Genetic characterization of Thermotolerant rice genotypes with allele coding at the seedling development stage

Bandi Arpitha Shankar and Srividhya Akkareddy

Abstract
Rice is the most critical crop for food security on the planet. Heat is a major constraint on rice production. Industrialisation has had a significant impact on climate change, necessitating the development of more heat-resistant strains and methods for filtering a large number of genotypes for high temperature tolerance. The purpose of this article is to describe the process by which the Temperature Induction Response (TIR) method for identifying thermotolerant rice genotypes was standardized. Rice's phenotypic characteristics are determined using percentage germination, seedling development, and molecular analysis. Heat stress is applied to the plants via the TIR procedure, which involves adjusting the temperature in a TIR chamber to fatal (55 °C) and sub-lethal (38-55 °C) levels while varying the humidity. In response to elevated temperatures, 14 of the 74 genotypes tested exhibited thermal tolerance. Tolerant and susceptible genotypes were classified according to their survival percentages. The tolerant class is determined by comparing the growth and development of high-survival genotypes, as well as their shoot and root lengths, fresh and dry weights, to the heat tolerant controls N22, Dular, and Nipponbare. These genotypes may be used as donors in breeding efforts aimed at mitigating global warming, owing to their inherent heat tolerance. The molecular markers associated with the heat tolerant class via allele coding are extremely useful and may be used in marker-assisted breeding to enhance the heat tolerance of farmed cultivars.

Keywords: Rice genotypes, global warming, thermo tolerance, sub-lethal and lethal temperatures, lethal temperatures, SSR primers, allele code

Introduction
Rice is the most significant and essential cereal food grain domesticated all through the world particularly in Asia and Africa (Krishnan et al., 2011) [31]. The name wild rice is generally utilized for types of the genera Zizania and Porteresia, both wild and tamed, albeit the term may likewise be utilized for crude or crude assortments of Oryza (Lafarge et al., 2017) [34]. Rice, a monocot, is regularly developed as a yearly plant, albeit in tropical regions it can get by as a perennial and can deliver a ratoon crop for up to 30 years (Huang et al., 2012) [36]. Rice development is appropriate to nations and areas with low work expenses and high precipitation, as it is work concentrated to develop and requires sufficient water. The rice plant can develop to 1–1.8 m (3 ft 3 in–5 ft 11 in) tall, every so often relying upon the assortment and soil richness. It has long, slim leaves 50–100 cm (20–40 in) long and 2–2.5 cm (3⁄4–1 in) wide (Shi et al., 201; Kesh et al., 2021) [27]. Rice enhancement for wetland rice fields is acknowledged to be answerable for 11% of the anthropogenic methane outpourings. Methane conveyed is achieved by long stretch flooding of rice fields cuts the earth off from ecological oxygen and causes anaerobic maturing of regular matter in the soil (Kesh and Kaushik, 2020) [26]. Methane creation from rice improvement contributes ~1.5% of complete anthropogenic nursery gases. Methane is on numerous occasions more impressive an ozone-exhausting substance than carbon dioxide (Yu et al., 2014) [66].

