SCREENING AND CHARACTERISATION OF RICE GENOTYPES FOR ABIOTIC STRESS TO DEVELOP CLIMATE RESILIENT VARIETY

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled **"Screening and characterisation of rice genotypes for abiotic stress to develop climate resilient variety"** 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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Nayana E. M.

DEDICATED TO MY PARENTS, TEACHERS AND FRIENDS

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CERTIFICATE

Certified that this thesis entitled "**Screening and characterisation of rice genotypes for abiotic stress to develop climate resilient variety**" is a record of research work done independently by Ms. Nayana E. M. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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LIST OF ABBREVIATIONS

%	Percentage
bp	Base pair
℃	Degree celcius
CTAB	Cetyl trimethyl ammonium bromide
et al.	et alia
HSP	Heat shock proteins
IRRI	International Rice Research Institute
MAS	Marker assisted selection
Mha	Million hectares
mM	milli molar
Mt	Metric ton
O. *sativa*	*Oryza sativa*
PCR	Polymerase chain reaction
QTL	Quantitative trait loci
RARS	Regional Agricultural Research Station
SSR	Single sequence repeat
TAE	Tris acetate EDTA
UV	Ultraviolet
V	Volt
µl	Microlitre

1. INTRODUCTION

In almost all countries in the world, rice is a staple meal. In more than 100 countries, it is grown commercially. Around 90 per cent of total production is accounted for by Asian countries. Rice is by far the most important food crop, especially in low- to middle-income countries. China is, at the same time, the largest user and manufacturer of rice in the world. In recent years, it has also become one of the world's fastest growing economies, with agriculture being one of the most important pillars of the economy. In fact, the consumption of rice is 142,700 metric tonnes per year, equivalent to almost 30 per cent of global rice production. India falls second in overall rice consumption at 97,350 metric tons. Other grains, which include wheat, sorghum and maize, are produced by India. Almost 50 per cent of Indians are relying on rice as a staple food, and through procurement and the implementation of distribution programs, the government plays an inevitable role in production of rice for consumers. In the developing world, rice is essentially the most important food crop of key importance, especially in Asia, with other countries, including Bangladesh, Vietnam, and the Philippines, where the availability of rice is equated with food security and is even related to political stability. This essentially suggests that production should continue to increase to compensate for population growth both now and in the future, and rice production needs to increase by 0.6-0.9 percent annually until 2050 to meet demand (Carriger and Vallee, 2007). As almost all of China's rice area is irrigated, China's rice production is higher because of higher productivity, while less than half of India's rice area is irrigated. In Kerala, the region of rice cultivation in 2015-16 decreased from 196900 hectars to 171400 hectars in 2016-17. But it is nice to note that in 2017-18 it has risen to 189,100 hectors.

The word stress is also defined physiologically, as a reaction to various circumstances. Stress is usually a shift in physiological conditions induced by factors that appear to affect the plant's stability (Singh *et al*., 2016). By the end of the 21st Century, due to both anthropogenic and natural causes, the earth's climate is expected to warm by an average of 2-4 ° C (IPCC 2007). Studies have shown that at the International Rice Research Institute, Manila, Philippines, the annual mean maximum and minimum temperatures have risen by 0.35 and 1.13

° C respectively, for the period 1979-2003 (Lafitte *et al*., 2004). This increase in temperature has exposed most of the world's crops to heat stress during some stages of their life cycle. The difficulty in precise prediction of the projected agricultural impacts of climate change further adds to the uncertainty (Zhang *et al.,* 2009). Most rice is currently grown in regions where the current temperatures for rice production are already close to optimal. Any further increases in mean temperatures or brief episodes of high temperatures during sensitive phases may therefore be super-optimal and may reduce the yield of grain.

By the end of the 21st century, rice yields were expected to decrease by 41 per cent (Ahuja *et al*. 2010). There is ample evidence that growing night-time temperatures have been the main cause of global mean temperature rises since the middle of the 20th century, and are thus the main contributor to the decrease in yield (Lafitte *et al*., 2004). In comparison, temperature rises in some areas will boost the establishment of rice crops, for example in Mediterranean areas, where cool weather typically causes poor establishment of crops (Zeng *et al.* 2003). But the negative effects associated with the rise in temperature greatly outweigh the positive ones. Thus, although some temperate zone countries may benefit from climate change, many tropical and subtropical countries tend to be more vulnerable to the possible impacts of global warming. It has been documented that rice is responsive to concentrations of salt. Many metabolic alterations are caused by saline soil and crop yield is reduced. It also results in a decreased photosynthesis rate caused by ionic imbalance. A major decrease in development was also noted earlier. In salinity susceptible areas, mineral shortages and toxicities are commonly seen. For salinity caused by low groundwater quality or inadequate irrigation, there may be related problems such as alkalinity, zinc and phosphorus deficiency, or boron toxicity. In the case of coastal salinity, toxicity and acidity are seen in aluminium and iron. It also affects the length of panicles, the number of spikelets and the yield of grain. Rice has the semi-aquatic evolutionary specificity of being. As a result, it has relatively few adaptations to water-limited circumstances and is highly sensitive to stress from drought. Drought is one of the major abiotic stresses that severely affect up to 70 percent of food crop yield and productivity worldwide. Plants' reaction to drought stress is complicated and includes changes in their

morphology, physiology and metabolism. It was estimated that the optimum amount of water required for irrigation to produce 1 kg of rice was about 3000 litres. Depending on the plant's growth stage, yield loss can rise up to about 100 percent as a result of drought stress. In order to help poor rice farmers in developing countries and for food sustainability to cater to the growing human population, yield losses must be minimised. Reducing plant growth is the most typical symptom of drought stress. The global decrease in the production of rice due to drought averages 18Mt annually. Therefore, this abiotic stress is a major restriction on the production of rice in water-limited environments. A total of 23 Mha of rice fields (10 Mha in the uplands and 13 Mha in the lowlands) are estimated to be drought-prone in Asia alone. With more than 10 Mha of drought-prone uplands and lowland fields, where yield losses due to drought are reported to cost an average of US $250 million annually, drought is a particularly important production constraint in eastern India. Drought disproportionately influences the poorest farmers. It is not simply the lack of water that reduces yield potential, but also the timing and duration of phenological process-related drought stress. Using three different strategies, rice, like other crops, can potentially resist drought stress: drought escape, drought avoidance, or drought tolerance. In situations where the timing of drought is mostly unpredictable, rice varieties that have drought avoidance and tolerance mechanisms are required.

Techniques in molecular biology and the fingerprinting of rice genome helps plant scientists for locating the DNA that determines the physiological feature that dictates the tolerance of salt in rice. Several researchers have carried out QTL analysis of physiological characteristics related to abiotic stresses (Lin *et al*. 2004 and Lee *et al*. 2007). In a single genotype the issue of climate change forces breeders to combine multiple abiotic characteristics (salinity and submergence, submergence and drought, salinity and drought, and salinity-submergence-drought), because these characteristics coexist in one cropping season. Rice scientists can explore radical approaches to designing the next generation of rice varieties that are climate-change-resilient and acceptable to farmers with the advent of recent biotechnological instruments, knowledge of genetic mechanisms and new sources of tolerance (Gregorio *et al*., 2013).

Under these conditions, the objective of this study is to screen and characterize 12 rice varieties for multiple abiotic stress to develop climate resilient variety.

Some of the genotypes selected in this study are released varieties from RARS Pattambi and RRS Vyttila and other genotypes are in breeding pipeline.

2. REVIEW OF LITERATURE

2.1 Rice

Rice is an annual grass having 2n=24 chromosomes. The genus Oryza includes 21 wild species and 2 cultivated species, *Oryza glaberrima* and *O. sativa.* Whereas *O. glaberrima* is cultivated in restricted areas of western Africa, *O. sativa* is cultivated globally. The cultivation of rice showed migration with the migrating population resulted in the spreading of Asian cultivated varieties across the globe. Currently Antartica is the only continent without rice and rice is the crop which feeds more than half of the total population. An increase of 0.6%–0.9% in annual rice production in each year has been calculated until 2050 to feed the exploding population (Carriger and Vallee, 2007). Thus climate resilient varieties with higher productivity remains a must to tackle the upcoming food scarcity.

2.2 Abiotic stress

Most of the plants are autotrophic in nature. They produce their own food from raw materials like water, carbon and mineral nutrients in presence of sunlight as the energy source.

Plants prone to a diverse range of environmental extremities which results in reduction and limiting of productivity in crops. Environmental stresses can be classified into two such as (1) Abiotic stress and (2) Biotic stress. Various non-living factors possess importance in the yield of crops (Gull *et al.,* 2019).

The stresses caused by abiotic factors are due to the change in the optimum level of them. Plants respond to abiotic stress through different mechanisms. Plants are non-motile. So when an abiotic stress is present they have to face it and develop mechanisms accordingly to survive. They either implement avoidance mechanisms or tolerance.

To adapt to the troublesome development conditions, plants react with a progression of morphological, biochemical, and molecular adjustments, targeting shielding the essential metabolic exercises. It is presently notable that the stress signal is first seen at the membrane level by the receptors and

afterward transduced into the cell to turn on the pressure responsive qualities for interceding stress resilience (Basu and Roychoudhari, 2014).

For example, abiotic stresses, such as low or high temperatures, insufficient or excessive water, high salinity, heavy metals, and UV radiation, are threatening the development and advancement of plants, prompting extraordinary penalties for harvest yields around the world. In order to relieve the weight of natural changes and to satisfy the need for population growth, it is becoming fundamental to provide crops with multi-stress resilience, as different abiotic stresses emerge together in the field. Achievability is increased as land plants, together with unsaturated fats, reactive species scavengers, molecular chaperons, and solutes inside cells, have actually established increasingly summarised guards against abiotic stresses, including plant cuticles. They are organised by a complex administrative system for stress reaction, including upstream signalling particles, including stress hormones, reactive oxygen species, gas transmitters, polyamines, phytochromes, and calcium, as well as downstream factors for gene regulation, particularly translation factors (He *et al*., 2018)

The plants may be healed from injuries whether the stress is moderate or short-term because the impact is transient whereas extreme stresses trigger crop plants to die by stopping flowering, seed development and inducing senescence. (Gull *et al*., 2019).

Abiotic stresses like salinity, chilling, water deficiency, and heavy metals antagonistically influence the development and a few physiological procedures of plants. So decrease in temperature primarily brings about mechanical constraints, while salinity and water deficiency apply their malignant impact by disturbing the ionic and osmotic balance of the cell. The adverse impacts of abundance salts are the outcomes of water deficiency that outcomes from diminished osmotic/water potential of soil solution because of high solute concentration in it, and ionic stress due to changed Na^+/K^+ proportions and Na^+/Cl^- proportions that are antagonistic to the plants (Basu and Roychoudhari, 2014)

In the field, plants are rigorously prone to an unpredictable blend of different kinds of stresses unlike a single one, which is even bad in the context of climate alterations, soil salinization and pollution in environment. Specifically, on

current scenario of population growth, it is getting vital to develop plants with multistress tolerance (He *et al*., 2018)

2.3 Temperature stress

Plants routinely face high temperature all over their multi-seasonal life cycle. It suggested that the driving forces for crop development are temperature and photoperiod (Kropff *et al*., 1995). 27 to 32 °C is the optimum temperature for rice development (Yin et al., 1996). Scientists have already predicted a rise of 2-4℃ by the end of 21st century. The rise in day temperature is more severely affecting the crop production than in night temperature. In a way, it is said that a transient temperature rise, probably 10-15 ° C above optimum, is heat shock or heat stress. Temperature stress, however, is a complex function of intensity (temperature in degrees), length, and temperature rate of increase. An average value of daily temperature from which a notable reduction in growth begins is known as the threshold temperature. Through controlled research facilities and field tests, higher and lower developmental limit temperatures for some plant species have been resolved. One below which plant development and enhancement stop is a lower developmental limit or a base temperature. Essentially, the temperature above which development and improvement stop is an upper developmental threshold. In physiological research, as in crop development, information on lower threshold temperatures is important (Wahid *et al*., 2007).

