MOLECULAR CHARACTERIZATION OF RICE GENOTYPES HAVING VARIABILITY IN HEAT TOLERANCE

by

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THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"Molecular characterization of rice genotypes having variability in heat tolerance"** 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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LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Per cent
µg	Microgram
µl	Micro litre
µM	Micro Molar
AFLP	Amplified Fragment Length Polymorphism
bp	base pair
cm	Centi Metre
cM	CentiMorgan
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleotide Triphosphate
et al.	And other co workers
etc	Etcetera
g	Gram
g-1	Per gram

hrs	Hours
kb	Kilo base
M	Molar
MAS	Marker Assisted Selection
mg	Milli gram
Min	Minutes
ml	Millilitre
mM	Millimolar
nm	Nanometre
°C	Degree Celsius
OD	Optical Density
PCR	Polymerase Chain Reaction
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
sec	Seconds
SNP	Single Nucleotide

	Polymorphism
SSR	Simple sequence repeats
STS	Sequence Tagged Site
UV	Ultra Violet
V	Voltage

INTRODUCTION

1. INTRODUCTION

Rice is an important crop and consumed widely across the globe as staple food. India is one of the world's largest producer of rice and occupies second position in it's production. It accounts about 20% of the world rice production. India has the largest area under rice cultivation with an area of 44Mha. It is the dominant crop of the country and contributes 43% of the total food.

To meet the demand of the growing population and to achieve food security in country, the production levels need to be increased by 2mt every year. Current IPCC projections indicate that the mean global temperature will rise 0.2°C per decade in coming years. For every 1°C increase in temperature there will be 10% decrease in grain yield. High temperature especially high night temperature stress causes chalkiness and spikelet sterility in rice and contributes for the reduced grain yield.

The crop grows optimally between 20°C to 35°C and increase in ambient temperature of more than 10-15°C relative to the optimum growing temperature can constitute heat stress (Wahid *et al.*, 2007). Climate change will likely result in more extreme climatic conditions in irrigated lowland and rainfed upland regions in tropical countries. Heat injury can result in high spikelet sterility. Even just an hour of exposure to heat stress at anthesis could induce sterility and result in grain yield reduction (Jagadish *et al.*, 2007). The sterility mainly results from reduced anther dehiscence, low pollen production and low numbers of germinating pollen grains on the stigma (Prasad *et al.*, 2006).

Rice in Kerala is grown under diverse ecologies from irrigated lowland, rainfed upland, rainfed wetland and deep water condition. High temperature induced sterility has not been an important problem in irrigated systems earlier. But at present the temperature may go up to 39°C during second/third crop at Palakkad, Thrissur and Kuttanad tract of Alappuzha. These areas are the main rice growing part of Kerala. Ilangovan *et al.* (2011) found that the decreased trends of rainfall and temperature were changed abnormally and influenced the soil fertility and rice yield in Pattambi. The last five years (2003-2008) the annual mean minimum temperature have increased by 0.5°C and 0.42°C, respectively during the month of July and June months when compared to 58 years of data. Kole wet lands; the rice bowl of central part of Kerala had experienced a drastic reduction in yield during the second crop season of 2009-2010. The study conducted revealed that the yield decline was due to high temperature accompanied with pest and diseases and weed infestation (Nandini *et al.*, 2010). Hence, it is urgent and need of time to elucidate the morphological, biochemical and physiological basis of heat tolerance in rice.

Genetic improvement of any plant system depends upon the existence of genetic variability available for manipulation. Considerable genetic variability exists for tolerance to high temperature stress in rice which can be exploited to develop high temperature tolerant cultivars. Identifying genotypes that can withstand high temperature during anthesis by maintaining high spikelet fertility (heat-tolerant genotypes) would help breeders to develop varieties better adapted to future climates. The understanding of the mechanisms by which rice crop

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withstand high temperature is of critical importance for the improvement of rice production and productivity for the upcoming climate change. Considering the current and predicted rates of increase in night temperature, the negative impact of diurnal temperature on rice production is likely to be felt on a much wider scale, with significant yield losses. However, phenotypic selection for secondary traits is labour intensive. Molecular marker technology serves as a tool for selecting such complex traits and allows breeders to track genetic loci controlling drought resistance traits without having to measure the phenotype, thus reducing the need for extensive field testing over space and time. Molecular markers are also not environmentally regulated and can be detected at all stages of plant growth.

Therefore, the present study was undertaken with the following objective:

To study the variation in rice genotypes for heat tolerance using microsatellite markers.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Rice (*Oryza sativa* L.) is the major cereal crop grown worldwide and is the staple food for about a half of the world's population. It occupies 154 million hectares annually with a total production of 600 million tons all over the world which accounts for about 35–75% of the total calories consumed by more than 3 billion Asians (Khush, 2005). FAO's latest projection indicates that total rice consumption would increase from the present 472 to 533 million metric tons in 2030. India annually plant rice on a total area of 43.9 million hectares, which produces an average of 130 million metric tons of rice. Under current consumption rate of rice, the production needs to reach 158 million metric tons in next 10 years, with an annual growth rate of 2.4%, to satisfy the population, which will grow at 1.9% (Robin *et al.*, 2010).

There are considerable risks for rice production due to abiotic stresses that will aggravate more under future climate change factors like high temperature, drought, salinity, and submergence. Higher temperatures can adversely affect rice yields through two principal ways, namely

(i) high maximum temperatures that cause spikelet sterility and adversely affect grain quality

(ii) increased night time temperatures that may reduce assimilate accumulation.

On the other hand, some rice cultivars are grown in extremely hot environments, so that the development of rice germplasm with improved heat resistance can capture an enormous genetic pool for this trait. Rice is highly sensitive to heat at reproductive stages. High temperature often coincides with

other stresses in rice production, namely drought in inland areas or submergence in coastal areas. There is vast information available on the impacts of high temperature stress on growth, physiological and yield characteristics of rice and has been reviewed for all the related parameters under following sub headings.

2.1. EFFECTS OF HIGH TEMPERATURE IN RICE

2.1.1. Vegetative phase

During vegetative stage, rice can tolerate relatively high temperatures (35/25°C; day/night temperature regime). Increase in temperatures beyond this critical level could reduce plant height, tiller number and total dry weight (Yoshida *et al.,* 1981). There was a decline in the photosynthesis that can be attributed to structural changes in the organization of thylakoids due to loss of stacking of grana in the chloroplast or its ability to swell (Wahid *et al.,* 2007). Moreover, membranes that house these cell organelles, are extremely important and sensitive to high temperatures, as it can increase the kinetic energy, and in turn the molecular movements, to loosen the bonds between biological membranes (Wahid *et al.,* 2007). Moisture deficit stress during vegetative stage can cause reduction in the tillering and rooting capacities, root function, leaf senescence or even death, and result ultimately in decrease of effective heads and yield loss (Mackill *et al.,* 1996).

2.1.2. Reproductive phase

Reproductive stage in rice is more sensitive to heat than the vegetative stage (Yoshida *et al.,* 1981). Anthesis/flowering, identified with the appearance of the anthers, is the most sensitive process to high temperature during reproductive

stage (Nakagawa *et al.,* 2003; Satake and Yoshida, 1978) followed by microgametogenesis. Reciprocal studies with manual shedding of pollen from control plants onto the stigma exposed to high temperature and vice versa showed that the ability of the pistil to be fertilized remained unaffected even over a period of 5 days at 41°C (Yoshida *et al.,*1981). Hence, the male reproductive organ is mainly responsible for spikelet sterility under high temperature and has been targeted for increasing tolerance to warmer climates. Processes closer to the meiotic stage during tetrad formation and young microspore stage are most sensitive to high temperature during microsporogenesis (Yoshida *et al.,* 1981), similar to drought (Liu *et al.,* 2006). A significant reduction in pollen production at 5°C above ambient air temperature (Prasad *et al.,* 2006) was attributed to impaired cell division of microspore mother cells. Heat stress during anthesis leads to an irreversible effect with stagnation in panicle dry weight even with subsequent improvement in the environment. Water deficit (Liu *et al.,* 2006) during flowering causes spikelet sterility in rice by affecting anther dehiscence, pollination and pollen germination. Mackill *et al.* (1996) reported that if the drought occurs at critical reproductive stage of rice, damage to panicle initiation and the meiosis of pollen mother cells occurs. They have also reported that under moisture deficit, spikelet and anthers dessicate, anther dehiscence and pollen shedding is disturbed and panicle exertion is inhibited, which result in severe reduction of seed setting.

2.2. EFFECT OF HEAT DEFICIT STRESS ON ANTHESIS

Rice genotypes can either escape or avoid high temperatures during anthesis, by heading during the cooler periods of the season (macroescape), by anthesing during cooler hours of early morning (microescape, *O. glaberrima* sp. Yoshida *et al.,* 1981), altering flowering pattern or by increased transpirational cooling of the canopy. Advancing peak anthesis toward early hours of the morning (Prasad *et al.,* 2006), is an efficient strategy to escape high temperatures during later hours of the day. Significant genotypic variation for early morning peak anthesis exists in rice germplasm and can be utilized. Moreover, rice has the ability to monitor and control the rate of flowering as an escape mechanism under high temperature (Jagadish *et al.,* 2007). Rice plants when exposed to high temperatures during critical stages can avoid heat by maintaining their microclimate temperature below critical levels by efficient transpirational cooling. Lower relative humidity of 60% at 38°C leads to a higher vapor pressure deficit of 2.65 facilitating the plant to exploit its transpiration cooling ability (Jagadish *et al.,* 2007). Moreover, Weerakoon *et al.,* (2008) using a combination of high temperatures (32-36°C) with low (60%) and high (85%) RH recorded high spikelet sterility with simultaneous increase in temperature and RH. Thus, the reduction in spikelet temperature in relation to RH is avoidance while the performance of a variety at a given spikelet temperature is true tolerance (Wassmann *et al.,* 2009). Heat avoiding genotypes thrive well in hot and dry rice cultivation regions of the world while for hot and humid regions either heat escape or true tolerance is essential to maintain productivity. However, with

predicted increased mean surface air temperature rather than just increased maximum temperature, the rice plant could be exposed to higher day and night temperatures further indicating the importance of true heat tolerance (Wassmann *et al.,* 2009). Increased heat tolerance is most needed in *O. sativa* spp., compared to *O. glaberrima* spp., as *sativa* spp. exhibit peak anthesis during late morning till mid afternoon (Yoshida *et al.,* 1981), exposing the heat sensitive reproductive organs to high temperatures, invariably leading to increased spikelet sterility (Jagadish *et al.,* 2008; Prasad *et al.,* 2006). Moreover, *O. sativa* spp., occupy major rice growing regions of Asia and is exponentially increasing in the African continent. High temperatures induce sterility, if the sensitive physiological processes (anther dehiscence, pollination and pollen germination on the stigma, pollen tube growth or the early events of fertilization) are affected. Anthesis in rice is extremely sensitive to hourly time course of high temperature sensitivity and spikelet opening on any flowering day during the flowering period (5-7 days) could be affected differently depending on the duration of exposure (Jagadish *et al.,* 2007). Drought stress delayed the flowering time of genotypes on average by 3±0.22 days. This delay in flowering time was associated with greater reduction in grain yield, harvest index, and filled grain percentage (Pantuwan., 2002). Pantuwan. (2002) also showed a strong association between delay in flowering time with low panicle water potential (r = -0.387); and hence genotypes with larger delay in flowering time suffered more drought stress since they flowered when available soil water was lower. The stage of pollen meiosis has been suggested as a very drought sensitive stage. This stage occurs about 10 days

before anthesis, and stress at that time is expected to reduce the number of normal pollen grains. In pot experiments with water stress beginning 7 days before flowering, anther dehiscence declined sharply at midday leaf water potentials of less than -1.5 MPa, and pollen viability also decreased (Ekanayake *et al.,* 1990). Genotypes that were able to maintain high panicle water potential (PWP) during the drought stress period that developed just before flowering time produced higher grain yield, harvest index, filled grain percentage, and fertile panicle percentage (Pantuwan. 2002).

2.2.1 Anther dehiscence

Anther dehiscence is the most susceptible process during anthesis under high temperature (Matsui *et al.,* 1999b). The increased vapor pressure deficit and enhanced evaporation from the anthers, caused by high temperatures, deprives the crucial moisture needed for pollen grain swelling leading to poor anther dehiscence. Also, increased pollen stickiness in water stressed anthers under heat and drought stress may lead to poor dehiscence (Liu *et al.,* 2006). With incomplete anther dehiscence, only a few pollens are able to reach the stigma surface, thereby leading to enhanced spikelet sterility.

2.2.2. Pollen viability

Rice pollen is extremely sensitive to temperature and relative humidity (Matsui *et al.,* 1997b) and loses its viability within 10 min of shedding (Song *et al.,* 2001). The presence of >20 germinating pollen on the stigma was shown to be associated with fertility at high temperature of 38°C (Matsui *et al.,* 1997a).

Developmental processes beyond pollen germination may also be sensitive to heat and have been shown in rice (Wassmann *et al.,* 2009).

2.3. EFFECT OF HEAT DEFICIT STRESS ON PHYSIOLOGICAL PARAMETERS

Rice, like other cultivated crops, has relatively variable temperature preferences over the growing season. The response of rice to high temperatures and severity of the possible damage differs according to the developmental stage (Wahid *et al.,* 2007). High temperatures are known to have deleterious effects on photosynthesis, respiration and reproduction. One can refer to threshold temperature as the value of daily mean temperature at which a detectable reduction in growth begins. The threshold values have been determined both for higher and lower temperatures through various experiments both at greenhouse and field levels. The magnitude of heat stress rapidly increases as temperature increases above a threshold level and complex acclimation effects can occur depending on the temperature and simultaneous presence of other stresses.

