T8**- Control (Ambient temperature + Normal irrigation).

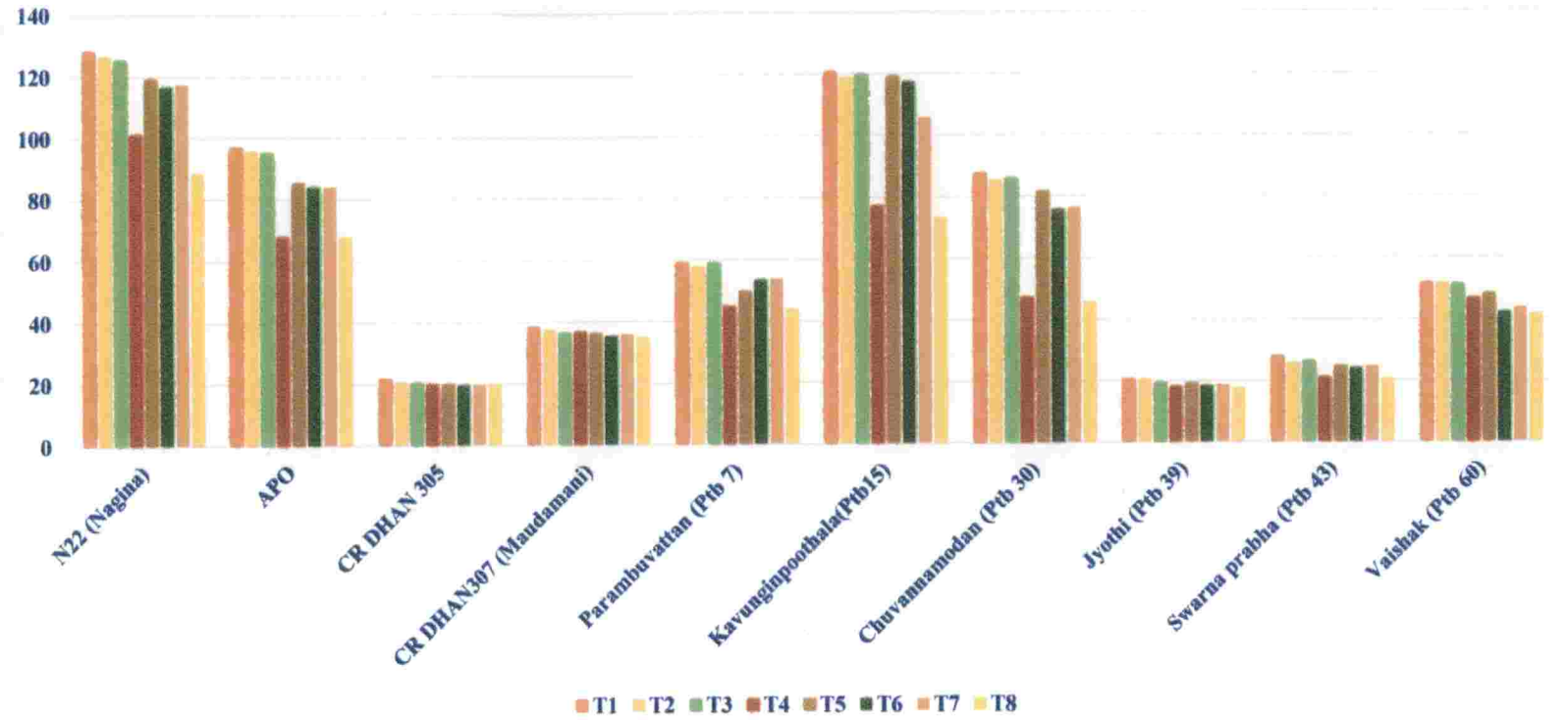


Figure 15. Variation in proline content (µg /g fresh tissue) of different genotypes under stress conditions.

T1- TIR treated seeds + Drought + Heat; **T2-** TIR treated seeds + Drought; **T3-** TIR treated seeds + Heat; **T4-** TIR control; **T5-**Germinated seeds under ambient temperature + Drought + Heat; **T6-**Germinated seeds under ambient temperature+ Drought; **T7-**Germinated seeds under ambient temperature + Heat; **T8-** Control (Ambient temperature + Normal irrigation).

31.9) and TKM9 (31.8, 36.7) compared to susceptible genotypes White Ponni (6.0, 12.0) and Moroberekan (10.0, 8.6) in two different seasons.

Cao *et al.* (2009) explained that high activity of antioxidants in plants might be one of the physiological mechanism for stress tolerance in rice. In the present study, peroxidase and SOD activity was increased under drought and considerable decrease was observed under heat stress and combined drought and heat stress. Variation in peroxidase activity and SOD activity under stress were depicted in figure 16 and 17 respectively. Induced plants showed higher activity of peroxidase and SOD under different stress conditions. Madhusudhan *et al.* (2003) observed increased cytosolic peroxidase activity led to decrease in H_2O_2 concentration in tobacco. Reduction of peroxidase activity under heat stress can be supported by the findings of Zhao *et al.* (2017), they found that the activity of SOD and peroxidase was reduced on exposure to high temperature in rice. Karuppanapandian *et al.* (2011) also reported reduction in SOD activity under high temperature.

5.3. EFFECT OF DROUGHT AND HEAT STRESS ON YIELD PARAMETERS.

Early Morning Flowering (EMF) trait is exhibited by a few wild rice species like *Oryza officinalis*. It is less explored, is to breed cultivars that escape heat at flowering because of their early morning flowering (EMF) trait (Satake and Yoshida, 1978). Spikelets are highly susceptible to heat stress at flowering; however, they remain fertile when flowering occurs 1h prior to heat stress, because fertilization is completed within 1h after the onset of flowering (Satake and Yoshida, 1978). In the present study flower opening time of N22 was recorded as 9.27 am. Still 9.27 am is not an EMF trait contributing more spikelet fertility to N22. The recorded reduction in spikelet fertility of varieties depicted in figure 18 and this reduction can be attributed to exposure of pollen and stigma to higher temperatures during anthesis.

