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Genotypic variation for cellular thermotolerance in *Aegilops tauschii* Coss., the D genome progenitor of wheat

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Abstract Heat stress is a major productivity lowering factor in wheat. Wild progenitor species offer a wide spectrum of adaptation traits and can serve as valuable donors of stress tolerance. In the present study, genetic variation in 129 accessions of *Aegilops tauschii* Coss., the D genome donor of wheat, was evaluated for two heat tolerance related traits viz., cell membrane stability (CMS) and TTC (2,3,5-Triphenyl tetrazolium chloride) based cell viability. Cell membrane stability in the *Ae. tauschii* accessions at vegetative stage ranged from 15.24 to 80.39%. Nineteen *Ae. tauschii* accessions were superior to the tolerant bread wheat control (C 273). At anthesis stage a similar spectrum of variation was observed with twenty three accessions showing higher cell membrane stability than C 273. The average CMS level of entire germplasm set at anthesis (47.61%) was lower than at vegetative stage (58.89%). Clear genotypic differences were also observed for TTC based cell viability test. *Ae. tauschii* accessions displayed a range from 18.73 to 84.39% with eight genotypes excelling over tolerant bread wheat. Correlation of CMS values recorded at two stages was significant but of low predictive value ($r^2 = 0.137$). Similarly significant but moderate correlation was obtained between CMS and TTC test ($r^2 = 0.325$). Consequently all the three parameters were used to derive a cell thermotolerance index which was in turn used to identify ten tolerant *Ae. tauschii* genotypes. The identified accessions were re- evaluated for 1 more year and the three parameters viz., CMS at vegetative ($r^2 = 0.954$) and anthesis stage ($r^2 = 0.932$) and TTC cell viability at vegetative stage ($r^2 = 0.888$) showed high correlation Strategy for use of identified accessions as donors is discussed.

Keywords *Aegilops tauschii* Coss · Cell membrane stability · Genetic variation · Heat tolerance · TTC cell viability test · Wheat

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Introduction

Wheat is a cool season crop, but its cultivation extends well beyond its typical adaptation zone. Globally, terminal heat stress is estimated to affect wheat productivity on millions of acres. In India alone, about 13.5 million ha of wheat crop (about half of the total acreage) is estimated to be heat stressed (Joshi et al. 2007). With the current trends of climate change, the heat stressed wheat production

environments around the world are apprehended to increase about three fold by 2050 (Trethowan et al. 2005). The projected scenario challenges the sustenance of current productivity levels, and makes progressive increase in productivity to meet the future food requirements even harder. Adoption of heat stress tolerance as an important breeding objective has become imperative in wheat. Assessment of genetic variation for heat tolerance traits and identification of donor germplasm thus becomes an essential pre-requisite. Cultivated germplasm would be the preferred donor option, keeping in view ease of subsequent utilization. The wild germplasm, on the other hand, owing to its diverse ecogeographical distribution and adaptation to various stress prone environments, may throw up novel and hitherto untapped genetic variation. For instance, *Aegilops geniculata* accessions were found to possess relevant variation for improvement of heat and drought stress tolerance in wheat (Zaharieva et al. 2001). Skovmand et al. (2001) recommended the search for new variation in the progenitor gene pool represented by the wild diploid donors of the three wheat genomes for heat stress tolerance traits including leaf photosynthetic rate, stomatal conductance, stem reserve mobilization. Similarly, Valkoun (2001) has emphasized gene introgression from *Triticum boeoticum*, *Triticum urartu* and *Aegilops taushii* for tolerance to biotic and abiotic stresses as well as productivity.