Siddique *et al.,* (1999) reported that under high temperature plants may divert photosynthates to cope with heat stress leaving limited amount for reproductive development, which can diminish seed set and seed yield and reduce weight. Another effect of heat stress in many plant species is induced sterility when heat is imposed immediately before or during anthesis (Jagadish *et al.,* 2007). Jagadish *et al.,* (2007) reported that that spikelet tissue temperature above the threshold temperature of 33.7°C for an hour at anthesis was sufficient to induce spikelet sterility. Variable heat tolerances among rice genotypes during

flowering are known (Yoshida *et al.,* 1981, Matsui *et al.,* 2001, Prasad *et al.,* 2006), which can be attributed, to their different temperature thresholds (Nakagawa *et al.,* 2003). However, since Prasad *et al.,* (2006) identified heat tolerance in both sub spp. of *O. sativa*, no generalization can be made that either *indica* or *japonica* sub spp. are more tolerant than the other contrary to the earlier observation by Satake and Yoshida (1978). Also, experimental evidence for interaction between high temperature and duration of exposure was recently documented (Jagadish *et al.,* 2007).

2.3.1. Relative water content

Plant water content is the most important variable under changing ambient temperatures (Mazorra *et al.,* 2002). Under ample moisture, plants are able to maintain stable tissue water content even at higher temperatures. However, under moisture limiting conditions this tendency is largely impaired and the plants may show altered leaf water potential and its components. This may be due to the detrimental effect of heat on root hydraulic conductance (Wahid *et al.,* 2007). High temperatures seem to cause water loss in plants more during daytime than during nighttime, as rice plants when exposed to high temperatures tend to avoid heat by maintaining their microclimate temperature below critical levels by efficient transpiration cooling. This may lead to decrease in water potential and perturbation of many physiological processes. In wheat genotypes, the relative water content was found to decrease with increase in atmospheric temperature and the decrease was more pronounced in susceptible cultivars, particularly under late sown conditions (Sairam *et al.,* 2000). Sakamoto and Murata (2002) have reported

the accumulation of compatible osmolytes such as glycine-betaine under heat stress, so as to buffer cellular redox potential. The accumulation of such solutes may also have a bearing on tissue water potential.

2.3.2. Membrane thermo stability

Cell membrane stability or the reciprocal of cell membrane injury is a physiological index widely used for the evaluation of drought and temperature tolerance. Cell membrane thermostability can be related to the capacity for sustained grain filling under heat stress. Quantitative electrolyte leakage or cellular membrane thermostability (CMT) has been used as a measure of heat tolerance during the vegetative stage in many crops (Prasad *et al.,* 2006; Tripathy *et al.,* 2000). The membrane stability index decreased under heat stress treatment in rice genotypes and a greater reduction was observed in susceptible varieties (Tripathy *et al.,* 2000). There is also a strong correlation between cellular membrane stability and grain weight. Leaf membrane injury affects carbon production, consumption, transport and accumulation. In wheat, susceptible genotypes showed high thermal sensitivity concerning membrane lipid peroxidation and membrane permeability (Dias *et al.,* 2010). Heat stress accelerates the movement of molecules across membranes and loosens the chemical bonds making the lipid bilayer of biological membranes more fluid by either denaturation of proteins or an increase in unsaturated fatty acids.

2.4 HEAT TOLERANCE

Episodes of high temperature at anthesis in rice crop, which is the most sensitive stage to temperature are expected to occur more frequently in the future. These changes occurring in the climate would affect the spikelet fertility, sink size, shortening of growth period and increase in maintenance respiration. In addition to yield reduction, several quality traits like head rice recovery (HRR), chalkiness, amylose content and gelatinization temperatures are also affected. Hence, it is necessitated to identify the genotypes possessing heat tolerance, especially at anthesis. Keeping the afore said in view, it is proposed to screen the rice germplasm to identify the genotypes with desirable heat tolerance levels at different stages of crop growth in order to tailor those traits in developing climate resilient rice genotypes to mitigate the changes in climate. Sato (1979) studied high temperature damage to ripening in rice. He concluded that there is reduction in 1000-kernel weight in plants treated with high temperature, two to one week before flowering. Mackill *et al.* (1982) determined the combining ability of six rice lines for high temperature tolerance at anthesis. They reported that the tolerant lines N-22, IR-2006 and IET 4658 had general combining ability effects of 6.8, 4.08 and 3.02, respectively and the susceptible lines IR28, IR1561 and IR52 had general combining ability effects of -3.4, -4.92 and -5.58, respectively.

Sarwar *et al.* (1985) evaluated 1089 rice germplasm for high temperature tolerance. They reported that only 4 lines from Punjab showed panicle sterility under 10% and 164 (76-s coarse rice), 223 (saunfia), 242 (coarse variety), 245 (Baggi) were relatively better tolerant to high temperature.

Matsui *et al.* (1997) studied the effects of high temperature and CO_2 concentration on spikelet sterility in indica rice. They reported that the cause of sterility in heat sensitive cultivars is reduction in the number of deposited pollen grains on a stigma, although at higher temperatures (>40^{0}C) female sterility can also occur. Gui *et al.* (1995) evaluated 320 accessions in the greenhouse and identified four accessions as highly heat resistant, 50 accessions as moderately resistant. Matsui *et al.* (2001) studied the relationship between morphological characteristics of anthers and fertility in japonica rice cultivars subjected to high temperature at flowering. They concluded that the tight closure of the locules by the cell layers delayed locule opening, and decreased fertility at high temperatures.

Yu *et al.* (2004) developed near isogenic rice lines expressing heat tolerance during the grain filling stage using Hsien pure line BG33-2 (strong heat tolerance) as donor, and Ganzaoxian38 (heat sensitive) as the recurrent parent.

Matsui *et al.* (2005) reported that the relationship between the length of dehiscence at the basal part of thecae and the viability of pollen in 18 cultivars of rice subjected to a hot and humid condition for three days at flowering. Their results suggested that the low pollen viability in the non japonica type cultivars is associated with their small basal dehiscence on the thecae, and the length of basal dehiscence can be used as a selection marker of high temperature tolerance.

Prasad *et al.* (2006) studied decrease in spikelet fertility at high temperature and reported that the cultivar N-22 was most tolerant, while cultivars L-204, M-202, Labelle, Italica Livorna, WAB-12, CG-14 and CG-17 were highly

susceptible and cultivars M103, S102, Koshihikari, IR-8 and IR-72 were moderately susceptible to high temperature.

Jing *et al.* (2006) reported that Z2001 and 868 were highly tolerant, whereas Nongkeng 57 and Wuxiangjing 14 were moderately tolerant to heat out of 16 rice cultivars and concluded that increasing the rate of N, P, K or P + K applied at the heading stage was not effective in delaying spike initiation.

Jagadish *et al.* (2007) studied the effect of high temperature at anthesis on spikelet fertility in IR64 (low land indica) and Azucena (upland japonica) at 29.6°C (control), 33.7°C and 36.2°C tissue temperature. They concluded that in IR64, there was no interaction between temperature on duration of exposure, and spikelet fertility but in Azucena there was a significant interaction.

Jagadish *et al.* (2008) reported that exposure to high temperatures for 6 hours reduced spikelet fertility and fertility was lower at 38°C than at 35°C.They concluded that the check cultivar N22 was the most heat tolerant genotype and cultivars Azucena and Moroberekan the most susceptible.

Ying *et al.* (2008) reported that heat stress significantly reduced anther dehiscence and pollen fertility rate of Shuanggui 1 (heat sensitive), while it much less affected those of Huanghuazhan (heat tolerant). They also reported that the number of spikelets per panicle, seed-setting rate, and 1000-grain weight were significantly decreased under heat stress in both cultivars, leading to a significant reduction in grain yield, with more reduction in Shuanggui 1 than in Huanghuazhan and heat stress significantly reduced root oxidation activity and ribonucleic acid (RNA) content of young panicles, and significantly increased

malondialdehyde (MDA) content of leaves and ethylene evolution rate of young panicles, and the extent of the decrease or increase was more in Shuanggui 1 than in Huanghuazhan.

Singh *et al.* (2008) reported that high temperature tolerance has been genetically engineered in plants mainly by over expressing the heat shock protein genes or indirectly by altering levels of heat shock transcription factor proteins. They also noticed that apart from heat shock proteins, thermo tolerance has also been altered by elevating levels of osmolytes, increasing levels of cell detoxification enzymes and through altering membrane fluidity.

Ying *et al.* (2009) reported that the relative high yield in heat tolerant genotypes under high temperature stress is associated with low leaf temperature, high root activity, high levels of ATP ase activity in grains, photosynthetic rate, and activities of antioxidant enzymes in leaves.

Min *et al.* (2009) reported that heat treatment could up-regulate the expression of all six genes, and the expression of these genes also increased in filial generations after heat treatment, indicating that these genes could be inherited and play important roles for rice under high temperature stress in filial generations.

Kobayashi *et al.* (2011) reported that the percentage of sufficiently pollinated treatment. At a daytime temperature of 37.5°C, %SPF varied widely among the cultivars and was highly correlated with the length of dehiscence formed at the basal part of the theca and the percentage of dehisced thecae. The factor that better explained the variation in %SPF shifted from the length of the

basal dehiscence to the percentage of dehisced thecae with increasing duration of high temperature treatment.

Shah *et al.* (2011) reported that flowering at cooler times of day, more pollen viability, larger anthers, longer basal dehiscence and presence of long basal pores are some of the phenotypic markers for high temperature tolerance and protection of structural proteins, enzymes and membranes and expression of heat shock proteins (HSPs) are some of the biochemical processes that can impart thermo tolerance.

2.5 GENETIC DIVERSITY

Many approaches have been suggested for selection of diverse parents for hybridization programme *viz., per se* performance, eco geographical diversity, genotypic value, combining ability, multivariate analysis etc. Earlier, geographical diversity has been considered as a remarkable index of genetic diversity (Joshi and Dhawan, 1966). Now with the development of biometrical techniques, it is possible to assess the divergence based on genetic criteria for a set of characters utilizing multivariate analyses like distant analysis, canonical analysis, factor analysis and cluster analysis. In addition to estimation of genetic diversity multivariate analysis and cluster analysis also help in selection of diverse parents for hybridization programme, to know the characters contributing for maximum diversity.

In general the clustering techniques in a cluster analysis may be hierarchical or non hierarchical. For biological solutions hierarchical clustering techniques are most commonly used for grouping the data. In a hierarchical

classification the data is subjected to series of partitions that run from a single cluster containing all individuals to n clusters each containing a single individual. These hierarchical clustering techniques can be further sub divided into agglomerative and divisive methods, which separate n individuals successively into finer groupings. Among the agglomerative and divisive methods the former is most commonly used than the latter. Some of the agglomerative hierarchical clustering techniques that are most widely used are single linkage clustering or nearest neighbouring technique (Sneath, 1957 and Johnson, 1967), complete linkage clustering, group average clustering, centroid clustering, median clustering and Ward's clustering method (Ward, 1963). Divisive clustering techniques are essentially of two types, monothetic that divide the data on the basis of single specific attribute, and polythetic, where divisions are based on the values taken by all attributes.

Hierarchical clustering techniques impose a hierarchical structure on data and it is usually necessary to consider whether this is merited or whether it introduces unacceptable distortions of the original relationships amongst the individuals. The method most commonly used for assessing the match between the dendrogram and distance matrix is 'Cophenetic correlation coefficient'.

2.6 MOLECULAR MARKERS BASED GENETIC DIVERSITY IN RICE

Mackill *et al.* (1996) compared a level of polymorphism on a sample of 14 rice accessions including several closely related japonica cultivars and two indica cultivars using AFLP, RAPD and microsatellite markers. For AFLP, using 17 primers combination with seven primer and two enzymes (Eco RI, Mse I) 529

bands were generated out of which 147 were polymorphic accessions. With 21 RAPD primers, 43 bands showed polymorphism out of total 103 amplified. For microsatellite markers, the number of alleles per locus ranged from one to six with average of 3.3. All marker types gave same classification of rice accession into sub species. Within japonica cultivars the average per cent polymorphism between any two accessions was 29, 33 and 47 percent for AFLP, RAPD and microsatellite respectively. The average per cent polymorphism between indica and japonica accessions was 65 per cent for AFLP, 34 per cent for RAPD and 76 per cent for microsatellite respectively.

Akagi *et al.* (1997) studied the polymorphism of microsatellite loci using 59 japonica cultivars including both domestic and modern Japanese cultivars. Microsatellites consisting of AT repeat showed high gene diversity even within genetically closely related cultivars. A percent diagnosis using 17 microsatellite loci demonstrated that plants that carried desired chromosome region had been selected in breeding programmes. These hyper variable microsatellites consisting of AT repeats should promote the selection of plants, which carry desired chromosome from genetically similar parents. Back crossing could help to eliminate unnecessary chromosome regions with microsatellite polymorphism at an early stage in breeding programme.

Sebastein *et al.* (1998) assessed the nuclear diversity of 78 rice cultivars developed by IRRI, the Philippines Rice Research Institute Bureau of Plant Industry and University of Philippines from 1968 to 1995 using 41 microsatellite markers representing 41 loci with a total of 174 alleles. Over the 30 years of

improvement, the molecular diversity of material being produced had increased slightly and the number of frequently occurring alleles had decreased. The diversity was highest in irrigated lowland rice cultivars; intermediate in the upland material and lowest in the rainfed low land cultivars.

Kwon *et al.* (1999) estimated the genetic diversity within 25 japonica and six indica varieties using a combination of 19 microsatellite and 28 random decamer primers. Microsatellites showed polymorphism among all the varieties by revealing 91 alleles with an average of 48 bands per primer. The decamer primer generated 114 nonredundant bands with a mean of 4.07 bands per primer. They concluded that the microsatellite primers detected more alleles than random primers, although the mean difference was statistically non-significant.

Garland *et al.* (1999) used ten microsatellite markers for analyzing diversity in a set of 43 cultivars from Australia and concluded that the microsatellite markers are useful for cultivar identification and assessment of genetic relationships.

Sun *et al.* (2000) evaluated the genetic diversity of cultivated rice and common wild rice from 10 countries in Asia by RFLP analysis using 44 probes of single copy. They reported that the common wild rice from China has the largest genetic diversity in 10 countries and the secondary is the common wild rice from India.

Davierwala *et al.* (2000) studied the genetic diversity among 42 indian elite rice varieties with three different types of markers (RAPD, ISSR and STMS). A total of 168 bands were amplified out of which 153 bands (91per cent) showed

polymorphism, when data for all the three markers were considered together and concluded that three molecular marker systems together provide wider genome coverage and are therefore, the better indicator of genetic relationships among 42 elite rice cultivars than those revealed by individual molecular markers.