2.3.1 Effect of temperature stress

The stages of development most susceptible to temperature in rice is supposed to be flowering (anthesis and fertilization) and to a lesser extent the preceding stage booting (microsporogenesis) (Farrell et al. 2006). Plants became completely sterile on exposure to 41 °C for 4 h at flowering (IRRI 1976), whereas it had no effect on spikelet fertility (Yoshida *et al.,* 1981). Some of the phenotypic markers for high temperature tolerance include flowering at cooler times of day, more viable pollen, bigger anthers, longer basal dehiscence and presence of large basal pores. Protection of structural proteins, enzymes and membranes and expression of heat shock proteins (HSPs) are some of the

biochemical processes that can promote thermo-tolerance (Shah *et al*., 2011). During flowering and grain filling periods, elevated temperatures lead to heat stress, resulting in enormous decreases in spikelet fertility and decay in rice quality (Shi *et al*., 2016). Summer heat has been elevated as a result of climate change and it is the main limiting factor in crop production (Espe *et al*, 2017). Amplification of spikelet deterioration, which is more in the *Japonica* variety than in *Indica*, has been shown at a daily temperature of up to 38 ° C. Wu *et al.* (2017) stated that high temperature restricted spikelet formation is related with the production and decomposition of cytokines. Peroxide accumulation in the spikelets is also caused by high temperature, which can lead to cellular destruction (Fu *et al*, 2016) and reduction in number of spikelet. Anther filling at the panicle initiation stage is also inhibited by high temperature, which may decrease pollen activity (Wang Y. L. *et al*., 2016).

The reactive oxygen species (ROS) age of overabundance, which induces oxidative stress, is one of the important effects of high temperature stress. Plants are continuously battling for stamina under varying conditions of environmental tension, including elevated temperatures. A plant is able, by physical changes within the plant body and much of the time through making signals for evolving metabolism, to somewhat withstand heat stress. In the light of high temperatures, plants modify their metabolism in various ways, in particular by producing compatible solutes that can organise proteins and cell structures, preserve cell turgor by osmotic alteration, and adjust the antioxidant mechanism to restore cell redox parity and homeostasis (Hazzanusman *et al.*, 2013).

Elevated temperatures results in significant pre-and post-reap harms, including dying of leaves and twigs, burns from the sun on leaves, branches and stems, leaf senescence and abscission, shoot and root development restraint and decreased yield. Significant effect of high temperatures on shoot development is an extreme decrease in the main internode length bringing about unexpected passing of plants. There is a general tendency of decreased cell size at the whole plant level, stomata conclusion and abridged water loss, increased stomatal and trichomatous densities, and wider root and shoot xylem vessels (Shah *et al*., 2011).

2.3.2 Cop up mechanisms of plants

The three separate methods of heat resistance in rice are heat defence, heat avoidance and heat tolerance. Heat defence is the morphological growth conditioning and leaf transpiration mechanism to reduce the temperature of the panicle and limit damage from elevated temperatures. Heat avoidance is a change to regulate spikelet flowering time by short flowering period and early flowering, which is considered a favourable function of heat resistance in rice (Bheemanahalli *et al*, 2017). Heat tolerance is defined as the preservation of normal life activity under high temperatures. For eg.in African wild rice, which preserves the balance of cell metabolism and allows plants to thrive under extreme high temperatures, Li *et al* (2015) isolated and cloned thermo-tolerance, a significant quantitative trait locus.

2.3.2.1 Morphological mechanisms

Regular heat induced conditions in plants are closure of stomata and decreased water loss, increased stomatal and trichomatous densities and larger xylem vessels (Srivastava *et al*., 2012). Plants that grow in a hot atmosphere stay away from heat stress by reducing sunlight assimilation. The presence of small hairs (tomentoses) that structure a thick coat on the outside of the leaf as a cuticle, a protective waxy covering, supports this ability. Leaf blades often get some distance from light in such plants and arrange themselves in line with sun beams (paraheliotropism). Increased transpiration keeps leaves from heat stress in all hydrated plants, and the temperature of the leaves could be 6 °C or even 10-15 °C lower than the temperature. Many organisms have produced accounts of life that allow them to maintain a strategic distance from the most sweltering time of the year. By fulfilling the entire regenerative cycle in the course of the cooler months, this can be done by leaf abscission, leaving heat tolerant buds, or in desert annuals (Fitter and Hay, 2002).

High temperature stress can also be kept away from the board's crop activities, such as selecting suitable planting methods, planting date decision, cultivars, water system techniques, and so on. Supplanting heat-sensitive cultivars with heat-tolerant ones, altering planting time, selection of genotypes with a growth span that allows peak stress periods to be avoided, and prominent use of plant

hormones are some of the adaptive characteristics that will help to moderate the expected reduction in yield due to climate change (Shah *et al.*, 2011).

The capacity to grasp the high temperature signal, produce and pass on the signal, and start fitting physiological and biochemical changes relies on plant endurance under heat stress. Resistance is also considerably enhanced by high temperature-incited gene expression and metabolite synthesis. Dynamic research regions are the physiological and biochemical responses to temperature stress, and molecular approaches are being implemented in plants to improve high temperature tolerance (Hasanuzzaman *et al.,* 2013).

Different genes and/or major genes quantitatively restrict temperature in rice, and a few quantitative attribute loci (QTLs) that regulate heat resilience have been distinguished in rice (Guan-fu *et al*., 2015).

Mukamuhirwa *et al.* (2019) assessed the impact on growth, grain yield, and quality characteristics of seven Rwandan rice cultivars raised in climate chambers of simultaneous drought and temperature stress. During different plant developmental stages, two temperature ranges were applied, 23/26 °C night / day and 27/30 °C night / day, along with single or recurring drought treatments. Plant production and yield were heavily influenced by drought, while the quality attributes were affected by genotype. For all measured cultivars, the combination of a high temperature with drought at the seedling and tillering stages resulted in zero panicles.

2.4 Salinity stress

Salinity is a major reason for yield loss in rice being second to drought (Flowers and Yeo, 1995). In particular, at its young stage, rice was categorized as the salt-susceptible cereal (Lutts *et al*., 1995) and salinity limits the production efficiency at the adult stage (Todaka *et al,* 2012). Concentration of NaCl above 200mM can't be tolerated by most of the plants (Zhou *et al*., 2016). Seed germination, establishment of seedling, vegetative growth, and flower fertility are severely affected (Guo *et al*., 2018) as a bad outcome of ionic toxicity, osmotic pressure, oxidative damage, and nutritional shortage (Feng *et al*., 2014). It is important to note that, it is associated with drought, another global problem that can be intensified by severe temperatures (Slama *et al.*, 2015). The rice

growth process is important for salinity sensitivity (Zeng and Shannon, 2000). Germination is the stage where rice is most tolerant to salinity (Khan and Panda, 2008). It becomes more susceptible at seedling stage, gain tolerance during vegetative growth stage and then become more susceptible during maturation. Panicle length, spikelet number per panicle, and grain yield are the major parameters get affected with salinity (Zeng and Shannon, 2000), and emergence of panicle and onset of flowering get delayed (Khatun and Flowers, 1995). Pollen viability is decreased thus the yield is reduced (Khatun and Flowers, 1995). Obviously, under salt stress, shoot development is generally more influenced than root development. Studies showed that rice is more safe at regenerative and grain filling than at germination and vegetative stages. Two significant burdens are forced on plant tissues during salinity stress: osmotic stress because of the soil with high concentration of solute, and ion based stresses because of the modified concentration of potassium, sodium and chlorine particles. At the point when rice get presented to salt stress, as a fast recuperation procedure, transpiration and leaf expansion are hindered or diminished.

Soil salinity stress can also trigger nutrient abnormalities in rice varieties. The plant has close interaction with the growth medium and the rest of the plant. Among nutrients and plants under salt stress, it is called a scaffold. Na+ and Cl- accumulation in plant tissues and soil is the most serious adverse aspect of salinity stress (Platten *et al*., 2013). High Na+ fixation, which is a fundamental plant supplement for growth and plant growth, has an adverse effect on potassium (K+) ions (Jung *et al*. 2009). In addition, the plant's nitrogen take-up decrease was also seen under elevated salt conditions (Abdelgadir *et al*. 2005). Further studies have shown that salinity stress has an adverse effect on P, K+, Zn , Fe, Ca2 +, and Mn, but has a synergistic influence on rice crop nitrogen (N) and Mg (Jung *et al*. 2009).

2.4.1 Effect of salt stress

Under expanded salt stress, a crucial fall in average root length, average root numbers per plant, and shoot length occurred (Jamil *et al*., 2012; Jiang 2010). As a result, root and shoot lengths are two indicators of the reaction of rice plants to saline stress. Salt stress is really influenced by the division of rice cells and

cell elongation, which leads to a reduction in root, leaf growth, and yield (Munns 2002).

Salt stress induced panicle sterility in some rice varieties, especially at pollination and fertilisation times due to genetic characteristics and nutrient inadequacies due to high salinity impact (Hasamuzzaman *et al.* 2009). Different studies have shown that salinity stress can cause panicle sterility during fertilisation, leading to a decrease in grain environment, pollen bearing limit, and stigmatic surface reduction, or both (Khan and Abdullah, 2003). The fundamental thing that leads to decreased grain yield under salt stress is the absence of carbohydrate shift to vegetative growth and advancement of spikelets. For example, yield components, spikelets per panicle, length of panicle, number of tillers per plant , number of florets per panicle, and 1 000-grain weight seriously affect overall as a result of the the expanding scenario of salt stress (Hussain *et al*., 2017). One of the essential determinants of salt resilience in rice is plant vigour (Platten *et al*., 2013). Growth strength is such a factor that can maintain a strategic distance from salt stress's harmful impacts (Kumar *et al.,* 2013). In order to identify salt tolerance in countless rice genotypes, Yeo *et al*. (1990) assessed a wide range of physiological parameters, including shoot Na+ fixation, leaf-to-leaf compartmentation and plant vigour, and pointed out that the grouping of moved Na+ would be lower in rapidly developing genotypes than in gradually growing ones. Vigour is an aspect of avoidance rather than a method of resistance that most certainly fills in as efficiency matters most (Reddy *et al*., 2017).

When rice plants are faced with elevated salinity (NaCl > 50 mM), they experience a rapid and transitory decrease in stomatal conductance and rate of growth (Moradi and Ismail, 2007). After stress induced by NaCl (Rodriguez et al., 1997), or KCl or mannitol (Frensch and Hsiao, 1994), a rapid decrease in development is often seen at the root stage, affirming the prevalent function of osmotic disequilibrium. Moradi and Ismail (2007) suggested in this rapid reaction, extensive genotypical comparisons, with a marginally slower decrease in stomatal conductance rate in salt-delicate genotypes relative to salt-tolerant genotypes.

2.4.2 Effect in physiological parameters

A few physiological parameters, such as photosynthesis and plant growth, are affected over a period of weeks, as indicated by the intensity of salt stress. This is attributed to changes in the osmotic and ionic state of cells, elevated concentrations of organic osmolytes and hormones, such as ABA, decreased membrane permeability, decreased partial pressure of intercellular CO_2, decreased stomatal conductance, decreased photosynthetic apparatus efficiency and feedback inhibition due to decreased sink action (Munns and Tester, 2008).

2.4.3 Effect in morphology

The mortality rate of rice leaves increased in all rice varieties at the early stage of seedling growth with elevated salt stress (Shereen *et al.*, 2005). Due to salinity stress exposure after 1 wk, the mortality rate in leaves is around 0 to 300 percent. Salt stress indicates a decline in growth and improvement later in a few months (Munns 2002). It could cause leaves to move and decrease in the leaf region and eventually decrease the rate of plant photosynthesis (Amirjani 2011). Salt stress has an effect on the metabolism of plant cells, especially on leaf senescence. It can also disrupt the cells in transpiring leaves and cause hindrance to the growth of rice plants. The collection of salt in the old leaves causes the leaves to die, which is essential for a plant's endurance (Munns *et al*., 2006). If the rate of leaf death exceeds the rate of new leaf initiation and surface expansion, plant senescence can occur, as these provide the photosynthates necessary for further growth and improvement (Munns and Tester, 2008).