Heat tolerance is a complex trait and yield reduction under stress provides a convenient and useful measure of tolerance. This strategy, however, is not effective for screening of wild and locally unadapted germplasm (e.g., winter habit lines in environments suitable for spring wheat). Physiological and biochemical indices which have a bearing on tolerance serve as a useful alternative. While physiological and biochemical components of heat tolerance in wheat are yet to be fully delineated, useful physiological indices including canopy temperature depression (Blum et al. 1982; Reynolds et al. 2007) and stem reserve mobilization (Fokar et al. 1998; Blum 1998) have been shown to be associated with heat tolerance, though mainly in the context of cultivated germplasm. Carbon isotope discrimination also serves as a useful test of drought and heat tolerance (Reynolds et al. 2007) but may not be suitable for large number of samples. Cell membrane stability (CMS) and TTC (2,3,5-triphenyl tetrazolium chloride) based cell viability are used as indicators of heat tolerance mechanisms operating at cellular level. These two tests are rapid, provide flexibility in terms of stage of plant development and are amenable to use in breeding for heat tolerance. Unlike some other tests which require larger plots planted under commercial agronomic regimes, CMS and TTC can be easily applied to wild germplasm. The cellular membrane stability test estimates the amount of electrolyte leakage from heat stressed tissues in vitro using a simple conductometric technique. The TTC cell viability assay is based on the principles of tetrazolium salt reduction to formazan by dehydrogenase respiratory enzyme and thus indicates resilience of the mitochondrial component cell machinery when challenged with heat stress. (Chen et al. 1982; Porter et al. 1995; Fokar et al. 1998).

In the present study 129 accessions of *Ae. tauschii* Coss., the D genome donor of wheat were evaluated for cell membrane stability and TTC based cell viability. *Ae. tauschii* is regarded as a rich reservoir of genetic variation for improvement of bread wheat (Cox 1998). It has the greatest amplitude of genetic variation as well as the widest ecological adaptation among the three diploid progenitors of bread wheat (Zohary et al. 1969). Results pertaining to extent of variation observed for the two traits, and *Ae. tauschii* accessions identified as promising donors are presented.

Materials and methods

Plant material

A set of 129 *Ae. tauschii* accessions from the collection of about 250 being maintained at Punjab Agricultural University, Ludhiana were selected for the study, primarily on basis of resistance to stripe and leaf rust and robust growth habit. The PAU *Ae. tauschii* collection incorporates materials sourced from University of Missouri and Kansas State University, USA; CIMMYT, Mexico; ICARDA, Syria and IPK Gaterslaben, Germany.

Raising of plants and field layout

The experiment was conducted in the Wheat Section of Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana (30°54′ N

and 75°48′ E) during 2007–2008 and 2008–2009 crop seasons. *Ae. tauschii* seeds germinated in small plastic cups were vernalized in growth chamber maintained at 4–6°C with 8 h light/16 h dark photoperiod for about 6 weeks. The vernalized seedlings were transplanted in field. The natural day length was supplemented with artificial lighting to ensure 14–15 h of light per day. The plots consisted of a single row of 1 m length. Three replications were sown in a randomized complete block design. The transplanting was made in last week of October. The maximum temperature for 1 week prior to sampling of leaf tissues at vegetative stage (27.0–30.6°C in 2007–2008 and 27.2–31.5°C in 2008–2009) and anthesis stage (27.2–34.0°C in 2007–2008 and 26.8–33.2°C in 2008–2009) was adequate for heat hardening. Three cultivated varieties representing tolerant (C 273), widely cultivated (PBW 343) and new promising (PBW 550) genotypes were included to serve as checks.

Screening for cell membrane stability (CMS)