Luce *et al.* (2001) evaluated 419 cultivars of rice from gene banks in five European countries with reference to a collection of 57 Asian cultivars. The cultivars were examined using 16 microsatellite markers. The structure of rice from European collection is almost exclusively bipolae; 85 per cent of varieties are from the sub species japonica and 13 per cent from sub species indica. Average polymorphism depicted was 9.1 alleles per locus. All cultivars were identified and classified on the basis of their allelic patterns. A total of 338 accessions had different genotypes among 419.Groups with varietal affinities were identified using a method of hierarchial classification. The microsatellite polymorphism revealed that 82 cultivars displayed an intervarietal polymorphism with two alleles on one or more loci.

Qian *et al.* (2001) studied the genetic variation within and between five population of *O.granulata* from two different regions of China using RAPD and ISSR markers. A total of 61 polymorphic loci were detected out of 199 amplified bands using 20 RAPD primers. However, 52 polymorphic loci were detected from the 113 amplified bands using ISSR primers. Both the marker types used in the study revealed the low level of genetic diversity in wild population studied. As with RAPD marker it revealed 73 percent of total genetic diversity resided between the two regions, whereas, only 19 and 6 per cent were present between

populations within the region and within the population respectively. However, ISSR marker showed that a great amount of variation (49 per cent) was present between two regions, with 98 per cent between population within regions and 12 per cent within population. Result indicated that the per cent polymorphism detected by ISSR was higher than that detected by RAPD markers.

Genetic variation and clonal diversity of seven *Psammochloa villosa* (Poaceae) populations from northwest China were investigated using Inter Simple Sequence Repeat (ISSR) markers. Of the 84 primers screened, 12 produced highly reproducible ISSR bands. Using these primers, 173 discernible DNA fragments were generated with 122 (70.5%) being polymorphic, indicating the presence of considerable genetic variation at the species level. In contrast, there were relatively low levels of polymorphism at the population level with the Percentage of Polymorphic Bands (PPB) ranging from 6.1 to 26.8 (Li and Ge, 2001).

Ni *et al.* (2002) used microsatellite markers to evaluate the genetic diversity in rice. About 111 microsatellite markers distributed over the whole rice genome were used to evaluate 38 cultivars of particular interest to US breeding programmes and one accession each of the wild species *O.rufipogon* and *O.nivara*. A total of 753 alleles were detected. The number of alleles per marker ranged from 1 to 17 with an average of 6.8. A positive correlation was found between the number of alleles per locus and maximum number of repeats with in a microsatellite marker. Compared to indica cultivars, japonica group showed significantly higher genetic diversity on chromosome 6 and 7 and lower diversity on chromosome 2. All rice cultivars and lines could be easily distinguished and

resulting group corresponded exactly to indica and japonica sub species with japonica divided into temperate and tropical types. The study suggested that microsatellite could be efficiently used for estimation of genetic diversity and identification of cultivars.

Sarla *et al.* (2003) delineate geographically, diverse *O. nivara* accessions using ISSR and SSR markers based on AG and GA repeats. Genetic diversity among 24 accessions of *O. nivara* from 11 states of India and four *O. sativa* varieties, one each from Glaszmann's isozyme groups I, II, V and VI were analyzed using ISSR and SSR primers. The primers based on AG and GA repeats were informative, and their resolving power ranged from 4.2 to 10.8 and PIC value from 0.64-0.89. Ten alleles out of 40 amplified at six loci were unique to an accession. Two accessions each from Uttar Pradesh and Bihar and one from Madhya Pradesh were distinct from other accessions. *O.nivara* alleles in common with Jaya, Dular, Basmati 370 and Taipei 309 were identified.

Ravi *et al.* (2003) estimated genetic diversity among the 40 cultivated varieties including hybrids and their A, B and R lines and five wild relatives of *O.sativa L.*(One of *O.nivara*, three of *O.rufipogon* and one of *Portereisia coractata*) using SSRs and RAPD markers. A total of 36 decamer RAPD primers and 38 SSR primers were used to evaluate for polymorphism. A total of 499 RAPD loci were produced with a polymorphism percentage of 90. By using SSR the highest PIC value was 0.89. They concluded that the two-marker system contrasted most notably in pair by pair comparison of relationships and SSR analysis resulted in a more definitive separation of clusters of genotypes

indicating the higher level of efficiency of SSR markers for the accurate determination of relationships between accessions that are too close to be accurately differentiated by RAPD markers. Twenty two ISSR markers were used for diversity studies in rice, 34 polymorphic bands were generated. In collection of barley accessions, 137 polymorphic bands were yielded, and the PPB was 88.4%. The result from the clustering analysis by un-weighted pair group method arithmetic average (UPGMA) indicated that those accessions (rice or barley) from the same geographical location could be clustered into one group (Lin *et al.*, 2005).

Neeraja *et al.* (2005) studied a set of tall races of rice using gene derived simple sequence repeats and to compare the level of gene derived SSRs with other SSRs of rice. Experimental material comprises of a set of 21 tall rice landraces and a set of eight check genotypes, which includes five semi dwarf/semi tall non lodging, high yielding varieties and three tall landraces which were not responsive to nitrogen under propped condition in terms of grain yield. These were characterized using 17 gene derived SSRs and eight other genomic SSRs. Gene derived SSRs had lower polymorphism information content (0.68) than the other SSRs (0.87). UPGMA analysis of gene derived SSR data separated tall landraces and high yielding varieties which were nitrogen responsive into major cluster and the other three landraces which were non responsive to nitrogen into minor cluster at 0.37 similarity. The use of gene derived marker that target functional loci appears to be an effective strategy in characterizing landraces for optimizing choice of parents for hybridization programmes.

Ram *et al.* (2005) analysed genetic diversity among 35 rice accessions which included 19 landraces, 9 cultivars and 7 wild relatives using SSR markers. They reported that the genetic diversity was the highest among wild relatives (0.436) followed by land races (0.356) and the lowest for cultivars.

Lapitan *et al.* (2007) analysed twenty four rice cultivars carrying good quality traits for genetic diversity using 164 markers. They concluded that between the two rice subspecies, indica gave more alleles compared to japonica and likewise displayed a higher genetic diversity.

Alvarez *et al.* (2007) studied microsatellite polymorphism in a sample of 39 traditional rice (*Oryza sativa* L.) varieties and 11 improved varieties which are planted in Cuba. They concluded that Higher heterozygosity (H) was found in traditional varieties (HTV = 0.72) than in improved varieties (HIV = 0.42), and 68% of the total microsatellite alleles were found exclusively in the traditional varieties.

Rabbani *et al.* (2008) evaluated the genetic polymorphisms and identities of 10 traditional, 28 improved and 2 Japanese cultivars of rice using the random amplified polymorphic DNA technique. They reported that 40 cultivars were grouped into 3 main clusters corresponding to aromatic, non-aromatic and japonica groups.

Herrera *et al.* (2008) used a set of 48 simple sequence repeat (SSR) markers to assess the genetic diversity of 11 Venezuelan rice cultivars, and concluded that Venezuelan rice cultivars are showing low diversity.

Seetharam *et al.* (2009) characterized thirty rice genotypes comprising land races, pure lines, somaclones, breeding lines and varieties specifically adapted to costal saline environments by SSR markers. They reported that out of 35 primers of SSR markers, 28 were found to be Polymorphic and there was overlapping of tolerant genotypes and susceptible genotypes with in cluster. The genetic diversity in 370 aromatic rice germplasms introduced from home and abroad, was investigated by using a total of 60 SSR markers. There was significant differentiation between Southern China and South western China, Central China and South western China, respectively. Cluster analysis showed that 370 aromatic rice varieties were mainly classified into indica and japonica, and aromatic rice varieties from the same areas or the neighbouring provinces were basically assigned to the same group (Tang *et al.*, 2009).

Padmaja *et al.* (2011) analyzed the genetic diversity using twenty two SSR markers out of which seven markers exhibited polymorphism by generating 20 alleles. The result concluded that the use of SSR markers in genetic diversity studies helped in grouping the genotypes according to their genetic relatedness.

Singh *et al.* (2011) analyzed the genetic diversity of a set of 50 elite rice genotypes through SSR analysis. SSR marker analysis revealed distinct polymorphism among the cultivars with 28 primers and the polymorphic information content (PIC) value ranged from 0.139-0.99 with an average of 0.589 per primer. UPGMA Cluster analysis grouped the rice genotypes into eight clusters in which genetically similar type clustered together.

Federaci *et al.* (2011) analyzed the genetic diversity of 26 weedy accessions of weedy rice and 6 Uruguayan cultivars using AFLP methodology. They revealed three groups of samples and they reported that one group had a black hull, purple apex and long awn (wild type traits) while another group had straw hull and apex, and short or no awn (domestication traits) and the weedy rice in the third group is presumed to most closely mimic cultivated rice and may have recently evolved.

Ashfaq *et al.* (2012) analysed genetic diversity among rice genotypes, including 15 indica basmati advance lines and 5 basmati improved varieties by 28 SSR markers. They Reported that the dendrogram based on cluster analysis grouped the 20 genotypes of rice into five clusters based on their genetic similarity.

Choudhary *et al.* (2013) studied molecular genetic diversity of major Indian rice cultivars using 64 SSR primers. Cluster analysis clearly grouped 100 genotypes into their respective decadal periods i.e. 1970s, 1980s, 1990s and 2000s. Molecular marker analysis indicated that the genomic polymorphism between 703T and 704S was the smallest in the 6 pairs of rice lines, with only 16 polymorphic sites, including 22 different alleles.

Poli *et al.* (2013) reported that EMS induced mutant NH219 can tolerate heat stress more when compared with its wild type N22. NH219 showed lesser reduction in yield/plant and related traits compared to N22. Ribulose bisphosphate carboxylase large chain precursor (EC 4.1.1.39) was present in NH219 leaves and absent in N22 under ambient growth conditions. Both pollen viability and spikelet

fertility were significantly reduced in IR64 but not in N22 and NH219. Based on single marker analysis of F_2 mapping population from the cross between IR64 and NH219, it was observed that marker RM 1089 was associated with number of tillers and yield per plant, RM 423 with leaf senescence, RM 584 with leaf width and RM 229 with yield per plant.

Cluster analysis based on microsatellite allelic diversity clearly demarcated the landraces, cultivars and wild relatives into different groups. Seven rice landraces Rupsal, Nagalmutha, Ravana, Marishal, Polai and Talmugra were screened with three SSR markers (RM3735, RM3586 and RM6100) associated with heat tolerance at flowering stage. In PCR amplification, all seven genotypes showed monomorphic allele with RM3735 and RM3586 markers as well as polymorphic alleles with RM 6100 marker. This RM6100 marker was linked with a major quantitative trait locus (QTL) on chromosome 10 for heat stress tolerance at flowering stage (Bharathkumar *et al.*, 2014).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The study was conducted in the Department of Plant Physiology and the Department of Plant Biotechnology, College of Agriculture, Vellayani during August 2016 to August 2017 to study the "Molecular characterization of rice genotypes having variability in heat tolerance". The main objectives of this study was the variation in rice genotypes for heat tolerance using microsatellite markers.

3.1 PLANT MATERIALS

The material for this study contain 10 genetically diverse genotypes of rice which were received from National Rice Research Institute, Cuttack, Orissa and Regional Agricultural Research Station, *Pattambi* of these lines is given in Table 1.

Table 1. List of rice genotypes used for molecular diversity study

Sl.No.	Varieties	Sl.No.	Varieties
1	CR Dhan 307	6	PTB 7
2	APO	7	CR Dhan 202
3	NAGINA 22	8	CR Dhan 204
4	RAJALAXMI	9	CR Dhan 305
5	CR Dhan 701	10	AJAY

3.2 MOLECULAR DIVERSITY ANALYSIS OF RICE GENOTYPES USING SSR MARKERS

3.2.1 Plant materials

All the above mentioned 10 rice genotypes were sown in field in December 2016. About 21 days old plant was taken for further study.

3.2.2. DNA extraction

Total genomic DNA of 21 days old seedlings was extracted using CTAB method (Murray and Thomson, 1980) as described below:-

About 500-750 mg leaves were collected in paper bags. Washed under running tap water and dried over filter paper. Frozen leaf tissues (about 100 mg) were ground to a fine powder with liquid nitrogen using mortar and pestle and finely grinded powder was transferred to well labelled 2.0 ml autoclaved centrifuge tubes. 800 µl of CTAB buffer was added and incubated for 1 hour at 65^0C in water bath. During incubation, the contents were occasionally mixed four to five times by inverting the tubes gently. 800 µl of chloroform: iso-amyl alcohol (24:1) was added to the tubes and then put on the shaker for 30-40 minutes. 6. The mixture was then centrifuged in a microcentrifuge (Eppendorf Centrifuge 5415D) at 10,000 rpm for 15 minutes at room temperature. The upper aqueous phase was transferred to fresh tubes with the help of micropipette. Care was taken to avoid debris inclusion. About two- thirds volume of chilled isopropanol was added and mixed well by inverting the tubes. The samples were refrigerated at -20°C/-40^0C for 15 minutes followed by centrifugation at 10,000 rpm for 15 minutes to make a pellet. The supernatant was discarded. The DNA pellet was washed with 300 µl

70% ethanol and centrifuged at 10,000 rpm for 5 minutes. The ethanol was discarded and the pellet was retained. The pellet was air-dried and finally the purified DNA pellet was dissolved in 50- 100µl of TE buffer . It was kept overnight at room temperature. RNase treatment was given by adding 5µl of RNase at 37^0C for 1 hour. DNA pellet was stored at -20^0C for long term stability.

3.2.3 Quantification of extracted dna

Quantification of nucleic acids was performed by using 1000 spectrophotometer (ELICO, SL 218 UV-Vis Spectrophotometer). The optical surface of the spectrophotometer system was cleaned by putting 1-2µl deionised water on the lower optical surface. The lever arm was closed and tapped few times to clean the upper optical surface and both the optical surfaces were wiped with tissue paper by lifting the lever. A Blank (Water and TE buffer in which no DNA sample was dissolved) measurement was performed by loading 3ml of TE buffer. Measurement of the nucleic acid sample was done by loading 5µl of sample and selecting Measure. Pure nucleic acid typically yield a 260/280 ratio of ~1.80 for DNA. Value less than 1.8 indicates presence of significant concentrations of proteins and a ratio more than 1.8 indicates presence of RNA.

