PLANT SCIENCE TODAY
Vol 13(1): 1-7
https://doi.org/10.14719/pst.12222

eISSN 2348-1900

RESEARCH ARTICLE

Heat tolerance assessment and molecular diversity analysis of wheat (*Triticum aestivum* L.) genotypes using Heat Susceptibility Index (HSI) and SSR/STS markers

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Received: 11 October 2025; Accepted: 25 December 2025; Available online: Version 1.0: 21 January 2026; Version 2.0: 29 January 2026

Cite this article: Rajitha JN, Rajneesh K, Manoj KP, Rajesh A, Mishra CN, Upama M . Heat tolerance assessment and molecular diversity analysis of wheat (*Triticum aestivum* L.) genotypes using Heat Susceptibility Index (HSI) and SSR/STS markers. Plant Science Today. 2026; 13(1): 1-7. https://doi.org/10.14719/pst.12222

Abstract

This study was conducted during the *Rabi* season of 2020-21, aimed to assess the heat susceptibility and genetic diversity of 25 wheat genotypes under four distinct environmental conditions: timely sown conditions in LPU, Jalandhar, Punjab (E1) and ICAR-IIWBR, Karnal, Haryana (E2), as well as late sown conditions at both experimental sites i.e. E3 and E4 respectively. These genotypes, sourced from ICAR-IIWBR, Karnal, Haryana, were cultivated following an Alpha Lattice Design with two replications. The evaluation of heat susceptibility using heat susceptibility index (HSI) categorized the genotypes into two groups: tolerant and susceptible. Heat stress responses varied among genotypes and locations. At Jalandhar (L-1), DBW187, HD3298, HI1612, DBW303, PBW644, NIAW3170, HD3086, HD3118 and DBW88 showed tolerance, whereas DBW222, WB-2, DBW173, DBW90, PBW771, HD3043, HI1605 and HD3249 were susceptible. In contrast, DBW222, HD3086, DBW173, WB-2, DBW90, HI1605, DBW88, PBW771, HD3043, NIAW3170 and HD3249 were tolerant at Karnal (L-2), whereas DBW303, DBW187, HD3298, PBW644, HD3118 and HI1612 were susceptible. Across both locations, HD2967, WH1142, HD3171 and WH1270 consistently exhibited heat tolerance, while DBW71, K1317, WH1105 and HI1628 remained susceptible. Furthermore, an analysis of genetic diversity employing STS and SSR markers revealed a similarity range from 0.61 to 0.94, with an average similarity coefficient of 0.77. Remarkably, genotype pairs such as DBW187 and WH1270, HD3086 and WH1142, DBW173 and HD3298, as well as DBW90 and WH1105, exhibited the highest similarity index of 0.94. The construction of a dendrogram using NTSYSpc software delineated four major clusters, each of which was further subdivided. This research underscores the significance of genetic diversity in plant breeding, as it provides breeders with a rich pool of genetic resources to develop improved varieties endowed with various stress-resistant traits.

Keywords: genetic diversity; heat stress; heat susceptibility index (HSI); sequence tagged site (STS); simple sequence repeat (SSR markers)

Introduction

Wheat (*Triticum aestivum* L.) is one of the foremost food crops in terms of acreage, production and nutritive value globally. It is one of the major cereals consumed by nearly 40 % of the population and grown on approximately one-sixth of the total cultivable land worldwide (1). In India, during the 2024-25 crop year, wheat was grown on ~32.76 million ha with an estimated production of 117.51 mt and average productivity of ~3587 kg/ha (2). An increasing population and climate change pose threats to food security. Due to the global changes in climate, the effects and severity of environmental stress on crops are predicted to increase in the near future (3). According to climate predictions, the average temperature will rise by 1-4 °C by the end of the twenty-first century and wheat yield will decrease by 4.1-6.4 % (4). Heat stress is the most detrimental abiotic stress for the growth and development of crops, affecting their yield and the quality of produce. Multi-location trials conducted for wheat may encounter some heat stress, especially when sown under two dates of sowing, i.e. timely sown and late sown. Environmental stress adversely affects the survival of plants, plant growth and reproductive performance. Nowadays, new parameters of heat tolerance, such as the Heat Susceptibility Index (HSI), have been developed to identify genotypes with heat tolerance. This can help in increasing the efficiency of the breeding programme and helping plant breeders to produce new heat-tolerant genotypes.