Jagadish *et al.* (2010) reported that physiological processes during the sensitive stage negatively affects spikelet fertility under water stress and it was

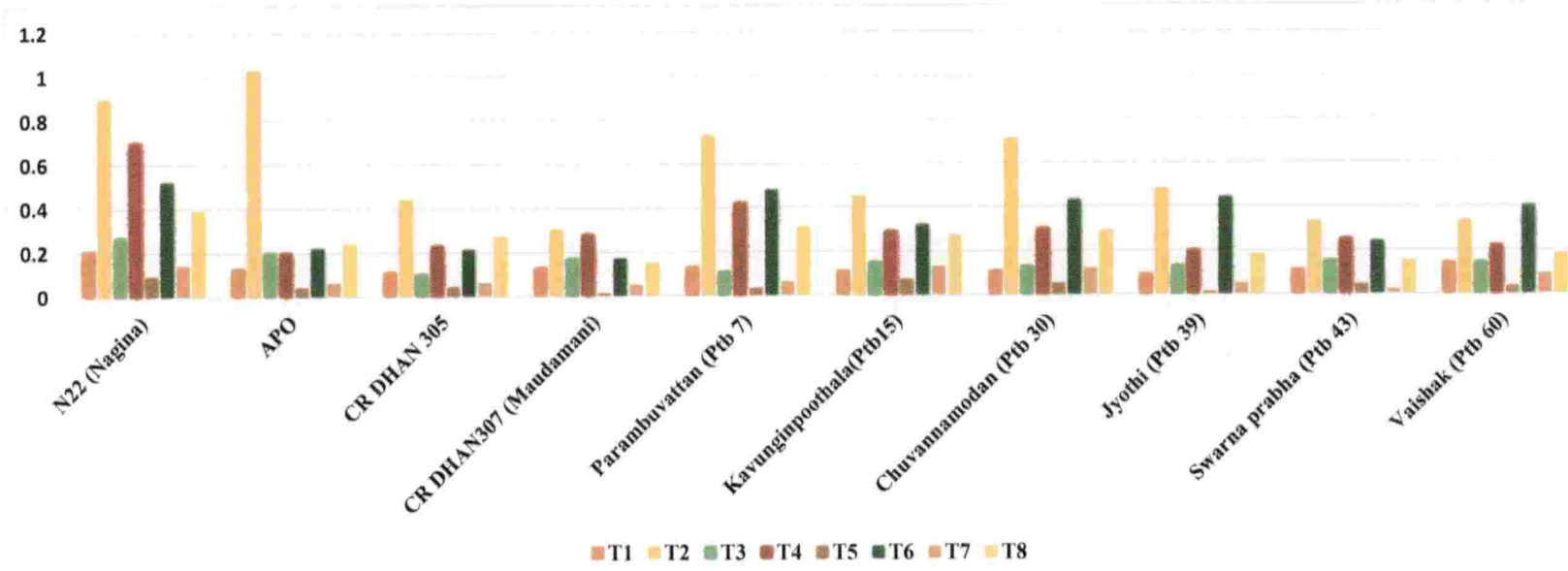


Figure 16. Variation in peroxidase activity (g^{-1} min^{-1}) of different genotypes under stress conditions.

T1- TIR treated seeds + Drought + Heat; **T2**- TIR treated seeds + Drought; **T3**- TIR treated seeds + Heat; **T4**- TIR control; **T5**-Germinated seeds under ambient temperature + Drought + Heat; **T6**-Germinated seeds under ambient temperature+ Drought; **T7**-Germinated seeds under ambient temperature + Heat; **T8**- Control (Ambient temperature + Normal irrigation).

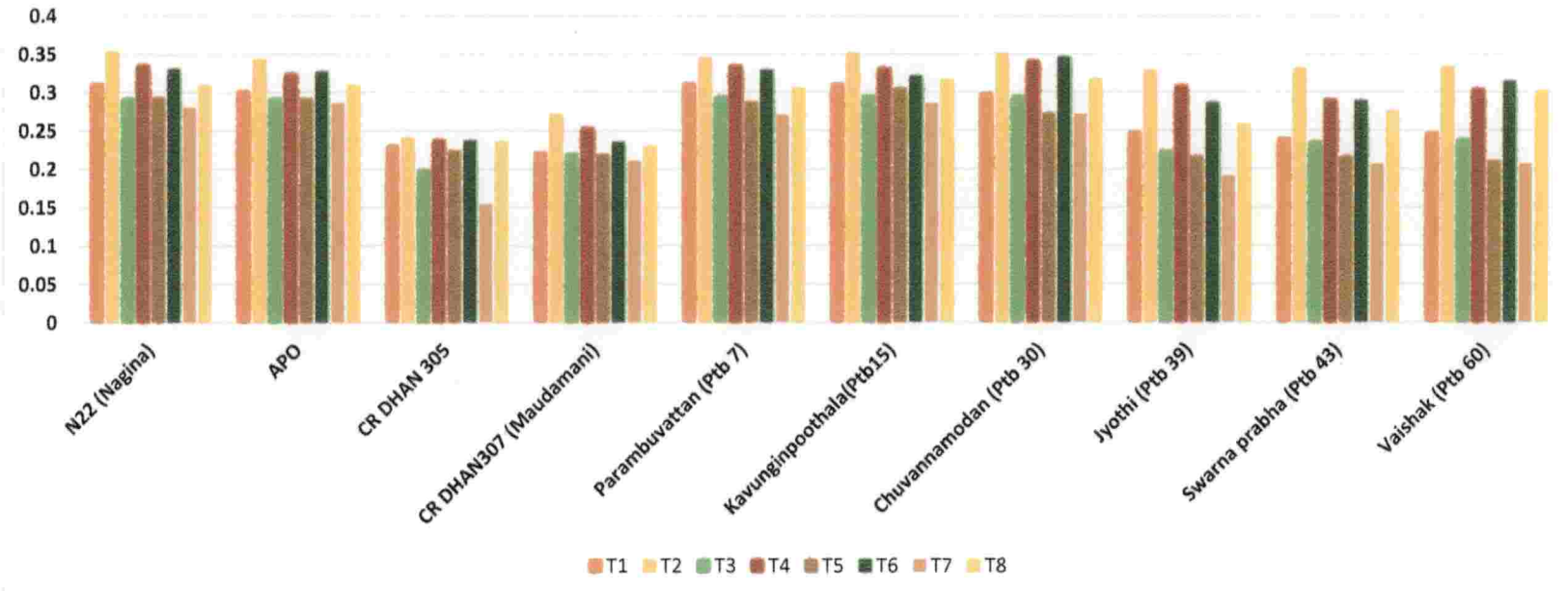


Figure 17. Variation in superoxide dismutase activity (g^{-1} min^{-1}) of different genotypes under stress conditions.

T1- TIR treated seeds + Drought + Heat; **T2**- TIR treated seeds + Drought; **T3**- TIR treated seeds + Heat; **T4**- TIR control; **T5**-Germinated seeds under ambient temperature + Drought + Heat; **T6**-Germinated seeds under ambient temperature+ Drought; **T7**-Germinated seeds under ambient temperature + Heat; **T8**- Control (Ambient temperature + Normal irrigation).

