Indian J. Agric. Res., 49 (6) 2015 : 522-527
Print ISSN:0367-8245 / Online ISSN:0976-058X

AGRICULTURAL RESEARCH COMMUNICATION CENTRE
www.arccjournals.com/www.ijarjournal.com

Evaluation of cellular thermotolerance and associated heat tolerance in wheat (*Triticum aestivum* L.) under late sown condition

Chubasenla Aochen[1] and Pravin Prakash*

Department of Plant Physiology, Institute of Agricultural Sciences,
Banaras Hindu University, Varanasi-221 005, India.

Received: 30-09-2014 Accepted: 23-03-2015 DOI: 10.18805/ijare.v49i6.6679

ABSTRACT

Fifty wheat genotypes were evaluated at the seedling stage of growth, for genetic variation in cellular thermotolerance by cell membrane thermostability (CMS) and Triphenyl tetrazolium choride (TTC) assays. A subset of eight genotypes was also evaluated at the anthesis stage using the same assays. Large and significant differences existed among wheat genotypes for TTC and CMS at the seedling and anthesis stages. Average thermotolerance declined from seedling to anthesis stage. Thermotolerance was well-correlated between growth stages among the eight genotypes for both CMS (r=0.95; p= 0.01) and TTC (r=0.92; p= 0.01). The correlation between TTC and CMS among the eight genotypes at seedling and anthesis stages was significant (r=0.95; p=0.01 and r=0.93; p= 0.01, respectively). The effect of heat stress on wheat genotypes selected on the basis of TTC and CMS thermotolerance ratings were evaluated. 1000-grain weight, grain filling duration (GFD) and grain filling rate (GFR) reduced under heat stress. The heat susceptibility index (S) revealed K-65 and Yangmai-6 to be susceptible and NW-1014 and DBW-14 to be moderately tolerant to heat stress. GFR and 1000-grain weight were found to have highly significant positive correlation with CMS and TTC ratings at both seedling and anthesis stages.

Key words*:* Cellular thermotolerance, Grain filling duration, Grain filling rate, Heat susceptibility index, Heat tolerance, Wheat.

INTRODUCTION

High temperature stress is a major environmental stress limiting wheat (*Triticum aestivum* L.) productivity. It is estimated that in India alone, around 13.5 million ha of area under wheat is heat stressed (Joshi *et al.*, 2007). According to the Recent Assessment Report of the Intergovernmental Panel on Climate Change, global earth temperatures have increased by 0.74ºC century, and are likely to increase by 1.1 to 6.4°C by 2100 which will further aggravate the problem (IPCC, 2007). Hence, detailed understanding of physiology and genetics of heat tolerance and proper selection method and germplasm will facilitate development of heat tolerant wheat cultivars.

Heat tolerance is generally defined as the ability of the plant to grow and produce economic yield under high temperatures. A cell-membrane system that remains functional during heat stress appears central to adaptation of plants to high temperature (Raison *et al.*, 1980). Several studies show that an acclimated plant survives when exposed to a temperature, otherwise lethal to a non-acclimated plant (Key *et al.*, 1981; Hong *et al.*, 2003). This phenomenon is the major aspect of acclimation response termed as acquired thermotolerance. Several reports indicated that genetic variation in heat tolerance as measured by the electrolyte leakage method exists in various crops (Kuo *et al.* 1992; Blum *et al.*, 2001; Thiaw and Hall, 2004). Chen *et al.* (1982) also reported that genotypic differences existed in the induction of acquired heat tolerance by TTC cell viability assay in four crops. The temperature sensitivity of higher plants is not constant throughout their life cycle (Levitt, 1980), with the seed germination and seedling establishment stages being especially susceptible.

Therefore, comparison of methods for measuring thermotolerance at different stages of development after imposing controlled heat hardening of whole plants in the growth chamber was undertaken. The main objective of this study was to compare the TTC and CMS assays in estimating genetic variability for heat tolerance, to compare thermotolerance ratings at seedling and anthesis stages and to validate whether the performance of wheat genotypes grown in the field, under heat stress, are associated with their thermotolerance ratings.

*Corresponding author's e-mail: prakashpbhu@gmail.com.

[1]Centre for Biotechnology, ICAR Research Complex-NEHR, Umiam-793103, Meghalaya, India.