A new report found that, due to rising temperatures and lessening sun-based radiation during the later extended lengths of the 20th century, the rice yield improvement rate has decreased in various bits of Asia, standing out from what may have been seen had the temperature and sun arranged radiation designs not happened (Kumar and Kaushik, 2021; Malhi et al., 2021) [32, 33, 41]. The yield improvement rate had fallen 10–20% at specific regions (Raza et al., 2019; Jain et al., 2018) [45, 48]. The examination relied upon records from 227 farms in Thailand, Vietnam, Nepal, India, China, Bangladesh, and Pakistan (Jain et al., 2019) [19]. The instrument of this...
falling yield was not palatable, anyway may incorporate extended breath during warm nights, which devours energy without having the choice to photosynthesize (Fu et al. 2019) [11]. In view of expanding temperatures by an unnatural weather change, plants are defenceless to intermittent warmth and dry spell pressure that generally influences the growth and development. Plants adjust to high temperature pressures with basal level resilience innate and can acquire resistance to serious temperature stress (Kim et al., 2011) [29]. Thermo tolerance acquired is very quick and has been demonstrated to be initiated during the phone's acclimation until the temperature time frame is very high. The temperature influences the expansive range of cell and digestion parts, and outrageous temperatures force the seriousness of factors relying upon the degree of progress in temperature, power, and length (Kumar et al., 2021) [32, 33]. The capacity to hold and change in accordance with the supra-ideal temperature results from both warmth harm avoidance and warmth touchy fix segments (Yufang et al., 2021) [67]. Seeds presented to the temperature of the sub-deadly prior to testing with weighty temperatures have a preferred development recuperation over the seeds tested straightforwardly to extreme temperatures (Satisrjay et al., 2016). Both outrageous conditions (dry seasons and floods), on the off chance that they surpass certain basic periods, will have significant ramifications for rice and can cause complete disappointment of rice plants during delicate stages either as water deficiencies or exorbitant splashing (Chaturvedi et al., 2017) [3]. So, there is a need to receive a diverse methodology while considering the effect of high temperature stress, likewise zeroing in on other ecological pressing factors, which might be similarly adverse to rice usefulness (Priyanka et al., 2021b, 2021a) [33, 44]. Resistance acquired for certain abiotic stress has been displayed to give cross security to different pressing factors like saltiness, cold temperatures, and dryness (Kaushik 2019a, 2020b) [34]. Along these lines, assessing the overall exhibition of rice genotypes for high temperature resistance utilizing TIR approach (Liu et al., 2013) [37].

The best temperature for rice germination is some place in the scope of 28 and 30 °C. High temperature impacts essentially all advancement periods of rice from germination to developing (Shah et al., 2011) [50]. The cut-off temperature at the seedling stage has been perceived as 35 °C; the essential sign of warmth stress is the vulnerable turn of events. By thinking about its significance, accessibility, uses and need for creation, heat pressure has acquired a substantial significance because of winning expanded temperatures (Burke et al., 2011) [3]. Warmth stress majorly affects every one of the phases of rice. By taking every single detail into thought, our investigation is focused on heat pressure lenient genotypes in rice accomplished by TIR convention so these genotypes can be additionally used to deliver heat open minded assortments either by reproducing program or by quality articulation examination (Sato et al., 2016) [49].

Material and Methods

Experiment design

The area selected for the experiment is Phenotypic lab at Institute of Frontier Technology, Regional Agricultural Research Station, Tirupati, using TIR (Temperature Induction Response) protocol. The experimental material includes 74 diverse rice genotypes taken from Nellore, Maruteru, several land races and African lines (NERICA) including proven varieties for heat tolerance such as N22, Dular and Nipponbare which were used as genotype checks to choose tolerant sets (Prashanth et al., 2012). These TIR approaches involve first identification of challenging temperature and induction temperatures and then they are standardized before using the germplasms for intrinsic tolerance. Phenotyping of rice genotypes for thermo tolerance utilizing TIR protocol was set up in this lab and the same technique was utilized in this investigation.

Table 1: Genotypes selected for heat tolerance study

<table>
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<tr>
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*, Check Varieties (reported as heat tolerant genotypes by earlier researchers)
Several genotypes were selected to carry out the work. A total of 74 genotypes which include wild relatives, land races, Aus groups, japonica lines etc., were selected for the work. The genotypes are tested for high temperatures to select heat tolerant lines along with proven check varieties.

