7. Heat stress tolerance

Parameswaran C, M Shahid, J Meher, P Swain and N Basak

7.1 Introduction

By the end of the 21st Century, the earth's climate is predicted to warm by 1.1-6.4 °C (IPCC 2007), due to both anthropogenic and natural factors (Eitzinger *et al.*, 2010). Emission of greenhouse gasses (GHG) such as carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) from agricultural systems is one of the major sources contributing to this global increase of temperature (Maraseni *et al.*, 2009; Smith and Olesen, 2010). It is reported that high temperatures would cause a marked decrease in world rice production. In tropical regions, high temperatures are a constraint to rice production and the most damaging effect is on spikelet sterility. Heat stress in rice affects the anthesis and grain filling stages of the crop. The optimum temperature for rice growth and development is 24-35°C. Even one-degree rise in temperature above the optimum temperature results in 7-10% loss in the yield of rice. Apart from yield, grain quality is also significantly altered during heat stress in rice. High temperature during anthesis severely affects the pollen viability and pollen germination on stigma. Thus, late sown *rabi* season rice crop and timely sown long duration cultivars are highly sensitive to heat stress in eastern India. Accordingly, several studies on heat stress in rice are undertaken for the screening of genotypes in field and control growth conditions for tolerance, physiological efficiency, and management effect on minimizing heat stress impact on rice.

7.2. Facilities for heat tolerance study

7.2.1 Temperature gradient tunnel (TGT)

The control system in the TGTs (Plate 7.1) includes exhaust fans, humidity controller and temperature measurement using thermometers. Studies related to screening of genotypes in heat stress, effect of B on high-temperature tolerance of rice genotypes are performed at TGT facility at NRRI.

Plate 7.1: Temperature gradient tunnels at NRRI

7.2.2 Open-top chamber (OTC)

These OTCs (Plate 7.2) are designed with feedback control system to maintain ambient or increased air temperatures within the chambers. The parameters such as temperature and carbon dioxide levels can be controlled in open top chambers. The other parameters are similar to the external field condition (Krishnan *et al.*, 2011). Experiments are regularly conducted related to effect of carbon dioxide and temperature on grain yield of rice.

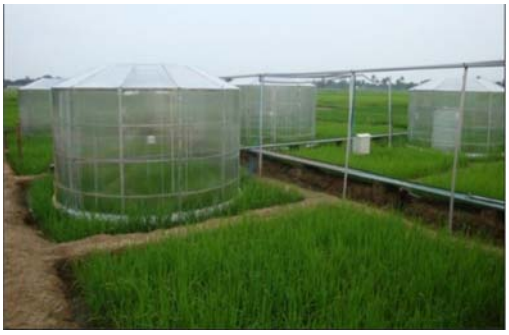

Plate 7.2: Open-top chambers at NRRI

Plate 7.4: Growth chamber facility at NRRI

7.2.3 Rapid generation advance (RGA)

The RGA facility (Plate 7.3) is used for the advancement of generations using modified single seed descent method. The parameters such as light quality, temperature, humidity can be controlled from the desktop in the lab. The temperature control systems are used for the administration of heat stress in rice at anthesis and grain filling stages (Pradhan *et al.*, 2016). Apart from advancement of breeding materials, experiments on other stresses are also performed at RGA facility.

Plate 7.3: Rapid generation advance facility at NRRI

7.2.4 Growth chamber

Growth chamber (Plate 7.4) can artificially replicate the conditions under which the plant material might be exposed. It has been optimized for allowing maximum growth area. Environmental parameters such as temperature, humidity and light can be controlled. Physiological studies related to heat stress are carried out in the growth cabinets.

7.3 Screening for heat stress tolerance

At present, there are only few donors are available for heat tolerance in rice. Also, screening of several genotypes in control condition such as RGA, phytotron, TGT has limitation of space and time. Thus, the strategy for heat tolerance screening in rice involves screening of genotypes in field condition under staggered sowing during dry season in which flowering time coincides with maximum temperature of the region. The better performing genotypes in field condition are evaluated in control condition for confirmation of the heat tolerance (Tenoria *et al.*, 2013).