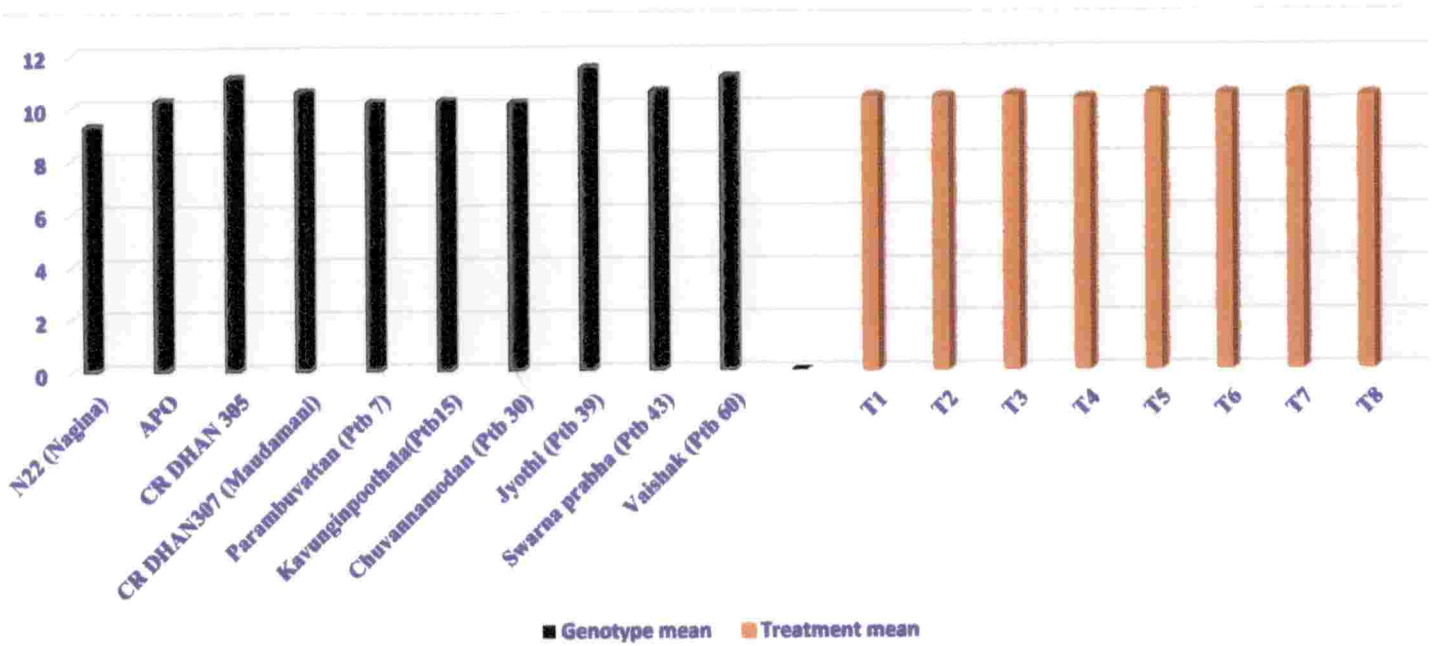


Figure 18. Variation in time of anthesis (am) among the genotypes and the treatments.

T1- TIR treated seeds + Drought + Heat; **T2**- TIR treated seeds + Drought; **T3**- TIR treated seeds + Heat; **T4**- TIR control; **T5**-Germinated seeds under ambient temperature + Drought + Heat; **T6**-Germinated seeds under ambient temperature+ Drought; **T7**-Germinated seeds under ambient temperature + Heat; **T8**- Control (Ambient temperature + Normal irrigation).

similar to heat stress. In the present study reduction in spikelet fertility was significant and percent reduction ranged between 15.5 to 26.2 % (figure 19). Induced plants recorded least percent reduction in fertility. These findings are in line with the findings of Das *et al.* (2014), where they observed that heat stress for three days during the panicle initiation led to spikelet sterility, they reported a decrease of 5.2 % per °C increase in temperature. Earlier studies revealed that drought stress during reproductive stage significantly reduces spikelet fertility (Praba *et al.*, 2009) and grain yield (Boonjung and Fukai, 1996). Bahuguna *et al.* (2015) reported that rice accession NL-44 recorded 50-60% spikelet fertility and the genotype N22 recorded 67-79% under controlled environment temperature of 38°C, although both had about 87% fertility under extremely hot field conditions and these genotypes displayed lower membrane damage and higher antioxidant enzymes activity across leaves and spikelets. Prasad *et al.* (2006) reported that high temperatures at anthesis can result in poor anther dehiscence, poor pollen germination and retarded pollen tube growth and finally reduced spikelet fertility. Findings of Boominathan (2013) reported that the mean reduction in spikelet fertility was 39 to 40 per cent under high temperature stress compared to control.

Pollen viability of genotypes was affected significantly under stress (figure 20). Induced plants under different stress condition showed least percent reduction than non-induced plants. Recorded reduction was 23, 25.7 and 25 % under drought, heat and combined stress in non-induced plants, whereas in induced plants the reduction was 15.2, 16.3 and 17.7 % respectively. This findings was in line Chandola (2015) they observed that the effect of thermal induction was found to be clearly observed under pollen characters. All the parameters like pollen produced, pollen released, pollen germination and pollen viability were found to be increased after thermal induction as compared to non-induction. Liu *et al.* (2006) reported reduced pollen viability and yield under drought. Earlier studies in plants reported possible mechanisms responsible for decreased pollen viability under drought and heat stress, developmental abnormalities in anthers

