
Studies to Improve Wheat for High Temperature Stress Areas

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Abstract: Heat stress is one of major limitations in wheat (*Triticum aestivum* L.) productivity in arid, semiarid, tropical and semi tropical regions of world. Wheat is grown as winter cereal crop in subtropical countries like India. The crop experiences chronic high temperature at most of phenological stages of growth. A detailed understanding of genetic variation and mechanisms of heat tolerance in wheat would facilitate development of intrinsically thermotolerant genotypes. To understand thermotolerance of currently grown wheat varieties, studies on physiological assays at seedling stage, canopy temperature depression (CTD), dry matter translocation (DMT) were carried out along with estimation of field performance. Seedling screening assays identified varieties showing thermotolerance at seedling stage and showed association with high temperature tolerance at adult plant stage. Advanced generation selections were developed from the crosses involving these genotypes, which showed yield advantage over standard check in rod row trials at three different locations. The thousand-kernel weight for these selections ranged from 47.7 to 50.1g with average increase of 13% over the parental mean at Pune location. Studies on an alternative dwarfing gene *Rht8*, known to reduce plant height by 10 percent without significant reduction in yield under high temperature was also undertaken. Genotypic and phenotypic analysis of 92 genotypes confirmed the absence of *Rht8* gene in the cultivated varieties of India. *Rht8* gene was transferred to tall genotypes viz. Ajantha and MP3054 from donor Chuan Mai18. Studies on canopy temperature depression on a set of varieties in complementation with dry matter translocation established that cooler canopies with a longer grain-filling period of 40-42 days after ear emergence showed advantage under continual heat stress throughout growth period. Further research is in continuation to dissect and understand the genetic basis to variation in heat stress tolerance exhibited by different varieties.

Keywords: Heat Stress, Wheat, Canopy Temperature, Dry Matter Translocation, *Rht8*

1. Introduction

High temperature stress is a major environmental constraint limiting wheat productivity in many parts of the world. The optimum temperature for all physiochemical processes of wheat from vegetative to reproductive stage is 20°C or lower [1]. The bread wheat, which originated in Mediterranean region, migrated to the cooler climate of temperate regions. It also migrated to warmer regions and conquered the subtropical areas particularly after development of suitable semi-dwarf varieties. Wheat cultivation continue to spread in non-traditional areas. Further spread in subtropical/tropical areas will depend upon ability of this crop to tolerate warm climate. The global warming is likely to change the climate of conventional wheat growing areas and the wheat varieties should have

tolerance to higher temperature.

India is the second largest producer of wheat. In India, spring wheat is cultivated, which is sown at the beginning of winter and harvested in the beginning of summer. The total area under wheat cultivation is divided in to six agro-climatic zones. Wheat crop experiences high temperature stress throughout crop season in warmer areas (Central and Peninsular zones) and at or after anthesis in North Western Plains Zone (NWPZ) and North Eastern Plains Zone (NEPZ). Heat stress damages the plant system in a complex manner by disrupting cell membranes [2], enzyme deactivation, and disruption of metabolic pathways. This damage affects photosynthetic activity, accelerate leaf senescence [3] and also affects quality by lowering the glutenin to gliadin ratio [4]. Genetic basis of thermotolerance has been established and genes have been localised on chromosomes 3A, 3B, 4A, 4B and 6A [5].

Table 1. MTS and TTC values (%) and their correlation with yield and biomass.

Parameter	MTS values	TTC values
Range	21.7-73.2%	14.1 – 61.3%
Mean	46.4	38.0
C.V. (%)	11.6	16.3
Correlation with grain yield per plant	0.366 ^{ns}	0.760*
Correlation with grain yield per metre	0.232 ^{ns}	0.756*
Correlation with biomass per plant	0.430 ^{ns}	0.762*
Correlation with biomass per metre	0.346 ^{ns}	0.758*

**significance at 1% level

*significance at 5% level

Genetic improvement of wheat crop for thermotolerance would require evaluation of cultivated wheat germplasm for heat tolerance. Indian wheat cultivars are developed to suit the requirements of different agro climatic zones and represent wide variability in terms of form and characteristics. However, this variability has been largely exploited for improving yields in favourable environments and for biotic stresses particularly foliar diseases. The stability of wheat grain production and further improvements in the yield potential will depend upon ability of the crop to withstand high temperature stress and fluctuations in the temperatures. There is a need to thoroughly assess these germplasm for heat tolerance and to mine the genes governing high temperature tolerance. In this paper, the studies conducted to evaluate wheat germplasm for high temperature tolerance and techniques used are discussed.