Genetic diversity is an important component in plant

breeding as diverse plant genetic resources provide an opportunity for plant breeders to produce better varieties coupled with various stress-resistant characters. Analysis of genetic diversity using molecular markers has proved to be a cornerstone for determining the diversification and conservation of genetic variation in developing approaches for plant propagation. Genetic diversity is important for the survival and adaptability of wheat genotypes against biotic and abiotic stress. Simple sequence repeats (SSR) and sequence tagged site (STS) markers have been confirmed as an effective tool for estimating genetic diversity in wheat (5). The ability to select a superior variety increases with genetic diversity present among the population. Hence, the discovery of such a trait becomes a significant tool in the amelioration of plant breeding programme.

Materials and Methods

The experiment was carried out under timely (E1 and E2) and late (E3 and E4) sown conditions at both agricultural experimental fields of the Genetics and Plant Breeding Department, School of Agriculture, Lovely Professional University, Jalandhar, Punjab and ICAR-IIWBR, Karnal, Haryana. The experimental design comprised 25 different genotypes of wheat grown in an Alpha Lattice Design with two replications during *Rabi* 2020-21. Standard agronomic practices, including stage-wise irrigation (Crown root initiation, tillering, jointing, flowering and grain filling) and fertilizer application (Nitrogen Phosphorous Potassium (NPK) at 120:60:40 kg/ha) and manual pest and weed management, were followed uniformly across all environments. For timely sowing, seeds were sown in rows, spaced 20 cm apart, whereas for late sowing, the row spacing was reduced to 18 cm. Each plot consisted of three rows of 3 m length, resulting in a plot size of 1.8 m^2. The list of genotypes is given in Table 1.

HSI evaluates the performance of a genotype under a stressful environment compared to its maximum potential under a normal environment. HSI was calculated using the formula (6):-

$$H = (1-YD/YP)/D$$

Table 1. List of genotypes used in the study

Sr. No	Name of genotypes	Parentage/ Pedigree	Origin	Condition
1.	DBW 303	WBLL1*2/BRAMBLING/4/BABAX/LR42//BABAX*2/3/SHAMA*2/5/ PBW343*2/KUKU NA*2//FRTL/PIFED	ICAR-IIWBR, Karnal	ES, HF, NWPZ
2.	DBW 187 (KARAN VANDANA)	NAC/TH.AC//3*PVN/3/MIRLO/BUC/4/2*PASTOR/5/KACHU/6/KACHU	ICAR-IIWBR, Karnal	ES, HF, NWPZ & TS, IR, NWPZ,NEPZ
3.	WH 1270	SHA7//PRL/VEE#6/3/FASAN/4/HAAS8446/2*FASAN/5/CBRD/KAUZ/6/ MILAN/AMSE L/7/FRET2*2/KUKUNA/8/2*WHEAR/SOKOLL	CCSHAU, Hisar	ES, HF, NWPZ
4.	DBW 222	KACHU/SAUAL/8/ATTILA*2/PBW65/6/PVN//CAR422/ANA/5/BOW/ CROW//BUC/PVN/3/ YR/4/ TRAP#1/7/ ATTILA/ 2*PASTOR	ICAR-IIWBR, Karnal	TS, IR, NWPZ
5.	HD 3086 (PUSA GAUTAMI)	DBW14/HD2733//HUW468	ICAR-IARI, New Delhi	TS, IR, NWPZ, NEPZ
6.	HD2967	ALD/COC//URESH/HD2160M/HD2278	ICAR-IARI, New Delhi	TS, IR, NWPZ/NEPZ
7.	DBW 173	KAUZ/AA//KAUZ/PBW602	ICAR-IIWBR, Karnal	LS,IR, NWPZ
8.	HD 3298	CL1449/PBW343//CL882/HD2009	ICAR-IARI, New Delhi	VLS, IR, NWPZ
9.	WB-2	T.DICOCCONCI9309/AE.SQUARROSA (409)/3/MILAN/S87230// BAV92/4/2*MILAN/S87320//BAV92	ICAR-IIWBR, Karnal	TS, IR, NWPZ and Bihar
10.	DBW 88	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES	ICAR-IIWBR, Karnal	TS, IR, NWPZ
11.	DBW 90	HUW468/WH730	ICAR-IIWBR, Karnal	LS, IR, NWPZ
12.	DBW 71	PRINIA/UP2425	ICAR-IIWBR, Karnal	LS, IR, NWPZ
13.	WH 1105	MILAN/S87230//BABAX	CCSHAU, Hisar	TS, IR, NWPZ
14.	PBW 771	BW 9246/2*DBW17	PAU, Ludhiana	LS, IR, NWPZ
15.	HD 3171	PBW 343/HD 2879	ICAR-IARI, New Delhi	RF, TS, NEPZ
16.	HI 1628 (Pusa Wheat 1628)	FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/PFAU/WEAVER// BRAMBLING	ICAR-IARI, RS Indore	TS, RIR, NWPZ
17.	PBW 644	PBW175/HD2643	PAU, Ludhiana	RF NWPZ
18.	WH 1142	CHEN/AEGILOPS SQUARROSA(TAUS)//FCT/3/2*WEAVER	CCSHAU, Hisar	RF NEPZ
19.	K 1317	K0307/K9162	CSAUA&T, Kanpur	RF NEPZ
20.	NIAW 3170	SKOLL/ROLF07	MPKV, Niphad	RIR NWPZ
21.	HD 3118	ATTILA*2/PBW65//WBLL1*2/TUKURU	ICAR-IARI, New Delhi	IR LS, NEPZ
22.	(HI 1612) Pusa Wheat 1612	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES	ICAR-IARI, RS Indore	TS,RI NEPZ
23.	HI 1605 (Pusa Ujala)	BOW/VEE/5/ND/VG9144//KAL/BB/3/YACO/4/CHIL/6/CASKOR/3/ CROC_1/AE.SQUARROSA(224)//OPATA/7/PASTOR/MILAN/KAUZ/3/ BAV92	ICAR-IARI, RS Indore	TS,RIRPZ
24.	HD3043	PJN/BOW//OPATA*2/3/CROC_1/Ae.squarrosa(224)//OPATA	ICAR-IARI, New Delhi	TS, RIR NWPZ
25.	HD3249 (Pusa Wheat 3249)	(PBW343*2/KUKUNA//SRTU/3/PBW343*2KHV/AKI)	ICAR-IARI, New Delhi	IR NEPZ