Concentration of the DNA was calculated by the formula shown below:

Concentration of the DNA µg /ml of sample = Optical density at 260nm X 50 X Dilution factor

3.2.4 Estimation of quality of DNA

Quality of DNA was checked by agarose gel electrophoresis. For agarose gel electrophoresis, 3 g of agarose was dissolved in 300 ml of 1X TBE electrophoresis buffer. The mixture was heated till the agarose dissolved completely, i.e. when the solution became transparent. It was cooled down to 60°C with constant stirring and 4-5µl of ethidium bromide (10mgml-1) was added to buffer. The agarose solution was then poured into already prepared gel mould with combs and was left for 30-40 minutes for solidification. DNA samples were prepared by adding loading dye (6X loading dye consists of 0.25% w/v bromophenol blue, 0.4% w/v sucrose or 30% glycerol in sterile water) to the DNA such that the final concentration of loading dye was 1X. After solidification combs were removed gently and the gel was placed in the gel tank for electrophoresis. DNA samples were loaded into the wells. After loading, the gel was subjected to electrophoresis at constant voltage of 5V/cm for about 1 hour. After electrophoresis, the gel was visualized under UV trans-illuminator and photographed with gel documentation system. The observations on the intactness of bands of DNA samples were taken which revealed the quality of our DNA.

3.2.5 Dilution of original genomic DNA

The concentration of DNA of germplasm lines was diluted to adjust the concentration to 25ng/µl for use in polymerase chain reactions.

3.2.6 PCR Analysis

3.2.6.1 Selection of primers

The primer sequences were synthesized from Promega as 25 nmol in dry form. These primers were diluted to 10μM working concentration using double distilled water. For the present study 50 SSR markers (Table 2) are used. These primers were evenly distributed on 12 rice chromosomes.

Table 2. List of primers used for genetic diversity

Sl.No.	Primer	Forward sequence	Reverse sequence
1	RM1003	GATTCTTCCTCCCCTTCGTG	TTCCTGTCAGAACAGGGAGC
2	RM169	TGGCTGGCTCCGTGGGTAGCTG	TCCCGTTGCCGTTCATCCCTCC
3	RM474	AAGATGTACGGGTGGCATTC	TATGAGCTGGTGAGCAATGG
4	RM525	GGCCCGTCCAAGAAATATTG	CGGTGAGACAGAATCCTTACG
5	RM167	GATCCAGCGTGAGGAACACGT	AGTCCGACCACAAGGTGCGTTGTC
6	RM263	CCCAGGCTAGCTCATGAACC	GCTACGTTTGAGCTACCACG
7	RM280	ACACGATCCACTTTGCGC	TGTGTCTTGAGCAGCCAGG
8	RM302	TCATGTCATCTACCATCACAC	ATGGAGAAGATGGAATACTTGC
9	RM271	TCAGATCTACAATTCCATCC	TCGGTGAGACCTAGAGAGCC
10	RM484	TCTCCCTCCTCACCATTGTC	TGCTGCCCTCTCTCTCTCTC
11	RM303	GCATGGCCAAATATTAAAGG	GGTTGGAAATAGAAGTTCGGT
12	RM7117	AGTTGGCTGGTTGCTACCAC	AGGGTTCCCTGGCTACTCAC
13	RM348	CCGCTACTAATAGCAGAGAG	GGAGCTTTGTTCTTGCGAAC
14	RM552	CGCAGTTGTGGATTTCAGTG	TGCTCAACGTTTGACTGTCC
15	RM1090	GTTATAGCGCACCCTGGATG	GAACCGAAGGGACATGTGTG
16	RM120	CACACAAGCCCTGTCTCACGACC	CGCTGCGTCATGAGTATGTA
17	RM80	TTGAAGGCGCTGAAGGAG	CATCAACCTCGTCTTCACCG
18	RM6836	TGTTGCATATGGTGCTATTTGA	GATACGGCTTCTAGGCCAAA
19	RM122	GAGTCGATGTAATGTCATCAGTGC	GAAGGAGGTATCGCTTTGTTGGAC
20	RM237	CAAATCCCGACTGCTGTCC	TGGGAAGAGAGCACTACAGC
21	RM84	TAAGGGTCCATCCACAAGATG	TTGCAAATGCAGCTAGAGTAC
22	RM527	GGCTCGATCTAGAAAATCCG	TTGCACAGGTTGCGATAGAG

23	RM241	GAGCCAAATAAGATCGCTGA	TGCAAGCAGCAGATTTAGTG
24	RM11	TCGTCTACTGTTGGCTGCAC	ATAGCGGGCGAGGCTTAG
25	RM254	AGCCCCGAATAAATCCACCT	CTGGAGGAGCATTTGGTAGC
26	RM6	GTCCCCTCCACCCAATTC	TCGTCTACTGTTGGCTGCAC
27	RM1201	TTACCGCGCCACATATACAC	CGTACGAGCCCTAGTTACCG
28	RM1130	AGATCGGATTGGGATGGC	ACCCAACCAATTAGTGCCAC
29	RM232	CCGGTATCCTTCGATATTGC	CCGACTTTTCCTCCTGACG
30	RM255	TGTTGCGTGTGGAGATGTG	CGAAACCGCTCAGTTCAAC
31	RM271	TCAGATCTACAATTCCATCC	TCGGTGAGACCTAGAGAGCC
32	RM201	CTCGTTTATTACCTACAGTACC	CTACCTCCTTTCTAGACCGATA
33	OSR13	CATTTGTGCGTCACGGAGTA	AGCCACAGCGCCCATCTCTC
34	RM6100	TCCTCTACCAGTACCGCACC	GCTGGATCACAGATCATTGC
35	OSR16	AAAACTAGCTTGCAAAGGGGA	TGCCGGCTGATCTTGTTCTC
36	OSR17	GCTGGTTGATTCAGCTAGTC	GCCTCGTTGTCGTTCCACAC
37	RM1920	CAAACACAGTGTTGACAGAA	GCTATTGACTTATCCGTTCA
38	RM1925	AATTCATTCAAGCCTTGATA	ATTAGTTTCACCAAAGCAAC
39	RM1940	ACTATCGATCAAAATGCTAG	AAACGAATGGTTAAATGTTA
40	RM1942	CTGCTCAATGATACAGGA	GGCATCCACTAAATTTAGATA
41	RM1869	CGTTTCACAATGTAAGACTT	CTCCGTTTTACAATGTAAGA
42	RM1896	GGACAGGGTAAAGTGTTAGA	CCTAAGACCTATCAACTCCA
43	RM1937	AATAAATAAAAATCCAGCAC	AGATCAGATATGGCATTAAG
44	RM2770	TAGGCCCTGATTAGTTTCC	ATATATGTGTCCCTTCTCCATAC
45	RM2814	AATACCTGTTTGTATGTGTC	CACTTATAGGTTAATTATGTGA
46	RM2819	AATGTTGCTAGATTTAAAAC	CAGTAGGATATCTTACAACC
47	RM2887	GATCAATATGATTTTTTTCA	TAGTCGATTACTATTGGGTA
48	RM2972	GAGCCAATATGTTGTCTTGA	GTTCAGATCATGATGCCTAC
49	RM3103	CAGACAACTTGTAATGTACG	ATGTCATGGGAGATAATTAA
50	RM212	CCACTTTCAGCTACTACCAG	CACCCATTTGTCTCTCATTATG

3.2.6.2 Dilution of primers

Each primer was dissolved in 100µl of 1X Tris EDTA (TE) buffer and diluted further with deionized water to the working concentration of 10µM. The primers were diluted as per following formula:

µM of oligo in 100l of solution = OD/10

3.2.6.3 PCR Amplification

In-vitro amplification using Polymerase Chain Reaction (PCR) was performed in an Eppendorf Master cycler to study the parental polymorphism. PCR analysis was carried out using 50 SSR primers. PCR analysis was carried out in the reaction volume of 25µl containing the 1µl template genomic DNA, 1.2 µl of forward and reverse primers, 2.5µl of 2.5mM dNTPs, 2.5µl of 10X PCR buffer and 0.03µl of *Taq* polymerase (Promega) (5U/ µl). PCR profile and reaction mixture is given in Table 3 and Table 4 respectively.

Table 3. PCR reaction mixture

Components	**Stock Concentration**	**Volume (µl)**
Sterile water	-	16.57
PCR buffer with salt	10X	2.5
dNTPs	2.5 mM	2.5
Primer Forward	10 µM	1.2
Primer Reverse	10 µM	1.2
Taq Polymerase	5 units/µl	0.03
DNA Template	25 ng/µl	1
Total		25 µl

Table 4. Temperature profile used in PCR

Step No.	Cycling conditions	Temperature	Time
I	Initial Denaturation	94°C	3 Minutes
II	Denaturation	94°C	1 Minute
III	Annealing	50-55°C	1 Minute
IV	Extension	72°C	1Minute
V	Go to step II		35 cycles
VI	Final extension	72°C	5Minutes
VII	Store	4°C	∞

3.2.6.4 Visualization of pcr amplified product

After PCR, 2µl of 6X loading dye was added to each of the amplified products and mixed thoroughly. Agarose gel (3.5%) was prepared by dissolving agarose powder and after Ethidium bromide 4-5 µl/ 100 ml of gel in 1X TBE buffer (Table 3.8). About 10µl of each sample mixed with loading dye was loaded into the wells in the gel.

A permanent standard marker 100bp producing fragments between 100bp to 3000bp was loaded with sample. After loading, the gel was subjected to electrophoresis at constant voltage of 5 V/cm for about 1 hour. After

electrophoresis, the gel was visualized under UV trans-illuminator and photographed using Syngene G box Documentation System.

3.2.7 Scoring of SSR alleles

The SSR allele sizes were determined by the position of bands relative to the DNA ladder. Total number of alleles was recorded for each microsatellite marker in all the genotypes under study by giving the number to amplified alleles as 0 for absence and 1 for presence of allele. The amplicon size was made out from the 50 or 100 bp ladder run along with the PCR products on the gel.

Polymorphic information content (PIC) that provides an estimate of the discriminatory power of a locus or loci, by taking into account not only the number of alleles that are expressed, but also relative frequencies of those alleles, was estimated using the following equation of Botstein *et al.* (1980).

$$PIC = 1 - \sum P_{ij}^2$$

where Pij is the frequency of jth allele in ith primer and summation extendes over n pattern.

3.2.8 Genetic diversity analysis

The genetic diversity among the genotypes was carried out by computer software programme - DendroUPGMA. The data were subjected to unweighted pair groups method with arithmetic mean (UPGMA) analysis to generate dendrogram.

RESULTS

4. RESULT

The main objective of the study was to study the variation in rice genotypes for heat tolerance using microsatellite markers. The material for this study contains 10 genetically diverse genotypes of rice which were received from National Rice Research Institute, Cuttack and Regional Agricultural Research Station, *Pattambi*. The DNA of all genotypes were isolated and subjected to marker analysis. The results obtained in the present study are given below.

4.1 DNA Extraction

The genomic DNA isolated from PTB 7, CR Dhan 202, CR Dhan 204, CR Dhan 305, CR Dhan 307, CR Dhan 701, Ajay, APO, Nagina 22, Rajalaxmi were confirmed by agarose gel electrophoresis. All the isolated DNAs appeared in 0.8% gel indicating good quality DNA (Plate 1).

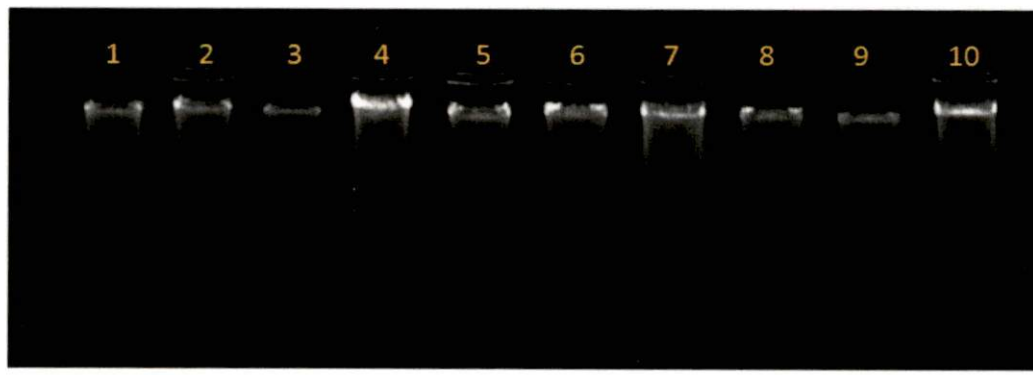


Plate 1: Genomic DNA isolated from 10 Genotypes (1- PTB 7, 2- CRDhan 202, 3- CRDhan 204, 4- CRDhan 305, 5- CRDhan 307, 6- CRDhan 701, 7- Ajay, 8- APO, 9- Nagina 22, 10- Rajalaxmi)

4.2 DNA Quality Confirmation

The quality of isolated DNAs was confirmed by measuring the absorbance at 260nm and 280nm (Table 5). The DNA samples showed 1.8 as A_{260}/A_{280} ratio it indicates the 100% purity, the ratio below 1.8 indicated the presence of proteins contamination and above 1.8 indicated the presence of RNA contamination in the sample.

4.3 Quantification of DNA

Quantity of DNAs was calculated based on the absorbance of sample at 260nm are given in Table 5. The Quantity of the isolated DNAs were ranges from 1563ng/µl to 3270ng/µl.