7.3.1 Daytime heat stress treatment at flowering stage

In initial field screening of genotypes for heat tolerance, staggered sowing is followed so that the flowering time coincides with the maximum temperature of the region. Based on number of genotypes, two or three staggered sowing can be done (Table 7.1). For all the sowing window, adequate susceptible and tolerant checks are taken. The maximum temperature at the time of flowering or anthesis of each genotype is noted down for the staggered sown lines. At

Table 7.1: The maximum temperature observed during 50% flowering of three different staggered sowing periods in NRRI from 2000-2015.

Sl No	Staggered sowing (SS)	Time of 50 % flowering	Average maximum temperature at 50 % flowering
1	SS1	2nd and 3rd week of March	32°C
2	SS2	4th week of Mar and 1st week of April	33.8 °C
3	SS3	2nd and 3rd week of April	37 °C
4	SS4	Last week of April and 1st week of May	39.6 °C

physiological maturity or harvest, three to five panicles from a hill and three hills in a genotype are harvested and spikelet sterility percentage calculated. Based on effective tillers, 1000 grain weight, harvest index and spikelet sterility genotypes are selected for further screening in control condition.

For screening of genotypes in control conditions, seeds of selected genotypes showing tolerance at field screening are sown in trays and transplanted in pots for screening. The transplanted pots are kept in trays to ensure same amount of water and nutrients are available for all the seedlings. Due to space factor, for all the genotypes, three replications for treatments and one for control are used. Also, during the tillering stage, only the main stem and two tillers are maintained to avoid overcrowding. The heat stress schedule for flowering stage heat stress is given in Table 7.2.

The high-temperature treatment schedule should be followed until anthesis of all the spikelets in the panicles is completed. After heat stress treatment, the plants are moved back to net house condition and spikelet sterility is measured. Then, the spikelet sterility data of field stress and control condition must be compared for the identification of best donor for heat tolerance.

Table 7.2: Environmental settings for high-temperature treatment under controlled condition

Step	Time	Duration	Tm (°C)	RH (%)	Light intensity (µmol m^{-2} s^{-1})
1	06:30-07:00	30 min	27	75	330
2	07:00-07:30	30 min	30	75	460
3	07:30-08:00	30 min	35	70	580
4	08:00-08:30	30 min	38/39	70	580
5	08:30-14:30	360 min	38/39	70	580
6	14:30-15:30	60 min	35	70	580
7	15:30-16:30	60 min	30	70	580
8	16:30-17:30	60 min	27	75	460
9	17:30-18:30	60 min	24	75	330
10	18:30-06:30	720 min	24	75	0

(Tenoria *et al.*, 2013)

Tm = temperature, RH = relative humidity. Note: Maximum temperature of 38°C is used for treatment at flowering stage and 39°C for booting stage.

7.3.2 Nighttime heat stress treatment in rice

Optimum night temperature for proper grain filling in rice is 22-26°C. High night temperature *i.e.* more than 27°C reduces the grain weight in rice. The night heat stress treatment is given for 5-6 hours for the anthesis to grain filling stages of crop. The control pots will be kept in day/night temperature of 30/24°C. For heat stress, the pots will be maintained at 30/30°C. The timing of night heat stress treatment is from 00:00 hours to 05:30 hours every day during the duration of the treatment. In dawn, the temperature will be slowly increased to simulate the natural condition and will be maintained at 30°C up to 14:30 hrs. After treatment, the spikelet fertility will be calculated at the physiological maturity of the crop (Coast *et al.*, 2015).

7.4 Phenotyping for heat stress tolerance

7.4.1 Spikelet sterility and grain weight

Spikelet sterility is measured by following the method given by Jagadish *et al.* (2007) with some modifications. Rice panicles are harvested during the maturity stage of the crop. Three hills are randomly selected from each genotype and three - four panicles per hill are collected for the measurement of spikelet sterility. Similarly, thousand grain weight is also measured from panicles collected from three different hills in the field. Generally, the weight of 100 grains are measured from a hill and converted into thousand grain weight.