Cell membrane stability was measured at both vegetative and anthesis stage corresponding to Zadoks growth stages GS30 and GS65 respectively (Zadoks et al. 1974). The assay was performed according to Sadalla et al. (1990a) with some minor modifications. Six leaves (7 cm long) per accession were excised, kept in stoppered glass vials and washed for 2–3 times with de-ionized water. The water was drained off but desiccation of samples was prevented by stoppering the vials. Samples were then kept for 1 h in water bath preheated to 49°C for administering the heat shock. After treatment, three replications (two leaves per vial) for each accession were made and 15 ml of de-ionized water was added to each vial making certain that cut ends of leaf samples were submerged. All vials were then placed for incubation at about 10°C for 24 h. After incubation samples were equilibrated for 1 h at room temperature and the conductivity (μ siemens) recorded using a digital conductivity meter (Model CON 510, Eutech Instruments, India). After the measurements were taken, vials were autoclaved for 15 min at 121°C/0.10 MPa and their conductance was measured again. CMS was expressed in percentage units as per Ibrahim and Quick (2001),

$$\text{CMS}\ (\%) = [1 - (T_1/T_2)] \times 100$$

T_1: conductivity reading after heat shock at 49°C, T_2: conductivity reading after autoclaving.

Screening for TTC based cell viability

TTC based cell viability was measured at anthesis, corresponding to Zadoks growth stages GS65 (Zadoks et al. 1974). This test was performed with some minor modifications in method followed by Ibrahim and Quick (2001). Two sets of two leaves (3.5 cm long) per accession were excised, placed in a test tube containing 0.1 ml deionized water and washed for 2–3 times with de-ionized water. First set served as a control and was left at 25°C for 1 h. Second set of samples which served as treatment were kept for 1 h in water bath preheated at 49°C for administering the heat shock. After 25 and 49°C treatments, 8 ml of TTC solution (0.8% 2,3,5- triphenyl tetrazolium chloride in 0.05 M $NaPO_4$ buffer, pH 7.4) was added in each test tube and vacuum infiltrated for 10 min. The tissue was incubated in TTC solution for 24 h at 25°C in the dark. After incubation, leaf samples were washed 2–3 times with de-ionized water and formazan was extracted with 3 ml of 95% ethanol for 24 h at 25°C in darkness. The amount of formazan dye produced by TTC reduction was determined spectrophotometrically at 530 nm. Cell viability, as a measure of thermotolerance, was determined as follows:

$$\text{TTC}\ (\%) = (\text{ODh}/\text{ODc}) \times 100$$

ODh: mean optical density for heat stressed sample (49°C for 1 h), ODc: mean optical density for control sample (25°C for 1 h).

Results and discussion

The screening of *Ae. tauschii* accessions revealed ample genetic variation for cellular membrane stability (Table 1). The CMS values at vegetative stage ranged from 15.24% (PAU acc. 14194) to 80.39% (PAU acc. 14202). This spectrum was wider than the CMS recorded for the bread wheat controls (Fig. 1), PBW 550 with a stability value of 36.90% showed sensitivity and C 273 represented the tolerant check at 64.80% CMS. Nineteen *Ae. tauschii* accessions

Table 1 Cell membrane stability (CMS %), TTC cell viability (TTC %) and cell thermotolerance index (CTI) in a set of *Ae. tauschii* Coss. accessions evaluated in 2007–2008