Table 5.**Concentration and purity of isolated DNA identified by spectrophotometric method**

Plant Name	A_{260} Value (nm)	A_{280} Value (nm)	A_{260}/A_{280} (ratio)	Quantity of DNA (ng/µl)
CRDhan 202	0.0773	0.0407	1.89	2319
CRDhan 204	0.069	0.038	1.82	2070
CRDhan 305	0.0521	0.0276	1.88	1563
CRDhan 307	0.087	0.047	1.85	2610
CRDhan 701	0.109	0.06	1.82	3270
Ajay	0.096	0.054	1.79	2880
APO	0.102	0.059	1.73	3060
Nagina 22	0.0801	0.0455	1.76	2403
Rajalaxmi	0.0604	0.0305	1.98	1812
PTB7	0.098	0.059	1.66	2940

4.4 Screening of primers by Polymerase Chain Reaction

PCR reactions were performed using selected primer by providing appropriate PCR conditions. Out of fifty primers, 11 of them showed

polymorphism in 3.5% agarose gel electrophoresis and other primers were monomorphic in nature (Plate 13, Plate 14, Plate 15, Plate 16 and Plate 17). RM1003, RM167, RM474, RM303, RM302, RM484, RM7117, RM271, RM525, RM6100 and RM236 were the polymorphic markers. RM1003 showed polymorphic bands with ~128bp length, RM167 showed polymorphic bands with ~111bp length RM474 showed polymorphic bands with ~252bp length RM303 showed polymorphic bands with ~200 bp length, RM302 showed polymorphic bands with ~156bp length, RM484 showed polymorphic bands with ~299bp length, RM7117 showed polymorphic bands with ~158bp length, RM271 showed polymorphic bands with ~101bp length, RM525 showed polymorphic bands with ~131 bp length RM236 showed polymorphic bands with ~191bp length and RM6100 showed polymorphic bands with ~152bp length. Banding pattern of amplified products with RM1003, RM167, RM474, RM303, RM302, RM484, RM7117, RM271, RM525, RM6100 and RM236 in 3.5% agarose gel is shown in plate 2, plate 3, plate 4, plate 5, plate 6, plate 7, plate 8, plate 9, plate 10 , plate 11 and plate 12 respectively.

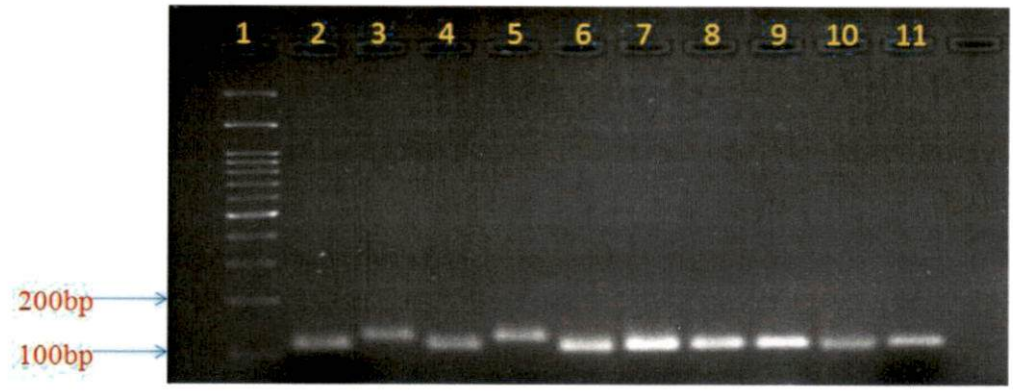


Plate 2: Amplification pattern of 10 rice genotypes obtained by SSR marker RM 1003 (Lane 1- 100bp ladder, Lane 2- PTB 7, Lane 3- CRDhan 202, Lane 4- CRDhan 204, Lane 5- CRDhan 305, Lane 6- CRDhan 307, Lane 7- CRDhan 701, Lane 8- Ajay, Lane 9- APO, Lane 10- Nagina 22, Lane 11- Rajalaxmi)

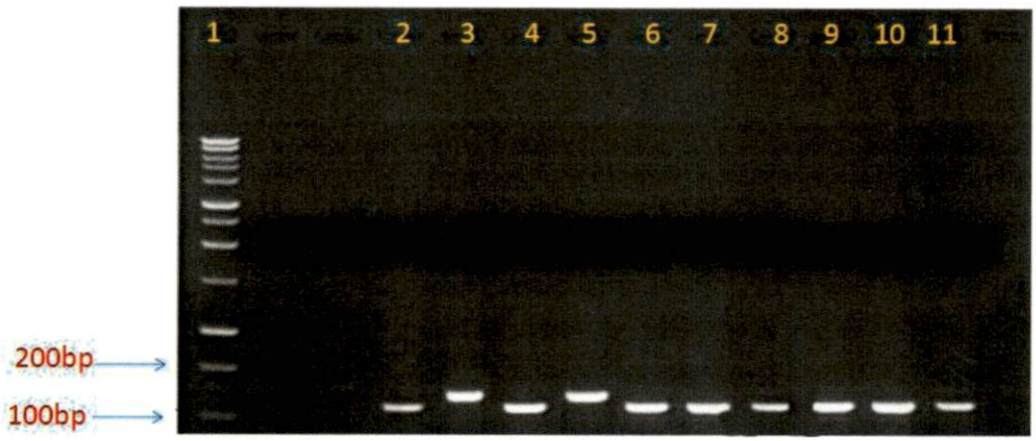


Plate 3: Amplification pattern of 10 rice genotypes obtained by SSR marker RM 167 (Lane 1- 100bp ladder, Lane 2- PTB 7, Lane 3- CRDhan 202, Lane 4- CRDhan 204, Lane 5- CRDhan 305, Lane 6- CRDhan 307, Lane 7- CRDhan 701, Lane 8- Ajay, Lane 9- APO, Lane 10- Nagina 22, Lane 11- Rajalaxmi)

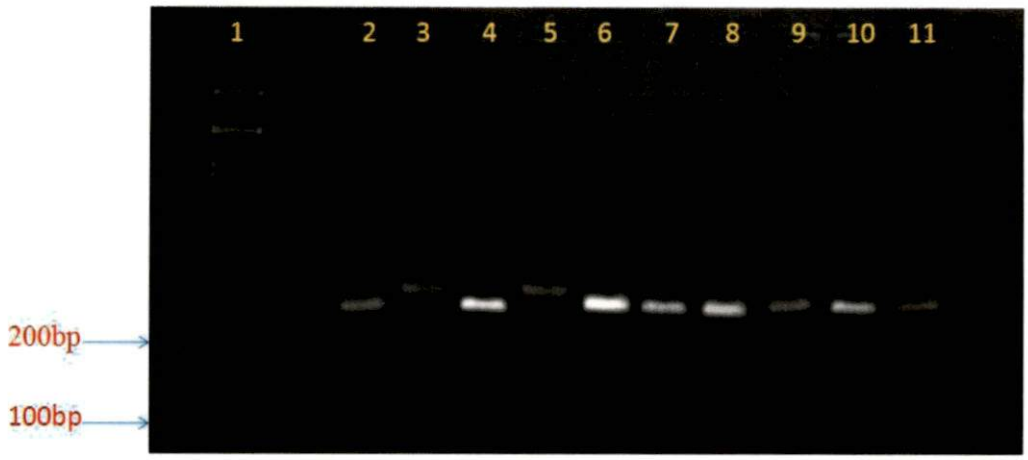


Plate 4: Amplification pattern of 10 rice genotypes obtained by SSR marker RM 474 (Lane 1- 100bp ladder, Lane 2- PTB 7, Lane 3- CRDhan 202, Lane 4- CRDhan 204, Lane 5- CRDhan 305, Lane 6- CRDhan 307, Lane 7- CRDhan 701, Lane 8- Ajay, Lane 9- APO, Lane 10- Nagina 22, Lane 11- Rajalaxmi)

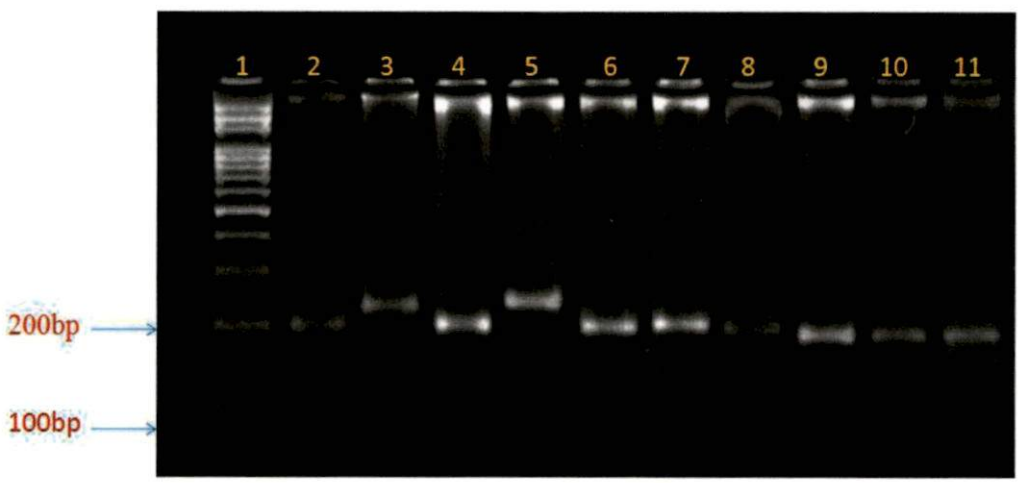


Plate 5: Amplification pattern of 10 rice genotypes obtained by SSR marker RM 303 (Lane 1- 100bp ladder, Lane 2- PTB 7, Lane 3- CRDhan 202, Lane 4- CRDhan 204, Lane 5- CRDhan 305, Lane 6- CRDhan 307, Lane 7- CRDhan 701, Lane 8- Ajay, Lane 9- APO, Lane 10- Nagina 22, Lane 11- Rajalaxmi)

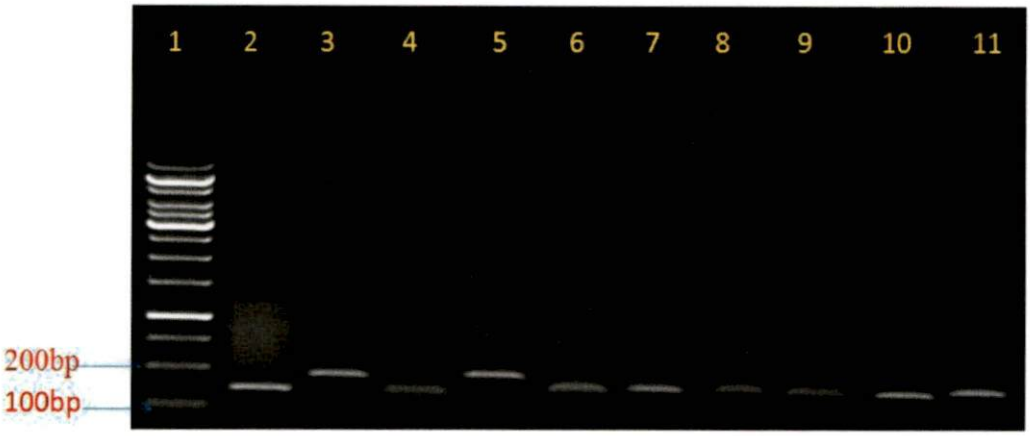


Plate 6: Amplification pattern of 10 rice genotypes obtained by SSR marker RM 302 (Lane 1- 100bp ladder, Lane 2- PTB 7, Lane 3- CRDhan 202, Lane 4- CRDhan 204, Lane 5- CRDhan 305, Lane 6- CRDhan 307, Lane 7- CRDhan 701, Lane 8- Ajay, Lane 9- APO, Lane 10- Nagina 22, Lane 11- Rajalaxmi)

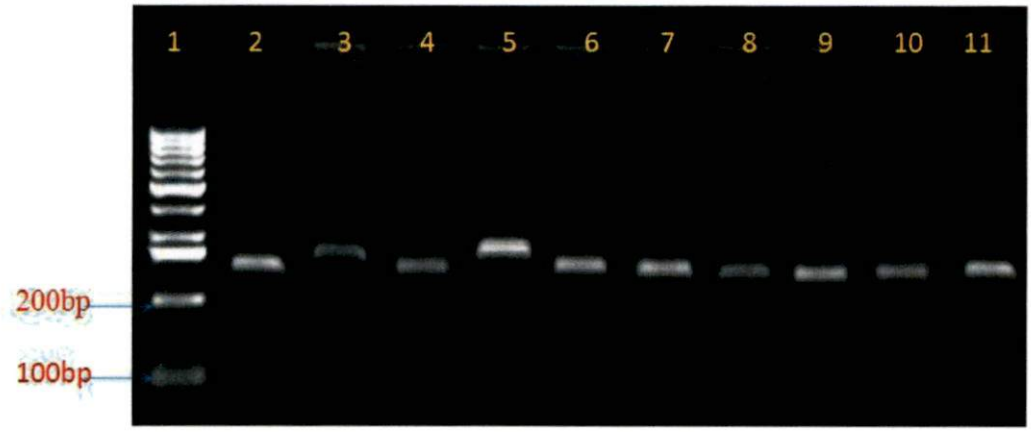


Plate 7: Amplification pattern of 10 rice genotypes obtained by SSR marker RM 484 (Lane 1- 100bp ladder, Lane 2- PTB 7, Lane 3- CRDhan 202, Lane 4- CRDhan 204, Lane 5- CRDhan 305, Lane 6- CRDhan 307, Lane 7- CRDhan 701, Lane 8- Ajay, Lane 9- APO, Lane 10- Nagina 22, Lane 11- Rajalaxmi)

Plate 8: Amplification pattern of 10 rice genotypes obtained by SSR marker RM 7117 (Lane 1- 100bp ladder, Lane 2- PTB 7, Lane 3- CRDhan 202, Lane 4- CRDhan 204, Lane 5- CRDhan 305, Lane 6- CRDhan 307, Lane 7- CRDhan 701, Lane 8- Ajay, Lane 9- APO, Lane 10- Nagina 22, Lane 11- Rajalaxmi)

Plate 9: Amplification pattern of 10 rice genotypes obtained by SSR marker RM 271 (Lane 1- 100bp ladder, Lane 2- PTB 7, Lane 3- CRDhan 202, Lane 4- CRDhan 204, Lane 5- CRDhan 305, Lane 6- CRDhan 307, Lane 7- CRDhan 701, Lane 8- Ajay, Lane 9- APO, Lane 10- Nagina 22, Lane 11- Rajalaxmi)