7.4.2 Pollen viability and pollen germination

Pollen viability and pollen tube growth studies are performed based on the report of Chunn *et al.* (2007). Briefly, six anthers are collected from a plant before anthesis and stored in 70% ethanol for further analysis. Pollen viability analysis is done for three plants in a genotype. The anthers are crushed with 10 µ of 1% (v/v) of I2 in 3% (v/v) KI solution and 1 µ is taken in glass slide for viability measurements. Pollen germination studies are performed by staining through aniline blue dye. The collected pollens are dusted on the stigma and after 30 minutes of dusting, the stigma is viewed under a light microscope for measuring the number of pollens germinated on the stigma.

7.4.3 Antioxidant enzymes assay

Various antioxidant enzymes implicated in stress physiology like superoxide dismutase, catalase and peroxidase can be estimated for tolerant and susceptible genotypes.

Superoxide dismutase assay is based on the capacity of the extracts to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) in the presence of the riboflavin–light-NBT system. (Beauchamp and Fridovich, 1971). Briefly, samples are extracted with 10 mL of distilled water and the supernatant obtained after centrifugation is used as enzyme source. The reaction mix contains phosphate buffer, methionine, NBT, EDTA, riboflavin and enzyme extract. The reaction is started by placing the reaction mix under a light source. After 10 min, the reaction is stopped by switching off the lights and the absorbance is read at 560 nm. A non-irradiated reaction mixture will have zero absorption at 560 nm, while the reaction mixture lacking enzyme will develop the most intense colour. The volume of enzyme extract producing 50% inhibition of the reaction has to be calculated. One unit of superoxide dismutase activity is defined as the enzyme which causes 50% inhibition of the initial rate of reaction in the absence of enzyme.

Catalase activity is estimated following the method of Kar and Misra (1976). The

sample is ground with 0.1 *M* phosphate buffer of pH 7.0, centrifuged and the supernatant is utilized as enzyme source. Phosphate buffer, 0.05 *M* H_2O_2 and the enzyme source is pipette out in a conical flask and incubated for three min. After three min, the reaction is stopped by addition of 0.7 *N* H_2SO_4. This reaction mixture is then titrated against 0.01 *N* $KMnO_4$ to find out the residual H_2O_2, the end point being the appearance of light violet colour which persists for at least 15 seconds. The blank is prepared by adding the enzyme source to phosphate buffer, 0.05 *M* H_2O_2 and 0.7 *N* H_2SO_4 are added to it and immediately titrated against $KMnO_4$. The enzyme activity is expressed as units. One unit of catalase activity is defined as the amount of enzyme which is needed to destroy 1µ*M* of H_2O_2 per minute per gram of fresh tissue under the assay condition.

For estimation of peroxide activity, the sample is extracted with 10 mL Tris-HCl buffer (pH 7.6) and centrifuged. The supernatant so obtained is used as an enzyme source. The reaction mixture consisting of H_2O, sodium acetate buffer and o-dianisidine is added to the enzyme source and thoroughly mixed. 0.1 mL of H_2O_2 is added and the change in absorbance per minute is monitored at 430 nm at intervals of 1-3 min by the method of Shannon (1966). Tris-HCl buffer is used as a blank.

7.4.4 Cell membrane thermo-stability

Heat stress increased the respiration rates and can lead to production of reactive oxygen species (ROS) in plants. A common method of evaluating damage to membranes is by examining cell membrane thermostability (CMS), which measures electrolyte leakage from tissues, such as leaves, subjected to stress (Fokar *et al.*, 1998). Briefly, to measure CMS, samples are selected with similar leaf size at different growth stages and cut into small pieces (3.5 cm long). These pieces, in three replicates, are then taken in a closed tube with 1 mL distilled water. The treatment tubes are placed in a water bath at 52°C for one hour (T1), while the controls should be kept at 10°C (C1). After treatment, 9 mL of distilled water is added to all tubes (treatment and control) and incubated at 10°C for 24 h. The tubes are brought to room temperature, and the conductance of the solution is measured with a conductivity meter (T1, C1). After the measurements are taken, the tubes are autoclaved at 100°C for 15 min and their conductance measured again (T2, C2). CMS is then calculated as by following formula