A low degree of stress induced as well as constitutive ABA in leaves is observed in the salt tolerant varieties (Zhang *et al.*, 2006).

2.4.4 Tolerance against salt stress

For the most part, rice plants suffer from salt by two primary strategies, ion exclusion and osmotic resilience (Munns and Tester, 2008). In addition, these mechanisms can be categorised into ion exclusion, osmotic tolerance and resistance to tissue (Roy *et al*., 2014). The exclusion of ions primarily involves the transport of Na+ and Cl-in roots, which prevents excessive accumulation of Na+ and Cl-in leaves. It requires Na+ recovery from the xylem, and ion efflux

back to the soil. Osmotic resilience is regulated by significant distance flags that decrease the output of shoots and are enabled before amassing Na+. Osmotic resilience involves the ability of the plant to withstand the drought aspect of salinity stress and to sustain leaf growth and stomatal conductance (Rajendran *et al.,* 2009). Tissue resistance involves vacuole sequestration of Na+, manufacture of compatible solutes and synthesis of catalysts that detoxify reactive oxygen species.

2.4.4.1 Crop improvement

Genetic modification of rice against natural burdens is important in order to increase the potential production of rice crops. We may change salt tolerant characteristics in our ideal rice cultivar using this technique. We can also use advanced procedures, similar to gene sequencing, QTL mapping, and marker-assisted breeding, to upgrade rice resilience to stress (Sing and Sengar 2014).
In field conditions, QTL mapping will pick a better salt tolerant genotype that is not affected by salt. Through this approach, several salt tolerance genes can be found (Flowers, 2004). QTL mapping allows researchers to sift through adaptive, tolerance genes and transformation of salt or other abiotic stresses. For example, AFLP, RFLP, and the most recent marker range of high density DNA microsatellite markers can help analysts sort out characteristics of interest in crop salt resilience (Sing and Sengar 2014).

2.5 Drought stress

Drought is a meteorological term and is commonly defined as a lack of accessibility of water, including periods without enormous precipitation that affects the growth of plants and keeping capacity of soil water, and occurs when the accessible water in the soil is reduced and conditions cause continuous loss of water through transpiration or evaporation. In virtually all plants, drought stress tolerance is seen, although its degree fluctuates from species to species, even within the species. The production of more than 23 million hectares of rice is affected by water deficit tension. (Kumbhar *et al*., 2015).

2.5.1 Effects of drought stress

Rice is susceptible to drought stress and different morphological and molecular mechanisms have been evolved to thrive with it (Henry *et al*., 2016). Reduction in plant height, leaf rolling, leaf senescence, stomatal closure, decreased leaf elongation and lower production of dry matter are morphological mechanisms (Kumar *et al.,* 2015). The leaf area, cell size and intercellular volume are reduced by water stress. Rice's vulnerability to drought or water stress, however, depends on timing, length, drought stress intensity, variety, and rice's growth stage (Sokoto, 2014). Because of the decrease in turgor pressure during dry stress, cell growth in rice is seriously impaired (Taiz and Zeiger, 2006). Cell elongation is more prone to stress from drought than division of cells (Jaleel *et al.,* 2009). The two main factors in water stress resistance are seed germination and early seedling production (Bunnag and Pongthai, 2013). In conditions of water stress, the total production of biomass and the fresh and dry weight of seedlings are greatly reduced (Ji *et al.,* 2012). By influencing multiple physiological and biochemical processes, such as photosynthesis, respiration, translocation, ion absorption, carbohydrates, nutrient metabolism and growth promoters, this stress decreases plant growth.

There are two forms of conditions for drought, known as terminal and intermittent. Intermittent drought conditions are not necessarily fatal, unlike terminal drought stress. In relation to other stress components that promote signal transduction pathways, the degree of sensitivity or resistance of rice to many drought conditions is coordinated by the action of various drought-responsive genes (Oladosu *et al.,* 2019)

2.5.2 Tolerance against drought

Drought tolerance is the plant ability to maintain plant tissue under high water potential and avoid the effect of water stress. Mechanisms of drought tolerance, including morphological adaptations, physiological acclimation, and cellular changes, are regulated at various stages by genetic factors (Sahebi *et al.,* 2018). For drought tolerance, a variety that is more resistant to water flow from the stomata into the atmosphere is regarded as good. The decrease in soil moisture may have resulted in lower leaf water content, causing guard cells to lose turgor

pressure and thus decreasing the size of stomatal pores (Tezera, *et al*., 2002) and/or causing stomatal closure. By closing the stomata, the reduction in stomatal conductance reduces transpiration, resulting in a prolongation of plant survival by extending the time of availability in the root zone of critical soil water reserves. Stomatal closure also helps retain a high content of leaf water and hence a higher capacity for leaf water, resulting in a decrease in photosynthetic activity. Delay leaf rolling is used as an essential selection criterion for rice drought tolerance, which could be enhanced by integrating the gene(s) into those lines / varieties that perform better under irrigated conditions but not well under conditions of water stress. In turn, higher photosynthetic rates may encourage greater biomass and crop yields. The presence of cuticular wax is also relevant for water stress and, as compared to irrigated rice, is more important in dry land adopted rice, resulting in thick and leathery leaves that prevent water loss from the rice plant surface (Singh *et al*., 2012). To improve drought tolerance in crops, leaf attributes can be implemented.

A strong selection criteria for selecting the dryness tolerance line or varieties is also the form of root system. The deep root system has been established as the goal for change in drought tolerance (Boyer, 1996). Cultivars with deep and dense roots are good for the situation of drought stress and are positively associated with the area of the xylem vessel, which is essential to the conductance of water from the soil to the upper sections of the plants to meet the evaporative demand. Plants affected by drought-affected typically display limited root system structure and the reduction in root system size is directly proportional to the magnitude of water storage in several reasons.

2.5.3 Morphological adaptations

Increased root thickness and volume, waxy or / and dense leaf coverings, decreased weight and size of the leaves, smaller epithelial cells, delayed leaf senescence, and increased area of green leaves are morphological adaptations. Physiological acclimation consists of the following: higher stomatal density and conductance; decreased transpiration rates; decreased and early asynchrony between flowering and maturation of females and males; and increased development, aggregation, assimilation, and partitioning of seed and biomass yield. Finally, increased chlorophyll content, particle numbers, and harvest

index and lower osmotic potential are needed by cellular adjustments for desiccation tolerance (Sahebi *et al.,* 2018). Mechanisms of drought tolerance, including morphological adaptations, physiological acclimation, and cellular adjustments, are regulated at various stages by genetic factors. Due to variations in plant phenology, the mechanism of drought tolerance is complex; in addition, several quantitative trait loci (QTLs) regulate drought traits.

100 genotypes were tested at 0, 5, 10, 15 and 20 percent against five levels of drought stress placed by Polyethylene glycol 6000 (PEG-6000). Germination in all genotypes decreased from 95.8 percent in control to 6.6 percent in the maximum stress (20 percent PEG) stage with increasing water stress. With the rise in water stress level, seedling height and dry weight in all rice genotypes also decreased (Islam *et al.,* 2018).

2.6 Microsatellites

Microsatellites are tandem repeats with 1-10 bp length. Tandem repeats are unstable so that they are relevant in the process of evolution. They undergo mutation in a rapid rate for each cell generation (Gemayel *et al.,* 2012).

Due to the fact that SSR markers are highly insightful genetic markers with characters such as co-dominance, multi-allelic etc. they have been used extensively for fingerprinting plants for more than 25 years. They can be transferred between related species and are highly reproducible (Mason, 2015). Microsatellites were originally formed from plant genome coding and non-coding areas, and many sources were used to scan for SSRs, including a number of DNA libraries (genomic, SSR genomic, bacterial artificial chromosome and cDNA libraries) and public databases (Hanai *et al*., 2007).

They are markers based on polymerase chain reaction (PCR) and need prior knowledge on sequence structure before it is used as a molecular marker. Considering all other molecular markers, microsatellite markers are widely used in past few decades because they are PCR based dispersed and abundant in genome; highly mutagenic, polymorphic, and informative; co-dominant, suitable for detecting heterozygotes, and multi-allelic; highly reproducible; cost- effective and easy to detect; require small quantity of sample DNAs etc.

In addition, when a reference genome is missing, microsatellites are especially useful in constructing a genetic map of large genomes. (Vieira *et al*., 2016)

RM547, RM212, RM566, RM127, RM225 and RM242 were validated by Mohapatra *et al.* (2018) for high temperature stress. They could screen rice varieties and categorized them into susceptible and tolerant.

2.7 QTLs

One approach to dissecting the complex question of plant stress tolerance is the application of quantitative trait loci (QTL) mapping. This method will be of great significance to breeding for abiotic stress tolerance in plants when fully evolved (Flowers T J, 2004). Many crops have been reported to have QTLs associated with abiotic stress tolerance (e.g. salt stress in rice (Singh *et al*., 2016), drought stress in cotton (Saranga *et al.*, 2004).

Based on a broad range of important traits such as osmotic change, photosynthesis, shoot and root responses, hormonal responses and whole plant response to drought tolerance, most QTLs in rice have been established (Sahebi *et al*., 2018). The fined mapped QTLs regulating abiotic stress tolerance (e.g. drought, salinity, P deficiency, and submergence) can be the first tier of marker-assisted selection (MAS) traits, thus providing a greater opportunity to reduce the development of high-risk rice in lowland rainfed areas. In a single genotype (salinity and submergence, submergence and drought, salinity and drought, and salinity-submergence-drought), the problem of climate change forces breeders to combine several abiotic characteristics since these characteristics co-exist in one cropping season. Rice scientists will explore radical approaches to developing the next generation of rice varieties that are climate-change-resilient and appropriate to farmers with the advent of recent biotechnological instruments, knowledge of genetic mechanisms and new sources of tolerance (Gregorio *et al*., 2013).

On chromosomes 1, 4, 6, and 7, QTLs for salt stress have been observed repeatedly. On chromosomes 8 and 11, none were found, and very few on chromosomes 2, 3, 5, 9, 10, and 12 (Negrao *et al*., 2011)

In 2009, Venuprasad *et al.* suggested that in other populations near, RM520, RM511, and RM6703 quantitative trait loci (QTLs) largely affecting yield under

upland water deficit stress have already been found previously. Thus, these regions are important in explaining genetic variation for upland drought tolerance in rice.

3. MATERIALS AND METHODS

The study was conducted in experimenting area and Molecular Biology laboratory of Department of Biotechnology, RARS Pattambi during September 2019 to September 2020 to screen and characterize the 12 rice genotypes. The materials and methods utilized for the study are discussed below.

3.1 Materials

3.1.1 Plant materials

The seed materials for this study comprised of 12 genotypes of rice (*Oryza sativa* L.). The different varieties were received from collection maintained in midterm storage system at Regional Agricultural Research Station, Pattambi, Palakkad.

Table 3.1 List of Rice Genotypes used for the study

PTB-61 Supriya
PTB-62 Akshaya
Manurathna
Vyttilla-9
Culture-06-7
Kalluruli selection
J S-1
NPT Culture 1
M-4
M-9
N-22 (Check)
Pokkali

3.1.2 Chemicals, plasticwares and glasswares

All the chemicals used in the study were good quality (AR/GR) obtained from various firms such as Invitrogen, MERK India Ltd., HIMEDIA, SRL. Glasswares were supplied by Borosil and plasticwares were supplied by Tarson India Ltd.

3.1.3 Equipments and machinery used

The facilities available at RARS Pattambi were utilized for the conduction of this work. The autoclave in which sterilization carried out was of EQUITRON. Waterbath used in the experiment were of Rotek. Refrigerated centrifuge (Model C-24BL) was used for the centrifugations. Storage of samples and other reagents were in Refrigerator. The microwave oven used was of LG. PCR was done using thermal cycler of the company Eppendorf. Agarose gel electrophoresis for the separation of DNA and PCR products was carried out in horizontal gel electrophoresis system manufactured by BIO-RAD (Sub-Cell Model 192, USA). The imaging and documentation of the gel were done using Gel Doc system of Medicare (Model- GELSTAN 4X Advanced). Temperature Induction Response reaction were carried out in Hot air oven of Rotek. Spinner of Tarsons India Pvt. Ltd. was used.