MATERIALS AND METHODS

Sampling procedure for acclimation at seedling stage: Fifty wheat genotypes of diverse origin were used in this study. Seedlings were raised in small pots, one plant per pot, under well-watered conditions and ambient temperature of 25°C. Ten-days-old seedlings were then transferred to a growth chamber for heat acclimation at 34°C under high relative humidity (>80%) for 24 hours. Plants were then sampled for assay immediately after acclimation. Triphenyl tetrazolium chloride (TTC) assay was done by the method of Porter *et al.* (1994). The level of acquired thermotolerance (ATT) was determined by measuring the percentage reduction of TTC to formazone, spectrophotometrically at 530 nm, using the following formula: Acquired thermotolerance (%) = (OD 34ºC'!50ºC / OD 34ºC) x 100. Cell Membrane Thermostability (CMS) assay was done by the method of Fokar *et al.* (1998). CMS was calculated as a reciprocal of cell membrane injury after Blum and Ebercon (1981): CMS (%) = $[1-(T_1/T_2) / 1-(C_1/C_2)] \times 100$ where T and C represent treatment and control respectively, and 1 and 2 refer to initial and final conductance readings respectively.

Sampling procedure for acclimation at anthesis stage: From the fifty wheat genotypes, eight were selected based on their divergent thermotolerance ratings at the seedling stage. Plants were grown in 4.0 L pots, one plant per pot, with the required fertilization. Mean temperature ranged between 20 and 22°C during the plant development. Plants were watered daily. At anthesis, plants were transferred to a constant temperature (34°C) and high humidity (>80%) in a growth chamber to acclimate for 24 h. Flag leaves were then sampled for TTC and CMS assays using the methods as described at the seedling stage. Comparisons were made for TTC and CMS thermotolerance ratings at seedling and adult plant stages.

Field experiment: Four wheat genotypes, NW-1014 and DBW-14 (high thermotolerance rating) and K-65 and Yangmai-6 (low thermotolerance rating) were grown in a field at Agricultural Farm, Institute of Agricultural Sciences, Banaras Hindu University Varanasi at three dates of sowing (DOS): 01 December (DOS I), 20 December (DOS II) and 20 January (DOS III) during the *rabi* season of 2009-10. The experiment was laid in randomized complete block design with three replications. The recommended dose of fertilizers and irrigations were given to the crop. Mean growth cycle temperature recorded an increment of 2.5 to 3°C and mean grain filling temperature showed an increment of 3.5 to 4°C at DOS III with respect to DOS I. The date at which 50% of the spikes flowered was recorded as the date of anthesis, and the date at which grains attained maximum dry weight was recorded as the date of maturity of the crop. Weather data was collected from the meteorological observatory located near the experimental field. Heat degree days [HDDs = Σ (mean daily temperature-base temperature)] were calculated using base temperatures of 7.5°C for grain filling duration. For each replication, a 25 cm row length was harvested at maturity to determine 1000-grain weight. The heat susceptibility index (S) was calculated for grain yield and 1000-grain weight as described by Fisher and Maurer (1978): $S = (1-Y/Y_p)/ (1-X/X_p)$, where Y = mean grain yield/ 1000-grain weight of a genotype in a stress environment; Y_p = mean grain yield/ 1000-grain weight of a genotype in a stress-free environment; X = mean Y of all genotypes; X_p = mean Y_p of all genotypes. S is the relative heat stress tolerance of wheat varieties ($S \leq 0.5$, highly stress tolerant; $S > 0.5 \leq 1.0$, moderately stress tolerant, and $S > 1.0$, susceptible)

RESULTS AND DISCUSSION

Significant differences existed among wheat genotypes tested for TTC and CMS at the 10-days old seedling and the anthesis growth stages (Table 1 and 2). Mean TTC values ranged from16.25% in Sonalika to 68.03% in UP-2338, whereas, mean CMS values ranged from 26.27% in Yangmai-6 to 77.27% in NW-1014 at the seedling stage (Table 1). At anthesis stage, mean TTC values ranged from17.46% in Yangmai-6 to 60.83% in DBW-14 and mean CMS values ranged from 21.3% in Yangmai-6 to 73.16% in NW-1014 (Table 2). Average TTC for all genotypes at seedling and anthesis stages was 39.29% and 38.78%, respectively, which did not differ much between growth stages. But, average CMS was found to decrease from the seedling (51.52%) to the anthesis (45.63%) growth stage. This decrease in thermotolerance may be due to loss of membrane function with plant aging. Similar findings of decrease in thermolerance have been documented earlier (Blum and Ebercon, 1981; Fokar *et al.*, 1998, Ibrahim and Quick, 2001).