$$CMS\ (\%) = \frac{1 - \frac{T_1}{T_2}}{1 - \frac{C_1}{C_2}}$$

7.4.5 Photosynthesis rate, transpiration rate and stomatal conductance

The photosynthesis rate, transpiration rate and stomatal conductance are measured by Infrared Gas Analyzer (IRGA). IRGA are used for the measurement of a wide range of hetero atomic gas molecules including CO_2, H_2O, NH_3, CO, SO_2, N_2O, NO and gaseous hydrocarbons like CH_4. Hetero atomic molecules have characteristic absorption spectrum in the infra-red region, therefore, absorption of radiation by a specific hetero atomic molecule is directly proportional to its concentration in an air sample. Infra-red gas analyzers measure the reduction in transmission of infra-red wavebands caused by the presence of gas between the radiation source and a detector; this reduction in transmission is a function of the concentration of the gas.

In brief, the working procedure of an IRGA is as outlined: one pair of CO_2 and H_2O analyzers defined as reference measures the

CO_2 and water vapour concentration in the ambient air that is sent into leaf chamber, similarly the second pair; the analysis chambers measure the CO_2 and water vapour concentrations in the air that is coming from the leaf chamber. The difference between the reference and the analysis IRGAs are computed. Parameters recorded from IRGA include photosynthetic rate in µmole CO_2 m^2 s^{-1}, stomatal conductance in mole H_2O m^2 s^{-1} and transpiration rate in mmole H_2O m^2 s^{-1}.

7.5 Screening for heat stress tolerance

7.5.1 Markers associated with heat tolerance

There are several QTL has been identified for heat tolerance in rice (Table 7.3). The associated markers can be utilized for introgression of genomic regions into high yielding variety. The marker closely associated with the QTL region are given below:

7.5.2 Breeding strategy for heat tolerance in rice

The breeding strategy and QTL mapping for heat tolerance is given in Fig. 7.1. Breeding rice varieties for heat stress tolerance is based on selection of grain yield, spikelet fertility and 1000 grain weight. Marker assisted breeding involves mapping of QTL which open some new avenue for targeted breeding programme which can reduce the breeding cycle.

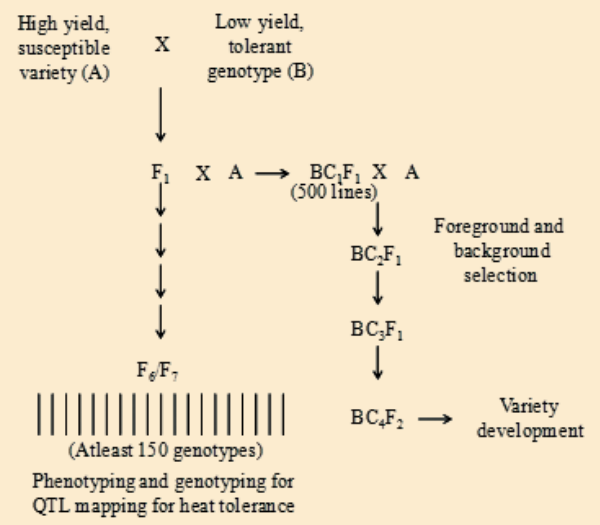

Figure 7.1: Breeding strategy for heat tolerance in rice

7.5 Genotypes and varieties

Staggered sowing based screening for thermotolerance performed for seven hundred genotypes and three genotypes found highly tolerant to heat stress with SES score 1 were AC39843, AC39834 and AC39969 with more than 80% spikelet fertility that was *at par* or better than the tolerant check Annapurna and N 22 were identified (Table 7.3).

