

Deciphering genetic variation of terminal heat stress-responsive traits in Nepalese wheat germplasm using phenotyping and functional marker analysis

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Abstract. Khanal N, Panthi S, Pandey MP, Luitel H, Poudel RR, Bhusal N. 2024. Deciphering genetic variation of terminal heat stress-responsive traits in Nepalese wheat germplasm using phenotyping and functional marker analysis. *Asian J Agric* 8: 134-141. Development of heat-tolerant wheat cultivars is becoming more essential for the food security due to the increasing impact of global warming. In the present study, Nepalese bread wheat accessions, along with the check varieties were evaluated under late-sown heat stress conditions, followed by genotyping of selected accessions using trait-based functional markers. The findings showed a reduction in Thousand-Grain Weight (TGW), Grain Weight per Spike (GWS), and Grain Filling Duration (GFD), due to the late-sown heat-stressed environment. The reduction for GFD and GWS among the wheat accessions ranged from 22.2 to 62.3% over the heat-tolerant check genotypes (NL-971 and Aditya), respectively. The correlation and principal component analysis showed that TGW, GWS, and GFD were positively associated with each other. The analysis of functional markers CWI21 and CWI22 (linked to TaCwi-A1 and TaCwi-A2 genes) and GS7D (linked to TaGS-D1a or TaGS-D1b genes) showed an association with the TGW. The accessions NGRC-02584 and NGRC-02602 possessed the positive alleles of markers CWI22 and GS7D for a higher TGW, indicating that these accessions have the potential to withstand terminal heat stress and could be included in breeding programs after validation.

Keywords: Accessions, molecular markers, terminal heat stress, thousand-grain weight, wheat

Abbreviations: CTAB: Cetyltrimethylammonium Bromide, CWI: Cell Wall Invertase enzyme, DM: Days to Maturity, GFD: Grain Filling Duration, GNS: Grain Number per Spike, GWS: Grain Weight per Spike, MTA: Marker-Trait Association, NGRC: National Agricultural Genetic Resources Centre, PIC: Polymorphic Information Content, TGW: Thousand-Grain Weight

INTRODUCTION

The demand for wheat is increasing multiplefold in comparison to the production projection, which could increase by 40% by 2050. To fulfil the rising demand, about 2.4% average annual growth rate over the yield of 2005 is necessary (Ray et al. 2013; Mottaleb et al. 2023). With the increasing impact of climate change, production of wheat has been affected by the incidence of various biotic (rusts, loose smut, Karnal bunt, etc) and abiotic (heat, drought, salinity, etc) stresses. The previous conservative predictions indicated that an increase in 2°C global mean temperature could cause a change in the timing, severity, and geographic distribution of both biotic and abiotic stresses (Deutsch et al. 2018; IPCC Secretariat 2021; Robles-Zazueta et al. 2024). The projections also indicated that due to abiotic stresses alone, about 50 percent of the wheat yield could be lost mainly due to heat stress (20%) followed by salinity (10%), drought (9%), low temperature (7%), and other form of stresses (4%) (Kajla et al. 2015). However, the amount of loss varies with the geographical conditions, incidence, and intensity of the

stress. High temperature (heat stress) during crop season causes a detrimental effect on wheat production. It is a combined effect of many factors like high heat intensity above the normal temperature, duration of exposure, rate of rise in temperature, and plant response at each development stage (Mishra et al. 2014). The effect of heat stress is complex, resulting in retardation of growth and development with the changes in physiological functions and reduction of grain formation and yield (Mondal et al. 2013). It has been estimated that each 1°C increase in temperature could cause an average of 7% yield loss (Liu et al. 2016; Zhao et al. 2017). In the case of wheat-growing areas of South Asia, terminal heat stress (particularly during the reproductive phase of the wheat) is a major problem. The projected rise in mean daytime temperature up to 4°C by the end of this century could lead to an increase in the intensity of heat stress, which may accelerate the loss of wheat yield (IPCC 2022).