S. no.	PAU accession no.	CMS (%)		TTC (%)	CTI
		Vegetative	Anthesis		
1	3627	49.58	69.60	52.25	57.14
2	3733	63.23	55.98	62.17	60.46
3	3735	56.33	45.75	45.17	49.08
4	3743	53.28	48.32	40.93	47.51
5	3747	55.60	41.77	46.98	48.12
6	3753	64.27	63.37	57.30	61.65
7	3759	69.22	36.01	52.19	52.47
8	3761	66.51	53.85	53.57	57.98
9	3769	73.83	70.05	75.17	73.02
10	3786	68.92	65.72	77.59	70.74
11	3826	35.13	67.52	62.90	55.18
12	5516	45.64	48.37	27.30	40.44
13	9385	62.81	35.38	53.22	57.77
14	9783	55.20	54.19	40.91	50.47
15	9790	61.07	48.38	63.85	50.10
16	9792	41.70	50.31	47.13	46.38
17	9796	37.83	41.47	51.10	43.47
18	9802	40.97	42.89	35.79	39.88
19	9804	29.66	36.41	39.45	35.17
20	9810	29.05	56.70	32.99	39.58
21	9814	33.86	45.01	49.67	42.85
22	9815	77.58	70.50	84.39	77.49
23	9821	55.72	52.43	56.66	54.94
24	9825	71.97	51.97	65.00	62.98
25	9826	54.78	51.84	42.35	49.66
26	9827	69.50	39.85	32.50	47.28
27	9828	55.20	51.84	61.50	56.18
28	13761	62.91	45.30	58.68	55.63
29	13781	58.23	54.76	64.20	59.06
30	14088	51.43	32.04	59.34	47.60
31	14091	19.48	13.51	39.13	24.04
32	14100	25.43	16.87	33.44	25.25
33	14102	55.77	18.99	59.64	44.80
34	14106	52.76	12.27	72.25	45.76
35	14109	41.67	35.98	58.52	45.39
36	14110	41.40	48.39	59.57	49.79
37	14113	52.33	30.08	66.32	49.58
38	14114	61.30	48.57	65.13	58.33
39	14117	50.43	32.65	41.24	41.44
40	14122	39.50	33.28	60.37	44.38
41	14128	35.57	23.73	34.17	31.16
42	14130	59.66	30.68	40.32	43.55
43	14135	48.53	40.08	70.53	53.05
44	14136	54.47	58.53	40.88	51.29
45	14145	52.33	38.86	42.24	44.48
46	14147	53.85	67.76	42.40	54.67
47	14150	55.79	47.67	66.55	56.67
48	14155	45.23	45.67	49.01	46.64
49	14156	41.92	38.89	48.27	43.03
50	14157	47.53	44.00	58.17	49.90
51	14158	40.06	41.35	41.58	41.00
52	14159	49.55	44.08	66.35	53.33
53	14165	70.48	72.90	80.43	74.60
54	14166	54.93	33.98	40.40	43.10
55	14173	52.47	40.79	52.94	48.73
56	14175	54.33	43.92	42.88	47.04
57	14178	52.32	54.62	54.27	53.74
58	14180	75.00	70.58	71.40	72.33
59	14185	70.53	51.01	68.67	63.40
60	14186	72.12	47.93	73.02	64.36
61	14187	57.40	50.62	52.76	53.59
62	14189	42.54	54.37	37.46	44.79
63	14190	18.98	41.33	25.18	28.50
64	14191	72.58	70.10	72.73	71.80
65	14194	15.24	34.31	31.40	26.98
66	14197	74.33	52.01	70.51	65.62
67	14200	71.12	67.88	80.63	73.21
68	14201	65.68	52.57	83.40	67.22
69	14202	80.39	64.62	71.67	72.23
70	14203	66.43	44.37	68.14	59.65
71	14204	56.53	44.92	76.09	59.18
72	14206	55.32	51.07	53.37	53.25
73	14208	52.93	42.87	41.54	45.78
74	14209	64.25	46.58	50.93	53.92
75	14210	57.60	53.95	37.94	49.83
76	14213	59.97	27.66	36.27	41.30
77	14214	70.42	67.41	74.33	70.72
78	14217	75.31	35.51	54.31	55.04
79	14223	75.22	33.05	45.30	51.19
80	14225	58.43	36.69	38.10	44.41
81	14227	61.98	44.33	75.10	60.47
82	14229	65.45	53.86	46.12	55.14
83	14232	53.80	47.96	78.13	59.96