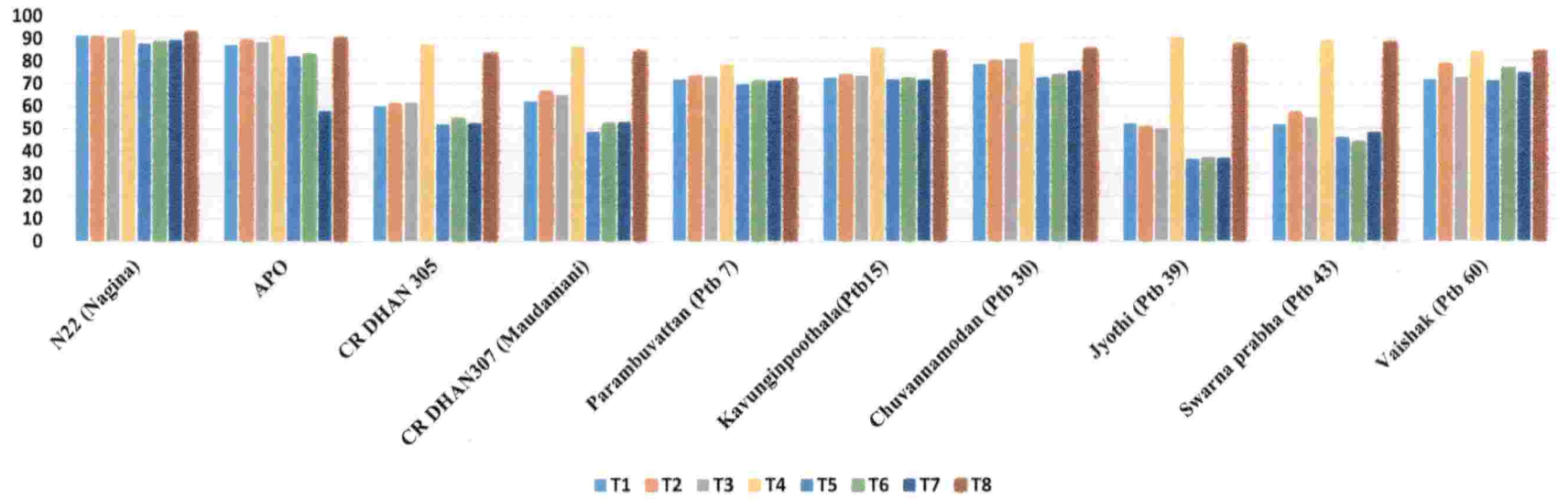


Figure 19. Variation in spikelet fertility (%) of different genotypes under stress conditions.

T1- TIR treated seeds + Drought + Heat; **T2**- TIR treated seeds + Drought; **T3**- TIR treated seeds + Heat; **T4**- TIR control; **T5**-Germinated seeds under ambient temperature + Drought + Heat; **T6**-Germinated seeds under ambient temperature+ Drought; **T7**-Germinated seeds under ambient temperature + Heat; **T8**- Control (Ambient temperature + Normal irrigation).

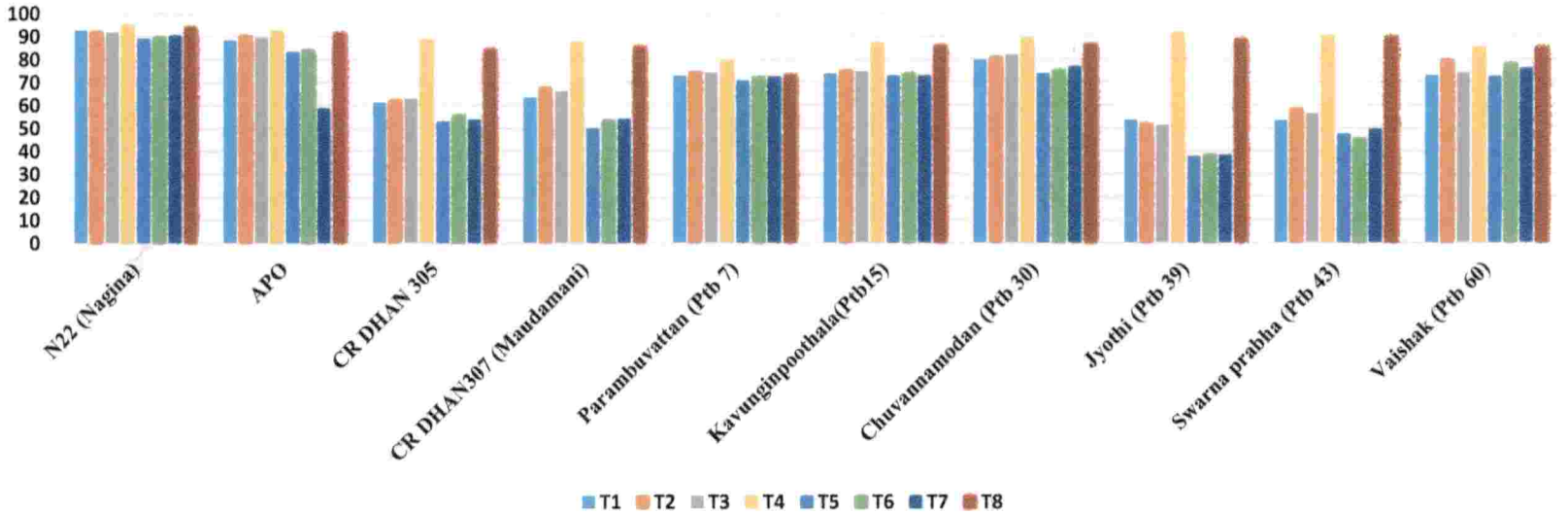


Figure 20. Variation in pollen viability (%) of different genotypes under stress conditions.

T1- TIR treated seeds + Drought + Heat; **T2**- TIR treated seeds + Drought; **T3**- TIR treated seeds + Heat; **T4**- TIR control; **T5**-Germinated seeds under ambient temperature + Drought + Heat; **T6**-Germinated seeds under ambient temperature+ Drought; **T7**-Germinated seeds under ambient temperature + Heat; **T8**- Control (Ambient temperature + Normal irrigation).

leading to dislocation of premature microspores (Saini *et al.*, 1984); abnormal vacuolization led to dysfunction ot tapetal cells (Lalonde *et al.*, 1997); degeneration of tapetal cells and lack of endothecial development (Ahmed *et al.*, 1992); altered carbohydrate accumulation and metabolism (Jain *et al.*, 2007); and oxygen starvation in the developing microspores which could lead to loss of pollen viability. Wahid *et al* (2007) also reported that high temperature stress affects concentration of starch and soluble sugar content in mature pollen. These probably led to a reduction in pollen viability.

In the present study, days to 50% flowering was advanced in plants exposed to drought condition and shown in figure 21. Similar results were obtained by Rejeth (2017) and he reported that early flowering under drought stress can be attributed to drought escape mechanism in rice. Late flowering was observed in Ptb-39 and Ptb-43, these results were in line with findings of Pantuwan *et al.* (2002), he opinioned that delay was associated with drought susceptibility in rice. In CR Dhan 305, CR Dhan 307, Ptb-30 and Ptb-60 flowering period was extended under heat stress and combined drought and heat stress. Rang *et al.* (2011) also got similar results in rice under drought, heat stress and combined stress. Among the genotypes early flowering was showed by N22, Apo, Ptb-7 and Ptb-15 under all the stress conditions. These findings can be supported by study conducted by Sailaja *et al.* (2015), where reduction in number of days to 50% flowering was observed in temperature tolerant genotypes like N22.