**Treatments:** Rice seeds were washed with distilled water 2-3 times and are stored for germination at room temperature. After 42 hours, the seedlings that attain 0.5 cm uniformly were selected and planted in an aluminium tray containing blotter paper moistened with water (Bado et al., 2016) [2]. Plates with these seeds are liable to sub-deadly (sub-lethal) temperatures (expansion in slow temperatures for each half an hour from 38 °C to 55 °C for 4 hours in this atmosphere - 'Labline' - (Humidity controlled chamber). Then these seeds are exposed to a deadly (lethal) temperature (55 °C) (induced) for 2 hours. Sub-set of other seeds are exposed directly to the deadly temperature (non-induced). The seeds of the rice induced and non-induced are permitted to recover at room temperature for one week. A control tray is maintained at room temperature, which are not exposed to the temperature of the sub-lethal and deadly (Liu et al., 2017) [38] conditions.

The treatment for the just sprouted seedlings is carried out in a special chamber called TIR (Thermal Induction Response) chamber where the plates are arranged according to the treatment. The chamber contains temperature adjustment along with humidity maintenance. The treatment varieties are tested along with the check varieties to compare the heat tolerance capacity in different varieties.

**Phenotypic Analysis**
Highly vigour seedlings are selected as tolerant types because certain seedlings do not respond properly towards heat stress. The susceptible varieties did not germinate even under suitable conditions. Along with the germination the shoot and root lengths of seedlings are taken into consideration to select tolerant varieties. The maximum root length and shoot lengths of the growing seedlings are compared with each other and the highly tolerant, medium tolerant and susceptible varieties are segregated.

**Molecular Analysis**
Out of 74, a set of 14 genotypes each under tolerant and control conditions were selected based on their survival percentage under sub-lethal conditions and are allowed for Selective line genotyping by comparing with the three checks i.e., N22, Dular and Nipponbare.

**DNA isolation and quantification**
The tender, sprouted seedlings were maintained for 15 days at room temperature thereafter, the complete genomic DNA content of the seedlings was extracted using the CTAB
method. The purified DNA pellet which was obtained through CTAB method is air-dried and dissolved in 50-100μl of TE (Tris base-1M, EDTA-0.5M) buffer (Tenorio et al., 2017). A proper DNA quantification was done using NanoDrop™ 1000 spectrophotometer (ThermoScientific, Wilmington, USA). Quantification of pure nucleic acid for DNA yielded a value of 260/280 ratio of ~1.80 which is proper for a DNA whereas the value more than 1.8 indicates the presence of RNA and less than 1.8 indicates the occupancy of proteins (Jagadish et al., 2012) [18].

Selection of primers
In General, 51 primers were chosen out of which 43 were SSRs reported for heat resilience and 8 were genic markers out of which 4 were brought to light from rice database www.gramene.org, and rest were designed from Primer 3 software. The microsatellite region of candidate genes were identified using SSRIT tool (http://archive.gramene.org/db/markers/ssritool) and then primer designing was done using primer-3 v.4.0.0, a primer designing tool (http://bioinfo.ut.ee/primer3-4.0.0/) (Shammughavadiel et al., 2017) [18]. Reported heat tolerant SSRs and genic SSRs used in the present study are provided in Table S1 and Table S2.

PCR amplification and product isolation
The PCR amplification was performed using an Eppendorf Master cycler to understand the polymorphism. The reactions were performed with standard temperatures and are repeated for 35 times. Agarose gel was prepared to understand the detailed amplification with a permanent marker of 50bp or 100bp which was loaded along with the samples (Xie et al., 2014) [63]. For gel perception UV trans-illuminator was utilized and Alpha Innotech Multi Imager gel documentation framework program from Alpha Innotech, California, USA was used for photography (Vallejos et al., 2007) [59].

Results
It was noted that all the 74 genotypes which were involved in sub-lethal temperatures were considered a major importance involving both tolerant and sensitive classes (Table 2). For all these genotypes the survival percentage was seen as a primary target, where all the 74 genotypes were segregated depending upon their survival ability and growth (Table 2). The survival percentages of all the genotypes were checked by taking their germination and growth into consideration. The survival percentage was calculated in two major classes i.e., SP between 80-100% and another one 60% or less than 60% (Table 2). The genotypes that fall between the SP range of 80-100% were chosen as heat tolerant class and genotypes other than tolerant class were considered as heat sensitive class, depending upon their germination and growth after heat treatment in TIR chamber (Table 2).