The association between thermotolerance at the seedling stage and thermotolerance at anthesis stage across cultivars was linear and highly significant for both the TTC assay (R^2=0.85; Figure 1) and the CMS assay (R^2=0.92; Figure 2). Similar findings were earlier reported by Fokar *et al.* 1998. Therefore, genotypic variations for cellular thermotolerance can be evaluated at seedling stage which will be more economical and will take relatively less time and space. The association between TTC and CMS across eight genotypes was found to be correlated at both seedling (r = 0.95; p=0.01) and anthesis (r=0.93; p=0.01) stages (Figure 3). Some of the genotypes such as DBW-14, NW-1014, and UP-2338 were relatively thermotolerant, whereas,

TABLE 1: Acquired thermotolerance (ATT%) as estimated by cell viablility (TTC) and cell membrane thermostability (CMS) assays in fifty wheat genotypes at ten-days-old seedling stage

Genotypes	ATT (%)	Genotypes	CMS (%)
UP-2338	68.03 ± 1.72 *a**	NW-1014	77.27 ± 1.84 *a*
DBW-14	65.44 ± 1.37 *ab*	HD 2285	74.55 ± 1.80 *a*
HD 2402	63.71 ± 1.91 *abc*	Calingiri	73.41 ± 1.78 *a*
PBW-343	62.53 ± 2.14 *bcd*	DBW-14	72.92 ± 1.61 *a*
NW-1014	62.09 ± 2.15 *bcd*	UP-2338	72.50 ± 1.33 *a*
Lok 1	59.20 ± 2.67 *cde*	HD 2402	67.21 ± 1.69 *b*
Calingiri	57.46 ± 2.14 *def*	PBW-343	66.90 ± 1.41 *bc*
HD 2285	55.23 ± 1.74 *efg*	HP 1209	65.71 ± 1.19 *bcd*
Chiriya 3	54.05 ± 2.03 *fgh*	HP 1744	65.20 ± 1.49 *bcde*
HUW 609	53.83 ± 1.78 *fgh*	BAZ	64.51 ± 1.71 *bcde*
HUW 234	52.24 ± 1.67 *fghi*	Chiriya 3	64.07 ± 1.33 *bcde*
UP 262	50.50 ± 2.30 *ghij*	Lok 1	63.52 ± 1.40 *bcdef*
KENPHAD 32	49.77 ± 2.22 *hijk*	HUW 609	62.76 ± 1.98 *bcdef*
HP 1209	48.38 ± 1.87 *ijkl*	HUW 234	62.73 ± 1.21 *bcdef*
Kalyansona	46.74 ± 2.29 *jklm*	Krichauff	61.80 ± 0.85 *cdef*
SAMNYT 19	45.18 ± 1.82 *klmn*	Lehar	61.30 ± 1.26 *def*
Lehar	44.70 ± 1.77 *klmn*	WL 711	61.30 ± 1.83 *def*
HUW 468	43.94 ± 1.29 *lmno*	CIANO 79	60.70 ± 1.50 *defg*
BAZ	43.90 ± 0.63 *lmno*	NW-1012	60.31 ± 1.29 *efgh*
CIANO 79	42.71 ± 1.37 *mnop*	SAMNYT 19	58.60 ± 1.86 *fghi*
HP 1744	41.30 ± 1.31 *nopq*	HD 2329	58.51 ± 1.66 *fghi*
Labh	40.22 ± 1.23 *nopqr*	UP 262	58.32 ± 1.76 *fghi*
ATTILA	38.70 ± 1.08 *opqrs*	KENPHAD 32	56.11 ± 1.75 *ghij*
WL 711	38.20 ± 0.67 *pqrst*	Kalyansona	55.40 ± 1.22 *hij*
HY 65	37.80 ± 1.20 *pqrst*	WH 542	53.72 ± 1.37 *ijk*
NW-1012	37.68 ± 2.28 *pqrst*	Vorobey	53.40 ± 1.17 *jk*
Krichauff	36.16 ± 1.75 *qrstu*	HUW 468	51.51 ± 1.87 *jkl*
HD 2329	35.84 ± 2.06 *qrstu*	Westonia	50.30 ± 1.87 *kl*
Westonia	35.63 ± 1.06 *qrstu*	Sokoll	49.82 ± 1.62 *klm*
HUW 206	35.27 ± 1.48 *qrstu*	Labh	48.11 ± 2.02 *lmn*
HUW-213	34.27 ± 1.41 *rstuv*	Nacozari	45.36 ± 1.86 *mno*
A-115	33.70 ± 1.26 *stuvw*	HY 65	44.50 ± 1.31 *no*
NIPHAD 4	33.40 ± 1.81 *stuvw*	Tammarin Rock	43.70 ± 1.38 *nop*
Sokoll	32.75 ± 1.73 *tuvwx*	ATTILA	43.61 ± 1.68 *nop*
WH-542	31.59 ± 2.51 *uvwxy*	Veery 42	41.53 ± 1.49 *opq*
Veery-42	30.78 ± 2.07 *uvwxy*	RAC 1262	39.42 ± 1.06 *pqr*
Vorobey	30.16 ± 1.49 *vwxy*	HUW -213	39.26 ± 1.58 *pqr*
Raj 3814	29.41 ± 1.56 *wxyz*	HUW 206	38.51 ± 1.42 *qrs*