ES: Early Sown, **TS**: Timely Sown, **LS**: Late Sown, **VLS**: Very Late Sown, **HF**: High Fertility, **IR**: Irrigated, **RF**: Rainfed, **RIR**: Restricted Irrigation, **NWPZ**: North Western Plains Zone, **NEPZ**: North Eastern Plain Zone, **PZ**: Peninsular Zone.

Where, YD = Mean of the genotypes in the stress environment

YP = Mean of the genotypes in a normal environment

D = 1 – (Mean YD of all genotypes/ mean YP of all genotypes)

In the present study, HSI value for grain yield was calculated for 25 genotypes sown in both locations under timely and late sown conditions.

Genotypes were classified into 4 different categories as follows: -

1. HSI < 0.50: highly heat-tolerant
2. HSI 0.51-0.75: heat-tolerant
3. HSI 0.76 -1.00: moderately heat tolerant
4. HSI > 1.00: heat susceptible

All 25 wheat genotypes were subjected to STS and SSR assays to analyze the genetic diversity. 1 STS and 15 SSR markers were used for genetic diversity analysis. The present experiment was conducted by following the standard protocol of DNA extraction and Polymerase chain reaction amplification in the laboratory of ICAR-IIWBR, Karnal, Haryana. The details of the markers/primers used in the study are shown in Table 2.

DNA isolation

Genomic DNA was extracted from young leaves using the CTAB (Cetyl Trimethyl Ammonium Bromide) protocol described by previous researchers (7).

STS and SSR genotyping

PCR was performed in a 10 µL reaction using a Life ECO thermal cycler (Bioer Technology), utilizing both STS and SSR markers. To ensure consistent results across the 25 genotypes, a bulk master mix was prepared containing 0.5 µL each of forward and reverse primers and 3.5 µL of nuclease-free water. Each reaction tube received 9 µL of this mix and 1 µL of genomic DNA. The optimized thermal profile included an initial denaturation at 94 °C (5 min), followed by 35 cycles of denaturation (94 °C), primer-specific annealing (51-61 °C) and extension (72 °C), with a final extension of 10 min. STS and SSR sequence information for these primers is also available in Grain Genes, a database for *Triticeae* and *Avena* (USDA). STS and SSR amplicons were resolved on a 1.5 % agarose gel prepared in 1xTBE buffer and stained with Ethidium bromide. Electrophoresis was carried out at a constant voltage and band sizes were estimated using a 100bp DNA ladder. The gels were visualized in a gel documentation system and the bands were scored visually for their presence [1] and absence [0]. The scored data were analysed in NTSYSpc software to generate a dendrogram illustrating the genetic relationships among the genotypes (8).