<table>
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<th>SP/SL* (%)</th>
<th>S. No.</th>
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* - Survival percent (SP) under sub-lethal (SL) conditions, ** - reported heat tolerant genotypes, # - NL- NERICA Lines: derived from crosses involving O. glaberrimal/O. sativa as parents. LR-Landrace.

A total of 11 genotypes were recorded in the class 0-20% SP (Figure 2) which means no germination at all or less germination, 6 genotypes were recorded in the class 20-40% SP where germination is very less, 4 genotypes were recorded in the class 40-60% SP where the germination is a bit good, later 10 genotypes were recorded in the class 60-80% SP where the germination rate is good and a total of 43 genotypes were recorded in the class 80-100% SP where the germination rate is more and growth is observed in the genotypes.
Certain phenotypic parameters were also taken to understand the survival percentage and growth of the survived seedlings post treatment under sub lethal conditions and compared with the control, which included maximum shoot length (cm) and root length (cm) of both control and sub lethal genotypes (Table 3). Different parameters were taken into consideration to select the heat tolerant genotypes, Survival percentage, Maximum root lengths (MRL) of both control and sub lethal genotypes and also Maximum shoot lengths (SHTL) of both control and sub lethal genotypes (Table 3). The mean, standard deviation, skewness and kurtosis were calculated for all the genotypes under sub-lethal conditions and also for control plates. The skewness and kurtosis were maximum for MRL – control and SHTL - control with the values of 1.45, 1.72 and 8.54, 5.54 respectively (Table 3).

A total of 52 genotypes were recorded in the class of 6.0-8.0 root length in cm in control and maximum of 22 genotypes were recorded in the class of 4.0-6.0 root length in cm in sub-lethal conditions (Table 4). Only one genotype was seen in maximum root length of 12.0-14.0 in control whereas 5 genotypes were observed in the root length class of 10.0-12.0 cm for sub-lethal conditions. Similarly, a maximum of 41 genotypes were considered under 6.0-8.0 shoot length in cm for control whereas 40 genotypes were recorded under 3.0-6.0 cm for shoot length for sub-lethal conditions (Table 4). Altogether only 2 genotypes were recorded in 14.0-16.0 cm for shoot length in control and a maximum of 5 genotypes were observed in 9.0-12.0 cm shoot length in sub-lethal conditions (Table 4).

Heat tolerant genotypes

After checking for the survival percentage of the selected genotypes i.e., 43 which were recorded under 80-100% SP, only 14 were proven to show high root and shoot growth. Later it was found that FR13A and Swarna Sub 1 have performed well and gave good results than the already proven check Dular in terms of germination, survival, shoot length and root length (Table 4). All the 14 genotypes gave good performance at higher temperatures. Although 3 genotypes like BPT 1235, JGL 3855 and Basmati 386 showed 80% SP, the others showed 100% SP at sub-lethal temperatures (Table 4). It was observed that maximum root length is more for BPT 1235 (check) which is around 10.74 cm whereas it is less for VL Dhan 16 i.e., 4.80 in sub-lethal conditions (Table 4). Also, it was observed that maximum shoot length is more for BPT 1235 i.e., around 11.75 cm and less for Vajram which is 5.23 in case of sub lethal conditions. In comparison of both the root and shoot lengths with control and sublethal the relativity is calculated i.e., relative lengths of root and shoot in which relative root length is maximum for FR 13A which is around -31.59 (Table 4). Also, the relative shoot length is maximum for FR 13A which is around -59.11 proving that FR 13A is giving good results similar to that of the checks and compared with all the 14 varieties (Table 4).
The relative root and shoot lengths were calculated using maximum root and shoot lengths of control and sublethal. Out of all the three checks taken relative root and absolute shoot length is maximum in case of Dular whereas the relative root length and the relative shoot length of FR 13 A is maximum i.e., 62.53 and 106.20 respectively compared to other selected and tolerant varieties. Interestingly the values of FR13A were more than that of check variety Dular proving it to be highly tolerant.