Table 7.3: List of single sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers for use in breeding program

Chromosome	QTL	Marker	LOD*	Phenotypic variation (%)
1	qHTSF1.2	id1013342	5.16	13.30
3	qHT3	RM570-RM148	13.60	11.40
4	qHTSF4.1	id4005120	4.06	17.60
5	qHTH5	RM592-RM7921	8.90	110.70
6	qHTSF6.1	id6007495	4.45	20.60
11	qHTSF11.2	id11003281	10.65	15.20

*LOD: Logarithm of odds

Table 7.3: Evaluation of rice genotypes based on spikelet fertility in heat stress

Sl No	SES score	Spikelet fertility %	Accession name	Heat tolerance
1	1	More than 80%	AC39843, AC39834, AC39969, N22, Annapurna, US 312, IET 24120	Highly tolerant
2	3	61-80 %	AC39975, AC11069, AC39935, AC10925, ADT43	Moderately tolerant
3	5	41-60 %	Pusa44, Ratna, Tapaswini, Surendra	Susceptible
4	7	11-40%	IR64, Naveen, Satabdi, Lalat	Highly susceptible
5	9	Less than 11%	-	Highly susceptible

References and further reading

Beauchamp C, Fridovich I. 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry*. **44**(1): 276-287.

Chhun T, Aya K, Asano K, Yamamoto E, Morinaka Y, Watanabe M, Kitano H, Ashikari M, Matsuoka M, Ueguchi-Tanaka M. 2007. Gibberellin regulates pollen viability and pollen tube growth in rice. *The Plant Cell*. **19**(12), 3876-3888.

Coast O, Ellis RH, Murdoch AJ, Quiñones C, Jagadish KS. 2015. High night temperature induces contrasting responses for spikelet fertility, spikelet tissue temperature, flowering characteristics and grain quality in rice. *Functional Plant Biology*. **42**(2), 149-161.

Fokar M, Blum A, Nguyen HT. 1998. Heat tolerance in spring wheat. II. Grain filling. *Euphytica*. **104**(1): 9-15.

Ishimaru T, Hirabayashi H, Sasaki K, Ye C, Kobayashi A. 2016. Breeding efforts to mitigate damage by heat stress to spikelet sterility and grain quality. *Plant Production Science*. **19**(1): 12-21.

Jagadish SVK, Craufurd PQ, Wheeler TR. 2007. High temperature stress and spikelet fertility in rice (*Oryza sativa* L.). *Journal of Experimental Botany*. **58**(7): 1627-1635.

Kar M, Mishra D. 1976. Catalase, peroxidase, and polyphenoloxidase activities during rice leaf senescence. *Plant Physiology*. **57**(2): 315-319.

Krishnan P, Ramakrishnan B, Raja Reddy K, Reddy VR. 2011. High-temperature effects on rice growth, yield, and grain quality. *Advances in Agronomy*. **111**: 87.

Krishnan P, Swain DK, Bhaskar BC, Nayak SK, Dash RN. 2007. Impact of elevated CO_2 and temperature on rice yield and methods of adaptation as evaluated by crop simulation studies. *Agriculture, Ecosystems & Environment*. **122**(2): 233-242.

Pradhan SK, Barik SR, Sahoo A, Mohapatra S, Nayak DK, Mahender A, Meher J, Anandan A, Pandit E. 2016. Population Structure, Genetic

Diversity and Molecular Marker-Trait Association Analysis for High Temperature Stress Tolerance in Rice. *PloS One*. **11**(8): e0160027.

Shannon LM, Kay E, Lew JY. 1966. Peroxidase isozymes from horseradish roots I. Isolation and physical properties. *Journal of Biological Chemistry*. **241**(9): 2166-2172.

Tenorio FA, Ye C, Redoña E, Sierra S, Laza M, Argayoso MA. 2013. Screening rice genetic resources for heat tolerance. *SABRAO Journal of Breeding and Genetics*. **45**(3): 371-381.

Ye C, Tenorio FA, Argayoso MA, Laza MA, Koh HJ, Redoña ED, Jagadish KS, Gregorio GB. 2015. Identifying and confirming quantitative trait loci associated with heat tolerance at flowering stage in different rice populations. *BMC Genetics*. **16**(1): 1. DOI: 10.1186/s12863-015-0199-7.❄