3.2 Methods

3.2.1 Screening of rice genotypes for water deficit stress and salinity stress by hydroponics

3.2.1.1 Sterlization of rice seeds:

- About 25 seeds of the rice genotypes was taken in petriplates
- 0.01% of mercuric chloride solution was prepared by dissolving 0.01mg mercuric chloride in 100 ml of distilled water.
- The mercuric chloride solution was added to the petriplates in such a way that the seeds are dipped in it.
- It was kept for 5 minutes with intermittent mixing by swirling the plates
- The solution was removed and the seeds were washed with the autoclaved distilled water for three times, one minute each.
- Water was completely removed from seeds.

- The seeds were placed in the sterilized cottons and placed them directly in the growth media.
- Twelve bottles were used to grow control plants and the other thirty six were used to grow different treatment varieties.

Table 3.2 Composition of yoshida solution (Yoshida *et al.*, 1976**)**

MACRONUTRIENTS	**SOURCE**	**g / 500**
Nitrogen (N)	Ammonium Nitrate (NH_4NO_3)	45.700
Phosphorus (P)	Sodium Dihydrogen Phosphate (NaH_2PO_4)	17.800
Potassium (K)	Potassium Sulphate (K_2SO_4)	35.700
Calcium (Ca)	Calcium Chloride ($CaCl_2.H_2O$)	58.675
Magnesium (Mg)	Magnesuim Sulphate($MgSO_4 .7H_2O$)	162.00

MICRONUTRIENTS	**SOURCE**	**g / 500**
Manganese(Mn)	Manganese Chloride ($MnCl_3.4 H_2O$)	0.750
Molybdenum(Mo)	Ammonium Molybdate 4 Hydrate ($NH_4Mo_7O_4.4H_2O$)	0.0375

Zinc (Zn)	Zinc Sulphate 7 Hydrate ($ZnSO_4.7H_2O$)	0.0175
Boron(B)	Boric Acid (H_3BO_3)	0.467
Copper (Cu)	Cupric Sulphate, 5 Hydrate ($CuSO_4$. $5H_2O$)	0.0155
Iron (Fe)	Ferric Chloride ($FeCl_3.6H_2O$)	2.310
Citric Acid	Citric Acid	5.950

The stock solution were prepared as above. 1.2 millilitre from each of the stock solution was taken and made up to 1 litre to serve as the culture solution. The pH of the solution was maintained at 5.0 using 0.01M NaOH and 0.01M HCl. Each week the culture solution was renewed and every third day the pH adjusted to 5.5.

3.1.1.3 Hydroponics

Four sets of 12 varieties were taken in bottles. About 35 ml of Yoshidha solution was added to control plants. One per cent Mannitol solution was used for inducing drought stress was prepared by dissolving 5 grams of D –Mannitol in 500 ml of nutrient solution (Water potential= -0.15MPa). This solution was added to each of the 12 bottles. 100mM NaCl solution for the induction of salinity stress was prepared by adding 2.922g of Sodium chloride into 500 ml of nutrient solution (Water potential= -0.3MPa). It was added to each of the 12 bottles. Simultaneous stress was induced by pouring equal amount of 1% Mannitol and 100mM NaCl solutions into each of the 12 bottles. The plants were maintained for 25 days and observations were recorded on the 25th day. The seedling length and germination percentage of both control and treated plants were recorded and Vigour Index is calculated using the formula given

below. The selected varieties were then grown in pots for molecular charcterisation.

Vigour index = Germination percentage x **Seedling length**

The selected varieties are then grown in pots to get enough leaf sample for DNA isolation.

3.1.2 Assessing intrinsic tolerant traits of genotypes by Temperature Induction Response (Senthil-kumar and Udayakumar, 2004)

The experiment was conducted following completely randomized design with three replication and 12 varieties. Two days old seedlings belonging to 12 varieties of rice were maintained in a growth chamber to standardize temperature induction response where in the temperature was raised from 32 ^{0}C to 42 ^{0}C for 5 hours gradually and then challenged by a lethal temperature of 49 ^{0}C for 3 hours. Then the seedling are allowed to recover for 72 hours at 30 ^{0}C in a controlled growth chamber.

At the end of recovering period the genotypes will be classified into susceptible, moderately tolerant and tolerant genotypes based on actual growth. The plant without any treatment will serve as control. The seedling length and Germination percentage of both control and treatment was measured and Vigour Index was calculated as stated above.

3.1.2 Molecular chracterisation

3.1.2.1 Isolation of DNA from leaves of rice

The sample for DNA isolation was the tender leaves of rice. They were collected using scissors and brought to lab by putting inside a transparent plastic bag. For better yield, the samples were stored inside the refrigerator before grinding. The CTAB method was followed for the isolation. The different reagents used were

a. 4% CTAB extraction buffer
b. β mercaptoethanol
c. Chloroform: Isoamyl alcohol (24:1)
d. 3M sodium acetate
e. Chilled isopropanol
f. 70% ethanol

g. Sterile double distilled water

The procedure is as follows

1. 0.1g of young rice leaf was weighed and frozen quickly in liquid nitrogen. Then was ground to fine powder in a mortar and pestle. Leaves can be stored in -80ºC either before or after grinding if needed.
2. The fine powder was transferred into a 2ml microfuge tube and was added with 1ml of CTAB extraction buffer (pre-heated at 65ºC) and 2µl of β-mercaptoethanol.
3. The tubes were shaken for proper mixing and were incubated in water bath set at 65ºC for one hour. Intermittently inverted the tubes for proper mixing.
4. The tubes were centrifuged at 7300rpm for 20 minutes at 4ºC
5. The supernatant was decanted and equal amount of chloroform:isoamylalcohol (24:1) to each tubes were added
6. The content was mixed by gentle inversion.
7. The tubes were kept in a shaker for 25 minutes for proper uniform mixing.
8. The tubes were centrifuged at 7300rpm for 20 minutes at 4ºC
9. The above aqueous layer was pipetted out carefully without disturbing the bottom layers to a microfuge tube.
10. Equal volume of chilled isopropanol was added and the tubes were incubated at -20ºC for overnight.
11. The tubes were centrifuged at 13000 rpm for 10 minutes at 4ºC. The supernatant was gently decanted.
12. The pellet was air dried and added with 30µl of 3M sodium acetate
13. The tubes were centrifuged at 10000rpm for 5 minutes at 4ºC
14. The supernatant was decanted and the pellet was dried.
15. The pellet was washed in 70% alcohol and air dried completely
16. The pellet was re dissolved in 50µl sterile double distilled water and was stored at -20º C

3.1.2.2 Agarose gel electrophoresis

The isolated DNA was separated on the basis of size by running from negative charge to positive charge in a horizontal gel electrophoresis system as DNA is negatively charged. 120 ml of 0.8% agarose gel was prepared and after

solidification, the gel was transferred into gel tank filled with 1X TAE buffer. 8µl of DNA from each sample mixed with 2µl of 1X gel loading dye was loaded in the well. The voltage was set to 70V and run for 2.5 hours. The image was taken using gel doc system.

3.1.3 Polymerase Chain Reaction

This reaction was carried out in sterile capped PCR tubes of 0.2ml.

Table 3.3 Primers used in PCR

Sl no	Name	Sequence	Chromosome on which it is located	Reference
1	RM316F	CTAGTTGGGCATACGATGGC	9	Temnykh *et al.*, 2000
2	RM316R	ACGCTTATATGTTACGTCAAC	9	Temnykh *et al.*, 2000
3	RM511F	CTTCGATCCGGTGACGAC	12	Temnykh *et al.*, 2001
4	RM511R	AACGAAAGCGAAGCTGTCTC	12	Temnykh *et al.*, 2001
5	RM302F	TCATGTCATCTACCATCACAC	1	Temnykh *et al.*, 2000
6	RM302R	ATGGAGAAGATGGAATACTTGC	1	Temnykh *et al.*, 2000
7	RM493 F	TAGCTCCAACAGGATCGACC	1	Temnykh *et al.*, 2001
8	RM493 R	GTACGTAAACGCGGAAGGTG	1	Temnykh et al., 2001
9	RM3412 F	AAAGCAGGTTTTCCTCCTCC	1	McCouch *et al.*, 2002
10	RM3412 R	CCCATGTGCAATGTGTCTTC	1	McCouch *et al.*, 2002

11	RM10793 F	GACTTGCCAACTCCTT CAATTCG	1	Sasaki *et al.*, 2005
12	RM10793 R	TCGTCGAGTAGCTTCC CTCTCTACC	1	Sasaki *et al.*, 2005
13	RM7076 F	ATCAACTCCGGCGTC AGAGACC	3	McCouch *et al.*, 2002
14	RM7076 R	GAGCAGGGTCCATGA AATTCTCC	3	McCouch *et al.*, 2002
15	RM5749 F	GTGACCACATCTATA TCGCTCG	4	McCouch *et al.*, 2002
16	RM5749 R	ATGGCAAGGTTGGAT CAGTC	4	McCouch *et al.*, 2002
17	RM26212 F	GTCGCTCCTCTCCTCC AATCC	11	Sasaki *et al.*, 2005
18	RM26212 R	GCTCGCTGCTTCTAAT CTCTTGC	11	Sasaki *et al.*, 2005

Table 3.4 Components of PCR reaction mix

Sl no.	**Components**	**Quantity in µL**
1	Sterile distilled water	3.95
2	10X Taq assay buffer (without $MgCl_2$)	1.00
3	dNTP mix (10mM each)	0.50
4	25 mM $MgCl_2$	0.25
5	3U Taq DNA polymerase	0.30
6	Forward primer (10µM)	0.50
7	Reverse primer (10µM)	0.50
8	DNA	3
Total		10 µL

Table 3.5 PCR Programme

Step	Temperature (℃)	Duration
Initial denaturation	95	3 minutes
Denaturation	94	50 seconds
Annealing		50 seconds
Extension	72	1 minutes
Final extension	72	10 minutes (35 cycles)

35 cycles were performed

3.1.4 PCR product separation by gel electrophoresis

The separation of amplified PCR products were done using agarose gel electrophoresis. 2% agarose gel was used. 2.4g of agarose was weighed and added into 120 ml of 1X TAE buffer. This was melted for 2 minutes in the microwave oven. After melting, a small quantity of ethidium bromide was added and mixed well by gently swirling the vessel and poured immediately to the gel casting tray fitted with comb plate for solidification. After 35-40 minutes, the solidified gel was transferred to the gel tank of electrophoresis unit filled with 1X TAE buffer. The whole tubes undergone PCR were mixed with 2µl each of gel loading dye and added to the wells. 4µl of 100bp DNA ladder was also added to one of the empty wells to determine the size of separated products. Voltage was set for 70V and running of gel was continued for 3 hours. After this the gel was documented using GEL DOC system.

4. RESULTS

The experiments regarding the study entitled "Screening and characterization of rice genotypes for developing climate resilient variety" were conducted in Regional Agricultural Research Station, Pattambi, Palakkad from September 2019 to September 2020.