Molecular analysis
All the 51 primers were standardized between the temperatures 55°C-59°C (Table 5). Most of the primers were standardized at 59°C temperatures but very few were observed at 57°C which include RM10115, RM10469, RM15087, RM282, RM17270 and RM17296 (Table 5). It was also shown that the product size is maximum for RM19715 which is 350 bp and it was observed that the product size of the primer is less for RM16216 which is around 90-110 bp (Table 5). We designed a set of 8 primers with respect to heat tolerance in rice using Primer 3 software (Table 6). These primers were designed from different HSP families and TT 1 (Thermal tolerance gene) gene targeted markers. We chose four polymorphic primers namely, RM16216, RM 17271, RM 5687 and a TT 1 gene targeted marker, TTC/TTM primers in the study (Table 5 and Table 6) for screening all the 14 genotypes each from heat tolerant and sensitive groups to understand the allelic patterns.
Several primers were selected from the literature based on their performance with respect to heat tolerance in several rice varieties. The product size of the primers were between 90 - 350 where the maximum size is observed in case of RM 19715 and minimum price is observed for RM 16216.

**Table 6:** List of genic primers, primer annealing temperature and their observed product sizes (bp)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Primer Name</th>
<th>Locus</th>
<th>Chr.</th>
<th>Tm</th>
<th>Product Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>RGNMS264</td>
<td>LOC_Os01g43650</td>
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<td>59</td>
<td>100</td>
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<tr>
<td>2</td>
<td>TTC/TTW</td>
<td>LOC_Os03g26970</td>
<td>3</td>
<td>59</td>
<td>130</td>
</tr>
<tr>
<td>3</td>
<td>TTC/TTM</td>
<td>LOC_Os03g26970</td>
<td>3</td>
<td>59</td>
<td>100 and 350</td>
</tr>
<tr>
<td>4</td>
<td>RGNMS2015</td>
<td>LOC_Os05g45410</td>
<td>5</td>
<td>59</td>
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</tr>
<tr>
<td>5</td>
<td>RGNMS2618</td>
<td>LOC_Os06g11610</td>
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<tr>
<td>6</td>
<td>SVHT801</td>
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<tr>
<td>7</td>
<td>RGNMS3524</td>
<td>LOC_Os11g32030</td>
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<td>59</td>
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<td>8</td>
<td>SVHT665</td>
<td>LOC_Os12g07665</td>
<td>12</td>
<td>59</td>
<td>200</td>
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</table>

The allelic patterns of the polymorphic markers were observed between checks, tolerant and sensitive genotypes (Fig 3). Several genotypes exhibited allelic patterns between 80-120 bp in case of tolerant group but not 120bp (Fig 3) with the primer RM5687.

**Fig 3:** Polymorphic amplification pattern generated by RM5687 marker between the selected heat tolerant and sensitive genotypic classes under study

~ 59 ~
Further, it was determined that both RM 17270 and RM 5687 were heat tolerant primers with polymorphic patterns, and also the distribution of genotypes between checks using these primers was understood (Table 7). The three major varieties like Basmati 386, FR13A, Swarna Sub1A were concluded to have high heat tolerance not only due to their phenotypic characters but also their polymorphic patterns were also considered with the checks. Basmati 386 gave less growth compared to the other two varieties when considered along with the phenotypic characters (Table 7).

**Analyzing the informativeness of polymorphic markers**

Each genotype was assigned an allele code generated by respective polymorphic marker based on their allele size and colour coded as shown in Figure 4. As many as a highest number of 100bp alleles were identified by TTC/TTM in the range of 100 to 350bp (Table 7). It was understood that the marker RM17270 was amplified at 190bp in N22, but no other genotype showed similar allele size to N22. The same marker was amplified at 195bp in Dular and Nipponbare genotypes, wherein Basmathi386, FR13A and Swarna Sub1A were also shown similar amplification pattern like Dular and Nipponbare (Table 7). The marker RM16216 showed three kinds of alleles i.e. 90bp, 100bp and 110bp. Of which, 90bp and 100bp are major alleles (Table 7).