Results

Heat Susceptibility Index (HSI)

HSI for grain yield per plot was estimated under timely and late sown conditions at two locations, LPU, Jalandhar, Punjab (L-1) and ICAR-IIWBR, Karnal, Haryana (L-2) (Table 3). The HSI values revealed

Table 2. STS & SSR primer used in present investigation

Sr. No.	STS & SSR Primer	Sequence of primers	Annealing temperature	References
1.	csLV34	F: 5'- GTT GGT TAA GAC TGG TGA TGG -3' R: 5'- TGC TTG CTA TTG CTG AAT AGT -3'	55	(9)
2.	GWM99	F: 5' AAGATGGACGTATGCATCACA 3' R: 5' GCCATATTTGATGACGCATA 3'	60	(10, 11)
3.	GWM 146	F: 5' CCAAAAAAACTGCCTGCATG 3' R: 5' CTCTGGCATTGCTCCTTGG 3'	60	(11, 12)
4.	GWM33	F:5' GGAGTCACACTTGTTTGTGCA 3' R:5' CACTGCACACCTAACTACCTGC 3'	60	
5.	WMC698	F: 5'GTGAAGGGAGAGCTAGCAA3' R: 5'ACAGTTGGCCCAGCTAGTA3'	51	
6.	WMC254	F: 5'AGTAATCTGGTCCTCTCTTCTTCT3' R: 5'AGGTAATCTCCGAGTGCACTTCAT3'	51	
7.	WMC313	F: 5'GCAGTCTAATTATCTGCTGGCG3' R: 5' GGGTCCTTGTCTACTCATGTCT3'	51	(10)
8.	WMC503	F: 5'GCAATAGTTCCCGCAAGAAAAG3' R: 5'ATCAACTACCTCCAGATCCCGT3'	61	
9.	WMC44	F: 5'GGTCTTCTGGGCTTTGATCCTG3' R: 5'TGTTGCTAGGGACCCGTAGTGG3'	61	
10.	WMC112	F: 5'TGAGTTGTGGGGTCTTGTTTGG3' R: 5'TGAAGGAGGGCACATATCGTTG3'	61	
11.	GWM583	F:5'TTCACACCCAACCAATAGCA3' R: 5' TCTAGGCAGACACATGCCTG 3	60	
12.	GWM325	F:5'TTTCTTCTGTCGTTCTCTTCCC3' R: 5' TTTTTACGCGTCAACGACG 3'	60	(11)
13.	GWM131	F:5'AATCCCCACCGATTCTTCTC3' R: 5' AGTTCGTGGGTCTCTGATGG 3'	60	
14.	BARC180	F:5'GCGATGCTTGTTTGTTACTTCTC3' R: 5' GCGATGGAACTTCTTTTTGCTCTA 3'	52	(13)
15.	BARC127	F:5'TGCATGCACTGTCCTTTGTATT3' R:5' AAGATGCGGGCTGTTTTCTA 3'	52	(13)
16.	BARC5	F:5'GCGCCTGGACCGGTTTTCTATTTT3' R:5' GCGTTGGGAATTCCTGAACATTTT3'	52	(10)

Table 3. Heat susceptibility index for grain yield per plot in Jalandhar, Karnal and both locations

HSI	Location		
	(L-1)	(L-2)	Genotypes common in both L-1 & L-2
Highly Heat tolerant (<0.50)	DBW187, HD3298, HI1612	DBW222, HD3086, DBW173, WB-2, DBW90, HI1605, DBW88, PBW771	HD2967
Heat tolerant (0.50-0.75)	DBW303, PBW644, NIAW3170	HD3043	-
Moderately heat tolerant (0.76-1.00)	HD3086, HD3118, DBW88	NIAW3170, HD3249	WH1142, HD3171, WH1270
Heat susceptible (>1.00)	DBW222, WB-2, DBW173, DBW90, PBW771, HD3043, HI1605, HD3249	DBW303, DBW187, HD3298, PBW644, HD3118, HI1612	DBW71, K1317, WH1105, HI1628

substantial variation among genotypes across environments, indicating differential responses to terminal heat stress.