*Mean values and standard error (±SE) followed by the same letters in ATT and CMS columns are not significantly different according to Duncan's multiple range test

TABLE 2: Acquired thermotolerance (ATT%) as estimated by cell viablility (TTC) and cell membrane thermostability (CMS) assays in eight wheat genotypes at anthesis stage

Genotypes	ATT (%)	Genotypes	CMS (%)
DBW-14	60.83 ± 1.36 *a**	NW-1014	73.16 ± 1.01 *a*
UP 2338	57.16 ± 1.24 *a*	DBW-14	68.40 ± 1.13 *b*
HD 2402	53.30 ± 1.55 *b*	UP 2338	63.43 ± 1.08 *c*
NW-1014	52.40 ± 1.02 *b*	HD 2402	60.70 ± 1.96 *c*
Sonalika	24.66 ± 1.12 *c*	K-8027	30.43 ± 1.96 *d*
K-8027	23.36 ± 1.19 *c*	K-65	25.30 ± 0.99 *e*
K-65	21.10 ± 1.41 *cd*	Sonalika	22.30 ± 1.30 *e*
Yangmai-6	17.46 ± 1.31 *d*	Yangmai-6	21.30 ± 1.19 *e*
Mean (%)	38.78	Mean (%)	45.63
C.V.	5.75	C.V.	5.25

*Mean values and standard error (±SE) followed by the same letters in ATT and CMS columns are not significantly different according to Duncan's multiple range test

Sonalika, Yangmai-6, and K-65 were relative poor in thermotolerance in terms of both TTC and CMS assays. Other genotypes differed in thermotolerance ratings with TTC and CMS. 1000-grain weight, GFD (days) and GFR (mg day^{-1} and mg HDD^{-1}) decreased under heat stress at late sown condition as compared to normal sowing (DOS I). The maximum reduction in 1000-grain weight was up to 50.5% in Yangmai-6, and least reduction up to 23.8% in NW-1014 (Table 3). Late sowing reduced GFD (in days) by 25.6% in K-65, and by 11.4% in NW-1014 (Table 3). Genotypic differences were observed in HDD accumulation during GFD under heat stress. Higher HDD accumulated during DOS II was due to increase in temperature but lesser decrease in GFD, for all genotypes. NW-1014 had lesser decrease in HDD under heat stress. Yangmai-6 showed the highest decrease in HDD (14.4%) under heat stress. Highest decrease in GFR in mg day^{-1} and mg HDD^{-1} was 34.7% and 49.8% observed in Yangmai-6 and K-65, respectively. DBW-14 had the least decrease in GFR (8.7% and 17.8% on mg day^{-1} and mg HDD^{-1} basis, respectively). Significant decrease in GFD and 1000-grain weight has been earlier documented under heat stress (Viswanathan and Khanna-Chopra, 2001; Modarresi *et al.*, 2010). Moniruzzaman (1986) had also reported heat stress to adversely affect grain filling rate. NW-