2. Physiological Studies to Understand Thermotolerance

Estimation of membrane thermostability and cell viability after heat stress treatment of hardened seedlings have been widely used for assessment of high temperature tolerance [6-11]. Cell membrane stability assay measures electrolyte leakage after heat stress treatment to leaf discs of hardened plants [12-15]. The triphenyl tetrazolium chloride (TTC) reduction assay measures the level of mitochondrial respiration activity [16], which serves as an indicator of cell viability. Fifty-six Indian bread wheat genotypes were assayed for acquired thermotolerance at seedling stage. Ten days old seedlings were hardened and then subjected to membrane thermo stability (MTS) and cell viability (TTC reduction) assays. Variability was detected among the 56 genotypes for acquired thermotolerance. The MTS values ranged from 21.7 to 73.2% and TTC values ranged from 14.1 to 61.3% (Table1). Significant correlation was observed between MTS and TTC values. TTC assay showed positive correlation with grain yield under high temperature stress and it could be used as a selection criterion in breeding for warmer areas.

The tolerant genotypes identified in this study were used as parents for developing high yielding and thermotolerant

selections. In segregating generations of a cross of HD2281 and NIAW34, the selection was done on grain yield per plant and grain yield per plot basis. A set of 15 selections obtained in F₄ generation were further advanced to F₈ generation by single plant selections made in subsequent generations. These F₈ generation selections showed higher thousand kernel weight (TKW) than parent HD2281, NIAW34 and checks used in trials at two locations, Trombay and Pune for three years. The thousand kernel weight (g) data for the year 2010-11 was presented in Table 2.

Table 2. Thousand Kernel Weight (g) of selections at two different locations (2010-11).

Selection /Genotype	Thousand Kernel Weight (g) at Trombay	Thousand Kernel Weight (g) at Pune
HD2281 (Parent1)	37.3±0.7	46.0±0.9
NIAW34 (Parent2)	25.7±0.4	39.2±1.0
Selection 1	41.0±0.7	47.7±1.5
Selection2	42.9±0.7	47.5±0.4
Selection 3	39.3±0.3	47.9±0.9
Selection4	40.6±0.6	48.0±1.4
Selection 5	38.4±0.8	47.4±0.9
Selection6	37.8±0.4	47.0±0.5
Selection 7	38.7±0.4	46.8±0.4
Selection8	40.3±0.1	50.1±1.6
Selection 9	41.3±0.1	48.9±0.7
Selection 10	38.0±0.3	48.0±0.2
Selection11	41.3±0.6	48.2±0.5
Selection12	39.3±0.06	48.2±1.1
Selection13	37.0±0.2	45.3±0.6
MACS6222 (Check1)	26.3±0.5	41.2±1.3
RAJ4083 (Check2)	23.9±0.2	36.6±1.2
MACS3125 (Check3)	26.9±0.9	49.3±0.8
Mean	36.8	46.6
LSD (5%)	1.49	2.78

3. Canopy Temperature Depression Studies

In this study, our hypothesis is that plant architecture can affect canopy temperature. Temperature differences between any two plant canopies with different morphological traits (plant color, waxiness, leaf size, spike size, and awns) may result from a difference in energy absorption. Canopy temperature depression (CTD), the difference between air temperature and canopy temperature as criteria to avoid evapotranspiration under heat stress is one of the parameter to measure tolerance [17]. An experiment was carried out at Gamma field, Bhabha Atomic Research Centre, Trombay to measure CTDs in 15 varieties, which include heat tolerant and sensitive genotypes with difference in tolerance level at different stages. The CTDs was measured with Infrared thermometer (IRT). CTDs were measured at different crop

growth stages from tillering to flag leaf senescence at weekly interval. Measurements were taken during midday when the temperature was reaching to a maximum in a day in a replicated manner. The genotypes showed difference for CTD at different stages. The data for all agronomic traits on five plants of each variety was recorded. The results showed that significant differences were present among varieties for CTD measured at tillering, boot stage, anthesis and grain filling (Table 3). The canopy temperature differences decreased as plant undergoes maturity. The average CTD values for all genotypes were maximum at tillering stage (7.24°C) followed by boot (6.12°C), anthesis (5.30°C) and grain filling stage (4.42°C), respectively. Further, correlation analysis showed association of canopy temperature depression at tillering and anthesis stage with grain yield per plant, grain yield per square metre and biomass per square metre. However, the canopy temperature depression at boot stage and grain filling have not shown any association with grain yield but CTDs at boot stage showed association with biomass per square metre (Table 4). In this study, positive association of CTD at tillering stage with grain yield implied that CTD can be used as selection criteria and can be measured at early stages (tillering) in the breeding programs.

Table 3. Canopy Temperature Depression (CTD) values at different growth stages.