Present study recorded reduction in productive tiller number in all the stress treatments (figure 22). Reduction under drought stress was also reported in the study conducted by Park *et al.* (1999) in rice. Similar findings of reduced tillers under heat stress was done by Mitra and Bhatia (2008); Kumar *et al.* (2011); Djanaguiraman *et al.* (2010). 40 % reduction in productive tiller number was reported by Rejeth (2017) under drought and the reduction can be connected with lower water status under drought.

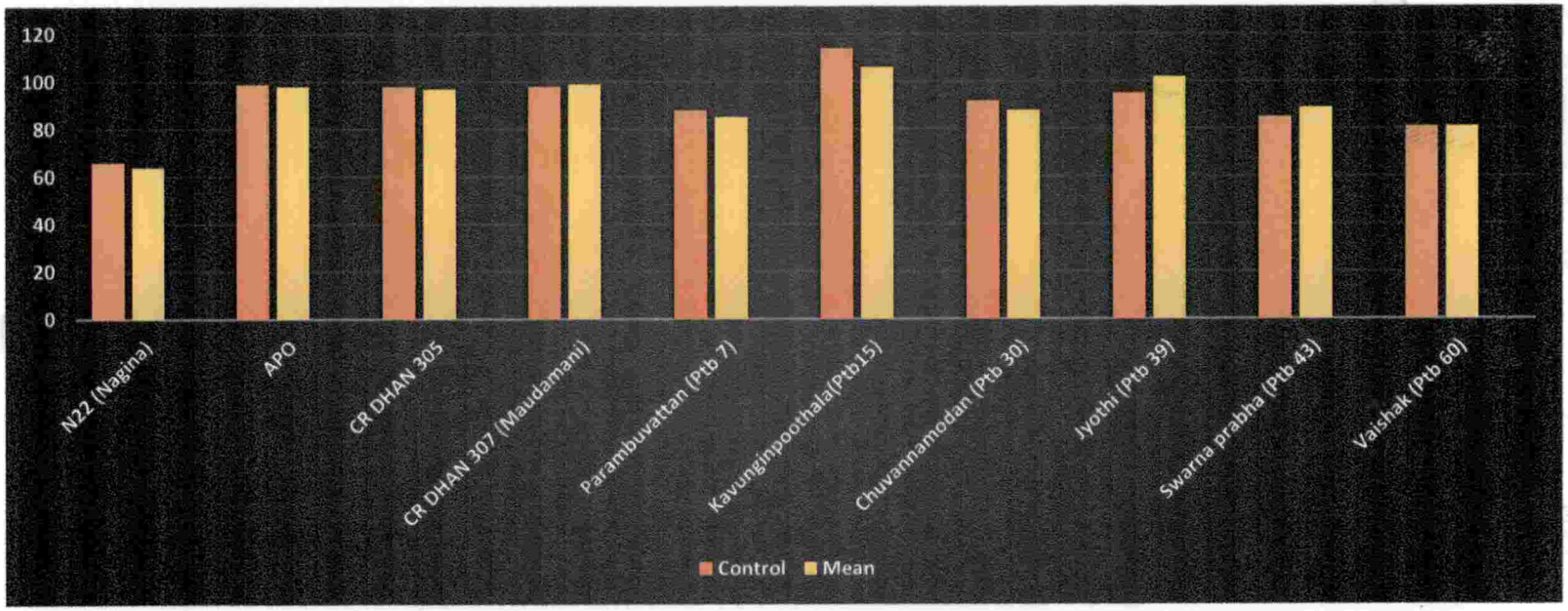


Figure 21. Variation in days to 50 % flowering (days) among the genotypes

T1- TIR treated seeds + Drought + Heat; **T2**- TIR treated seeds + Drought; **T3**- TIR treated seeds + Heat; **T4**- TIR control; **T5**-Germinated seeds under ambient temperature + Drought + Heat; **T6**-Germinated seeds under ambient temperature+ Drought; **T7**-Germinated seeds under ambient temperature + Heat; **T8**- Control (Ambient temperature + Normal irrigation).

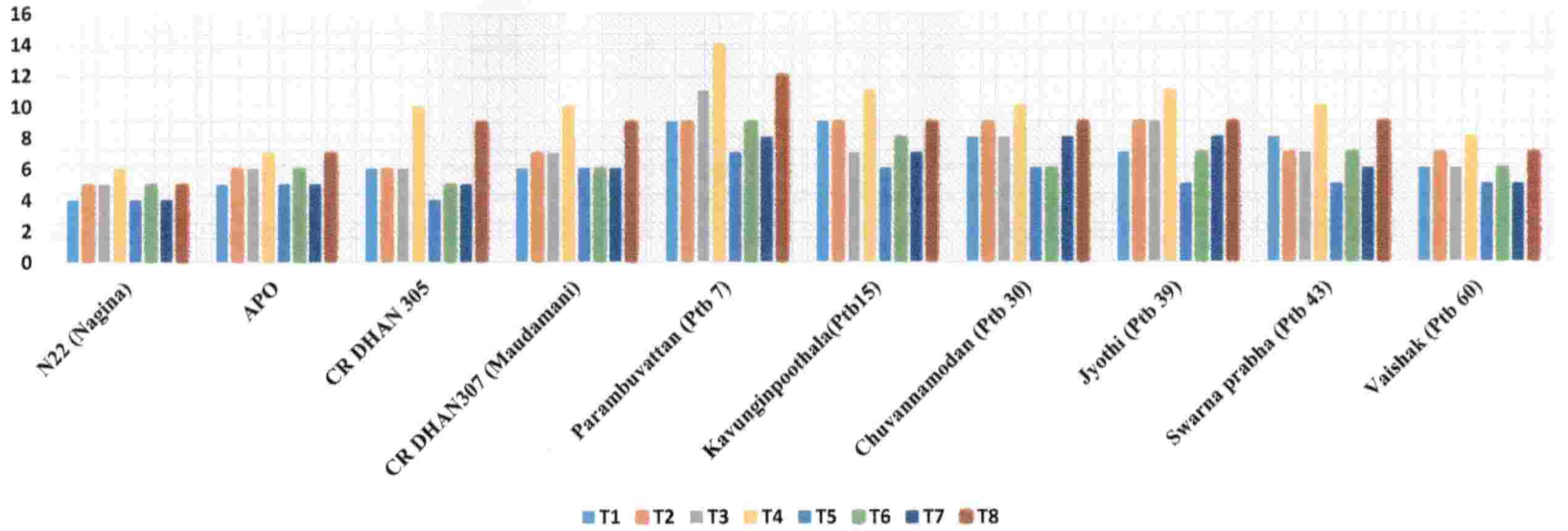


Figure 22. Variation in productive tiller number of different genotypes under stress conditions.