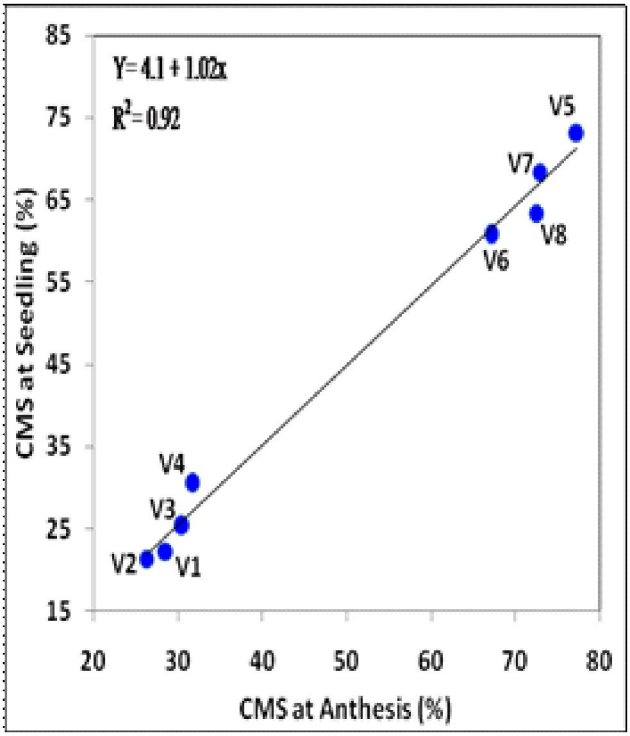

FIG 2: Relationship between thermotolerance, as estimated by CMS, at seedling and anthesis stages across eight genotypes (V1 = Sonalika, V2 = Yangmai 6, V3 = K- 65,V4 = K-8027,V5=NW-1014,V6=HD 2402,V7=DBW-14,V8=UP 2338)

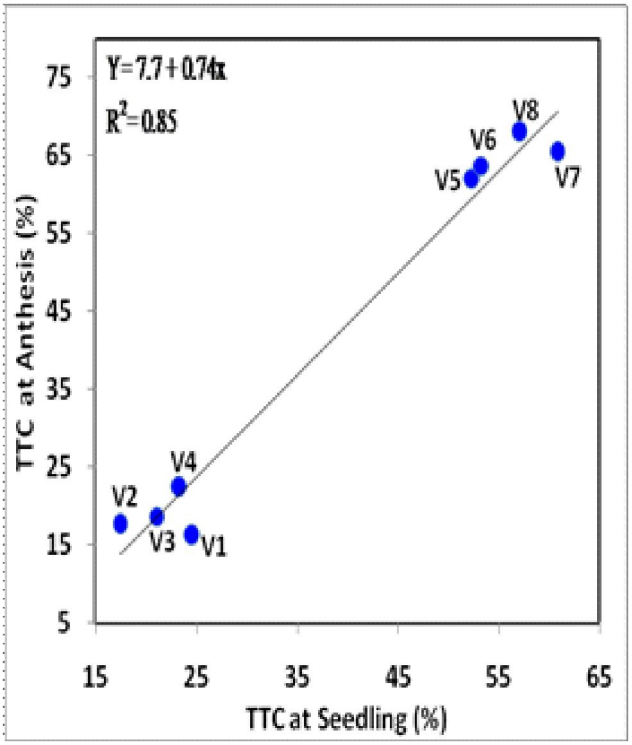

FIG 1: Relationship between thermotolerance, as estimated by TTC, at seedling and anthesis stages across eight wheat genotypes (V1 = Sonalika, V2 = Yangmai 6,V3 = K-65,V4 = K-8027,V5 = NW-1014,V6 = HD 2402,V7 = DBW-14,V8 = UP 2338)