In the major wheat-growing region of South Asia, i.e., Indo-Gangetic Plain areas, the majority of farmers adopt a rice-wheat system of production under which wheat sowing is frequently delayed due to the residual moisture in the

field after harvesting of rice. This leads to the shortening of the desirable 'cool period' for wheat production and adversely exposes the crop to progressively rising daily air temperature, causing heat stress during reproductive and grain-filling stages (Joshi et al. 2007). Currently, terminal heat stress prevails in around half of the major wheat-growing area of Nepal-Terai (plains) with western hot winds accompanied by the sudden temperature rise starting from mid-March (Puri et al. 2015). There is an urgent need to develop heat-tolerant wheat varieties to overcome the effect of heat stress in upcoming scenarios. The assessment of genetic potential of a large number of genotypes under stress conditions is the first strategy to know the response of plants under heat stress. Testing of potential genotypes with various morpho-physiological, biochemical, and yield component traits under stress conditions can be a starting step. The development and use of diagnostic markers associated with genes controlling for various characteristics have accelerated the utilization of crop germplasm (Choulet et al. 2014). A number of gene-based markers have been developed in wheat for grain weight and size (Jiang et al. 2011; Su et al. 2011; Yang et al. 2012; Zhang et al. 2014), grain quality (Distelfeld et al. 2006; Martin et al. 2006), diseases resistance (Brunner et al. 2010), abiotic stresses (Wei et al. 2009), and other traits. The gene bank accessions are the major sources of biotic and abiotic stress resistance (Aziz et al. 2018). Evaluation of wheat accessions under stress conditions and screening them for the presence of genes/markers associated with desirable traits enhance the breeding activity through the identification and incorporation of trait-rich landraces in the crossing scheme. In the present study, a large set of Nepalese wheat accessions were evaluated for terminal heat stress response and genotyped with diagnostic markers associated with kernel weight and drought stress to identify the potential wheat accessions that can withstand terminal heat stress.

MATERIALS AND METHODS

Phenotyping

Plant material and experiment location

A total of 92 accessions and four commercial varieties (check genotypes) of bread wheat were used in the present investigation. The 92 test genotypes were obtained from the National Agriculture Genetic Resources Centre (Genebank), Khumaltar, and commercial varieties from the National Wheat Research Programme (NWRP), Bhairahawa, Nepal. The phenotyping was conducted in the Research farm of the faculty of Agriculture, Agriculture and Forestry University, Rampur, Chitwan, Nepal (27°39' N and 84°21' E; 171 masl) during the crop season of 2019 (January to April).

Experimental design and evaluation for heat stress

The field experiment was conducted using an Augmented-Randomized Complete Block Design with five blocks. The seeds were hand-sewn in a 1m² plot with four rows of 1m length and 20 cm row-to-row spacing. The test

accessions were not replicated while check genotypes were replicated in each block. Sowing was done one month late than the normal sowing time, i.e., on 25th January, to expose the experimented crop to progressive heat during the grain filling period. The crop was irrigated twice during grain-filling period to keep soil moisture approximately 70-80% for maintaining field capacity to avoid any confounding effect of drought stress. The data were collected for different attributes: Days to Anthesis (DA-Z65, Zodaks scale), Days to Maturity (DM), Grain Filling Duration (GFD), Grain Weight per Spike (GWS), Grain Number per Spike (GNS), and Thousand-Grain Weight (TGW). The daily mean (maximum and minimum) temperature data during the crop season was obtained from an automatic weather station at the National Maize Research Program, Rampur.

Genotyping

Plant material and DNA isolation

Out of 92 accessions and four commercial varieties of wheat used in phenotyping, forty-five landraces and three commercial varieties were randomly selected for genotyping. The genomic DNA was isolated from 21 days old seedlings following the CTAB extraction method by Saghai-Marouf et al. (1984) with minor modifications. The modification includes 1) 2% polyvinylpyrrolidone was added instead of 0.1% 2-mercaptoethanol in the preparation of extraction buffer and 2) precipitated DNA was centrifuged at 5125 x g for 2 min instead of lifted out with a glass hook. The purity and quantity of isolated DNA were checked using a Nano DropTM 2000 Spectrophotometer (Thermo Fisher).