The present study revealed that the genotypes at Jalandhar, DBW187, HD3298, HI1612, DBW303, PBW644, NIAW3170, HD3086, HD3118 and DBW88 exhibited lower HSI values and were categorized as relatively heat-tolerant. In contrast, DBW222, WB-2, DBW173, DBW90, PBW771, HD3043, HI1605 and HD3249 showed higher HSI values and were classified as heat susceptible.

At Karnal, genotypes DBW222, HD3086, DBW173, WB-2, DBW90, HI1605, DBW88, PBW771, HD3043, NIAW3170 and HD3249 were identified as heat tolerant, whereas DBW303, DBW187, HD3298, PBW644, HD3118 and HI1612 showed susceptibility to heat stress. Notably, HD2967 displayed consistent heat tolerance across both locations, indicating stable performance under terminal heat stress.

Genetic diversity analysis

Genetic diversity among 25 wheat genotypes was assessed using 1 STS marker and 15 SSR markers. The SSR markers generated clear and reproducible polymorphic bands, confirming their suitability for diversity analysis in wheat. Gel results are shown in Fig. 1.

Similarity coefficients ranged from 0.61 to 0.94, with an average similarity of 0.774, indicating moderate to high genetic diversity among the genotypes. NTSYSpc UPGMA cluster analysis grouped the genotypes into four major clusters. Cluster 1 comprised 7 genotypes, Cluster 2 was the largest with 9 genotypes, Cluster 3 included 6 genotypes and Cluster 4 consisted of 3 genotypes. Further sub-clustering was observed within major clusters, reflecting close genetic relationships among certain genotypes (Table 4).

The highest similarity indices (0.94) were observed between the genotype pairs DBW187 and WH1270, HD3086 and WH1142, DBW173 and HD3298 and DBW90 and WH1105. In contrast, DBW303, HD3043, DBW71 and HI1605 remained isolated from the main clusters, indicating their genetic divergence with respect to other genotypes. A dendrogram has been created using NTSYSpc software, depicting the diversity graphically in Fig. 2.

Discussions

Heat Susceptibility Index and genotypic response

The significant variation in HSI across genotypes and locations highlights the strong influence of genotype × environment interaction on heat stress response in wheat. Genotypes showing lower HSI values maintained relatively higher yields under stress conditions, confirming the effectiveness of HSI as a selection criterion for identifying heat-tolerant genotypes. The differential classification of certain genotypes between Jalandhar and Karnal reflects the role of local climatic conditions and sowing environments in modulating heat tolerance expression. The stable heat tolerance of HD2967 across both locations suggests the presence of robust adaptive mechanisms, making it a valuable genetic resource for breeding programs targeting terminal heat stress.

A similar experiment with categorization of wheat genotypes into 4 groups of HSI, highly heat tolerant, heat tolerant, moderately tolerant and heat susceptible was reported earlier (14). An experiment on HSI in Rajasthan showed DBW 88 as susceptible for grain yield per plant, which contrasts with the current experiment. Although the genotype was heat-tolerant to other yield contributing traits, it was not studied in previous studies (15). HD2967 genotype showed heat tolerance in both locations, which is similar to the results obtained earlier (16). An analogous study conducted by previous researchers concluded that wheat genotypes show a significant amount of variation for yield according to their susceptibility and resistance to heat stress (17-21).

Genetic diversity and marker-based clustering

The moderate to high level of genetic diversity observed among the wheat genotypes demonstrates the effectiveness of SSR markers due to their co-dominant inheritance, high polymorphism and uniform genome coverage (22). The similarity coefficient range obtained in this study is comparable with earlier reports using SSR markers in wheat diversity analysis.

The formation of 4 distinct clusters suggests the presence of broad genetic variability, which can be strategically exploited in breeding programs. Genotype pairs showing high similarity may share common ancestry, whereas genetically divergent genotypes such as DBW303, HD3043, DBW71 and HI1605 represent valuable parental lines for hybridization to broaden the genetic base. Former researchers evaluated genetic diversity among 20 wheat genotypes using 18 SSR markers and reported the similarity coefficient from 0.69 to 0.89 (23). A similar range of coefficients of similarity (0.14-0.71) has been reported for 10 wheat genotypes using 15 SSR markers and comparable studies employing varying numbers of SSR markers have reported different ranges of similarity coefficients (24-28). The dendrogram generated in this study provides a clear visualization of genetic relationships, facilitating informed parent selection for wheat improvement under heat stress conditions.