**Table 7:**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Genotype</th>
<th>RM 17270</th>
<th>RM 16216</th>
<th>RM 5687</th>
<th>TTC/TTM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TOLERANT</td>
<td></td>
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</tr>
<tr>
<td>A</td>
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<td>A</td>
</tr>
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<td>Dular*</td>
<td>B</td>
<td>A</td>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td>C</td>
<td>Nipponbare*</td>
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<td>A</td>
<td>E</td>
<td>A</td>
</tr>
<tr>
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<td>A</td>
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<td>BPT1235</td>
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<td>A</td>
<td>B</td>
<td>G</td>
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<td>FR13A</td>
<td>B</td>
<td>A</td>
<td>C</td>
<td>A</td>
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<tr>
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<td>Jagannath</td>
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<td>A</td>
<td>E</td>
<td>A</td>
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<td>C</td>
<td>A</td>
<td>B</td>
<td>A</td>
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<tr>
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<td>C</td>
<td>G</td>
<td>A</td>
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<td>A</td>
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<tr>
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<td>B</td>
<td>B</td>
<td>A</td>
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<td>Vajram</td>
<td>C</td>
<td>B</td>
<td>C</td>
<td>A</td>
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<tr>
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<td>VL Dhan16</td>
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<td>B</td>
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<td>A</td>
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<tr>
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<td>A</td>
<td>C</td>
<td>A</td>
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<td>4</td>
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<td>A</td>
<td>C</td>
<td>A</td>
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<td>D</td>
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<td>B</td>
<td>G</td>
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<td>B</td>
<td>G</td>
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<tr>
<td>12</td>
<td>Udayagiri</td>
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<td>B</td>
<td>E</td>
<td>A</td>
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<tr>
<td>13</td>
<td>LN 409</td>
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<td>B</td>
<td>E</td>
<td>A</td>
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<tr>
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<td>NLR 30491</td>
<td>C</td>
<td>B</td>
<td>D</td>
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**Allele Size**

<table>
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<tr>
<th></th>
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<th>RM 5687</th>
<th>TTC/TTM</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>190</td>
<td>90</td>
<td>80</td>
<td>100</td>
</tr>
<tr>
<td>B</td>
<td>195</td>
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<td>350</td>
</tr>
<tr>
<td>C</td>
<td>200</td>
<td>110</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>205</td>
<td></td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>E</td>
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<td>F</td>
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<tr>
<td>G</td>
<td></td>
<td></td>
<td>120</td>
<td></td>
</tr>
</tbody>
</table>

Fig 4: Allele score as a check to detect heat tolerant varieties.
Table 7: Genotypes that showing similar allele pattern with reported heat tolerant sources for markers RM17270 and RM5687

<table>
<thead>
<tr>
<th>PRIMERS</th>
<th>N 22^</th>
<th>DULAR^</th>
<th>NIPPONBARE^</th>
<th>DULAR^/ NIPPONBARE^</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM 17270</td>
<td>-</td>
<td>-</td>
<td>Basmati 386, FR13A, Swarna Sub1A</td>
<td></td>
</tr>
</tbody>
</table>
| RM 5687  | Konark | Sathyam, Vajram, VLDhan16, Erramallelu\^
|          |       | Kab AusR270\^
|          |       | Jaganadh, Binhumgiri\^
|          |       | BPT 5204\^
|          |       | Lachit\^
|          |       | Udayagiri\^
|          |       | LN 409\^

^\*- reported heat tolerant genotypes, \#- heat sensitive genotypes

Discussions

High temperature stress (increase in more than tolerable air temperature) is perhaps the main natural element impacting crop development, advancement, and yield measures. This pressure initiates numerous biochemical, molecular and physiological changes and reactions that impacts different cells and entire plant measures that influence crop yield and quality (Wei et al., 2012) [62]. The impact of environmental stresses, particularly those of dry season and heat stress, has been studied separately. However, under natural conditions, both of these stresses often occur in union (Jagadish et al., 2010) [17]. The worldwide expansion in temperature will likewise expand the seriousness of other natural pressing factors like floods and dry seasons. Varieties in precipitation will cause flooding and more incessant dry seasons which are the main deterrents for the internal waters and vigorous planting (Veerasekaran et al., 2021).