4.1 Screening of rice genotypes for water deficit stress and salinity stress by hydroponics

Table 4.1 Total Seedling length

The seedling length measured are tabulated below

Variety	Treatment	Mean seedling length (cm)	Mean shoot Length (cm)	Mean root Length (cm)
N22	Control	24.82	15.92	8.9
	Drought	21.86	14.56	7.3
	Salt	16.06	9.5	6.56
	Multiple	23.23	13.67	9.56
Supriya	Control	25.06	15.36	9.7
	Drought	11.66	7.3	4.36
	Salt	14.6	8.95	5.65
	Multiple	21.5	15.6	5.9
Vyttila 9	Control	29.36	16.16	13.2
	Drought	22.32	12.02	10.3
	Salt	17.02	9.56	7.46
	Multiple	23.6	12.35	11.25
M-9	Control	37.88	20.62	17.23
	Drought	30.76	18.91	11.85
	Salt	22.98	12.29	10.69
	Multiple	34.94	20.89	14.05
Pokkali	Control	38.14	22.54	15.6
	Drought	33.82	19.26	14.56
	Salt	25.1	14.8	10.3
	Multiple	32.12	16.92	15.2
NPT culture 1	Control	20.98	12.68	8.3
	Drought	16.58	11.33	5.25
	Salt	15	9.75	5.25

	Multiple	21.82	12.36	9.46
Akshaya	Control	23.44	12.56	10.88
	Drought	19.36	12.11	7.25
	Salt	15.28	9.4	5.88
	Multiple	21.52	11.96	9.56
Culture-06-7	Control	28.76	16.4	12.36
	Drought	22.56	13	9.56
	Salt	16.18	8.68	7.5
	Multiple	21.54	12.02	9.52
M-4	Control	25.24	13.26	11.98
	Drought	27.76	16.07	11.69
	Salt	24.06	13.86	10.2
	Multiple	29.1	15.3	13.8
JS-1	Control	24.98	14.96	10.02
	Drought	24.38	14.13	10.25
	Salt	21.06	11.81	9.25
	Multiple	27.16	14.62	12.54
Manurathna	Control	23.46	12.61	10.85
	Drought	21.12	11.27	9.85
	Salt	19	10.1	8.9
	Multiple	22.28	14.36	7.92
Kalluruli	Control	42.54	24.12	18.42
	Drought	31.22	19.36	11.86
	Salt	18.9	10.96	7.94
	Multiple	32.04	19.85	12.19

The total seedling length was measured using meter scale from the tip of the root to the tip of the shoot. The length of five random seedlings were taken on the day 25 and the mean was calculated for each treatment.

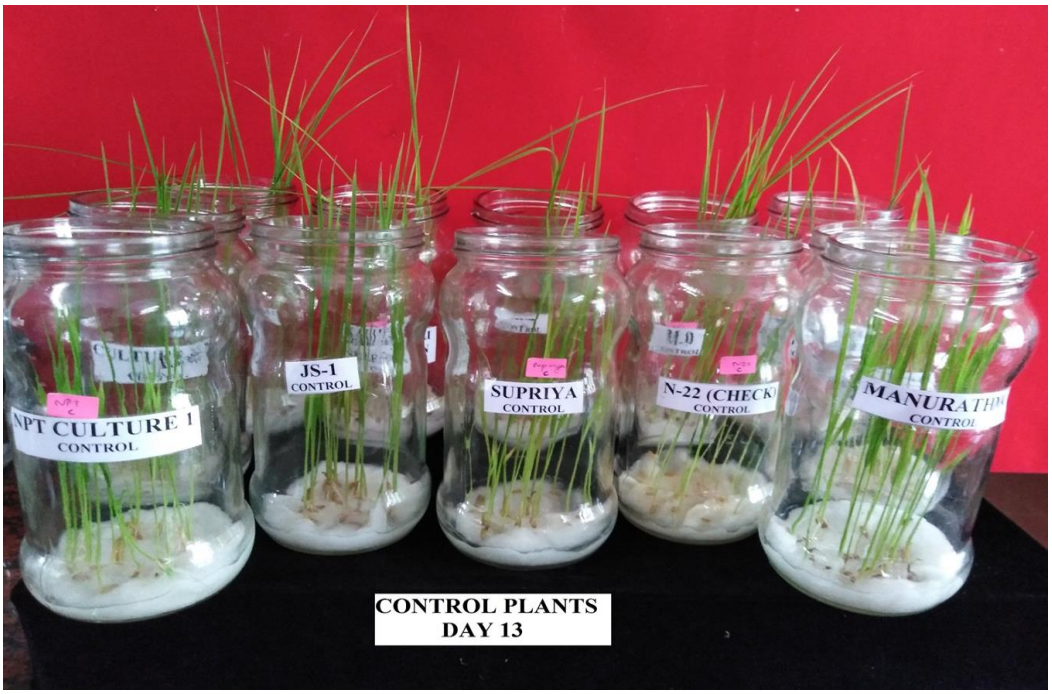

Plate 4.1 a: control plants in hydroponics- Day 13

Plate 4.1 b: Plants induced with drought stress- Day 13

Plate 4.1 c: Plants induced with salt stress- Day 13

Plate 4.1 d: Plants induced with multiple stress- Day 13

Table 4.2 Seedling vigour index

Variety	Treatment	Mean seedling length	Germination %	Seedling vigour
N22	Control	24.82	89	2208.98
	Drought	21.86	88	1923.68
	Salt	16.06	87	1397.22
	Multiple	23.23	87	2021.01
Supriya	Control	25.06	91	2280.46
	Drought	11.66	91	1061.06
	Salt	14.6	89	1299.4
	Multiple	21.5	89	1913.5
Vyttila 9	Control	29.36	90	2642.4
	Drought	22.32	89	1986.48
	Salt	17.02	89	1514.78
	Multiple	23.6	89	2100.4
M-9	Control	37.88	91	3447.08
	Drought	30.76	91	2799.16
	Salt	22.98	91	2091.18
	Multiple	34.94	91	3179.54
Pokkali	Control	38.14	93	3547.02
	Drought	33.82	93	3145.26
	Salt	25.1	90	2259
	Multiple	32.12	91	2922.92
NPT culture 1	Control	20.98	92	1930.16
	Drought	16.58	92	1525.36
	Salt	15	90	1350
	Multiple	21.82	90	1963.8
Akshaya	Control	23.44	91	2133.04
	Drought	19.36	91	1761.76
	Salt	15.28	91	1390.48
	Multiple	21.52	91	1958.32
Culture-06-7	Control	28.76	90	2588.4
	Drought	22.56	89	2007.84
	Salt	16.18	89	1440.02
	Multiple	21.54	90	1938.6

M-4	Control	25.24	93	2347.32
	Drought	27.76	93	2581.68
	Salt	24.06	90	2165.4
	Multiple	29.1	92	2677.2
JS-1	Control	24.98	93	2323.14
	Drought	24.38	92	2242.96
	Salt	21.06	90	1895.4
	Multiple	27.16	91	2471.56
Manurathna	Control	23.46	91	2134.86
	Drought	21.12	91	1921.92
	Salt	19	91	1729
	Multiple	22.28	91	2027.48
Kalluruli	Control	42.54	89	3786.06
	Drought	31.22	89	2778.58
	Salt	18.9	88	1663.2
	Multiple	32.04	88	2819.52

Seedling vigour index was calculated using the germination percentage and the mean seedling length. A total of 20 seeds each were taken to germinate. Germination percentage was calculated by taking the ratio of number of seeds germinated to the total number of seeds ie, 20. The seedling vigour index calculated as shown above (Table 4.2). The different varieties showed different level of growth in each treatment. The varieties showed significant growth on par with the control plants in the stress condition were selected. N22, Pokkali, Akshaya, M-4, and JS-1 showed tolerance to under water deficit stress. M-4 and JS-1 under salinity stress. M-4, JS-1 and NPT culture-1 showed tolerance against multiple abiotic stress.

4.1.3 Statistical analysis

The statistical analysis of the data was carried out by Paired T test using Microsoft excel software.

Table 4.3 Paired T test results

Variety	Paired t test result (p)		
	Drought stress	Salinity stress	Multiple stress
Akshaya	0.053347993	0.002426931	0.279845936
Supriya	0.000111236	0.0000877551	0.078677497
Manurathna	0.177643968	0.065228979	0.223667914
Vyttila-9	0.049353159	0.000201392	0.089253537
Pokkali	0.022933095	0.0003052	0.003590082
Kalluruli	0.003357255	0.0000801733	0.033100156
Culture-06-7	0.019818149	0.000382798	0.005114098
NPT Culture-1	0.002090536	0.000401785	0.149572831
N22	0.090739006	0.013427949	0.351257653
M-4	0.049961473	0.156361043	0.194800607
M-9	0.002182558	0.000605763	0.059456491
JS-1	0.382770413	0.020630479	0.169983206

Where $P< 0.05$ means the difference between control plants and treatment plants is significant. The varieties in which $P>0.05$ were selected as tolerant ones.

4.2 Screening of genotypes for temperature stress using Temperature Induction response technique

Screening of genotypes for temperature stress was done using TIR technique in controlled growth chamber. Seedling length was measured using scale and mean value was taken from 5 different observations. The seedling vigour index was calculated and tabulated

Table 4.4 Seedling vigour indices in TIR

Variety		Mean plant length	Germination %	Seedling vigour
Manurathna	Control	10.8	91	982.8
	Treatment	4.5	90	405
Akshaya	Control	10.2	91	928.2
	Treatment	8.54	91	777.14
Cul-06-7	Control	10.18	90	916.2
	Treatment	4.44	89	395.16
Pokkali	Control	10.5	93	976.5
	Treatment	5.56	93	517.08
JS-1	Control	8.04	93	747.72
	Treatment	7.56	93	703.08
Supriya	Control	14.56	91	1324.96
	Treatment	5.66	88	498.08
M-9	Control	9	91	819
	Treatment	6.88	90	619.2
Kalluruli	Control	9.98	89	888.22
	Treatment	5.9	88	519.2
N22	Control	10.7	89	952.3
	Treatment	9.18	89	817.02
Vytila- 9	Control	11.04	90	993.6
	Treatment	6.06	88	533.28
M-4	Control	10	93	930
	Treatment	4.3	88	378.4
NPT culture 1	Control	9.62	92	885.04
	Treatment	8.01	92	736.92

By comparing the seedling vigour indices of both control and treatment, the screening process was taken place. Akshaya, JS-1, M-9, N22 (universal check for temperature tolerance) and NPT culture-1 were selected as tolerant varieties

4.2.1 Statistical analysis

Statistical analysis of the data was done using paired T test.

Table 4.5 Paired T-test result

Variety	Paired t test result (p)
Akshaya	0.14359
Supriya	0.000439
Manurathna	0.002706
Vyttila-9	0.000192
Pokkali	0.001864
Kalluruli	0.023786
Culture-06-7	0.000974
NPT Culture-1	0.085207
N22	0.111541
M-4	0.001884
M-9	0.051654
JS-1	0.365807

Where $P < 0.05$ means the difference between control plants and treatment plants is significant. The varieties in which $P > 0.05$ were selected as tolerant ones.

4.3 DNA isolation

Each variety was grown in pots till 4-5 leaves stage. Tender leaves were cut off and used for isolation of DNA. High quality DNA was obtained from each of the 12 varieties using CTAB method.

4.4 Quantity and quality checking of isolated DNA

The amount of the DNA was checked using spectrophotometer. The quantity was calculated as follows

Table 4.6 Quantity and quality analysis of isolated DNA

Variety	$A_{(260/280)}$	Quantity (ng/µl)
M-4	1.86	1710
M-9	1.92	1650
N-22	1.94	1630
NPT culture 1	1.83	1750
Kalluruli	1.81	1810
Pokkali	1.85	1730
Manurathna	1.90	1680
JS-1	1.88	1690
Vyttila-9	1.75	1590
Akshaya	1.78	1610
Supriya	1.81	1810
Cul-06-7	1.83	1750

Agarose gel electrophoresis was used for the quality analysis. Single band of intact DNA with lesser protein and RNA contamination was obtained.