Conclusion

HSI revealed DBW187, HD3298, HI1612, DBW303, PBW644, NIAW3170, HD3086, HD3118 and DBW88 to be heat-tolerant genotypes in Jalandhar. DBW222, HD3086, DBW173, WB-2, DBW90, HI1605, DBW88, PBW771, HD3043, NIAW3170 and HD3249 were

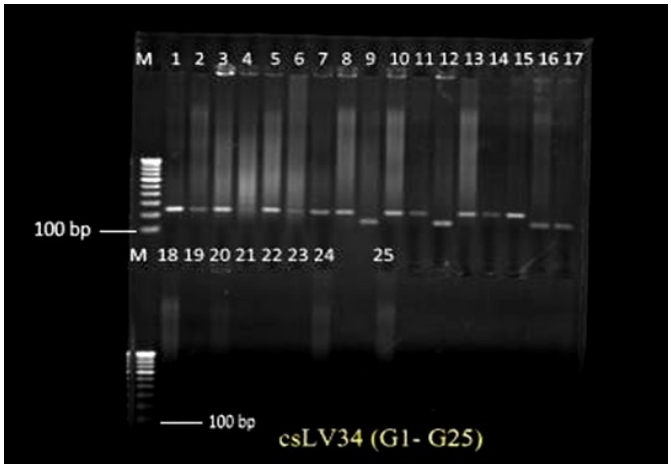

Plate 1. PCR amplification in 25 wheat genotypes using csLV34; M:100bp ladder; G1-G25: wheat genotypes.

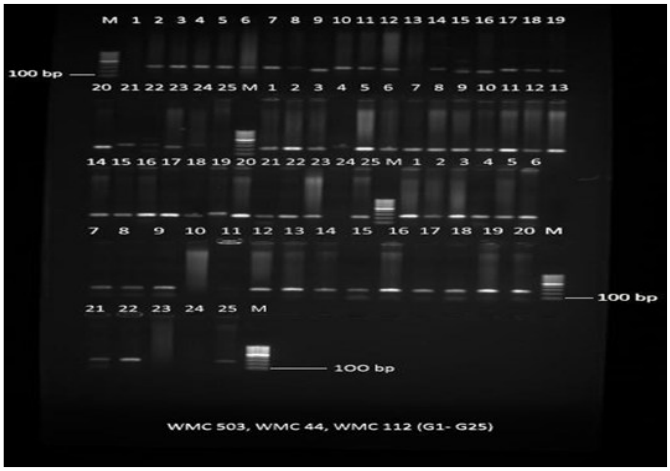

Plate 2. PCR amplification in 25 wheat genotypes using WMC503, WMC44, WMC112; M:100bp ladder; G1-G25: wheat genotypes.

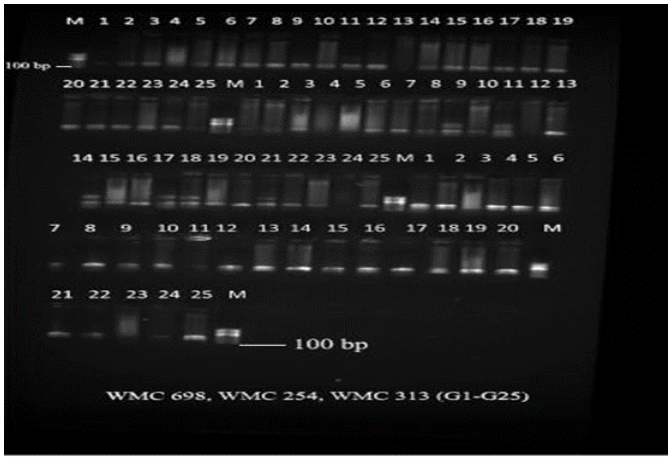

Plate 3. PCR amplification in 25 wheat genotypes using WMC698, WMC 254, WMC 313; M:100bp ladder; G1-G25: wheat genotypes.