The present investigation was, therefore, undertaken to study the effect of high temperature on survivability, growth and its component traits on different rice genotypes, to identify heat tolerant genotypes and to validate and tag the reported molecular markers and as well newly designed genic SSR markers among high temperature progressive and vulnerable genotypes (Chauhan et al., 2020; I. Dhaliwal et al., 2020; S. K. Dhaliwal et al., 2020; Kaushik, 2021, 2020) [6, 7, 8]. Also, genetic diversity is the foundation of the genetic improvement of crop plants as it serves as a reservoir for identifying superior lines that can withstand heat stress (Liang et al., 2010; Wanwarang et al., 2020) [35, 41]. Selective Line Genotyping (SLG) used in this study involves initial polymorphism study with extreme few numbers (two) of genotypes to allow/score remaining alleles (allelic variants) of high-types with polymorphic markers identified using extreme core bulks. Later we used those identified polymorphic markers to screen 14 genotypes each that fall in both extreme phenotypic classes individually (Yongyooa et al., 2019) [65]. Thus, SLG can be believed to be superior to BLA, as it arrives at identification of more kinds of alleles and allele patterns of genotypes; and also, their degree of trait association at particular locus. This is evident through different degrees of occurrence of each allele among high-types in the current study (Katherine et al., 2020). Therefore, polymorphic primers are considered very important to understand tolerant and sensitive genotypes because of their high informativeness (Ye et al., 2012).

Consequential variations exist among rice germplasm in response to maximum temperature stress viz. blooming at cooler occasions of day (early daytime blossoming), more dust feasibility, bigger anthers, longer basal dehiscence and presence of long basal pores, are some of the phenotypic markers for high temperature tolerance (Kheir et al., 2012) [28]. So, the development of more sustainable, resilient agricultural systems could be achieved by identifying heat tolerant rice genomes and development of new rice varieties for mitigating the yield losses under high temperature conditions during summer and kharif seasons (Kobayashi et al., 2011) [30]. The temperature above 35°C results in low seed setting rate, causes floret sterility and abnormal pollination (Lin et al., 2012) [36]. Day by day temperatures, higher than 30°C or every day greatest temperatures higher than 35°C during the blossoming time frame will bring about helpless anther dehiscence. Plants’ heat response is highly complex (Hedhly et al., 2011) [14]. In current days, some reputed techniques like gene editing are being used to address the heat tolerant/induced functional basis of the genotype. From the recent experiments conducted, it was proven that high temperature decreases the grain filling period in basmati rice from 32 to 26 days, reduced yield by 6%, and caused a decrease in absolute starch (3.1%) and amylose content (22%). Quantifiable exercises of key chemicals associated with sucrose to starch change, sucrose synthase, ADP-glucose pyrophosphorylase, starch phosphorylase and dissolvable starch synthase in endosperms created at 32°C were lower than those at 22°C contrasted and comparable aging stage on an endosperm premise. Specifically, granule-bound starch synthase (GBSS) action was fundamentally lower than comparing action in endosperms creating at 22°C during every formative stage (Ahmed et al., 2015) [1].