Table 1 continued

S. no.	PAU accession no.	CMS (%)		TTC (%)	CTI
		Vegetative	Anthesis		
84	14234	38.87	54.43	50.88	48.06
85	14236	48.04	48.72	50.81	49.19
86	14237	59.80	36.45	62.25	52.83
87	14238	47.87	62.80	78.54	63.07
88	14240	69.83	43.80	60.37	58.00
89	14242	38.28	59.87	70.27	56.14
90	14246	48.53	43.82	60.58	50.98
91	14247	54.73	53.54	61.90	56.72
92	14252	67.53	53.54	64.39	61.82
93	14253	27.53	33.65	30.50	30.56
94	14319	60.03	39.25	59.07	52.78
95	14321	71.68	76.20	79.50	75.79
96	14322	42.25	65.95	1.69	49.96
97	14330	55.35	45.33	46.68	49.12
98	14331	63.02	61.75	57.62	60.80
99	14332	50.23	64.11	60.64	58.33
100	14337	50.25	30.39	36.69	39.11
101	14339	22.94	32.99	51.43	35.79
102	14344	45.85	28.98	58.57	44.47
103	14345	60.90	47.49	75.84	61.41
104	14346	56.84	24.67	51.83	44.45
105	14349	59.93	58.93	63.74	60.87
106	14352	49.55	51.09	47.07	49.24
107	14355	56.73	55.07	74.57	62.12
108	14359	57.27	42.90	62.69	54.29
109	14361	28.65	55.48	49.82	44.65
110	14582	69.20	66.77	62.17	66.05
111	14586	54.20	47.00	64.49	55.23
112	14588	72.08	71.22	76.70	73.33
113	14589	55.54	41.57	51.53	49.55
114	14590	55.84	63.66	54.52	58.01
115	14591	35.53	61.47	53.69	50.23
116	14592	24.76	50.95	49.14	41.62
117	14594	62.77	47.15	60.73	56.88
118	14595	41.31	52.62	42.19	45.37
119	14596	55.38	34.29	53.17	47.61
120	14597	60.23	58.21	68.32	62.25
121	14598	52.87	57.17	49.80	53.28
122	14599	38.04	49.59	41.93	43.19
123	14600	62.58	69.27	68.51	66.79
124	14601	52.56	52.34	59.33	54.74
125	14602	53.73	39.72	55.55	49.67
126	14603	46.54	42.10	57.77	48.80

Table 1 continued

S. no.	PAU accession no.	CMS (%)		TTC (%)	CTI
		Vegetative	Anthesis		
127	14604	65.53	52.83	64.33	60.90
128	14605	49.70	39.57	52.43	47.23
129	14606	19.83	19.31	18.73	19.29
130	C 273	64.80	56.70	65.31	62.27
131	PBW343	57.50	54.20	75.21	62.30
132	PBW550	36.90	30.60	47.78	38.40
	CD (5%)	3.12	3.29	2.37	

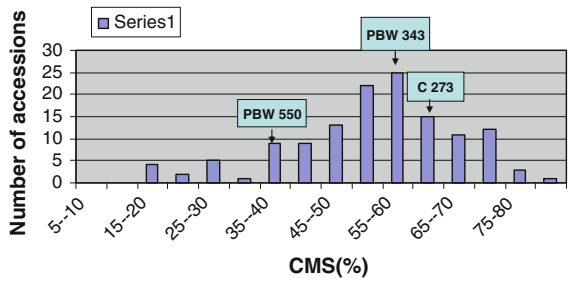

Fig. 1 Distribution of *Ae. tauschii* accessions for cell membrane stability (CMS) at vegetative stage

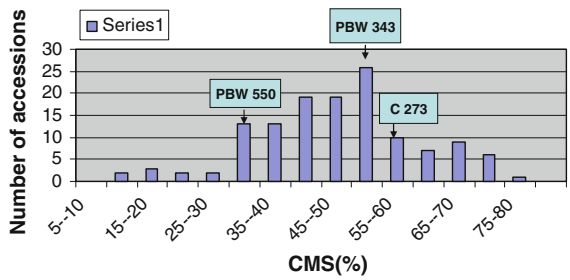

Fig. 2 Distribution of *Ae. tauschii* accessions for cell membrane stability (CMS) at anthesis stage