SSR markers and PCR amplification

A total of seven diagnostic DNA markers were used to check polymorphism among the selected wheat genotypes (Table 1). The PCR amplification was carried out in a thermocycler (Bio-Rad, USA) with 10 µL volume of PCR reactions containing- QIAGEN Multiplex PCR master mix (10mM Tris-HCL, pH 8.3, 50mM KCl, 1.5mM MgCl₂, 0.2mM dNTP mix, and 5U Taq polymerase), 1 ng of each primer, 50-60 ng genomic DNA. The thermocycler program used for the PCR reactions was 94°C for 5 min (Initial denaturation), 35 cycles of 94°C for 1 min (denaturation), 50/61°C for 1 min (annealing), and 72°C for 1 min (elongation), followed by 10 min at 72°C (final elongation) and stored at 4°C. The PCR products were electrophoresed using 2% agarose gel. The PCR products were scored based on the present (1) or absent (0) of bands by analyzing gel images using the software GelAnalyzer 19.1 Java version (www.gelanalyzer.com).

Statistical analysis

The phenotyping data were analyzed using a package "augmentedRCBD," by Aravind et al. (2021), functions in R Studio software (RStudio Team 2015). The polymorphism information content (PIC) of the genotyping data was calculated using the formula by Anderson et al. (1993). The diversity between accessions was determined by implementing DARwin software v.6 (Perrier and

Jacquemoud-Collet 2006). The UPGMA clustering of accessions and commercial varieties based on the markers alleles variation was performed using the Neighbor-Joining (NJ) method.

RESULTS AND DISCUSSION

The optimal temperature for wheat production lies between 12 and 28°C, while high temperature (>30°C) during grain filling duration causes terminal heat stress (Farooq et al. 2011). In the present study, average maximum and minimum temperatures during vegetative (25.04°C and 12.35°C), reproductive phase (31.28°C and 19.21°C) showed that the experimented wheat crop was exposed to terminal heat stress (Figure 1). The analysis of variance showed that test and check genotypes were highly significant, while, adjusted block effects were found to be non-significant for all the studied traits (Table 2). The mean grain filling duration ranged from 15 to 38 days among the accessions, while in commercial varieties, the average grain filling duration ranged from 20 days (Munal) to 32 days (NL971) (Tables 3 and 6). The variation in the grain filling duration may be due to the terminal heat stress causing force maturity in the studied genotypes. Among the studied accessions, thousand-grain weight ranged from 9.27 g (NGRC-00018) to 55.87 g (NGRC-02449), while it ranged from 29.18 (Munal) to 55.12 g (NL971) in check varieties (Table 3). The tested wheat accessions and check varieties recorded an average of 0.545±0.114g grain weight per spike with a 16.52 percent coefficient of variation. Similarly, in the tested accessions, grain weight per spike ranged from 0.06 g to 1.69 g, with NGRC-00199 having minimum and NGRC-02633 with maximum grain weight per spike.

In the present study, tested accessions showed lower grain weight/spike than the check varieties. The studied accessions showed 21.72, 26.69, 51.75, and 62.36% reduction from the average performance of heat tolerant check varieties (NL-971 and Aditya) for GFD, GNS, TGW, and GWS (Figure 3), respectively. In previous study, genotype Aditya (terminal heat tolerant check) showed an 8.56 percent reduction in TGW while testing under timely and late-sown conditions (Aryal et al. 2015). The tested accessions showed a reduction in their performance for the studied traits, indicating they may have varying levels of heat susceptibility. The previous findings of reduction in yield under controlled (6-51%), field (2-27%) (Bergkamp et al. 2018), and crop seasons (18.2% and 42.1%) (Sareen et al. 2014) indicated that the variable intensity and the duration of heat stress trigger the varying level of reduction of crop yield. Similarly, previous studies also indicated that more than 24°C during the reproductive stage could significantly reduce the production. The reduction in the growth period (Munsif et al. 2016; Thapa et al. 2019), grain weight per spike (Dias et al. 2008; Munsif et al. 2016; Abdelrahman et al. 2020; Yan et al. 2023), grain numbers (Ali et al. 2010; Mahrookashani et al. 2017), grain size (Fan et al. 2018; Yan et al. 2023), spike length (Mukherjee 2012), and grain yield (Prasad and Djanaguiraman 2014;