Plate 4.3 a: Different genotypes germinated in petriplates

Plate 4.3 b: Genotypes raised in pots for DNA isolation

4.5 Polymerase Chain Reaction

The standardization of primers was done first. The primers showed better amplifications in 55, 58 and 60 degree centigrade. (Table 4.7)

Table 4.7 Annealing temperature of primers used

PRIMER	ANNEALING TEMPERATURE (°C)
RM 316	55
RM 511	60
RM 302	60
RM 493	55
RM 3412	58
RM 10793	55
RM 7076	60
RM 5749	58
RM 26212	60

4.5.1 SSR-RM Markers specific for drought stress

Polymerase Chain Reaction was done using RM316, RM511 and RM302 which are already reported as drought tolerance specific. The expected product size is 192bp, 130bp and 156bp respectively. The details of amplification are tabulated below in table 4.8

Table 4.8 Drought specific SSR-RM markers

Primer	Genotypes with amplicons
RM316	M-4, JS-1, Manurathna, M-9, NPT culture-1
RM511	JS-1, Kalluruli, Vyttila-9
RM302	JS-1, Kalluruli, N22, NPT culture-1, Manurathna, Vyttila-9, Akshaya

4.5.2 SSR-RM Markers specific for salinity stress

The reported primer RM493, RM3412 and RM10793 were used for the characterization of salinity tolerant genotypes. The expected product size are 211bp, 211bp and 123bp respectively. The results are listed below in table 4.9

Table 4.9 Salinity specific SSR-RM Markers

Primer	Genotypes with amplicon
RM493	Pokkali, NPT culture-1, Vyttila-9
RM3412	M-4, Pokkali, JS-1, Kalluruli
RM10793	M-4, Pokkali, JS-1, M-9, Manurathna, Culture-06-7, Akshaya, Supriya

4.5.3 SSR-RM Markers specific for temperature stress

RM7076, RM5749 and RM26212 which are reported as specific for temperature tolerance were used for the molecular characterization. The expected product sizes were 172bp, 162bp and 180bp respectively. The results are given below in table 4.10

Table 4.10 High temperature specific SSR-RM markers

Primer	**Genotypes with amplicon**
RM7076	JS-1, Kalluruli, N22
RM5749	M-4, N22, Akshaya
RM26212	JS-1, N22, NPT Culture-1, Cul-06-7, Akshaya, Supriya

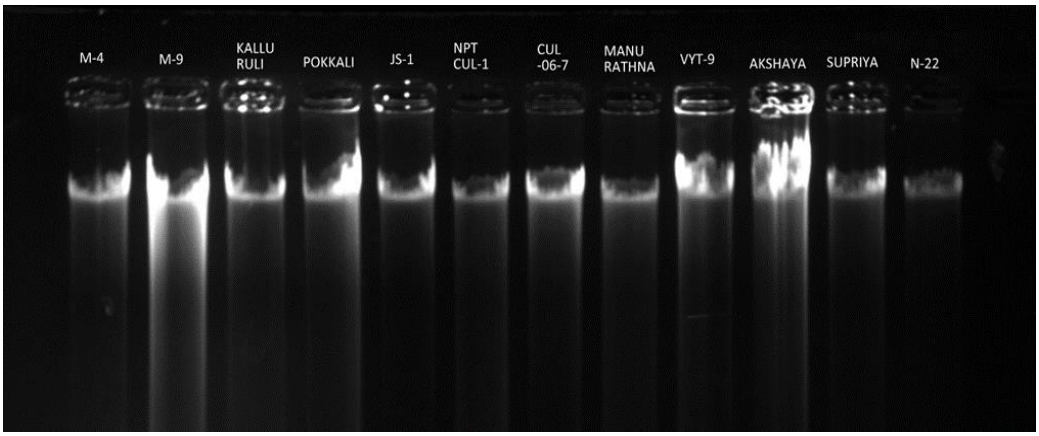

Fig 4.3 c: DNA isolated from 12 varieties

Fig 4.5.1a: Amplification profile of RM316

L: 100bp ladder 1. M-4 2. Pokkali 3. JS-1 4. Kalluruli 5. N22 6. M-9 7. NPT culture-1 8. Manurathna 9. Culture-06-7 10. Vyttila- 9 11. Akshaya 12. Supriya

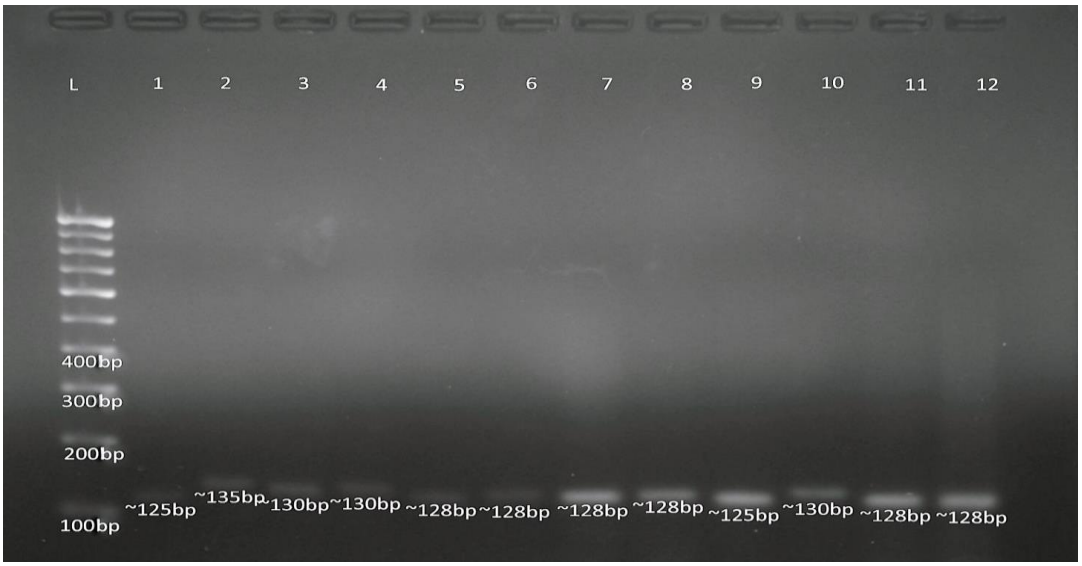

Fig 4.5.1b: Amplification profile of RM511

L: 100bp ladder 1. M-4 2. Pokkali 3. JS-1 4. Kalluruli 5. N22 6. M-9 7. NPT culture-1 8. Manurathna 9. Culture-06-7 10. Vyttila-9 11. Akshaya 12. Supriya

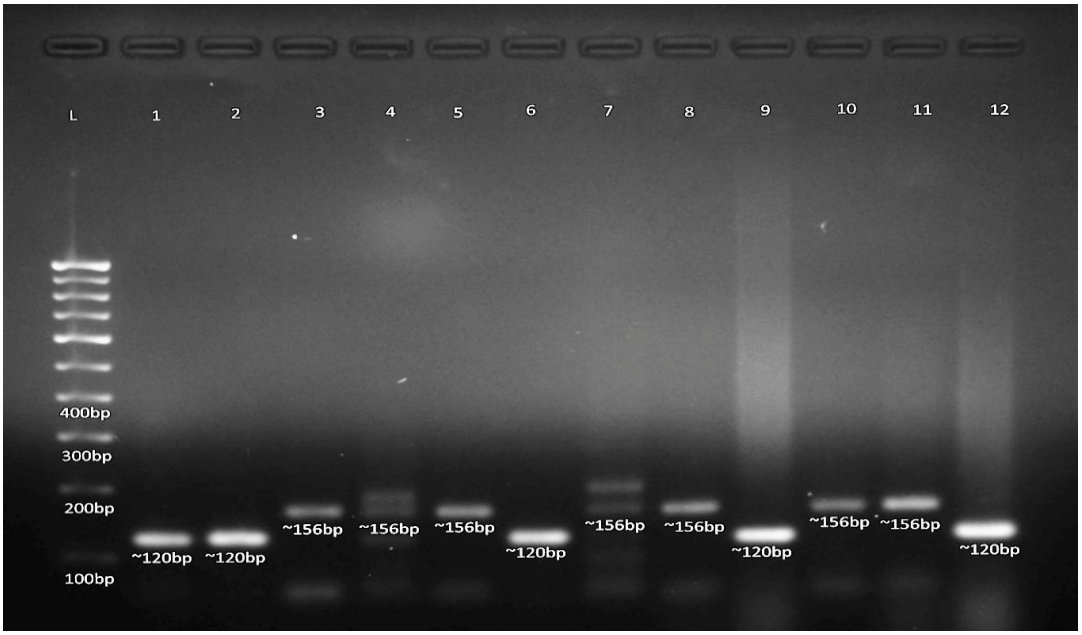

Fig 4.5.1c: Amplification profile of RM302

L: 100bp ladder 1. M-4 2. Pokkali 3. JS-1 4. Kalluruli 5. N22 6. M-9 7. NPT culture-1 8. Manurathna 9. Culture-06-7 10. Vyttila-9 11. Akshaya 12. Supriya

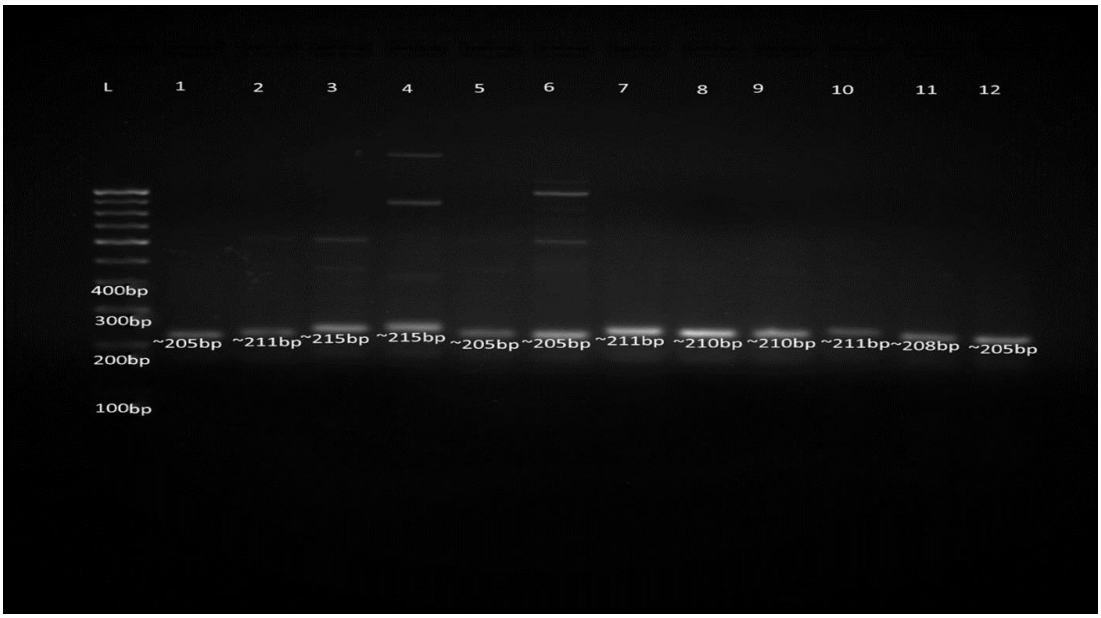

Fig 4.5.2a: Amplification profile of RM493

L: 100bp ladder 1. M-4 2. Pokkali 3. JS-1 4. Kalluruli 5. N22 6. M-9 7. NPT culture-1 8. Manurathna 9. Culture-06-7 10. Vyttila-9 11. Akshaya 12. Supriya

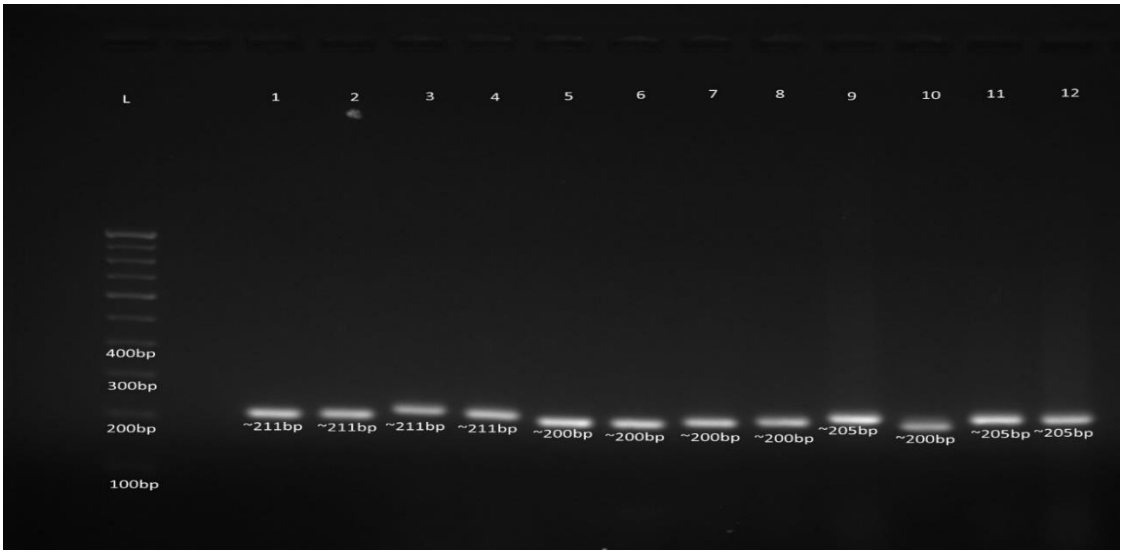

Fig 4.5.2b: Amplification profile of RM3412

L: 100bp ladder 1. M-4 2. Pokkali 3. JS-1 4. Kalluruli 5. N22 6. M-9 7. NPT culture-1 8. Manurathna 9. Culture-06-7 10. Vyttila-9 11. Akshaya 12. Supriya