were superior to the tolerant bread wheat control and eleven showed membrane stability lower than the sensitive control. A similar range of 12.27% (PAU acc. 14106) to 76.70% (PAU acc. 14588) was observed for CMS values at anthesis. C 273 again represented tolerant check (56.70% stability) and PBW 550 showing sensitivity with CMS of 30.60% (Fig. 2). Twenty three *Ae. tauschii* accessions were superior to best bread wheat, whereas seven *Ae. tauschii* accessions had CMS lower than sensitive control. The average CMS level at anthesis (47.61%) was, however, lower than the average of the

germplasm set at vegetative stage (58.89%). There seems to be a decline in CMS with plant age, a trend also reported by Blum and Ebercon (1981) and Fokar et al. (1998). It is important to know the extent to which relative ranking of genotypes are conserved across the two developmental stages. The correlation (r) of CMS values across two stages for the entire set worked out to be 0.37 which is highly significant ($P \leq 0.0001$) but not of great predictive value ($r^2 = 0.137$). A shift in tolerance pattern across the two stages is indicated and screening at one stage may not substitute for tests at the other stage. To qualify as thermostable in sub tropical and tropical environments, where stress prevails at vegetative as well as anthesis stages (Rane et al. 2007), a genotype may need to have high CMS at both stages.

The TTC based cell viability test conducted at vegetative stage also brought out clear genotypic differences (Table 1; Fig. 3). Cell viability values for set of accessions ranged from 18.73% (PAU acc. 14606) to 83.40% (PAU acc. 9815). Among the bread wheat checks PBW 343 (75.21%) showed higher percentage cell viability and PBW 550 (38.40%) showed sensitivity. C 273 (65.31%) which was highly tolerant for CMS test at both stages behaved moderately tolerant for this test. Eight *Ae. tauschii* accessions showed cell viability superior to PBW 343, the tolerant bread wheat check. On the other end of the distribution, thirty four accessions had percent cell viability scores below the sensitive cultivated wheat (PBW 550). Distribution pattern of CMS and TTC cell viability scores of *Ae. tauschii* accessions differs for number of lines transgressing the bread wheat checks on either extreme. In case of TTC cell viability, a relatively smaller number of accessions surpassed the tolerant bread wheat check and a larger group formed beyond the sensitive check. It might have thus been difficult to identify useful variation from a smaller set of *Ae. tauschii* accessions.

A significant correlation of $r = 0.57$ ($P \leq 0.0000001$) was obtained between CMS (vegetative) and TTC test (also conducted at vegetative stage). This is revealing in the sense that two different tests but carried out at one stage show a greater degree of association ($r^2 = 0.325$) than the same assay (CMS) conducted at the two stages ($r^2 = 0.137$). The association of TTC test at vegetative stage with CMS at anthesis is also positive and significant ($r^2 = 0.221$). Positive correlation between CMS and TTC tests have been reported by majority of the studies. Strong positive correlations were observed by Chen et al. (1982) on a set of dicotyledonous crops. In wheat, Sadalla et al. (1990b), Reynolds et al. (1994), Fokar et al. (1998) and Dhanda and Munjal (2006) obtained positive correlations between CMS and TTC tests. Positive correlations among the three tests conducted independently reflect an interlinked biological response to heat stress. The overlap however is not complete warranting the use of all three observations for selection of thermotolerant genotypes. A simple average of the three observations (CMS at two stages and TTC based cell viability) for each accession was worked out to give a composite cell thermotolerance index (CTI) which showed distribution of accessions beyond the bread wheat checks on either side (Fig. 4). On the basis of CTI, ten *Ae. tauschii* genotypes were identified. CMS and TTC cell viability values of these accessions were confirmed for one more season (2008–2009) and are listed in Table 2. Thermotolerance was well correlated across 2 years among the ten accessions