Bhusal et al. 2017; Kumar et al. 2020; Wang et al. 2021; Tian et al. 2023) due to the incidence of heat stress during the reproductive phase has already been observed. The reduction in production during this phase is mainly caused by the disruption in meiosis and mitosis, which affects fertilization, seed setting, and growth and limits the supply of carbohydrates (Semenov et al. 2014; Fan et al. 2018). In this study, a significant positive correlation has been recorded for all of the studied traits (Figure 2). The first principal component explained 72.56% variation of the studied traits (Table 7). The eigenvalues of the identified principal components ranged from 2.90 (PC-1) to 0.03 (PC-4). The important traits in the first PC were thousand-grain weight, grain weight per spike, grain filling duration, and number of grains per spike. Likewise, the number of grains per spike and thousand-grain weight were important traits for the second PC. The scattered plot of the principal component analysis showed that PC-1 and PC-2 explained a total of 89.04% phenotypic variation (Figure 2). The significant positive correlations among the traits, i.e., GFD, GNS, and TGW, with their association in the first principal components in this study, indicated that these traits are key in evaluation for heat stress conditions and could be used as selection criteria for heat stress tolerance. The findings of the previous studies also suggested that correlated traits can be simultaneously improved (Talukder et al. 2014; Balla et al. 2019; Schittenhelm et al. 2020).

Out of the seven markers used in the present investigation, only four markers were successfully assayed in tested genotypes. The markers CWI21 (associated with TaCwi-A1 gene) and CWI22 (associated with TaCwi-A2 gene) showed single alleles of 404 and 402 (bp), respectively. At the same time, markers GS7D (associated with TaGS-D1a or TaGS-D1b) and DREB1 showed two alleles of sizes 522 and 562 and 596 and 646 (bp), respectively (Table 4). Among the tested genotypes, markers CWI22 and GS7D detected positive alleles for higher thousand-grain weight in six accessions and one check genotype (Munal). The TaCwi-A1 and TaCwi-A2 are linked with grain size and weight (Su et al. 2011; Nadolska-Orczyk et al. 2017; Rasheed et al. 2019; Sehgal et al. 2019). A previous study suggested that the TaCwi-A1 gene encodes cell wall invertase enzyme (CWI) which is critical for sink tissue development and carbon partitioning (Ma et al. 2010) while the TaCwi-A2 gene is associated with the controlling of cell number in both the cell division and late grain-filling phases (Bednarek et al. 2012), and E3 ubiquitin ligase activity which is associated with heading and maturity in wheat (Su et al. 2011). Similarly, the GS7D marker produces TaGS-D1a or TaGS-D1b alleles, which are associated with the grain weight of wheat (Zhang et al. 2014).

The marker alleles and trait expression of the studied genotypes under terminal heat stress in the present study showed a significant association for GWS, GFD, and TGW. The marker CWI21 was associated with lower TGW (average: 19.04), while Marker CWI22 was associated with higher TGW (average: 33.66), contributing to 40 and 18 % phenotypic variations (Table 4), respectively. Similarly, for the GWS, these markers showed 25 and 17% phenotypic

variation, respectively. Similar findings were previously reported for these markers under normal growing conditions (Ma et al. 2012; Zhang et al. 2014; Rasheed et al. 2016). It has also been reported that TaGW2 is significantly associated with grain weight and width by controlling endosperm (Su et al. 2011; Yang et al. 2012).

The comparative genomic studies also suggested that TaGW2 is associated with grain weight (Sehgal et al. 2019; Rasheed et al. 2019). Similarly, GS7D associated with TaGS-D1 was significantly associated with grain weight and found in cultivars from various countries (Zhang et al. 2014). In the present study, the marker for the Dehydration-Responsive Element Binding (DREB) proteins- Dreb1 (P40/P18R) showed two amplified fragments of 596 bp and 646 bp, in which the 596 bp allele is present in 22 accessions and one check variety and 646 bp allele is present in all of the studied genotypes (wheat accessions and check varieties). The genotype possessing both alleles had low thousand-grain weight except for NGRC02584, NGRC00187, NGRC02601, and Gautam

(Table 5). The findings of the study suggested that these genotypes may possess the ability to withstand the combined effect of drought and heat stress.