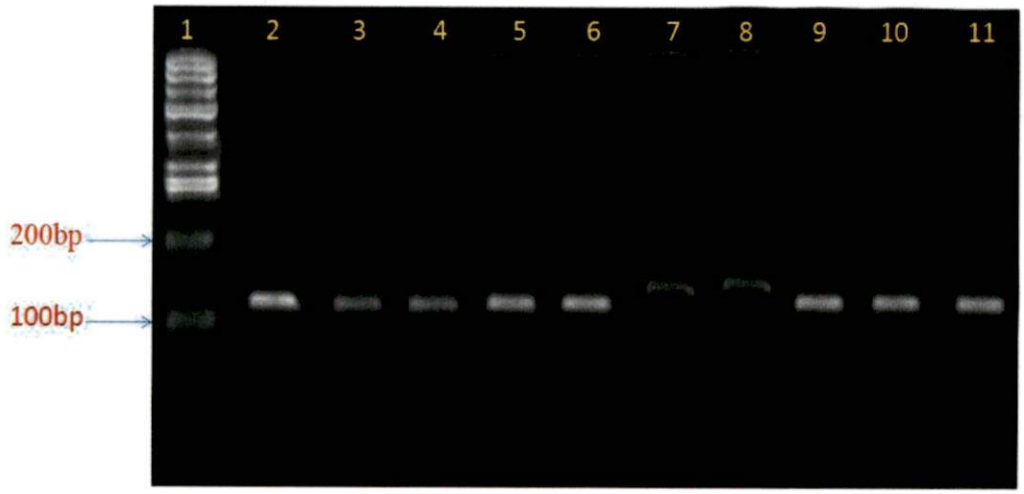


Plate 10: Amplification pattern of 10 rice genotypes obtained by SSR marker RM 525 (Lane 1- 100bp ladder, Lane 2- PTB 7, Lane 3- CRDhan 202, Lane 4- CRDhan 204, Lane 5- CRDhan 305, Lane 6- CRDhan 307, Lane 7- CRDhan 701, Lane 8- Ajay, Lane 9- APO, Lane 10- Nagina 22, Lane 11- Rajalaxmi)

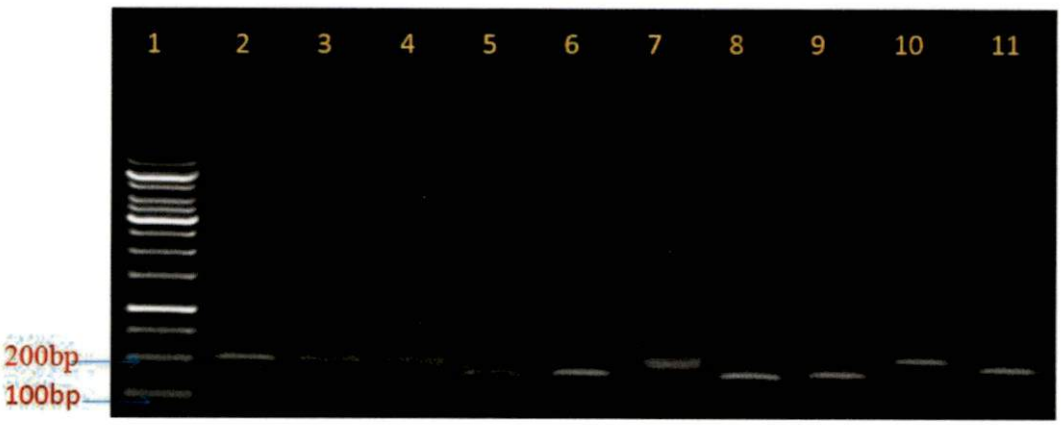


Plate 11: Amplification pattern of 10 rice genotypes obtained by SSR marker RM 236 (Lane 1- 100bp ladder, Lane 2- PTB 7, Lane 3- CRDhan 202, Lane 4- CRDhan 204, Lane 5- CRDhan 305, Lane 6- CRDhan 307, Lane 7- CRDhan 701, Lane 8- Ajay, Lane 9- APO, Lane 10- Nagina 22, Lane 11- Rajalaxmi)

Plate 12: Amplification pattern of 10 rice genotypes obtained by SSR marker RM 6100 (Lane 1- 100bp ladder, Lane 2- PTB 7, Lane 3- CRDhan 202, Lane 4- CRDhan 204, Lane 5- CRDhan 305, Lane 6- CRDhan 307, Lane 7- CRDhan 701, Lane 8- Ajay, Lane 9- APO, Lane 10- Nagina 22, Lane 11- Rajalaxmi)

Plate 13: Amplification pattern of 10 rice genotypes obtained by SSR marker RM 112~128bp length (Lane 1- 100bp ladder, Lane 2- PTB 7, Lane 3- CRDhan 202, Lane 4- CRDhan 204, Lane 5- CRDhan 305, Lane 6- CRDhan 307, Lane 7- CRDhan 701, Lane 8- Ajay, Lane 9- APO, Lane 10- Nagina 22, Lane 11- Rajalaxmi)

Plate 14: Amplification pattern of 10 rice genotypes obtained by SSR marker RM 80~144bp length (Lane 1- 100bp ladder, Lane 2- PTB 7, Lane 3- CRDhan 202, Lane 4- CRDhan 204, Lane 5- CRDhan 305, Lane 6- CRDhan 307, Lane 7- CRDhan 701, Lane 8- Ajay, Lane 9- APO, Lane 10- Nagina 22, Lane 11- Rajalaxmi)

Plate 15: Amplification pattern of 10 rice genotypes obtained by SSR marker RM 527 ~233bp length (Lane 1- 100bp ladder, Lane 2- PTB 7, Lane 3- CRDhan 202, Lane 4- CRDhan 204, Lane 5- CRDhan 305, Lane 6- CRDhan 307, Lane 7- CRDhan 701, Lane 8- Ajay, Lane 9- APO, Lane 10- Nagina 22, Lane 11- Rajalaxmi)

Plate 16: Amplification pattern of 10 rice genotypes obtained by SSR marker RM 255~144bp length (Lane 1- 100bp ladder, Lane 2- PTB 7, Lane 3- CRDhan 202, Lane 4- CRDhan 204, Lane 5- CRDhan 305, Lane 6- CRDhan 307, Lane 7- CRDhan 701, Lane 8- Ajay, Lane 9- APO, Lane 10- Nagina 22, Lane 11- Rajalaxmi)

Plate 17: Amplification pattern of 10 rice genotypes obtained by SSR marker RM 6~163bp length (Lane 1- 100bp ladder, Lane 2- PTB 7, Lane 3- CRDhan 202, Lane 4- CRDhan 204, Lane 5- CRDhan 305, Lane 6- CRDhan 307, Lane 7- CRDhan 701, Lane 8- Ajay, Lane 9- APO, Lane 10- Nagina 22, Lane 11- Rajalaxmi)

4.5 GENETIC DIVERSITY ANALYSIS

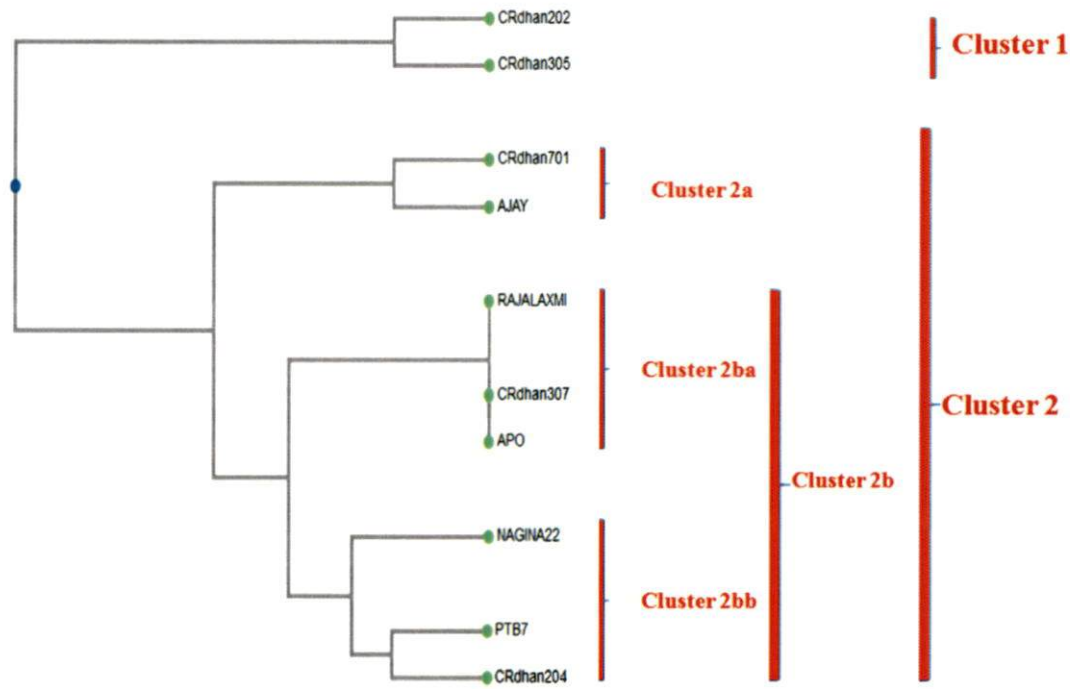
Polymorphic information content (PIC) value was calculated and given in Table 6.

The genetic diversity among the genotypes was carried out by computer software programme - DendroUPGMA. The data were subjected to unweighted pair groups method with arithmetic mean (UPGMA) analysis to generate dendrogram (Figure 1).

Table 6. Primers with their PIC values

Primer	PIC value
RM1003	0.66
RM167	0.66
RM474	0.66
RM7117	0.66
RM271	0.66
RM303	0.66
RM302	0.66
RM484	0.66
RM525	0.66
RM236	0.75
RM6100	0.71

Figure 1: UPGMA based dendrogram for all the 10 rice genotypes based on Scoring data



The dendrogram indicated that the 10 genotypes were clustered into two distinct clusters, Cluster 1 was the smallest with two genotypes and the cluster 2 divides two sub clusters, cluster 2a and cluster 2b. Cluster 2a was the smallest and comprised of two genotypes. Cluster 2b was the largest, contain two sub groups cluster 2ba and cluster 2bb, cluster 2ba contain three genotypes and cluster 2bb contain two sub groups. First sub group contain only one genotype and second sub group had two genotypes. The desirable heat tolerant genotypes which were identified and are scattered in all clusters but maximum desirable genotypes are present in cluster 2.

DISCUSSION

5. DISCUSSION

The DNA based markers are promising and effective tools for measuring genetic diversity in plants germplasm and elucidating their evolutionary relationships. DNA based markers remain unaffected across different stages, seasons, locations and agronomic practices (McCouch *et al.,* 1997).

The present study was carried out to study the variation in rice genotypes for heat tolerance using microsatellite markers. The results of the present study are discussed here:

5. 1 Polymorphism of SSR Markers

SSR markers are valuable as genetic markers because they detect high levels of allelic diversity, co dominant, easy and economically assayed by PCR (Weber and May, 1989), easily automated (Reed *et al.,* 1994 and Smith 1998), abundance and even genomic distribution (Weber and May, 1989) and high level of polymorphism (Maroof *et al.,* 1994). It has an average polymorphism at least 1.5 times higher than AFLP and RAPD markers (Mackill *et al.,* 1996). SSR's are highly polymorphic even between closely related lines (Gupta *et al.,* 1999). The polymorphism in SSR could be due to change in SSR region itself caused by the expansion or contraction of SSR or interruption (Li *et al.,* 2007).

In the present study, a total of 50 SSR markers which were dispersed throughout the 12 chromosomes were used to assess the extent of genetic diversity across 10 rice genotypes. RM1003, RM167, RM474, RM303, RM302, RM484, RM7117, RM271, RM525, RM6100 and RM236 were found to be polymorphic in nature. Out of these markers RM 6100 marker was specific for heat tolerance.

This RM6100 marker is linked with a major quantitative trait locus (QTL) on chromosome 10 for heat stress tolerance at flowering stage (Xiao *et al.*, 2011). Very recently, it was reported that induces of DREB2A gene expression also is associated to heat stress and it is reported as an important element of a transcriptional cascade in heat shock responses (Liu et al. 2011). Perhaps, expression of OsDREB2A may involve in the development of reproductive organs such as stamens during flowering stage under heat stress condition.

5.2 Polymorphism Information Content (PIC) Values

In the present study, the PIC values among the SSR loci tested are ranged from 0.66 to 0.75 , with an average of 0.67 per locus.

Polymorphism information content value is the reflection of allele diversity and frequency among the genotypes. To measure the informativeness of each SSR marker, PIC value was calculated. The PIC value is the indicator in predicting the usefulness of DNA markers for gene mapping, molecular breeding and germplasm evaluation. Markers with higher PIC values possess greater potential to reveal allelic variation. The average PIC value of SSR markers of different crops tested by different researchers varied based on number of SSR markers used and number of genotypes tested. The markers showed average PIC value of 0.67 that almost showed higher polymorphism, which confirms that SSR markers used in this study were highly informative for genetic studies and are extremely useful in distinguishing the polymorphic rate of a marker at a specific locus.

5.3 Genetic Diversity Pattern by Cluster Analysis

The clustering of accessions was done by using DendroUPGMA based on a similarity matrix using an unweighted pair group method with arithmetic mean (UPGMA) algorithm. The cluster analysis resolved the 10 rice genotypes into two major clusters. Dendrogram based on UPGMA analysis grouped the 10 genotypes into two clusters.

A dendrogram was generated with the aim of analyzing the relationships between the 10 genotypes tested. The genetic similarity index ranged from 0.1 to 0.833. The lowest value 0.1 was obtained between PTB7 and CR Dhan202 while highest similarity value (0.833) calculated was between the PTB7 and CR Dhan204 genotypes.

A dendrogram was generated by UPGMA to show the genetic relationships of the samples studied and is presented in Fig. 1. The dendrogram indicated that the 10 genotypes were clustered into two distinct clusters, Cluster 1 (CR Dhan 202 and CR Dhan 305) was the smallest with two genotypes and the cluster 2 divides two sub clusters, cluster 2a and cluster 2b. Cluster 2a was the smallest and comprised of two (CR Dhan 701 and AJAY) genotypes. Cluster 2b was the largest, contain two sub groups cluster 2ba and cluster 2bb, cluster 2ba contain three genotypes (CR Dhan 307, APO and RAJALAXMI) and cluster 2bb contain two sub groups. First sub group contain only one genotype (Nagina 22) and second sub group had two genotypes (PTB 7 and CR Dhan 204). The desirable heat tolerant genotypes which were identified and are scattered in all clusters but maximum desirable genotypes are present in cluster 2.