T1- TIR treated seeds + Drought + Heat; **T2**- TIR treated seeds + Drought; **T3**- TIR treated seeds + Heat; **T4**- TIR control; **T5**-Germinated seeds under ambient temperature + Drought + Heat; **T6**-Germinated seeds under ambient temperature+ Drought; **T7**-Germinated seeds under ambient temperature + Heat; **T8**- Control (Ambient temperature + Normal irrigation).

1000 grain weight was reduced among the genotypes under different stress treatments to an extent of 9.4 to 15.7 per cent compared to ambient conditions (figure 23). Drought and heat stress can negatively influence grain size and yield by reducing leaf area production and also green leaf area duration, thus decreasing the available photosynthates to seeds (Zhang *et al.*, 1998). Similar results were obtained by Boominathan (2013), where the reduction was found to be 6-8 per cent under heat stress. Rejeth (2017) reported 3.2 % reduction in 100 grain weight among the genotypes under drought. The present study showed the maximum number of fruits in thermally induced heat stressed plants and same treatment exhibited the maximum yield. Chandola (2015) reported maximum number of fruits and yield in thermally induced heat stressed plants in tomato. Similar results were found in the present study, where induced plants showed an average reduction of 10.6 % and non-induced plants showed 14.2 % under stress.

5.4. BASIS OF DROUGHT AND HEAT STRESS TOLERANCE IN RICE.

TIR induced plants showed better performance in terms of morphological, physiological, biochemical and yield attributes than non-induced plants. Similar results were also reported in tomato by Chandola (2015) and Boominathan (2013) in rice. This was supported with protein profiling and gene expression analysis. Relative expression of *PSTOL1* and *DRO1* were analysed in induced and non-induced plants of one tolerant (N22) and one susceptible genotype (Ptb-39), showing relatively higher expression of these genes in induced plants as compared to non-induced plants. Also *PSTOL 1* was only expressed in samples of tolerant genotype and in induced plants of susceptible genotype.

Uga *et al*. (2011) reported that *Dro1* can be used to improve drought avoidance of rice by changing its rooting pattern from a shallow to a deep system. Gamuyao *et al* (2014) observed that over expression of *PSTOL1* in rice varieties significantly enhances grain yield in phosphorus-deficient soil and *PSTOL1* helps in early root growth, thereby enabling plants to acquire more phosphorus and other nutrients.

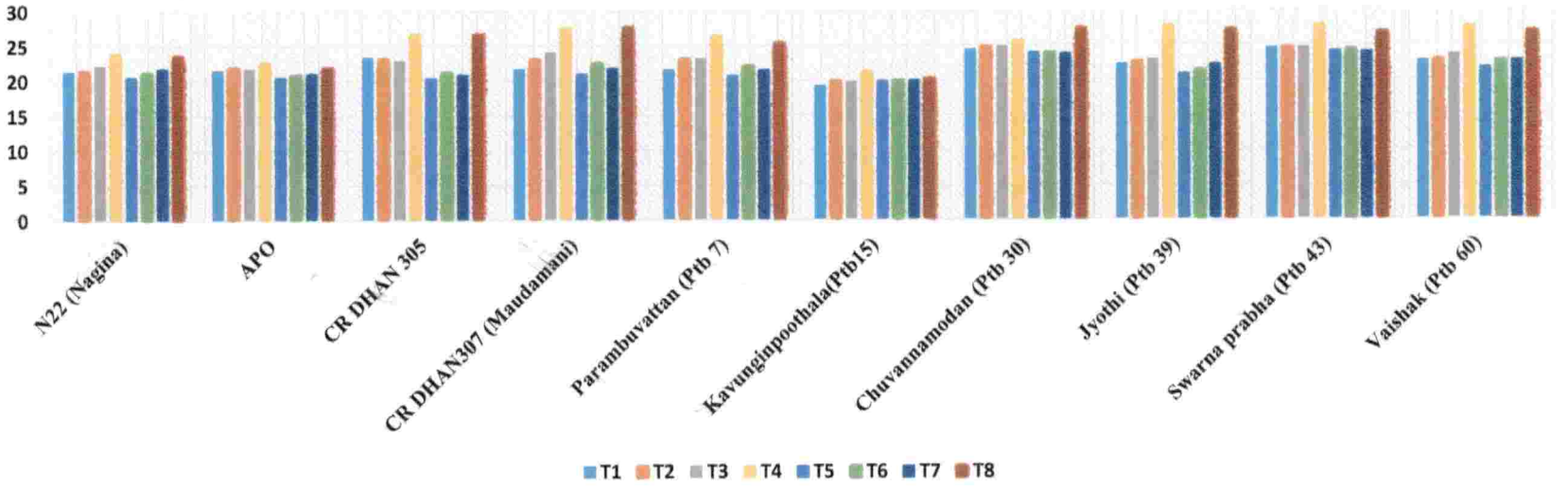


Figure 23. Variation in 1000 grain weight (g) of different genotypes under stress conditions.

T1- TIR treated seeds + Drought + Heat; **T2**- TIR treated seeds + Drought; **T3**- TIR treated seeds + Heat; **T4**- TIR control; **T5**-Germinated seeds under ambient temperature + Drought + Heat; **T6**-Germinated seeds under ambient temperature+ Drought; **T7**-Germinated seeds under ambient temperature + Heat; **T8**- Control (Ambient temperature + Normal irrigation).

In all the samples proteins bands of sizes around 20 and 75 kDa were found and the intensity was more in induced plants of tolerant genotype, followed by induced plants of susceptible genotype. Similar results were reported by Kumar *et al.* (1999), protein synthesis was maintained significantly higher in the acclimated seedlings compared to non-acclimated seedlings on being exposed to severe stress.

In tomato, heat treatment induced chloroplast HSP, HSP21, also named TOM111 and LeHSP23.8 by Lawrence *et al.* (1997) and Sun *et al.* (2002) respectively in leaves, flowers, and fruits. Joshi *et al.* (1997) reported that acquired thermo-tolerance in wheat is attributed to 26 kDa plastid localized heat shocked protein. Several HSPs with varying molecular weight and specific low molecular weight HSPs (17-14.2 kDa) were responsible for the heat shock response after heat shock treatment Del-Aquilla *et al.* (1998)

Similar results were reported by Nakomoto and Hiyama (1999) and Schöffl *et al.* (1999), they observed that increased production of HSPs occur when plants experience gradual increase in temperature. Larkindale and Huang (2005) reported that along with HSPs, several pathways like ABA, ROS and SA are involved in the development and maintenance of acquired thermo-tolerance.