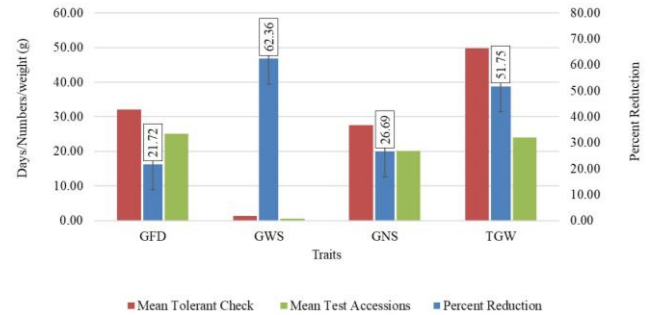


Figure 2. Mean performance and percent reduction in the tested accessions over the tolerant check varieties under late sown terminal heat stress

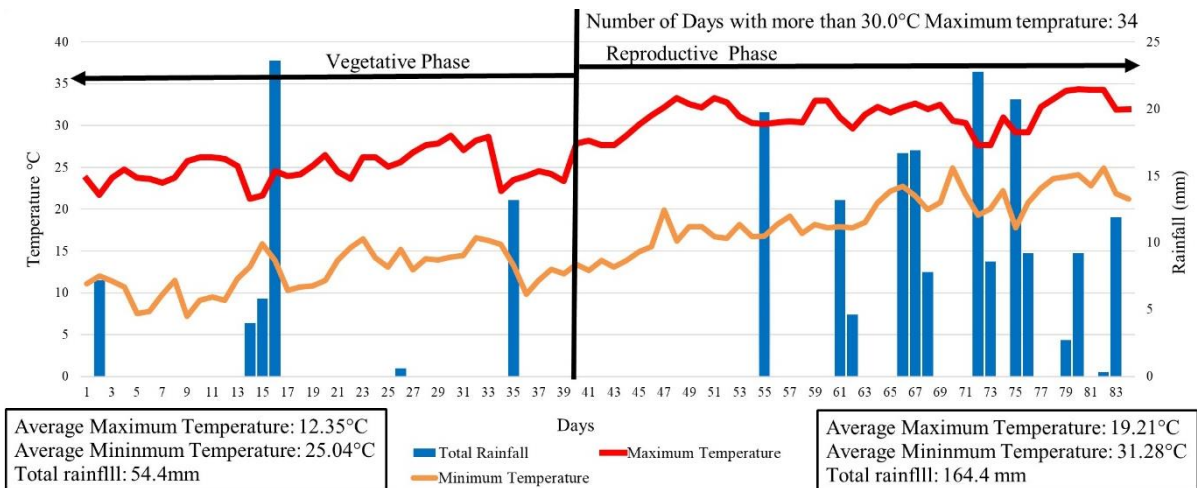


Figure 1. Daily minimum and maximum temperature and rainfall recorded during the crop growing period (Calendar dates)

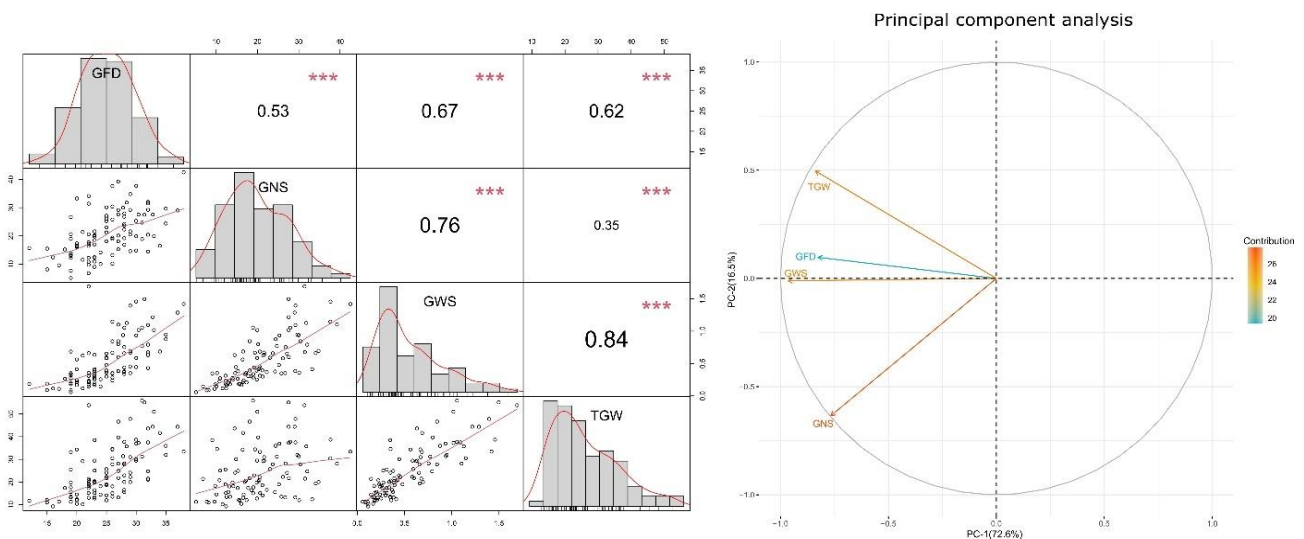


Figure 3. Correlation coefficients and principal component analysis of the studied traits