Nagina 22 a selection from landrace Rajbhog in Nepal is a well known drought and heat tolerant cultivar in Northern India. It has been used as a drought and heat tolerant donor for crop breeding, since last three decades in India. In this study revealed, two genotypes (PTB 7 and CR Dhan 204) were closely similar to Nagina 22, these genotypes were drought tolerant genotypes. Moreover, according to Mittler, (2006) high temperature is closely related to drought stress in natural environments and often occurs in combination and therefore, expression of DREB2A gene can be induced by drought or heat shock alone or by a combination of drought and heat shock.

Identification of heat tolerant genotypes is an essential requirement for developing heat tolerant varieties. In this study, more diversity was observed between heat tolerant and susceptible genotypes in SSR analysis. The marker RM6100 was found as a functional marker associated with heat tolerance in rice.

However, the genetic diversity analysis with SSR markers will contribute to maximize the selection of diverse parents in the future rice breeding program or development of heat tolerant cultivars. In addition, it will help in identifying efficient strategies for the sustainable management of genetic resources of rice crops to cope with the climate change. germplasm base in the future rice breeding program or development of the heat tolerant cultivars. In addition, it will help in identifying efficient strategies for the sustainable management of genetic resources of rice crops.

SUMMARY

6. SUMMARY

The study entitled "Molecular characterization of rice genotypes having variability in heat tolerance" was conducted at the Department of Plant Physiology, College of Agriculture, Vellayani, Thiruvananthapuram. The main aim of the study was to study the variation in rice genotypes for heat tolerance using microsatellite markers.

Genetic diversity is the foundation of the genetic improvement of crop plants. It serves as a reservoir for identifying superior alleles controlling key agronomic and quality traits through allele mining. Unlike morpho physiological traits used earlier to estimate genetic variability molecular markers have become quite handy in precisely understanding the extent of genetic divergence among varieties being chosen as parental sources in breeding programs.

In the present study, a set of 50 SSR primers were employed to assess the genetic diversity among the 10 genotypes. Out of 50 markers, 11 markers (RM 236, RM 484, RM 525, RM , RM 271, RM 302, RM 7117, RM 303, RM 1003, RM 167, RM 6100 and RM 474) showed polymorphism, the marker RM6100 was found as a functional marker associated with heat tolerance in rice.

The lower level of polymorphism may be attributed to a narrow genetic diversity. PIC is the reflection of allelic diversity and frequency among the varieties and varied greatly for all SSR loci tested. The PIC values varied from 0.66 to 0.75 with an average of 0.669. One SSR primer had PIC values higher than the average. Among these; RM 236, RM 484, RM 525, RM , RM 271, RM 302, RM 7117, RM 303, RM 1003, RM 167, RM 6100 and RM 474 showed PIC

values higher than 0.50. These markers are highly informative as they indicate high polymorphism. The PIC value can be looked as the measurement of usefulness of each marker in distinguishing one individual from another.

Genetic similarity was calculated from the matrix of binary data using software DendroUPGMA where 0 and 1 were standardized as the least and maximum of similarity respectively. To ascertain the statistical strength of genetic relationships identified through this analysis, bootstrapping of the data was performed. A dendrogram was generated with the aim of analyzing the relationships between the 10 genotypes tested.

The dendrogram indicated that the 10 genotypes were clustered into two distinct clusters, Cluster 1 was the smallest with two genotypes and the cluster 2 divides two sub clusters, cluster 2a and cluster 2b. Cluster 2a was the smallest and comprised of two genotypes. Cluster 2b was the largest, contain two sub groups cluster 2ba and cluster 2bb, cluster 2ba contain three genotypes and cluster 2bb contain two sub groups. First sub group contain only one genotype and second sub group had two genotypes. The desirable heat tolerant genotypes which were identified and are scattered in all clusters but maximum desirable genotypes are present in cluster 2. The genetic similarity index ranged from 0.1 to 0.833. The lowest value 0.1was obtained between PTB7 and CR Dhan202 while highest similarity value (0.833) calculated was between the PTB7 and CR Dhan204 genotypes. As such relationship cannot be correlated with heat tolerance but if primers associated with heat tolerance traits.

However, the genetic diversity analysis with SSR markers will contribute to maximize the selection of diverse parents in the future rice breeding program or development of heat tolerant cultivars. In addition, it will help in identifying efficient strategies for the sustainable management of genetic resources of rice crops to cope with the climate change. In addition, it will help in identifying efficient strategies for the sustainable management of genetic resources of rice crops.

REFERENCES

REFERENCES

Akagi, H., Yokozeki, Y., Inagaki, A and Fujimura, T. 1997. Highly polymorphic microsatellites of rice consist of AT repeats and a classification of closely related cultivars with these microsatellite loci. *Theoretical and Applied Genetics*. 94: 61-67.

Alvarez, A., Fuentes, J. L., Puldon, V., Gomez, P. J., Mora, L., Duque, M. C., Gallego, G and Tohm, J. M. 2007. Genetic diversity analysis of Cuban traditional rice *(Oryza sativa* L.) varieties based on microsatellite markers. *Genetics and Molecular Biology*. 30 (4): 1109-1117.

Ashfaq, M and Khan, A.S. 2012. Genetic diversity in Basmati rice (*Oryza sativa.L*) germplasm as revealed by microsatellite (SSR) markers. *Russian Journal of Genetics*. 48 (1): 53-62.

Bharathkumar, S., Pragnya, P. J., Jitendra, K., Archana, B., Singh, O. N., and Reddy, J. N. 2014. Identification of rice germplasms associated with microsatelite (SSR) markers for heat tolerance at reproductive stage and expression of heat stress related gene. *Indian Res. J. Genet. & Biotech*. 6: 424-427.

Botstein, D., Whire, R. L., Skolnick, M and Davis, R. W. 1980. Construction of genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics*. 32: 314-331.

Chand, S. P., Roy, S. K and Senapathi, B. K. 2005. Genetic divergence analysis in Aman rice under semi deep rainfed condition. *Crop Research*. 30 (1): 46-49.

Choudhary, G., Ranjitkumar, N., Surapaneni, M., Deborah, D. A., Vipparla, A and Anuradha, G. 2013. Molecular genetic diversity of major indian rice cultivars over decadal periods. *PLoS ONE* . 2 : 228-34.

Davierwala, A.P., Chowdari, K.V., Kumar, S., Reddy, A.P.K., Ranjekar, P.K and Gupta, V.S. 2000. Use of three different marker systems to estimate genetic diversity of Indian elite rice varieties. *Genetica*. 108: 269-84.

Dias, A.S., Barreiro, M. G., Campos, P.S., Ramalho, J.C. and Lidon, F.C., 2010. Wheat Cellular Membrane Thermotolerance under Heat Stress. *Journal of Agronomy and Crop Science*. 196(2):100–108.

Ekanayake, I.J., Steponkus, P.L., De Datta, S.K., 1990. Sensitivity of pollination to water deficits at anthesis in upland rice. *Crop Sci.* 30: 310–315.

Fedaraci, M. T., Vaughan, D., Tomooka, N., Kaga, A., Wang, X. W., Doi, K., Francis, M., Zorilla, G and Saldain, N. 2011. Analysis of Uruguayan weedy rice genetic diversity using AFLP molecular markers. *Electronic Journal of Biotechnology*. 4 (3): 130-145.

Garland, S. H., Lewin, L., Abedinia, M., Henry, R and Blankeney, A. 1999. The use of microsatellite polymorphism for the identification of Australian breeding lines of rice (*Oryza sativa* L.). *Euphytica.* 108: 53-63.

Gui, L.T and Shen, B. 1995. Appraisal of the heat tolerance of rice varieties in the flowering phase. *Crop Genetic Resources*. 1: 34-35.

Gupta, P.K., Varshney, R.K., Sharma, P.C and Ramesh, B. 1999. Molecualr markers and their application in wheat breeding: a review. *Plant Breeding.* 118: 369-390.

Herrera, T. G., Duque, D. P., Almeida, I. P., Nunez, G. T., Pieters, A. J, Martinez, C. P and Tohme, J. M. 2008. Assessment of genetic diversity in Venezuelan rice cultivars using simple sequence repeat markers. *Electronic Journal of Biotechnology.* 11 (5): 1-14.

80

Ilangovan, R., Beevi, P., Rao, G. S., and Moosa, P. P. 2011. Impact of climate change on soil degradation and rice productivity at Pattambi, Kerala in the humid tropics. In: Rao, G. S. L. H. V. (ed.), *Climate Change Adaptation Strategies in Agriculture and Allied Sectors*. Scientific publishers, India, pp. 220-225.

IPCC, 2007. Intergovernmental Panel on Climate Change. Fourth Assessment Report of the intergovernmental Panel on Climate Change: The Impacts, Adaptation and Vulnerability. Cambridge University Press, United Kingdom and New York, NY, USA.

Jagadish, S. V. K., Craufurd, P. Q and Wheeler, T. R. 2007. High temperature and spikelet fertility variety in rice (*Oryza sativa* L.). *Journal of Experimental Botany.* 58 (7): 1627-1635.

Jagadish, S.V. K., Craufurd, P. Q., Wheeler, T. R., 2008. Phenotyping parents of mapping populations of rice (*Oryza sativa* L.) for heat tolerance during anthesis. *Crop Sci.* 48: 1140–1146.

Jing, S., Gen, C. L., Ping, Z. P., Hong, X. X and Chu, Z. J. 2006. Responses of rice cultivars to high temperature at the heading stage and methods to enhance heat tolerance. *Jiangsu Journal of Agricultural Sciences*. 22 (4): 325-330.

Johnson, S. C. 1967. Hierarchical clustering schemes. *Psychometrika*. 32: 241-254.

Joshi, A. B and Dhawan, N. L. 1966. Genetic improvement in yield with special reference to self fertilizing crops. *Indian Journal of Genetics and Plant Breeding*. 26: 101-113.

Khush, G. S. 2005.What it will take to feed 5.0 billion rice consumers in 2030. *Plant Mol. Biol.* 59: 1-6.

81

Kobayashi, K., Matsui, T., Murata, Y and Yamamoto, M. 2011. Percentage of dehisced thecae and length of dehiscence control pollination stability of rice cultivars. *Plant Production Science.* 14 (2): 89-95.

Kwon, S. J., Ahn, S. N., Chol, H. C and Moon, H. P. 1999. Evaluation of genetic relationships and fingerprinting of rice varieties using microsatellite and RAPD markers. *Korean Journal* of *Crop Science*. 44: 112-116.

Lapitan, C.V., Darshan, S. B., Toshinori, A and Edilberto, D. R. 2007. Assessment of genetic diversity of Philippine Rice cultivars carrying quality traits using SSR markers. *Breeding Science.* 57: 263-270.

Li, A and Ge, S. 2001. Genetic variation and clonal diversity of *Psammochloa villosa* (Poaceae) detected by ISSR Markers. *Annals of Botany.* 87: 585-590.

Li, L., Liu, G., Gong, Y., Dai, W., Wang, Y., Yu, F and Ren, Y. 2007. Evaluation of genetic purity of F_1 hybrid seeds in cabbage with RAPD, ISSR, SRAP and SSR markers. *Horticultural Science.* 42: 724–727.

Lin, W. X., He, H. Q., Chen, X. X., Xiong, J., Song, B. Q., Liang, Y. Y and Liang, K. J. 2005. Use of ISSR molecular marker approach to estimate genetic diversity in rice and Barley allelopathy. *Proceedings of The 4th World Congress on Allelopathy*, Wagga Wagga, Australia. 168-174.

Liu, J. X., Liao, D. Q., Oane, R., Estenor, L., Yang, X. E., Li, Z. C., Bennett, J., 2006. Genetic variation in the sensitivity of anther dehiscence to drought stress in rice. *Field Crops Res.* 97: 87–100.

Liu, H. C., Liao, H.T., and Charng, Y.Y. 2011. The role of class A_1 heat shock factors (HSFA1s) in response to heat and other stresses in Arabidopsis. *Plant Cell Environ.* 34: 738–751.

82

Luce, C., Noyer, J. L., Tharreau, D., Ahmadi, N., Fety, H., Dore, C., Dosba, F and Baril, C. 2001. The use of microsatellite markers to examine the diversity of genetic resources of rice (*Oryza sativa*) adapted to European conditions. *Acta Horticulturae*. 546: 221-235.

Mackill, D. J., Coffman, W. R., and Garrity, D. P., 1996. Drought resistance in Rainfed lowland rice improvement. International Rice Research Institute, Manila, pp. 79–110.

Mackill, D. J., Coffman, W. R and Rutger, J. N. 1982. Pollen shedding and combining ability for high temperature tolerance in rice. *Crop Science.* 22: 730-733.

Mackill, D. J., Zhang, Z., Redona, E. D and Colowit, P. M. 1996. Level of polymorphism and genetic mapping of AFLP markers in rice. *Genome*. 39: 969-977.

Maroof, M. A. S., Yang, G. P., Zhang, Q and Gravois, K. A. 1994. Correlation between molecular marker distance and hybrid performance in U.S. Southern long grain rice. *Crop Science.* 37: 145–150.

Matsui, T and Omasa, K. 2001. Rice (*Oryza sativa* L.) cultivars tolerant to high temperature at flowering: Anther characteristics. *Annals of Botany.* 89: 683-687.

Matsui, T., Namuco, O. S., Ziska, L. H and Horie, T. 1997. Effects of high temperature and CO_2 concentration on spikelet sterility in *Indica* rice. *Field Crops Research.* 51: 213-219.

Matsui, T., Omasa, K., Horie, T., 1999a. Mechanism of anther dehiscence in rice (*Oryza sativa* L.). *Ann. Bot.* 84: 501–506.

Matsui, T., Omasa, K., Horie, T., 1999b. Rapid swelling of pollen grains in response to floret opening unfolds anther locules in rice (*Oryza sativa* L.). *Plant Prod Sci.* 2: 196–199.

Matsui, T., Omasa, K., Horie, T., 2001. The difference in sterility due to high temperatures during the flowering period among *japonica* rice varieties. *Plant Prod Sci.* 4: 90–93.