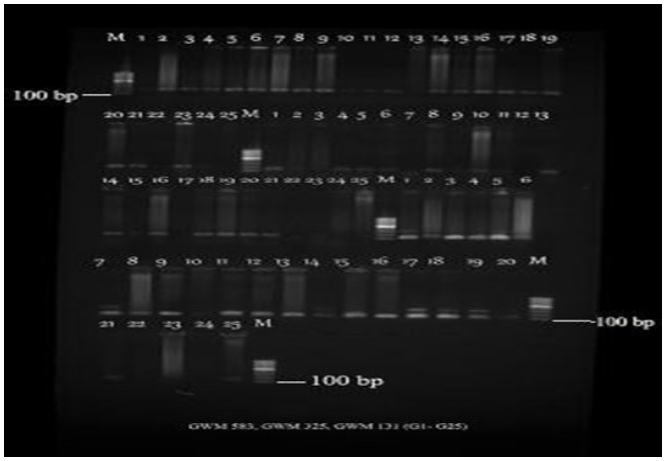

Plate 4. PCR amplification in 25 wheat genotypes using GWM 583, GWM 325, GWM131; M:100bp ladder; G1-G25: wheat genotypes.

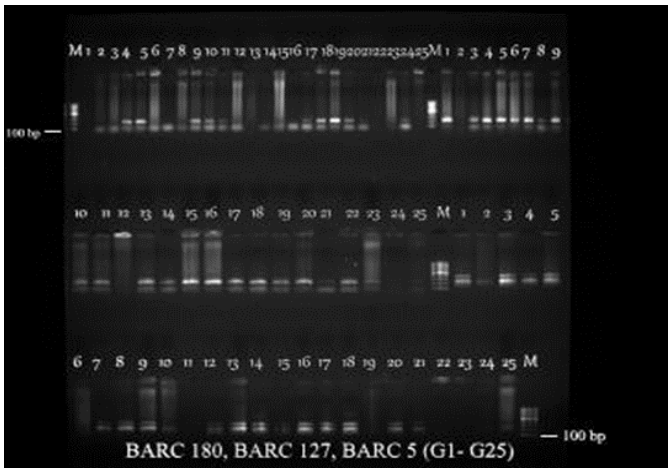

Plate 5. PCR amplification in 25 wheat genotypes using BARC180, BARC127, BARC5; M:100bp ladder; G1-G25: wheat genotypes.

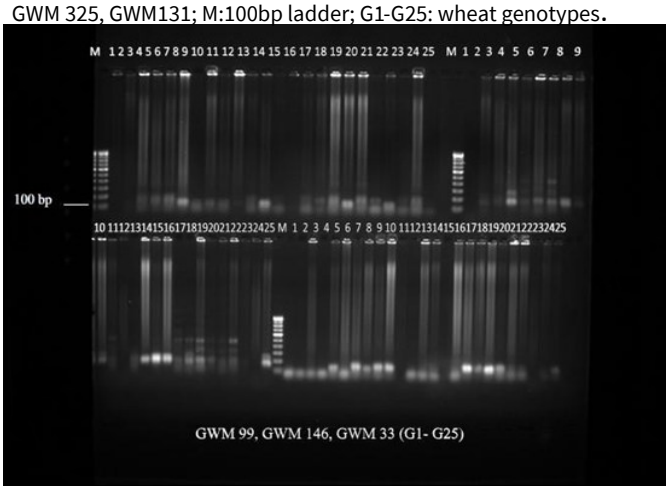

Plate 6. PCR amplification in 25 wheat genotypes using GWM99, GWM146, GWM33; M:100bp ladder; G1-G25: wheat genotypes.

Fig. 1. SSR marker banding pattern in 25 wheat genotypes.

Table 4. Clustering of wheat genotypes

S. No.	Cluster	Sub cluster	Genotypes
1.	Cluster 1	Sub cluster 1	DBW187, WH1270, DBW222, HD2967
		Sub cluster 2	HD3086, WH1142
		Divergent genotype	DBW303
2.	Cluster 2	Sub cluster 1	DBW173, HD3298, WB-2
		Sub cluster 2	DBW 90, WH1105, PBW771, HD3171, HI1628
		Divergent genotype	DBW71
3.	Cluster 3	Sub cluster 1	DBW88, K1317
		Sub cluster 2	HD3118, HI1612, HD3249
		Divergent genotype	HI1605
4.	Cluster 4	Sub cluster 1	PBW644, NIAW3170
		Divergent genotype	HD3043