Table 1. Details of the trait-based functional markers used for genotyping in the present study

Markers	F/R	Primer sequence (5'-3')	Band size (bp)	AT (°C)	Genomic region	Associated trait	Reference
CWI21	F	GTGGTGATGAGTTCATGGTTAAG	404	58	2A	Lower kernel weights	Ma et al. 2012
	R	AGAAGCCCAACATTAATCAAC					
CWI22	F	GGTGATGAGTTCATGGTTAAT	402	60	2A	Higher kernel weights	Ma et al. 2012
	R	AGAAGCCCAACATTAATCAAC					
GS7D	F	AACTTAGGGAGCGAAAACAA	522, 562	52	7D	Thousand kernel weight	Zhang et al. 2014
	R	CACCAAGACTGGAGATGAAA					
Sus-SNP-227/589 L2	F	CTATAGTATGAGCTGGATCAATGGC	381	60	2B	Thousand kernel weight	Jiang et al. 2011
	R	GGTGTCTTGAGCTTCTGA					
SAP39	F	GATCATCAAAGATTGCCCAAGTTC	1906	60	7A	Thousand kernel weight	Chang et al. 2014
	R	AGTCACGGACGGGCAGATG					
Dreb1	F	ATATGGATTGCCTTGATGCA	596, 616	55	3B	Drought tolerance	Wei et al. 2009
	R	TTGTGCTCCTCATGGTACTT					

Note: F/R: Forward and Reverse sequences, bp: Base pair, AT: Annealing Temperature

Table 2. Mean squares of analysis of variance for various traits of Nepalese wheat accessions and commercial varieties (checks) under late sown terminal heat stress 30.03

Source of variation	DF	GFD	GWS	GNS	TGW
Blocks (eliminating treatments)	4	2.07	0.026	7.50	58.00*
Treatments (test accessions)	91	24.73***	0.125 ***	62.50 ***	120.00 ***
Checks (commercial varieties)	3	138.53***	0.663 ***	92.50 **	631.00 **
Entries (ignoring blocks)	95	29.95***	0.204 ***	71.00 ***	186.00 ***
Checks vs. accessions	1	179.14***	0.663 ***	771.90 ***	4824.00 ***
Residuals	12	2.07	0.014	19.20	17.00

Note: *** Significant at (P<0.001); ** significant at (P<0.01); DF: Degree of Freedom, GFD: Grain Filling Duration and TGW: Thousand-Grain Weight, GWS: Grain Weight per Spike, GNS: Grain Number per Spike

Table 3. Mean, range, and CV of studied traits of Nepalese wheat accessions and commercial varieties (checks) under terminal heat stress

Traits	Grand mean (SE)	Check varieties				Accessions range				CV%
		Gautam	Aditya	Munal	NL-971	Minimum		Maximum		
						Value	Accession	Value	Acc. No.	
GFD	25.20±1.42	28.4± 0.81	32.8± 0.58	21.0± 0.54	31.4 ±0.60	15.00	NGRC-04431, NGRC-02455	38.00	NGRC-02561	5.60
GWS	0.55±0.11	1.12±0.05	1.22±0.08	0.62±0.05	1.49±0.02	0.06	NGRC-00199	1.69	NGRC-02633	18.97
GNS	20.76±4.0	31.82±1.90	27.56±1.99	21.37±1.46	27.51±1.81	5.10	NGRC-00199	42.50	NGRC-02561	20.45
TGW	24.77±4.699	35.73±2.22	44.39±2.33	29.18±1.69	55.12±2.90	9.27	NGRC-00018	55.87	NGRC-02449	15.073