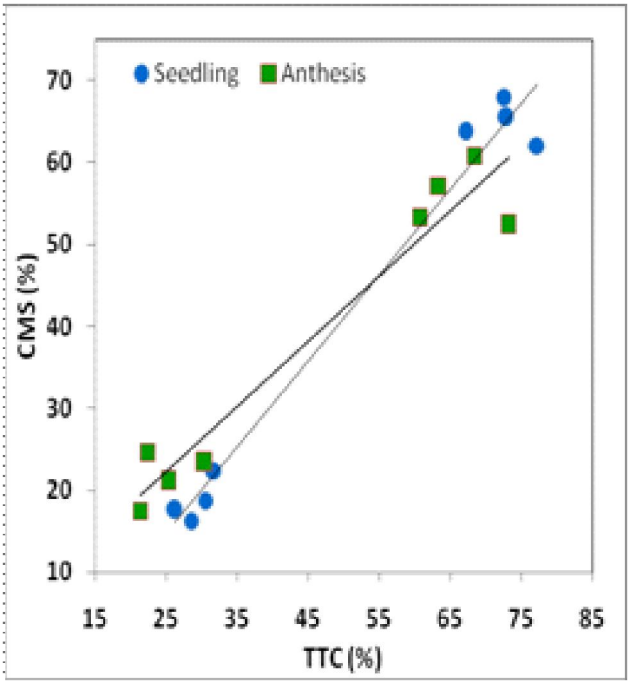

FIG 3: The association between TTC and CMS as measured at the seedling and anthesis growth stages across eight wheat genotypes. (Seedling stage: y= 11.9 + 0.93x, R^2 = 0.92; Anthesis stage: y= 2.4 + 0.79x, R^2 = 0.88)

TABLE 3: 1000-grain weight, grain filling duration [GFD, days and associated heat degree days (HDDs)] and grain filling rate (mg day^{-1} and mg HDD^{-1}) of four wheat genotypes across different dates of sowing

Genotype	DOS	1000-grain weight (g)	Grain filling duration		Grain filling rate	
			Days	HDDs	mg day^{-1}	mg HDD^{-1}
DBW-14	I	43.3	34	671.1	1.27	64.6
	II	38.1	31	671.6	1.23	56.8
	III	31.4	27	591.8	1.16	53.1
Yangmai-6	I	40.8	33	648.4	1.24	63.0
	II	34.4	30	653.7	1.15	52.6
	III	20.2	25	555.1	0.81	36.4
NW-1014	I	43.7	35	717.6	1.25	61.0
	II	40.2	34	732.6	1.18	54.9
	III	33.3	31	688.4	1.07	48.4
K-65	I	41.6	39	629.4	1.06	66.2
	II	34.5	38	800.3	0.91	43.1
	III	21.2	29	639.4	0.73	33.2
Mean		35.2	32.2	666.6	1.1	52.8
C.D. (0.05)		0.44	1.28	29.9	0.017	0.76

TABLE 4: Heat susceptibility index (S) of four wheat genotypes in terms of total grain yield and 1000-grain weight

Genotype	Heat Susceptible Index (Total grain yield)	Heat Susceptible Index (1000-grain weight)
DBW-14	0.87	0.73
Yangmai-6	1.25	1.35
NW-1014	0.74	0.63
K-65	1.18	1.3

S is the relative heat stress tolerance of wheat varieties ($S \leq 0.5$, highly stress tolerant;
$S > 0.5 \leq 1.0$, moderately stress tolerant, and $S > 1.0$, susceptible)

TABLE 5: Correlation of TTC and CMS at seedling and anthesis stages, with GFD, GFR, 1000-grain weight and total grain yield of four wheat genotypes.

	GFD	GFR	1000-grain weight	Total grain yield
TTC at seedling	0.42	0.97**	0.98**	0.95**
TTC at anthesis	0.38	0.97**	0.96**	0.92**
CMS at seedling	0.52	0.94**	0.99**	0.98**
CMS at anthesis	0.52	0.94**	0.99**	0.98**

** Correlation at 0.01 (2-tailed); r= 0.83

1014 and DBW-14 were found to be moderately heat stress tolerant and Yangmai-6 and K-65 to be heat susceptible genotypes on the basis of heat susceptibility index (Table 4). GFR, 1000-grain weight and total grain yield under heat stress conditions for four genotypes exhibited highly significant positive correlation with TTC and CMS values for the same genotypes at seedling and anthesis stages. GFD had positive correlation with TTC and CMS but was not significant (Table 5).

CONCLUSION

Therefore, it is revealed that the capacity for grain filling is associated with cellular thermotolerance, which is an heat-adaptive trait. These associations are more visible, especially when contrasting genotypes for cellular thermotolerance are used for study. Cellular thermostability or cellular viability in these genotypes could be either genetically linked or associated with starch synthesis at higher temperatures as there is lesser reduction in test weight and total grain yield in tolerant genotypes which further needs to be investigated.

ACKNOWLEDGEMENT

Financial assistance received from UGC, New Delhi to the first author is gratefully acknowledged.

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