Genotype	Tillering (°C)	Boot stage (°C)	Anthesis (°C)	Grain filling (°C)
C306	7.30	7.18	5.20	5.10
PBW373	9.25	8.00	5.32	4.88
HD2687	8.30	6.25	5.25	4.75
RAJ3777	5.20	4.80	4.15	4.00
RAJ4037	5.24	4.63	3.95	3.60
RAJ4083	5.48	5.15	4.05	3.90
PBW435	7.50	5.63	6.20	3.75
Kalyansona	6.08	5.65	3.60	3.50
HD2281	8.42	8.00	7.10	5.40
Ajantha	7.80	5.52	5.20	3.20
HD2189	8.00	6.20	5.80	3.75
NIAW34	5.10	4.21	3.60	3.55
NIAW917	7.30	7.20	6.12	3.60
MP3054	9.80	8.00	7.30	7.10
HW2003	7.90	7.65	6.80	6.15
Mean	7.24	6.12	5.30	4.42
LSD (5%)	0.35	0.81	0.35	0.40

Table 4. Correlation coefficients of CTD at different stages with all the plant traits.

Trait CTD	Plant height (cm)	Days to maturity	Days to anthesis	Grain number	Grain yield/spike	Grain yield /plant	Grain yield /sq. mt	Biomass/sq.mt
CTD (Tillering)	0.55*	0.59*	0.66*	0.33	0.73**	0.77**	0.64*	0.66**
CTD (Boot stage)	0.63**	0.82**	0.77**	0.25	0.36	0.36	0.18	0.57*
CTD (Anthesis)	0.53*	0.42	0.38	-0.04	0.55	0.72**	0.67*	0.83**
CTD (grain filling)	0.25	0.60*	0.46	0.16	0.27	0.46	0.25	0.48

**significance at 1% level

*significance at 5% level

4. Translocation of Dry Matter in 1B-1R and Non 1B-1R Genotypes Studies

High temperature stress accelerates the plant development and resulted in overall reduction in plant size [18]. A number of studies established an increase in respiration [19], reduction in photosynthesis [20], inhibition of starch synthesis in kernels [21], reduction in kernel number and weight [22] and overall acceleration of senescence as a result of heat stress. The final effect of all these physiological changes is reduction of yield under heat stress. One of the trait most affected is synthesis of pre anthesis and post anthesis assimilates in plant and further their translocation during grain filling. Studies in our lab from past few years showed lower thousand kernel weight in genotypes carrying 1B-1R translocation as compared non 1B-1R carrier genotypes under warmer climate. To find the evidence for

lower thousand-kernel weight, an experiment is planned to measure the pre anthesis and post anthesis dry matter production and its translocation under high temperature conditions in 1B-1R genotypes and non 1B-1R genotypes. An experiment was carried to study role of 1B-1R translocation on grain development and final grain size under heat stress. Eight varieties, of which four carried 1B-1R and four without 1B-1R translocation, were grown in winter, 2010-2011. Each variety was replicated five times in the experiment. Two tallest tillers of each variety from each replication were cut from base and collected from ear emergence to grain harvest at 10-day interval. These tillers were air and then oven dried (60°C). Each tiller was dissected to separate chaff, grains, leaves and stem. The chaff, grain, leaves and stem were collected and put into separate packets and their weights were taken. The weights of these plant parts were used to estimate translocation of stem reserve to developing grains at each stage.

Table 5. Translocation of dry matter (TDM), contribution of pre-anthesis assimilates to grain (CPA) and translocation efficiency (TE) in bread wheat genotypes with 1B-1R translocation and non 1B-1R translocation.

Genotypes	Total above ground dry matter produced (mg)								
	1B-1R genotypes	Anthesis (mg)	Maturity (mg)	Grain yield (mg/plant)	Grains/spike	TDM (mg)	TE (%)	1000 grain weight (g)	CPA (%)
WH542		2216	1674	1191	50.7	542	25.7	24.4	45.5
HD2687		2717	2062	1462	62.3	655	27.3	24.1	44.8
HUW206		3378	2868	1851	65.0	510	32.5	15.1	27.5
PBW343		2643	2018	1321	46.0	625	27.4	23.6	47.3
Mean		2738.5	2155.5	1456	56.0	553	28.2	21.8	41.3
LSD (5%)		686.5	485.6	212.0	18.0		3.10		
Non 1B-1R genotypes	Anthesis (mg)	Maturity (mg)	Grain yield (mg/plant)	Grains/spike	TDM (mg)	TE (%)	1000 grain weight (g)	CPA (%)	
Ajantha	3354	2297	1644	36.0	1057	31.5	40.7	64.3	
HD2189	2966	2175	1657	38.0	791	26.6	36.4	47.7	
PBW435	2360	1699	1812	44.3	661	28.0	43.8	36.5	
Sonalika	1875	1204	1360	32.0	671	35.8	42.3	49.3	
Mean	2638.75	1843.75	1618	37.6	795		40.8		
LSD (5%)	590.5	432.0	224	9.0			4.12		