Mazorra, L. M., Nunez, M., Echerarria, E., Coll, F., S´anchez-Blanco, M. J., 2002. Influence of brassinosteroids and antioxidant enzymes activity in tomato under different temperatures. *Plant Biol.* 45: 593–596.

McCouch, S. R., Chen, X., Panaud, O., Temnykh, S., Xu, Y., Cho, Y. G., Huang, N., Ishii, T and Blair, M. 1997. Microsatellite marker development, mapping and applications in rice genetics and breeding. *Plant Molecular Biology.* 35: 89-99.

Min, H., Yan, W., Lin, P., Ming, Y. C., Sun, Q., Peng, G., Ling, W., Ying, D. X and Qing, L. 2009. Screening and identification of heat tolerance genes of hybrid rice. *South west China Journal of Agricultural Sciences*. 22 (5): 1199-1203.

Mittler, R. 2006. Abiotic stress, the field environment and stress combination. *Trends Plant Sci*. 11: 15–19.

Mohamed , H. and Whaibi, A. 2011. Plant heat shock proteins. *J. King Saud University*. 23:139-150.

Nakagawa, H., Horie, T., Matsui, T., 2003. Effects of climate change on rice production and adaptive technologies. In: Mew, T.W., Brar, D.S., Peng, S., Dawe, D., Hardy, B. (Eds.), Rice Science: Innovations and Impact for Livelihood. International Rice Research Institute, Philippines, pp. 635–657.

Nandini. K., Jaikumaran. U, and Girija. T. 2010. Physiological analysis on yield decline in rice (*Oryza sativa*.L) with high temperature in kole wet lands of Kerala [abstract]. In: *Abstracts, National Conference of Plant Physiology on Physiological and Molecular Approaches for Crop Improvement under Changing Environment;* 25-27 November, 2010, Varanasi. Banaras Hindu University, Varanasi & Indian Society for Plant Physiology, New Delhi. Abstract No.3.97.

Neeraja, C. N., Hariprasad, A. S., Malathi, S and Siddiq, E. A. 2005. Characterization of tall landraces of rice (*Oryza sativa* L.) using gene derived simple sequence repeats. *Current Science*. 88: 149-152.

Ni, J., Colowit, P. M and Mackill, D. J. 2002. Evaluation of genetic diversity in rice sub species using microsatellite markers. *Crop Science*. 42: 601-607.

Padmaja, D., Radhika, K., Meenakshi Sundaram, Rao, L.V. S. 2011. Studies on genetic diversity in selected germplasm lines of rice (*Oryza sativa* L.) using SSR markers. *The Journal of Research* ANGRAU. 39 (3): 26-31.

Pantuwan, G., Fukai, S., Cooper, M., Rajatasereekul, S. and O'Toole, J. C., 2002. Yield responses of rice (*Oryza sativa* L.) genotypes to water deficit in rainfed lowlands. In: Saxena, N. P., O'Toole, J. C., John, C. (Eds.), Field Screening for Drought Tolerance in Crop Plants with Emphasis on Rice: Proceedings of an International Workshop on Field Screening for Drought Tolerance in Rice, 11- 14 Dec 2000, ICRISAT, Patancheru, India, pp. 54-58.

Poli, Y., Basava, R. K., Panigrahy, M., Vinukonda, V. P., Dokula, N. R., Voleti, S. R., Desiraju, S., and Neelamraju, S. 2013. Characterization of a Nagina22 rice mutant for heat tolerance and mapping of yield traits. Rice 6: 36.

Prasad, P.V.V., Boote, K. J., Allen Jr., L. H., Sheehy, J. E., Thomsa, J. M. G., 2006. Species, ecotype and cultivar differences in spikelet fertility and harvest index of rice in response to high temperature stress. *Field Crops Res.* 95: 398– 411.

Qian, W., Ge, S and Hong, D.Y. 2001. Genetic variation with in and among population of a wild rice *Oryza granulata* from China detected by RAPD and ISSR markers. *Theory of Applied Genetics*. 102: 440-449.

Rabbani, M. A., Hassan, Z., Pervaiz, M and Shahid, M. 2008. Genetic analysis of traditional and improved cultivars of Pakistani rice (*Oryza sativa* L.) using RAPD markers. *Electronic Journal of Biotechnology*. 11 (3): 1-10.

Ram, S. G., Thiruvengadan, V and Vinod, K. K. 2005. Genetic diversity among cultivars, landraces and wild relatives as revealed by microsatellite markers. *Journal of Applied Genetics*. 48 (4): 337-345.

Ravi, M., Geethanjali, S., Sameeyafarheen, F and Maheswaran, M. 2003. Molecular marker based genetic diversity analysis in rice (*Oryza sativa* L.) using RAPD and SSR markers. *Euphytica*. 133: 243-252.

Reed, P.W., Davies, J.L., Copeman, J. B., Bennett, S.T., Palmer, S. M., Pritchard, L. E., Gough, S.C.L., Kawaguchi, Y., Cordell, H. J., Balfour, K. M., Jenkins, S.C., Powell, E. E., Vignal, A and Todd, J. A. 1994. Chromosome specific microsatellite sets for fluorescence based, semi-automated genome mapping. *Nature Genetics*. 7: 390–395.

Robin, S., Kavithamani, D., Manonmani, S., Sundaram, M. K., and Thiyagarajan, K. 2010. Molecular tagging of a thermo-sensitive genic male sterile gene and identifying new TGMS lines in rice. In: *28th International Rice Research Conference; 8-12 November 2010, Hanoi, Vietnam OP12: Molecular Biology and "Omics" Technologies*, pp1-5.

Sairam, R. K., Srivastava, G. C. and Saxena, D. C., 2000. Increased antioxidant activity under elevated temperature: a mechanism of heat stress tolerance in wheat genotypes. *Biol. Plant.*43: 245–251.

Sakamoto, A and Murata, N. 2001. The use of bacterial choline oxidase, a glycinebetaine-synthesizing enzyme, to create stress resistant transgenic plants. *Plant Physiol.* 125:180-188.

Sarla, N., Bobba, S and Siddiq, E. A. 2003. ISSR and SSR markers based on AG and GA repeats delineate geographically diverse *Oryza nivara* accessions and reveal rare alleles. *Current Science*. 84: 683-690.

Sarwar, M and Avesi. 1985. Evaluation of rice germplasm for high temperature tolerance. *Pakistan Journal of Agriculture.* 6 (3): 162-164.

Satake, T and Yoshida, S., 1978. High temperature-induced sterility in indica rice at flowering. *Jpn. J. Crop Sci.* 47: 6-17.

Sato, K. 1979. High temperature damage to ripening in rice plant. *JARQ.* 13: 90-95.

Sebastein, L. S., Hipolito, L. R., Tabanao, D. A., Maramara, G.V and Caldo, R. A. 1998. Molecular diversity of Philippines bred rice cultivars. *SABARAQ Journal of Breeding and Genetics*. 30: 83-90.

Seetharam, K., Thirumeni, S and Paramasivam, K. 2009. Estimation of genetic diversity. *Current Science*. 84: 683-690.

Shah, F., Huang, J., Cui, K., Nie, L., Shah, T., Chen, C and Wang, K. 2011. Impact of high-temperature stress on rice plant and its traits related to tolerance. *Journal of Agricultural Science.*1-12.

87

Siddique, K. H. M., Loss, S. P., Regan, K. L. and Jettner, R. L., 1999. Adaptation and seed yield of cool season grain legumes in cool season grain legumes in Mediterranean environments of south-western Australia. *Aust. J. Agric. Res.* 50: 375-387.

Singh, A., Singh, S. K and Anil, G. 2008. Genetic engineering for heat tolerance in plants. *Physiology and Molecular Biology of Plants.* 14(2): 155-166.

Singh, B., Singh, S. P and Kumar, J. 2011. Assessment of genetic diversity of aromatic rices (*Oryza sativa* L.) using morphological, physiochemical and SSR markers. *Indian Journal of Genetics and Plant Breeding*. 71 (3): 214-222.

Smith, J. S.C and Register, J. C. 1998. Genetic purity and testing technologies for seed quality: a company perspective. *Seed Science Research*. 8: 285–293.

Sneath, P. H. A. 1957. The application of computers to taxonomy. *Journal of Genetics and Microbiology*. 17: 201-226.

Song, Z. P., Lu, B. R., Chen, J. K., 2001. A study of pollen viability and longevity in Oryza *rufipogon, O. sativa* and their hybrids. IRRN. 26: 31–32.

Strain, B. R and Cure, J. D. 1985. Direct effects of increasing carbon dioxide on vegetation. Washington, DC.286.

Sun, C. Q., Wang, X. K., Yoshimura, A and Iwata, N. 2000. A study of the genetic diversity of common wild rice (*O. rufipogon* Griff.) and cultivated rice (*O. sativa* L.) by RFLP analysis. *Yi Chuan Xue Bao*. 27 (3): 227-34.

Tang, A., Neng, S. G., Ai, J. G., Ju, L., Li, W. J., Qing, T. S and Song, H. P. 2009. Comparative assessment of SSR diversity in aromatic rice germplasm. *Hereditas.* 31(4): 412-419.

Tripathy, J. N., Zhang, J., Robin, S., Nguyen, T.T. and Nguyen, H.T. 2002. QTLs for cell membrane stability mapped in rice (*Oryza sativa* L.) under drought stress. *Theor. Appl. Genet.* 100: 1197-1202.

Wahid, A., Gelani, S., Ashraf, M., and Foolad, M.R., 2007. Heat tolerance in plants: an overview. *Environ. Exp. Bot.* 61: 199–233.

Ward, J. H. 1963. Hierarchical grouping to optimize an objective function. *Journal of American Statistical Association.* 58: 236-244.

Wassmann, R., Jagadish, S.V. K., Sumfleth, K., Pathak, H., Howell, G., Ismail, A., Serraj, R., Redona, E., Singh, R. K., Heuer, S., 2009. Regional vulnerability of climate change impacts on Asian rice production and scope for adaptation. *Adv. Agron.* 102: 93–105.

Weber, J. L and May, P. E. 1989. Abundant class of human DNA polymorphisms, which can be typed using the polymerase chain reaction. *American Journal of Human Genetics.* 44: 388-396.

Weerakoon, W. M. W., Maruyama, A., and Ohba, K. 2008. Impact of humidity on temperature-induced grain sterility in rice (*Oryza sativa* L.). *J. Agron. Crop. Sci.* 194: 135–140.

Xiao,Y., Pan, Y., Luo, L.; Zhang ,G., Deng,H., Dai, L., Liu, X., Tang, W., Chen, L., and Wang, G. 2011. Quantitative trait loci associated with seed set under high temperature stress at the flowering stage in rice (Oryza sativa L.). *Euphytica.* 178 : 331-338.

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Ying, C. A. O., Hua, D., Nian, Y. L., Qing, W. Z., Chuan, Z. S and Yang J. C. 2008. Effect of heat-stress during meiosis on grain yield of rice cultivars differing in heattolerance and its physiological mechanism. *Acta Agronomica Sinica.* 34 (12): 2134-2142.

Ying, C. A. O., Hua, D., Nian, Y. L., Qing, W. Z., Jun, L. L and Yang J.C. 2009. Effect of high temperature during heading and early filling on grain yield and physiological characteristics in *Indica* rice. *Acta Agronomica Sinica.* 35 (3): 512-521.

Yoshida, S., 1981. High temperature stress in rice. IRRI Research Paper Series . 67.

Yu, Z. H., Jin, H.Y., Huang, W. D., Xue, Q.Y., An, Z. P., Hua, L.G., Kai, L and Yun, H. 2004. A study on developing near isogenic lines of heat tolerance at grain filling stage in rice. *Acta Agriculturae Universitatis.* 26 (6): 847-853.

APPENDICES

APPENDIX I

CTAB Extraction Buffer (pH= 8,0)

C-TAB	2 %	
Tris- HCl (pH 8.0)	100 mM	
EDTA	20 mM	
NaCl	1.4 M	
β-mercaptoethanol	0.2 % (v/v)	freshly added prior to DNA extraction
PVP	4 % (w/v)	

APPENDIX II

TE buffer (pH= 8,0)

Tris- HCl (pH= 8.0)	10 mM
EDTA	1 mM

APPENDIX III

TBE Buffer (50X) for 100 ml solution (pH= 8.2-8.5)

Tris base	53.5 g
Boric acid	27.5 g
EDTA	4.9 g

APPENDIX IV

Gel loading Dye (6X) for 120 ml solution

Bromophenol blue	200 mg
Xylene cyanol	50 mg
Glycerol	90 ml
EDTA (0.5 M, pH= 8.0)	20 ml
Water	10 ml

MOLECULAR CHARACTERIZATION OF RICE GENOTYPES HAVING VARIABILITY IN HEAT TOLERANCE

by

NEETHU V. MOHAN
(2012-09-118)

Abstract of the Thesis

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ABSTRACT

ABSTRACT

The study entitled "Molecular characterization of rice genotypes having variability in heat tolerance" was conducted at the Department of Plant Physiology, College of Agriculture, Vellayani, Thiruvananthapuram.

Rice (*O. sativa*) is very sensitive to high temperature especially at the reproductive and grain filling stage which leads to higher spikelet sterility and ultimately yield losses. The present investigation was, therefore, carried out with the objective to study the variation in rice genotypes for heat tolerance using microsatellite markers. In the present study, a set of 50 SSR primers were employed to assess the genetic diversity among the 10 genotypes. Out of 50 markers, 11 markers showed polymorphism, the marker RM6100 was found as a functional marker associated with heat tolerance in rice, and is functional for further crop breeding programmes.

A dendrogram was generated with the aim of analyzing the relationships between the 10 genotypes tested. The genetic similarity index ranged from 0.1 to 0.833. The lowest value 0.1was obtained between PTB7 and CR Dhan202 while highest similarity value (0.833) calculated was between the PTB7 and CR Dhan204 genotypes.

However, the genetic diversity analysis with SSR markers will contribute to maximize the selection of diverse parents in the future rice breeding program or development of heat tolerant cultivars.

Keywords: *O. sativa*, SSR markers, high temperature, heat tolerance, spikelet fertility, genetic diversity