Chandola (2015) studied the effect of TIR in mitigating water and heat stress, and the results of protein profiling showed that during induction treatment in the variety NDTVR-60 proteins bands of sizes around 18 kDa and 20 kDa were found to be intense and in genotype GT, the intensity of protein bands are much higher in leaves after induction in comparison to control.

Srikanthbabu *et al.* (2011) reported that the induced seedlings accumulated higher levels of hsp18.1 and hsp70 transcripts as well as HSP104 and HSP90 proteins. Similar findings were reported by Malik *et al.* (1999), they found that in carrot expression of a low molecular weight HSP17.7 has been adapted to extremely high temperature signifies the relevance of these proteins in imparting tolerance.

SUMMARY

SUMMARY

The present programme was conducted to study the effect of Temperature Induction Response (TIR) technique in combined drought and heat stress. It was conducted in two experiments and the salient findings are given below.

In the first experiment both lethal and induction temperatures were standardized. 52°C for 3 hrs was selected as lethal temperature where 100% mortality was observed. 32-42°C for 5 hrs & 42-52°C for 30 min followed by 52°C for 3 hrs was selected as optimum induction temperature because maximum recovery growth and least percent reduction in recovery growth were observed under this temperature treatment.

In the second experiment ten genotypes were used to study the effect of TIR on combined drought and heat stress tolerance. Morphological, physiological, biochemical and yield parameters were studied in all the genotypes under eight treatments. Morphological parameters such as plant height, leaf area, shoot dry weight and the root traits such as root volume and root dry weight were decreased under different stress conditions *viz*, drought, heat stress and combined drought and heat stress. Root length was increased in stressed plants compared to control plants, especially under drought stress. All the morphological characters were recorded maximum in TIR induced plants without stress in all the genotypes. Canopy temperature was least in N22 and the highest in Ptb-39.

Physiological and biochemical parameters such as cell membrane stability, chlorophyll stability, stomatal conductance, photosynthetic rate, proline content, peroxidase activity and SOD activity were measured. All the parameters were recorded highest in TIR induced plants compared to non-induced plants and control plants. Cell membrane stability, chlorophyll stability, stomatal conductance and photosynthetic rate were decreased under drought, heat stress as well as under combined drought and heat stress. Induced plants maintained higher values under stress conditions. Proline

content was increased under stress condition in all the genotypes with maximum accumulation in N22. Among the treatments accumulation was more recorded in induced plants. Activity of antioxidants like peroxidase and SOD were enhanced under drought and reduction in activity was recorded under heat stress and combined stress. Activity was significantly higher in TIR induced plants in all the genotypes compared to non-induced plants.

Among the yield parameters, time of anthesis of tolerant genotype N22 observed at 9.27 am. Flowers of Ptb-39 opened in late hours (11.44 am) with respect to other genotypes. Spikelet fertility and pollen viability were significantly reduced under stress conditions especially under heat stress and combined stress. Induced plants of all the genotypes showed higher pollen viability and hence spikelet fertility. All the yield parameters were significantly affected by stress conditions in non-induced plants compared to induced plants. As a consequence of improvement in physiological, biochemical and morphological parameters in thermally induced plants, there was improved stress tolerance, which led to better performance under stress.

Relative expression of *PSTOL 1* and *DRO 1* were analysed in induced and non-induced plants of one tolerant (N22) and one susceptible genotype (Ptb-39), showing relatively higher expression of these genes in induced plants as compared to non-induced plants. Also *PSTOL 1* was only expressed in samples from tolerant genotypes and in induced plants of susceptible genotype. The root protein profile by SDS-PAGE in samples of induced and non-induced plants of two selected genotypes (tolerant- N22 and susceptible- Ptb-39) revealed that in all the samples proteins bands of sizes around 20 and 75 kDa were found and the intensity was more in induced plants of tolerant genotype, followed by induced plants of susceptible genotype. Protein synthesis was maintained significantly higher in TIR induced plants compared to non- induced plants on being exposed to severe stress.

It is, therefore, concluded that tolerance to drought, heat stress and combined drought and heat stress could be improved in rice genotypes by TIR technique at seedling stage. This technique not only improved better survival of plants under stress conditions but also improved yield parameters of the plant. It is also concluded that effect of TIR technique at seedling stage persists till maturity of the plant and reflected in terms of improved morphological, physiological and biochemical parameters of the plant under stress.

Future line of work

This investigation was conducted in ten rice genotypes in pots moreover in protected condition, so there is further need to revalidate the effect of thermal induction in stress tolerance when cultivated in the field. Also the effect of TIR technique in controlling biotic stress such as infection of pathogens and pest infestation can be studied. It is also proposed to investigate, if the effect of thermal induction in one generation persists in the subsequent generations.

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VALIDATION OF TEMPERATURE INDUCTION RESPONSE (TIR) TECHNIQUE FOR INDUCING DROUGHT AND HEAT STRESS TOLERANCE IN RICE (*Oryza sativa* L.)

by

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Abstract of the thesis

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ABSTRACT

The experiment entitled "Validation of Temperature Induction Response (TIR) technique for inducing drought and heat stress tolerance in rice (*Oryza sativa* L.)" was undertaken at the Department of Plant Physiology, College of Agriculture, Vellayani during 2016-18. The objective of the study was to standardize TIR protocol for rice and to study the effect of TIR technique for combined drought and heat stress tolerance in rice.

The investigation comprised of two experiments. In the first experiment, two rice varieties namely Jyothi (Ptb-39) and Vaishak (Ptb-60) were used for the standardization of lethal and induction temperature and in the second experiment ten rice genotypes (N22, Apo, CR Dhan 305, CR Dhan 307, Ptb-7, Ptb-15, Ptb-30, Ptb-39, Ptb-43 and Ptb-60) were used to study the effect of TIR technique on combined drought and heat stress tolerance. In the first experiment 13 different treatments (**T1-3**: 49°C for 2, 2½ and 3 h, **T4-6**: 50°C for 2, 2½ and 3 h, **T7-9**: 51°C for 2, 2½ and 3 h, **T10-12**: 52°C for 2, 2½ and 3 h, **T13**: control) were used to identify lethal temperature and induction temperature was standardized from four treatments (**T1**- 28-40 °C for 5 h & 40-52 °C for 30 min, **T2**- 32-40 °C for 5 hr & 40-52 °C for 30 min, **T3**- 32-42 °C for 5 h & 42-52 °C for 30 min, **T4**- Control) with three replications.