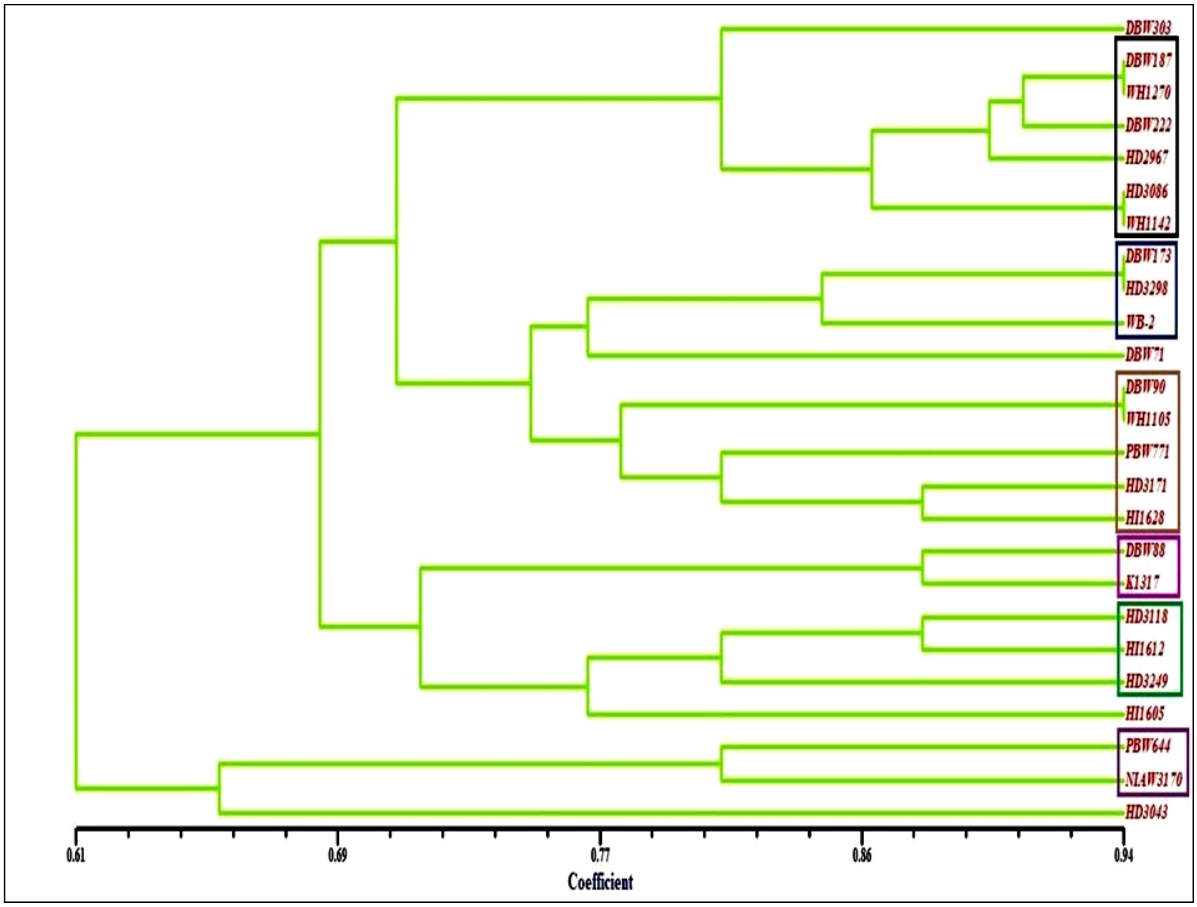

Fig. 2. Dendrogram showing genetic relationships among 25 genotypes based on the similarity index obtained using SSR markers.

highly heat tolerant genotypes in Karnal location. HD2967, WH1142, HD3171 and WH1270 were found to be heat-tolerant in both locations. However, HD2967 was highly tolerant at both locations. Hence, HSI helps in the efficient selection of tolerant genotypes under stress conditions. Genetic diversity study revealed a similarity coefficient that ranged from 0.61 to 0.94 with an average of 0.77. Genotype DBW187 and WH1270, HD3086 and WH1142, DBW173 and HD3298, DBW90 and WH1105 showed the highest similarity index 0.94.

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Authors' contributions

RJN prepared the original draft of the manuscript. MKP contributed to the conceptualization of the study. RK was involved in manuscript formatting and reviewing. CNM, RA and UM contributed to conceptualization and critical review of the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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