The data showed more accumulation of pre anthesis assimilates 2738.5mg in 1B-1R genotypes and the lesser accumulation in non 1B-1R genotypes of about 2638.75mg. However, the total matter translocated during grain filling is more in non 1B-1R genotypes as compared to 1B-1R genotypes. The genotypes Ajantha, HD2189, PBW435 and Sonalika had mean translocated matter of 795mg as compared to 553mg of 1B-1R genotypes during grain filling period (Table 5). Translocation efficiency and contribution of plant assimilates is higher in non 1B-1R genotypes as compared to 1B-1R genotypes. Consequently, the grain yield per plant and 1000-kernel weight is higher in non 1B-1R genotypes as compared to 1B-1R genotypes. The results of study showed that the genotypes with 1B-1R translocation were found to be poor in mobilization of dry matter under warmer climate.

5. Rht8 Gene Studies in Bread Wheat under Heat Stress

Productivity potential of bread wheat (*T. aestivum* L.) is not fully realized because of biotic and abiotic stresses, which the plant faces in field. In recent times, heat stress has emerged as a major problem in the sub-tropical wheat cultivation areas due to climate change. Therefore, agronomically desirable plant type with tolerance to heat stress has become a necessity. Earlier, significant improvement in yield was made with reduction in plant height associated with reduced lodging and improved partitioning efficiency. The GA₃ insensitive dwarfing genes (*RhtB1b* and *RhtD1b*) have contributed significantly to yield improvement in India [22-24] and worldwide [25-27].

However, the varieties carrying 'Norin 10' genes are sensitive to heat stress and show reduced fertility and yield under high temperatures as observed in Southern European environments [28, 29]. Short stature varieties grown in Southern European countries are responsive to GA₃ and carry a different semi-dwarfing gene, which reduces plant height by 10% without significant reduction in yield [30-32]. The microsatellite locus *Xgwm261* shows polymorphism with predominance of 164bp, 174bp and 192bp alleles. The 192bp allele in wheat varieties from Germany, UK and Yugoslavia showed that it reduces plant height by 10 and 8cm as compared to 164 and 174bp alleles respectively [33-35]. The semi-dwarfing effect of the 192bp allele is due to a tight linkage (0.6cM) with the gene *Rht8*. The 192bp allele has been used as a marker for *Rht8* to study its distribution worldwide and to demonstrate its effect on agronomic traits [36-40]. Screening of allelic variation at XGWM261 microsatellite locus showed that considerable variation is present at this locus however, the gene *Rht8* found to be absent. It was found that 192bp allele present in Indian genotypes was a height neutral allele as the genotypes possessing 192bp allele did not show height reduction and yield superiority in two environments [41]. A crossing programme was started to transfer 192bp linked *Rht8* gene from donor Chuan Mai 18 to Ajantha (165bp carrier at *Xgwm261* locus) and MP3054 (174bp at *Xgwm261* locus) to analyse the effect of *Rht8* gene. In F₆ generation, the segregants carry Rht8-192bp allele, 165bp or 174bp alleles were grouped, and the mean values for plant height and grain yield were calculated to study the effect of *Rht8* gene on plant height and grain yield. The results showed reduction in mean culm height and plant height (cm) in segregants of cross (Chuan Mai 18 with MP3054) over the tall parent,

MP3054 (Table 6). The segregants showed reduced spike length however, spikelet density found to be improved.

Table 6. Effect of 192bp-*Rht8* gene on plant height and other yield components.

Genotype at <i>Xgwm261</i> locus	Culm length (cm)	Plant height (cm)	Spike length (cm)	Spikelet number	Spikelet density
192bp- <i>Rht8</i> gene	50.0±1.19	58.5±1.29	8.45±0.21	20.0±0.40	2.37±0.07
174bp	54.4±0.92	64.5±1.00	10.0±0.28	20.5±0.39	2.07±0.05
ChuanMai18	40.9±0.79	48.6±0.94	7.70±0.15	20.6±0.33	2.70±0.04
MP3054	65.0±0.18	76.4±0.88	11.4±0.81	20.8±0.44	2.19±0.04

6. Conclusions

Heat stress is a complex trait. A thermotolerant genotype needs adaptation for all physiological traits at all plant developmental stages. However, data from seedling assays experiment showed that the seedling thermotolerance was correlated with adult plant performance. Canopy temperature depression (CTD), which is an integrative measure of plant's ability to counteract stress, showed that CTD measured at tillering and anthesis stage associated with grain yield and biomass in field studies. Introduction of milder reduced height gene *Rht8* showed that balanced plant architecture is the key to achieve final grain yield improvement particularly in taller genotypes. The studies implied that the breeding programme aimed to develop intrinsically thermotolerant genotypes should involve strategies to combine gene combinations for various plant physiological processes and selection should be based on final plant yield under heat stress.

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