The genotypes selected for the study include genotypes from Indica, Japonica Javanicas, Aus groups and wild relatives of rice. These genotypes are selected and are allowed to grow under sub-lethal conditions. The genotypes giving good performance i.e., survivability, maximum root and shoot lengths are selected and are separated from the entire genotype sets (Alberio et al., 2018) [4]. High temperatures are induced by using TIR protocol in which temperatures to a maximum level and control in humidity can be done (Sudhakar et al., 2013) [57]. TIR technique relies on genotype’s acquired tolerance, to determine their heat tolerance, wherein a gradual exposure of genotypes to a temperature regime over a period was used. Using TIR technique, it was proved that sufficient genetic variability was present among rice genotypes for high temperature tolerance. The percent survival of seedlings varied from 0 to 100% with a mean of 80.33%. The percent reduction in root growth varied from 0 to 73% with a mean of 20.89%. Four genotypes namely NLR 34242, NLR40066, NLR40059, NLR40050 also exhibited higher thermotolerance without reduction in root but slight reduction in seedling survival by 10%. Similar studies were also conducted by Vijayakshmi et al. (2015) [56]. Later molecular analysis was carried out to check the polymorphic patterns obtained by heat tolerance specific SSR primers. A total of 51 primers were selected out of which 43 are chosen from literature and 8 are designed by using Primer 3 software, these designed primers belong to different HSP (Heat Shock protein) families (Guo et al., 2020) [13]. Molecular markers are very important tools in the understanding the genetic variation and in interpretation of genetic relationships within and among different species (Zhu et al., 2017) [46]. Simple sequence repeats (SSRs), also known as the microsatellite DNA marker, is an effective tool for...
identifying genetic differences of germplasm resources with the advantages of ease, clarity and maximum polymorphism, co-dominance and its steadiness (Liang et al., 2010; Ma et al., 2010) [33]. There has been developing interest with the use of marker-based models in recent years. In the studies conducted by using these models, descriptions of the effect of allele coding system on inference and computations are often absent or missing (Lv et al., 2017) [39]. Also, several other common allele coding alters these regression coefficients by deducting a value from each marker such that the mean of regression coefficients is zero within each marker. It is also known as centered allele coding (Stranden et al., 2011) [56]. This kind of coding is highly useful to identify the genotypes that fall within similar allele size ranges for a major number of loci, to identify like allele pattern with proven genotypes (heat tolerant) and to group them under a class (Shim et al., 2020) [55].

Thus, keeping in lieu of the above identified potently reported polymorphic set of markers, irrespective of their allelic differences among the genotypes it can be concluded that the efficiency of selective line genotyping technique used in the current study is remarkable in associating effective markers to the trait (Sharma et al., 2019; 2020) [52, 53]. Further the efficiency of the technique is well explored with limited genotyping panel to arrive at small number of useful markers out of huge tests, for further studies (Gouda et al., 2020) [12]. The above outcomes propose that the TIR strategy is an amazing and productive method to recognize hereditary changeability in high temperature resistance in rice inside a brief timeframe and it is appropriate for screening an enormous number of genotypes (Hsuan et al., 2019) [10].

Conclusion
Rice occupies 23% of the total area under cereal production in the world. It is the staple nourishment for the greater part of the total populace with Asia addressing the biggest producer and consumer region. For the most part, rice is unfavourably influenced by high temperature in the lower heights of the tropical regions. Under molecular analysis carried out in the present study, a set of 51 reported and as well new SSR and genic markers were employed to assess the genetic diversity among the 74 rice genotypes. These markers gave good amplification with prominent alleles at 57˚C and 59˚C. By employing Selective line genotyping approach; both of each fourteen selected Tolerant and Sensitive genotypes along with three reported genotypes (N22, Dular and Nipponbare) were subjected for genotyping with a total of 51 markers. Out of 51 markers 92% were monomorphic between heat tolerant and sensitive classes, as well as compared to checks. Four markers i.e., RM17270, RM16216, RM5687, TTC/TTM, were polymorphic. Despite the fact that, allele coding is a viable strategy the alleles produced from various polymorphic markers were not unmistakably recognized lenient set from touchy arrangement of genotypes like TIR procedure. The recognized 14 genotypes of rice can be utilized as contributor hotspot for growing high temperature lenient rice genotypes to oppose worldwide ascent temperature.

References
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10.1016/j.fcr.2015.10.012.