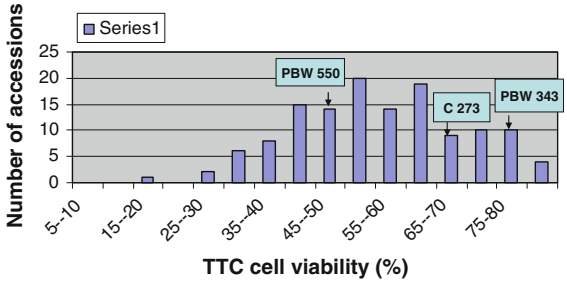

Fig. 3 Distribution of *Ae. tauschii* accessions for TTC cell viability

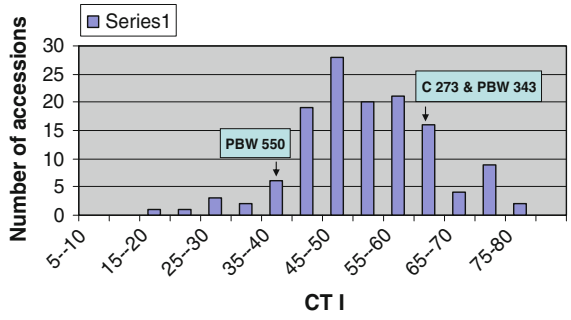

Fig. 4 Distribution of *Ae. tauschii* accessions for cellular thermotolerance index (CTI)

Table 2 Cell membrane stability (CMS %), TTC cell viability (TTC %) and cell thermotolerance index (CTI) in top ten *Ae. tauschii* Coss. accessions re-evaluated in 2008–2009

S. no.	PAU accession no.	CMS (%)		TTC (%)	CTI
		Vegetative	Anthesis		
1	3769	70.90	73.21	78.76	72.06
2	3786	65.27	69.09	75.27	67.18
3	9815	75.48	74.49	80.50	74.99
4	14165	72.73	70.73	82.04	71.73
5	14180	76.35	74.53	76.70	75.44
6	14191	73.89	73.52	77.35	73.71
7	14200	74.39	66.20	85.01	70.29
8	14202	84.67	62.26	76.12	73.47
9	14321	76.02	70.84	82.43	73.43
10	14588	75.59	74.81	72.95	75.20
11	C273	63.94	58.51	63.31	61.22
12	PBW343	53.84	56.48	74.69	55.16
13	PBW550	34.34	34.12	45.04	34.23
	CD (5%)	2.197	1.978	1.709	–

and three checks. High coefficient of determination between 2 years for CMS ($r^2 = 0.954$) and TTC ($r^2 = 0.888$) at vegetative stage and CMS at anthesis stage ($r^2 = 0.932$) showed the genetic component of variation to be strong. Further information on the ten identified accessions along with CTI averaged over 2 years is given in Table 3. Nine of ten accessions identified for high CTI belong to subspecies *strangulata* while one belonged to *Ae. tauschii* subsp. *tauschii* (Table 3). Subspecies *tauschii* is characterized by elongated cylindrical spikelets while *strangulata* has more quadrate spikelets, equally long as wide (Van Slageren 1994), besides a more robust plant type. Two of tolerant genotypes originated in Israel, two in Iran, one each in Turkminestan, Azerbaizjan and Afghanistan while three have an unknown geographic area of origin (Table 3).

The wheat checks were deliberately chosen to represent the wide range of variation in cultivated wheats. C 273 is a traditional, tall cultivar (now obsolete), which is known for adaptation to hot, dry environments and is regarded as a donor of drought and heat tolerance traits. In our studies (unpublished) C 273 represents the tolerant extreme for cell membrane stability among cultivated germplasm, making it a useful benchmark. Any variation beyond C 273 provides rationale for embarking on wide hybridization based transfer. PBW 343 (Attila), a

Table 3 *Ae. tauschii* Coss. accessions identified as potential donors for cellular thermotolerance traits