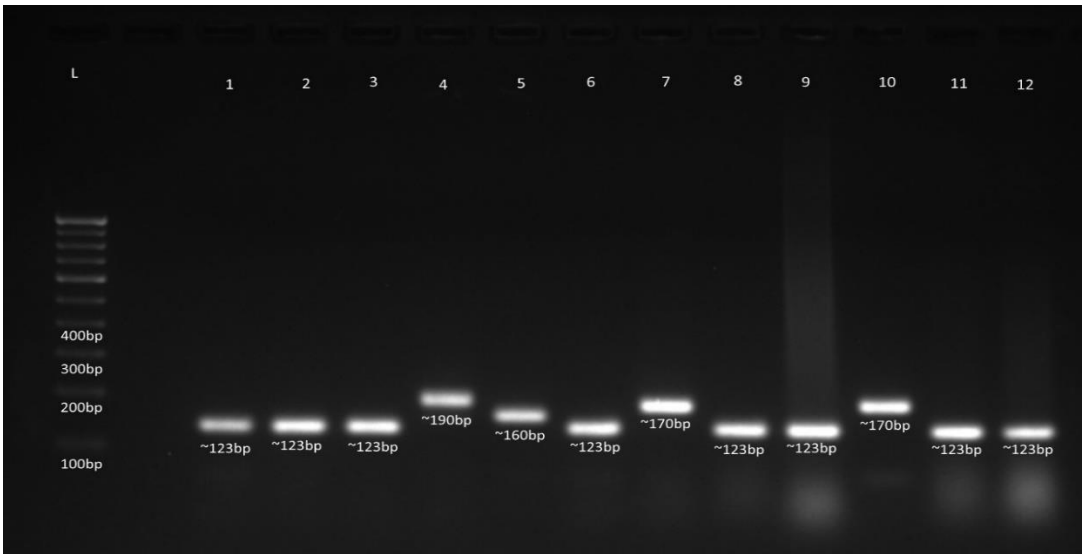

Fig 4.5.2c: Amplification profile of RM10793

L: 100bp ladder 1. M-4 2. Pokkali 3. JS-1 4. Kalluruli 5. N22 6. M-9 7. NPT culture-1 8. Manurathna 9. Culture-06-7 10. Vyttila-9 11. Akshaya 12. Supriya

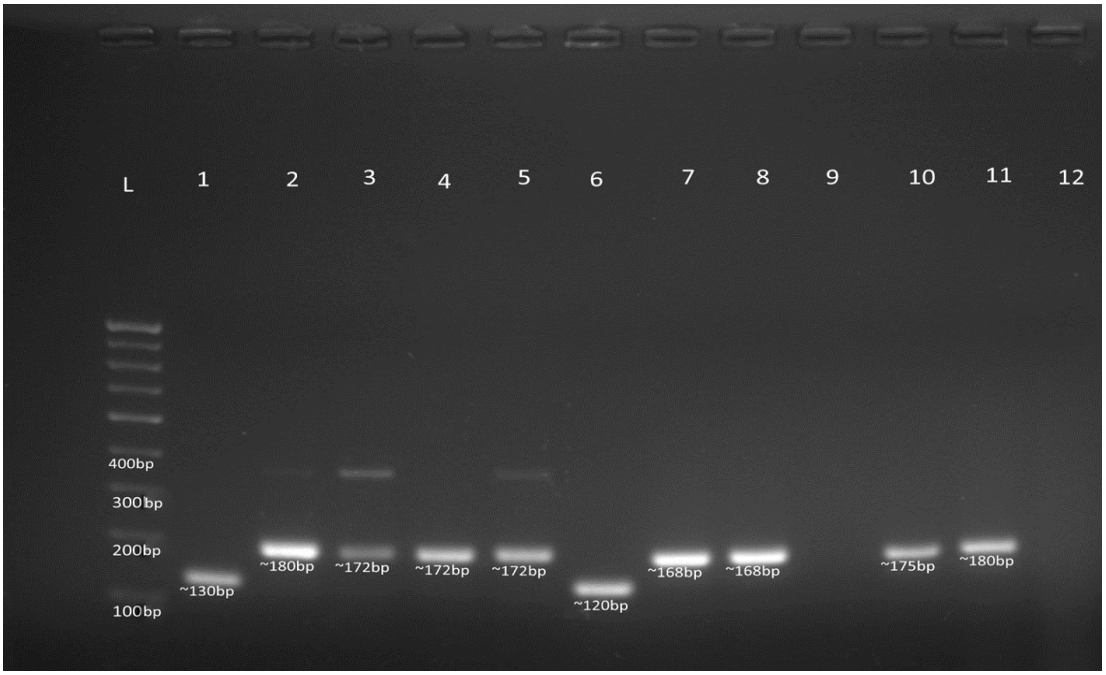

Fig 4.5.3a: Amplification profile of RM7076

L: 100bp ladder 1. M-4 2. Pokkali 3. JS-1 4. Kalluruli 5. N22 6. M-9 7. NPT culture-1 8. Manurathna 9. Culture-06-7 10. Vyttila-9 11. Akshaya 12. Supriya

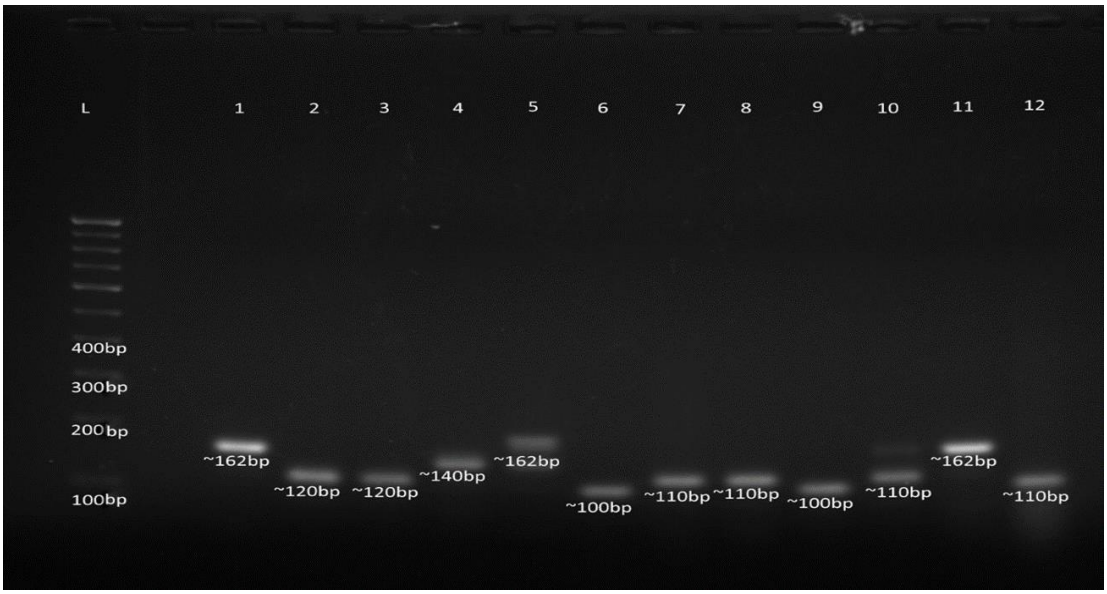

Fig 4.5.3b: Amplification profile of RM5749

L: 100bp ladder 1. M-4 2. Pokkali 3. JS-1 4. Kalluruli 5. N22 6. M-9 7. NPT culture-1 8. Manurathna 9. Culture-06-7 10. Vyttila-9 11. Akshaya 12. Supriya

Fig 4.5.3c: Amplification profile of RM26212

L: 100bp ladder 1. M-4 2. Pokkali 3. JS-1 4. Kalluruli 5. N22 6. M-9 7. NPT culture-1 8. Manurathna 9. Culture-06-7 10. Vyttila-9 11. Akshaya 12. Supriya

5. DISCUSSION

The work entitled 'Screening and characterization of rice genotypes for multiple abiotic stress for developing climate resilient variety' was carried out at Regional Agricultural Research Station, Pattambi, Palakkad during September 2019 to September 2020. The objective of the study was to screen given varieties by inducing abiotic stresses and to characterize them by molecular methods.

5.1 Screening of rice genotypes for water deficit stress and salinity stress by hydroponics

5.1.1 Varieties used in the study

Twelve varieties used in this study are M-4, M-9, JS-1, N22, Pokkali, Kalluruli, Manurathna, Akshaya (PTB-61), Supriya (PTB-62), NPT culture-1, Vyttila- 9 and Culture-06-7. M-4 and M-9 are mutant varieties developed at RARS pattambi which are in the breeding pipeline. They are developed from a variety named as Eravapandy (PTB-18) released by RARS Pattambi. JS-1 is selected from Jaya and it has moderate resistance against leaf folder. Early flowering is also a desirable character of this variety. Its performance is superior in *rabi* season than in *kharif.* It has an average grain yield of 7113kg/ha.

Akshaya and Supriya are recently released varieties from RARS Pattambi, specifically for Mundakan season having long term maturity and a yield of 6.5 to 7 ton per hector. They are resistant to lodging and remain healthy till harvesting which is an added advantage for higher yield capacity. They are moderately resistant to both high temperature and drought stress.

NPT culture-1 is undergoing field trials at RARS Pattambi. Parents of the culture are RPBio226/IRgC71598/MTU1010. Salient features of this are drought tolerance, non-lodging with higher yields and moderate resistance to major pest and diseases including leaf folder and neck blast. They found to perform well at upland conditions. In this study, they performed well in multiple abiotic stress condition which is a well desirable character.

Culture-06-7 is developed from a cross between Pranava and Vellari. It is high yielding, tall, non-lodging with high straw yield, specifically suited for the Mundakan season. It also shows resistance against leaf folder, blast, brown spot and sheath rot.

Pokkali is a variety having the status of geographical indication tag from 2007. It is the traditional variety used in Pokkali type of cultivation span over the coastal areas of Kerala. It grows better in low to moderate saline conditions. RARS Vyttila combined the characters of traditional varieties grown in saline soils such as Cheruvirippu, Karuka, Eravapandy with some high yielding varieties and released them as Vyttila-1 to Vyttila-9 (Sreelatha and Shylaraj, 2017).

5.1.2 Tolerance against abiotic stress

Hydroponics helps plants to use water efficiently and grow better with controlled climatic conditions. So laboratory studies can be performed in hydroponics (Trejo-Telles *et al.*, 2012).

In a study conducted by Amaravel *et al.* in 2019 ninety seven rice genotypes including traditional landraces were collected and subjected to screening under salt stress by hydroponics system on Yoshida nutrient solution. Among the 97 rice genotypes scored, 5% were tolerant, 21% were moderately tolerant, 54% genotypes were susceptible and 18% were highly susceptible. The rice genotypes viz., Pokkali, FL478, Kuliyadichan, Gurukot and IR12L-107 revealed significant level of tolerance to salt stress. In the current study Pokkali did not perform well. In another study Pradheeban *et al.* (2015) screened twenty two rice cultivars against five salt levels (0, 4, 8, 12 and, 24 dSm-1). Values of all tested variables decreased with increasing salt levels in all tested cultivars except for sodium to potassium ratio in shoots. Among the cultivated rice cultivars, based on shoot and root parameters and sodium to potassium ratio in shoots, Pachaperumal, Periavellai, At 303, Adakari, Bg 406 and CO 10 categorized as highly tolerant group while Bg 250, At 353, At 362, Modaikarupan, H4, Bg 304 and Morungan were grouped as tolerant. Also Bg 352 and At 308 found susceptible and Bg 360 seems very susceptible. The

varieties M-4 and JS-1 showed tolerance against salinity stress in hydroponics in the present study.