In the first experiment 100% mortality was observed for the treatment 52°C for 3 h and was selected as lethal temperature. Maximum recovery growth and least percent reduction in recovery growth were observed under **T3** (32-42 °C for 5 h & 42-52 °C for 30 min) and this treatment was selected as induction temperature.

The second experiment was laid out with 8 treatments [**T1**- TIR treated seeds + Drought + Heat, **T2**- TIR treated seeds + Drought, **T3**- TIR treated seeds + Heat, **T4**- TIR control,**T5**-Germinated seeds under ambient temperature + Drought + Heat, **T6**-Germinated seeds under ambient temperature + Drought, **T7**-Germinated seeds

under ambient temperature + Heat, **T8**- Control (Ambient temperature + Normal irrigation)] and three replications.

Among the genotypes, Ptb-15 recorded the highest plant height, shoot dry weight, root length and root volume. Leaf area was maximum for Ptb-43 and Apo recorded maximum root dry weight. Minimum canopy temperature was shown by N22 and Ptb-7. The genotype N22 showed the highest mean values for cell membrane stability index, chlorophyll stability index, stomatal conductance, photosynthetic rate, proline content, peroxidase activity, spikelet fertility and pollen viability. Early flowering and minimum days to 50% flowering also were recorded in N22. Productive tiller number was highest in Ptb-7 and 1000 grain weight was maximum for Ptb-43. The highest SOD activity was observed in Ptb-15. Ptb-39 (Jyothi) showed the minimum values for most of the stress related traits and yield components under various stress conditions and hence this genotype is selected as the most susceptible genotype towards both drought and heat stress conditions

Among the treatments, T4 (TIR Induced plants without stress) recorded maximum value and T5 (non-induced plants under combined drought and heat stress) recorded minimum value for all the above mentioned morphological characters except root length. Maximum root length was recorded in T2 (induced plants under drought) and minimum in T8 (control). Peroxidase and SOD activity were highest in T2 and minimum for T5. Maximum value for yield related traits were observed for T4. Plants under combined drought and heat stress without induction showed minimum value for all the physiological and yield parameters.

The results of the present study showed that TIR technique influenced all the genotypes at various stress levels. TIR induced plants exhibited better performance on biochemical and physiological traits than non-induced plants in all the genotypes that give rise to better stress tolerance. N22 and Apo were selected as the best genotypes for stress related traits. Apart from these two, Ptb-15, Ptb-7 performed

better under heat stress and drought respectively. For combined stress, Ptb-30 and Ptb-15 were showed better performance compared to other genotypes. Ptb39 was identified as the most susceptible genotype for both drought and heat stress.

TIR induced and non-induced plants of Ptb-39 (most susceptible) and N22 (tolerant) were selected to study the changes in protein profiling and gene expression level using SDS PAGE and RT PCR. The results revealed that protein profiling showed variation between tolerant and susceptible genotypes under induced and non-induced condition for the expression of 20 kDa and 75 kDa protein. Expression level of *PSTOL1* and *DRO1* also showed variation between induced and non-induced plants of tolerant and susceptible genotypes. Protein synthesis was maintained significantly higher in the induced plants compared to non-induced plants on being exposed to severe stress. Tolerance of genotypes towards stress has been attributed to changing transcript levels of stress induced genes.

In the present study, T3 (32-42°C for 5 hrs & 42-52°C for 30 minutes) and T12 (52°C for 3 hrs) were selected as the induction and lethal temperatures respectively. The study also revealed that TIR technique can be used as a potential tool for improving the performance of high yielding susceptible genotypes under stress conditions.

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

Buffers for biochemical analysis

1. Sodium Phosphate Buffer (0.1M)- pH. 6.5.

A: 0.1 M solution of NaH_2PO_4 - 1.39 g in 100 ml.

B: 0.1 M solution of Na_2HPO_4 - 2.70 g in 100 ml.

68.5 ml of solution A was mixed with 31.5 ml of solution B and the volume made upto 200 ml.

2. 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.

3. Protein denaturing buffer (pH. 8.0)- 100 ml.

10 M urea	- 48 g urea dissolved and made upto 80 ml.
1 M NaH_2PO_4. 2 H_2O	- 0.78 g dissolved and made upto 5 ml.
1 M tris-base	- 0.12 g dissolved and made upto 1 ml.
5 M NaCl	- 0.585 g dissolved and made upto 2 ml.
Distilled water	-12 ml.

APPENDIX-III

Stock solutions for SDS PAGE

a) Electrode buffer pH – 8.3

Tris base -9 g

Glycine -21.6 g

SDS -1.5 g

Make upto 1.5 L using double distilled water.

b) 0.5 M Tris- HCl pH- 6.8

Tris HCl - 7.8 g

Tris base - 0.45g

Make upto 100 ml using double distilled water.

c) 10 % SDS

SDS - 1 g

Double distilled water - 10 ml

d) 1.5 M Tris- HCl pH- 8.8

Tris HCl - 3.69 g

Tris base - 13.39g

Make upto 100 ml using double distilled water.

e) Acrylamide stock (30 %)

Acrylamide - 29.2 g

N'N'- methylene bisacrylamide - 0.8 g

Make upto 100 ml using double distilled water and kept in dark colour bottle at 4°C.

f) Sample buffer

0.5 M Tris HCl	- 0.12 ml
10 % SDS	- 0.2 ml
Glycerol	- 0.1 ml
0.5 % bromophenol blue	- 0.5 ml
Double distilled water	- 0.48 ml
2- mercapto ethanol	- 0.05 ml

Add mercapto ethanol just before use.

g) Polymerising agents

Ammonium per sulphate (APS) 10 % prepared freshly before use.

TEMED- Fresh from refrigeration.

h) Staining solution

Coomassive brilliant blue R 250	- 0.29 g
Methanol	- 56.75 ml
Glacial Acetic Acid	- 11.5 ml

Make upto125 ml using Double distilled water.

i) Destaining solution

Methanol	- 30 ml
Glacial Acetic Acid	- 10 ml
Double distilled water	- 60 ml

APPENDIX-IV

Gel solutions for SDS PAGE

a) Separating gel (10 %)

30 % acrylamide stock	- 3.3 ml
1.5 M Tris HCl	- 2.5 ml
10 % SDS	- 100 µl
Distilled water	- 3.99 ml
10% APS solution	- 100 µl
TEMED	- 10 µl

b) Stacking gel (5 %)

30 % acrylamide stock	- 510 µl
0.5 M Tris HCl	- 750 µl
10 % SDS	- 30 µl
Distilled water	- 1.66 ml
10% APS solution	- 40 µl
TEMED	- 7 µl