S. no.	PAU accession no.	Sub-species	Origin	Supplier	Average cellular thermotolerance index
1	3769	*strangulata*	Iran	University of Missouri, USA	72.54
2	3786	*tauschii*	Unknown	University of Missouri, USA	72.14
3	9815	*strangulata*	Iran	Kansas State University, USA Supplier's acc. no., TA 2529	72.17
4	14165	*strangulata*	Turkmenistan	IPK Gatersleben, Germany Supplier's acc. no. AE 250	74.79
5	14180	*strangulata*	Azerbaijan	IPK Gatersleben, Germany Supplier's acc. no. AE 267	73.16
6	14191	*strangulata*	Afghanistan	IPK Gatersleben, Germany Supplier's acc. no. AE 280	73.88
7	14200	*strangulata*	Unknown	IPK Gatersleben, Germany Supplier's acc. no. AE 425	72.75
8	14202	*strangulata*	Unknown	IPK Gatersleben, Germany Supplier's acc. no. AE 427	71.75
9	14321	*strangulata*	Israel	P.A.U Gene Bank Supplier's acc. no. 14321	74.61
10	14588	*strangulata*	Israel	P.A.U Gene Bank Supplier's acc. no. 14588	74.26

cultivar developed at CIMMYT, Mexico is derived from Veery group of wheats which in turn emerged from a winter wheat × spring wheat hybridization programme. In the irrigated, subtropical wheat zone of India, PBW 343 is the most widely grown cultivar covering about 7 million hectares. Its excellent adaptation is attributed to high stomatal conductance leading to cooler canopy, besides a stay green habit. For the two heat tolerance related traits discussed in this paper, it shows medium to high scores. PBW 550 is a recently released cultivar which is known for heat avoidance rather than tolerance on account of its early maturity. PBW 343 and PBW 550 are thus suitable recipients for improved CMS and TTC cell viability traits from *Ae. tauschii*. The transfer programme has been initiated using a diploid × hexaploid cross approach (Gill and Raupp 1987; Sehgal 2005). The D genome of cultivated wheats has a narrow genetic base owing to the evolutionary bottleneck that marks the advent of hexaploid wheat. For instance 326 bp DNA sequence at *Gss* locus showed *Ae. tauschii* to be 30 times more diverse than *Triticum aestivum* (Caldwell et al. 2004). Reports of direct use of *Ae. tauschii* germplasm for heat tolerance in wheat are not available. However evidence for high temperature stress tolerance in synthetic hexaploid wheats has been reported by Yang et al. (2002) and the use of synthetic hexaploids for improving environmental stress tolerance in wheat has been shown to hold great potential (Trethowan and Mujeeb-Kazi 2008).

Wheat breeding for heat tolerance based on selection for yield in stress environments has been shown to be effective (Joshi et al. 2007; Singh et al. 2007). This simple strategy however, may not be effective for screening of wild species germplasm which carries potential donors of heat tolerance related traits. The association of the two cellular thermotolerance tests with field performance under stress is not addressed by this study, primarily due to the fact that this wild, winter habit species does not lend itself to a meaningful yield trial, particularly in our environment. The relationship of variation in CMS with heat tolerance has been indicated by studies in several crops including soybean (Martineau et al. 1979), sorghum (Sullivan and Ross 1979), potato and tomato (Chen et al. 1982), cowpea (Ismail and Hall 1999) and cotton (Azhar et al. 2005). Similarly, TTC based cell viability assay has been implicated strongly as a heat tolerance component (Chen et al. 1982). In wheat Reynolds et al. (1994), Fokar et al. (1998), Ibrahim and Quick (2001) and Dhanda and Munjal (2006) demonstrated positive association of CMS and TTC with field performance. Work at our centre with a set of wheat cultivars, including the checks used in this study show association of these two parameters with field performance (Bala 2008)

Further, yield reduction under stress is hard to assess in the segregating phase, making early generations selection almost impossible for heat tolerance *per se*. Transfer of component traits such as CMS and TTC based cell viability, from wild donors can be facilitated by rapid single plant assays on segregating materials and along with selection for other component traits may raise the heat tolerance levels beyond those presently available in wheat.

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