Binodh *et al.* (2019) screened a total of 50 indigenous rice genotypes collected from rice growing regions of Tamil Nadu in hydroponics for their drought tolerance ability. The germination percent (GP) and mean plant lengths were significantly varied in stress and control plants. The results suggested that the genotypes kuliyadichan, chandaikar, mallikar, mattaikar, rajalakshmi and nootripattu are tolerant against drought stress. It is concluded that these genotypes can be used as donor candidates towards genetic improvement of drought tolerance (DT) in rice. In the current study genotypes *viz*, N22, Pokkali, M-4, JS-1 and Akshaya were selected as tolerant varieties for drought.

Almeida *et al.* (2016) studied about the screening methods for multiple stress tolerance in rice. For the salt and cold stress assays they used hydroponic cultures, while for the drought assay plants were grown in soil and subjected to water withholding. All setups had enabled visual score determination and were suitable for sample collection along the imposition of stress. The methodologies used were simple and affordable to implement in most labs, allowing the discrimination of several rice genotypes at the molecular and phenotypic level. In the current study in which hydroponic cultures were used for the screening of multiple abiotic stress tolerance, the selected varieties were JS-1, M-4 and NPT culture-1.

5.2 Screening of genotypes for temperature stress using Temperature Induction response technique

Using this standardized TIR protocol, highly thermo tolerant rice genotypes were screened from 72 rice germplasm by Sudhakar *et al.* in 2013. Among the genotypes, NLR-145 showed highest thermo tolerance in terms of 100 per cent seedlings survival and no reduction in root and shoot growth. NLR-40066, NLR-40070 and NLR-40050 also showed higher thermo tolerance in terms of seedlings survival and no reduction in root and shoot growth. This study revealed that TIR technique can be used for identification of thermo tolerant rice genotypes. The identified rice varieties can be used as donor source for

developing high temperature tolerant rice genotypes to withstand future temperature rise.

In the present study Akshaya, JS-1, and NPT culture-1 showed tolerance against high temperature. Their performance was comparable with the performance of N22. In an earlier study of landraces, Apo and Norungan exhibited lesser decrease in root growth and shoot growth with 12% mortality. So they were considered as thermo tolerant on comparison with N22 (Vijayalakshmi *et al.,* 2015). In another study Chenellu and Njavara showed less reduction in root and shoot growth along with lesser mortality rate. They exhibited high chlorophyll stability index and less lipid peroxidation in terms of malondialdehyde content values, which confirmed the physiological basis for resistance against temperature stress (Beena *et al.,* 2018).

N22 (Nagina 22) is considered as the universal check for high temperature tolerance (Ye *et al*., 2015). A number of heat shock proteins showed upregulation at anthesis for high temperature stress. The heat tolerance may be the result of this (Jagadish et al., 2010). N22 showed drought tolerance too in this study. N22 is reported as an early maturing, deep rooted, drought tolerant genotype adapted to upland conditions (Kumar *et al.,* 2015).

5.3 Molecular characterization of rice genotypes

RM316, RM302 and RM511 (Drought specific), RM493, RM3412 and RM10793 (salinity specific) RM7076, RM5749, RM26212 were the primers used in this study. The expected product size for each primer were retrieved from the Gramene database.

5.3.1 Drought tolerance

As JS-1 showed amplification for RM316, RM511 and RM302, it can be considered as drought tolerant. Phenotype study is also supporting this conclusion. Multiple abiotic stress tolerance was shown by NPT culture-1 in the screening process which is supported by the genotyping as it showed amplification for two of the primers.

Salam *et al.,* (2017) screened 21 varieties for drought stress using PEG and molecular characterization were done using markers RM103 and RM 212.

Polymorphism was obtained for RM 212 only. But linkage between marker and seedling vigour index was not significant. In this study also some varieties are not showing linkage between phenotyping and genotyping.

In a study conducted in Srilanka, yield components were studied for phenotyping. In order to determine the relation between diversity in alleles and tolerance against drought of conventional varieties of rice, the Simple Sequence Repeat (SSR) marker analysis was used. Suwandal, Suduru samba and Kirimurunga landraces were identified as drought tolerant. Meanwhile, the SSR marker RM252 were better at identifying tolerant varieties than all other markers checked (Munasinghe *et al.,* 2017).

5.3.2 Salinity tolerance

In the current study Pokkali was selected as salinity tolerant variety even though it do not correlates with the results of phenotype studies. JS-1 and M-4 showed amplicons in RM3412 and RM10793 which supports the conclusion of phenotyping.

In a previous study RM 3412 along with other primers such as RM10745, RM 10772 and RM 10764 were identified as specific for salt tolerance. A combination of either two markers (RM 1287 and RM 3412) or four (RM 1287, RM 3412, RM 10764 and RM 10772) reported to be highly effective in categorizing tolerant and susceptible varieties (Kumari *et al.,* 2019). Aliyu *et al*., in 2011 also identified RM493 and RM3412 as trait specific for salinity tolerance. They were also reported as better markers for classifying tolerant and susceptible varieties just below RM8094 (Islam *et al*., 2012)

5.3.3 High temperature tolerance

JS-1, Kalluruli and N22 had specific amplicons for RM7076. RM5749 had specific amplicons for N22, Akshaya and M-4. RM26212 showed specific bands in JS-1, N22, NPT culture-1, Culture-06-7, Akshaya and Supriya. So JS-1 and Akshaya can be considered as heat tolerant which correlates with the phenotyping.

An earlier study found that most of the markers for heat tolerance are held by chromosomes 11, 10, 8, 6, 4 and 3. For each QTL, the ratio of phenotypic

variance described were ranging from 17.1% for RM160 to 36.2% for RM3586. At the RM468-RM7076 and RM241-RM26212 intervals, four QTLs were identified for filled grains per panicle on chromosome 4, elaborating 13.1 and 31.0 percent of overall variance in phenotype, respectively. Two QTLs regulating the proportion of unfilled grain were also located on chromosome 3 at loci RM554 and RM3686, explaining 25.0 and 11.2 percent of the overall phenotypic variance. For 1,000-grain weight at locus RM103 on chromosome 6, one QTL was observed, describing 30.6% of the overall variance in phenotype. A QTL on chromosome 4 at the locus RM5749 was also found, describig 10.8 percent of the overall variance in phenotype of total yield of grain (Buu *et al.,* 2014)

JS-1 can be selected as the most promising variety as it performed well under drought and high salinity conditions and it also performed well in Temperature Induction Response technique during phenotyping. It also showed specific amplicons for all the primers used in this study except for RM5749 and RM493. NPT culture-1 can be selected as a promising variety too as it performed well in both phenotyping and genotyping

6. SUMMARY

The study entitled “Screening and characterization of rice genotypes for abiotic stress to develop climate resilient variety” was done at Regional Agricultural Research Station, Pattambi during the period September, 2019- September 2020.

Twelve KAU varieties released and varieties in breeding pipeline used in this experiment were Akshaya (PTB61), Supriya (PTB62), Vyttila-9, Manurathna, Kalluruli, Pokkali, N22, Culture-06-7, NPT culture-1, M-4 and M-9. Some of them are already released varieties and some of them are in pipeline. These varieties were screened for abiotic stress under laboratory conditions

Hydroponics technique was utilized for the phenotyping of these varieties. 4 sets of each variety were maintained for 25 days. After 25 days of observation, the total seedling length was measured and seedling vigour index was calculated. Considering the data obtained N22, Pokkali, M-4, JS-1 and Akshaya were selected as drought tolerant. Salinity tolerant varieties identified were M-4 and JS-1. Multiple abiotic stress tolerant varieties selected were NPT culture-1, M-4 and JS-1. Thermal tolerance study was carried out by Temperature Induction Response technique. Akshaya, JS-1, M-9, N-22(check), and NPT culture-1 were selected according to the seedling vigour index.

Molecular characterization of these genotypes were done by using already reported SSR-RM markers. RM316, RM511 and RM302 were markers related to QTL for drought tolerance, RM493, RM3412 and RM10793 were markers related to QTL for salinity tolerance and RM7076, RM5749 and RM26212 were markers related to QTL for high temperature stress.

JS-1 showed amplification at expected product size range for RM316, RM511, RM302, RM3412, RM10793, RM7076 and RM26212 which are specific for drought, high salinity and temperature tolerance. It correlates with the result obtained by phenotyping. Even though M-4 performed well in phenotypic studies, its performance was not good enough in genotypic studies. NPT culture-

1 amplified for RM316, RM302, RM493 and RM26212. They are specific for drought, salinity and temperature tolerance respectively. It also showed tolerance for multiple stress in phenotyping. Akshaya showed drought tolerance and high temperature stress tolerance in phenotyping where as in genotyping amplification was for temperature specific primers only. As per this study JS-1 and NPT culture 1 can be selected as most promising varieties for climate resilience. These varieties can be evaluated in field for yield attributes and can be used as donor parents in crop improvement programs.

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APPENDIX I

Composition of CTAB Buffer:

(i) CTAB (10%)

10g CTAB was dissolved in sterile distilled H2O and volume was made up to 100 ml with distilled water.

(ii) Sodium chloride (NaCl, 4M)

23.37g of NaCl was dissolved in distilled H2O and volume was made upto 100 ml. The solution was autoclaved prior to use.

(iii) Tris: Cl buffer (pH 8.0, 1M)

12.11 g of Tris salt was dissolved in distilled H2O and volume was made upto 100 ml and pH was adjusted to 8.0 using 1 N HCl. The solution was autoclaved prior to use.

(iv) Ethylene Diamine Tetra Acetic acid (EDTA, 0.5 M)

18.62 g EDTA was dissolved in sterile distilled H2O. The pH of the solution was adjusted to 8.0 using IN NaOH. The volume was made upto 100 ml using sterile distilled H2O and the solution was autoclaved.

(v) 2-Mercaptoethanol (2%)

2% solution provided by manufacturer was used directly.

APPENDIX II

Composition of buffers and dyes used for agarose gel electrophoresis (AGE)

1. TAE Buffer 50X

Tris base: 242 g

Glacial acetic acid: 57.1ml

0.5 M EDTA (pH=8): 100ml

2. Loading Dye (6X)

0.25% Bromophenol blue

0.25% Xylene cyanol

30% Glycerol in water

3. Ethidium bromide

The dye was prepared as a stock solution of Ethidium bromide (stock 10 mg/ml; working concentration 0.5 μg/ml (SRL) and was stored at room temperature in a dark bottle.

4. **Agarose -** 0.8 per cent (Genomic DNA) 2 per cent (for PCR samples).

"Screening and characterisation of rice genotypes for abiotic stress to develop climate resilient variety"

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ABSTRACT OF THESIS

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ABSTRACT

The study entitled as “Screening and characterization of rice genotypes for abiotic stress to develop climate resilient variety” was completed in Regional Agriculture Research Station, Pattambi, Palakkad during the period September 2019 to September 2020.

12 rice genotypes were screened morphologically using hydroponics. M-4, JS-1, Pokkali, Akshaya and N22 were selected as drought tolerant ones whereas M-4 and JS-1 alone were selected as saline tolerant ones. N22, M-9, JS-1, NPT culture-1 and Akshaya showed tolerance against high temperature in Temperature Induction Response reaction. Drought specific primers such as RM316, RM511 and RM302 were used for the charactrisation. JS-1 showed amplification for all the three primers whereas Kalluruli, NPT culture-1, Vyttila-9 Jyothsana and Manurathna had specific amplicons for two of the primers. Salinity specific primers used were RM493, RM3412 and RM10793. The variety Pokkali confirmed its tolerance against higher salt concentration by amplifying in all the three primers used. M-4 and JS-1 got amplified for two of the primers used. RM7076, RM5749 and RM26212 used as the screening sieve for high temperature tolerant varieties. N-22, being the universal check got amplified in all the three primers. JS-1 and Akshaya had amplicons for two of the primers which support the result of phenotyping. From the whole study, JS-1 was selected as the most promising genotype for multiple abiotic stress considering the performance in both phenotyping and genotyping. NPT culture-1 was selected as the second best performer in this study.