Note: SE: Standard Error, CV: Coefficient of Variation, GFD: Grain Filling Duration (days), GWS: Grain Weight per Spike (g), GNS: Grain Number per Spike, and TGW: Thousand-Grain Weight (g)

Table 4. Association of the key heat stress responsive traits with the gene-based makers analyzed in the present study

Markers	Associated gene	Allele size (bp)	Mean R ²			
			GFD	TGW	GNS	GWS
CWI21	Tacwi-A1a	404	-	(19.04) 0.18**	-	(0.429) 0.17**
CWI22	Tacwi-A1b	402	(29.27) 0.15**	(33.66) 0.40***	-	(0.801) 0.25**
GS7D	TaGS-D1a	522	-	(38.65) 0.23***	-	-
	TaGS-D1b	562	-	(20.05) 0.11*	-	-
Dreb1	DREB1	596 and 646	-	-	-	-

Note: *** Significant at (P<0.001); ** significant at (P<0.01), * significant at (P<0.05) percent, GFD: Grain Filling Duration, TGW: Thousand-Grain Weight, GNS: Grain Numbers per Spike, GWS: Grain Weight per Spike, R²: Coefficient of determination and the values in the parenthesis were average of performance of genotypes for the trait with positive alleles

Table 5. The marker allele variants of the studied accessions and thousand-grain weight and grain weight per spike under terminal heat stress

Test genotypes	Collection district	Accessions /pedigree	GWS	TGW	cwi21	cwi22	gs7da	gs7db	dreb1-569	dreb1-646
NGRC00179	Achham	Accessions	0.34	17.66	+	-	-	-	-	+
NGRC00180		Accessions	0.18	18.31	+	-	-	+	+	+
NGRC00182		Accessions	0.22	13.03	+	-	-	+	+	+
NGRC00198	Arghakhanchi	Accessions	0.09	11.30	-	-	-	+	+	+
NGRC00011	Baglung	Accessions	0.29	15.07	+	-	-	+	-	+
NGRC02607	Baitadi	Accessions	0.77	31.22	+	-	-	+	-	+
NGRC02609		Accessions	0.34	20.06	+	-	-	+	-	+
NGRC02589		Accessions	1.09	37.47	-	+	-	+	-	+
NGRC00190		Accessions	0.59	18.99	-	-	-	+	+	+
NGRC02571	Bajhang	Accessions	0.66	17.79	+	-	-	+	+	+
NGRC00178	Bajura	Accessions	0.58	12.28	+	-	-	+	-	+
NGRC02567		Accessions	0.43	20.98	+	-	-	+	-	+
NGRC02576	Dadeldhura	Accessions	0.31	16.77	+	-	-	-	+	+
NGRC02578		Accessions	0.24	14.48	+	-	-	-	-	+
NGRC02608		Accessions	0.41	12.39	+	-	-	+	-	+
NGRC04454	Dailekh	Accessions	0.37	16.52	+	-	-	+	-	+
NGRC02591	Darchula	Accessions	0.46	22.22	+	-	-	+	-	+
NGRC02612	Dolakha	Accessions	0.20	16.93	+	-	-	+	+	+
NGRC02616		Accessions	0.31	17.35	+	-	-	+	+	+
NGRC04441		Accessions	0.17	10.04	+	-	-	+	-	+
NGRC04443		Accessions	0.66	19.38	+	-	-	+	+	+
NGRC02596	Doti	Accessions	1.15	30.49	-	+	-	-	-	+
NGRC02603		Accessions	0.24	18.07	+	-	-	+	+	+
NGRC02573	Jajarkot	Accessions	0.48	18.99	+	-	-	+	-	+
NGRC02471	Jumla	Accessions	0.35	20.61	+	-	-	+	+	+
NGRC02601	Kailali	Accessions	0.67	34.81	+	+	-	+	+	+
NGRC02467	Kalikot	Accessions	0.79	24.71	+	-	-	+	+	+
NGRC02602	Kanchanpur	Accessions	0.64	39.29	-	+	+	-	-	+
NGRC00189	Kaski	Accessions	0.15	14.48	+	-	-	+	-	+
NGRC04449	Kavreplanchok	Accessions	0.59	25.70	-	-	+	-	+	+
NGRC02465	Mugu	Accessions	0.26	17.40	+	-	-	+	+	+
NGRC00187	Myagdi	Accessions	0.88	35.94	-	+	-	+	+	+
NGRC04418		Accessions	0.68	13.23	-	-	-	+	-	+
NGRC04400	Nuwakot	Accessions	0.28	13.18	+	-	-	+	+	+
NGRC00206	Parbat	Accessions	0.34	20.03	+	-	-	+	-	+
NGRC02633	Ramechhap	Accessions	0.35	16.91	+	-	-	+	+	+
NGRC04410		Accessions	0.38	15.57	+	-	-	+	+	+
NGRC02621	Rasuwa	Accessions	0.84	16.63	+	-	-	-	+	+
NGRC02544	Rolpa	Accessions	0.45	20.98	-	+	-	+	-	+
NGRC02553	Rukum	Accessions	0.36	11.96	+	-	-	+	-	+
NGRC04431	Salyan	Accessions	0.19	12.10	+	-	-	+	-	+
NGRC02450		Accessions	0.69	20.06	-	+	-	+	+	+
NGRC04450	Surkhet	Accessions	0.31	25.57	+	-	-	+	-	+
NGRC04452		Accessions	0.18	11.75	+	-	-	+	+	+
NGRC02584	Taplejung	Accessions	1.02	50.98	-	+	+	-	+	+
Gautam		SIDDHARTHA/NG 8319//NL 297	1.13	35.73	+	-	-	-	+	+
Munal		WAXWING*2/KIRITATI	0.62	33.00	-	+	-	+	-	+
Aditya		GS 348/NL746//NL748	1.22	44.39	+	-	-	+	-	+
Total positive alleles					36	8	3	41	23	48

Note: + indicated presence of the band, - indicated an absence of the band, GWS: Grain Weight per Spike, TGW: Thousand-Grain Weight

Table 6. Performance of the ten highest and lowest ranked accessions based on grain weight per spike

Genotypes	GFD	GWS	GNS	TGW
Ten high-performer accessions				
NGRC-02633	22	1.69	31.40	53.85
NGRC-04414	30	1.46	37.70	38.66
NGRC-02561	38	1.42	42.50	33.41
NGRC-00019	29	1.40	29.20	47.81
NGRC-00196	37	1.29	29.00	44.38
NGRC-02596	26	1.15	37.65	30.49
NGRC-00015	27	1.10	39.25	28.10
NGRC-02589	32	1.09	29.20	37.47
NGRC-02449	31	1.06	18.90	55.87
NGRC-02451	28	1.05	27.95	37.71
Ten Low-performer accessions				
NGRC-00199	19	0.06	5.10	11.27
NGRC-00198	19	0.09	7.70	11.30
NGRC-02455	15	0.09	8.15	10.98
NGRC-02456	17	0.11	9.60	11.35
NGRC-00018	16	0.12	12.40	9.27
NGRC-02579	21	0.12	11.55	10.17
NGRC-00189	24	0.15	10.05	14.48
NGRC-02666	22	0.15	6.80	21.40
NGRC-04441	20	0.17	16.10	10.40
NGRC-02552	23	0.17	12.50	13.36

Note: GFD: Grain Filling Duration, TGW: Thousand-Grain Weight, GNS: Grain Numbers per Spike, GWS: Grain Weight per Spike

Table 7. Eigenvalues and variability of principal components non-rotated component loadings

Traits	PC-1	PC-2	PC-3	PC-4
Eigenvalues	2.902	0.659	0.413	0.026
Proportion of variance explained	72.559	16.483	10.318	0.640
Components cumulative proportion	72.559	89.042	99.360	100.000
Grain filling duration (GFD)	0.682	0.010	0.308	0.000
Grain number per spike (GNS)	0.587	0.403	0.005	0.004
Grain weight per spike (GWS)	0.930	0.000	0.056	0.014
Thousand-grain weight (TGW)	0.703	0.247	0.043	0.007

In conclusion, a wide variation of kernel weight, a key heat-responsive trait in wheat, is demonstrated in Nepalese wheat germplasm under a terminal heat stress environment. The accessions NGRC-02584 and NGRC-02602 possess positive alleles of TGW-associated functional markers CWI22 and GD7D-a and have high TGW under late-sown heat stress conditions, indicating that these accessions have the potential to withstand terminal heat stress. These accessions could be used in the breeding program for terminal heat stress tolerance after further validation.

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