Indian J. Genet. Plant Breed., (2022) 82(1): 47-55
doi: 10.31742/IJGPB.82.1.7
ISSN: 0975-6906
www.isgpg.org

RESEARCH ARTICLE

Elucidating molecular diversity in spring wheat (*Triticum aestivum* L. em.Thell.) under terminal heat stress environment using morpho-physiological traits and SSR markers

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Abstract

A set of 36 wheat genotypes was analysed for genetic diversity and terminal heat tolerance under diverse environmental conditions for two years. Based on a minimum mean stress susceptibility index over the seasons, five genotypes, namely, HD3090, HD3086, HD3266, DW1636 and DW1638 emerged as terminal heat tolerant. The D^2 analysis grouped the 36 genotypes into eight and six clusters in 2016-17 and 2017-18, respectively. The result of the principal component analysis was also in agreement with the results of hierarchical cluster analysis. Out of 82 SSR markers used to study the molecular diversity, 45 were polymorphic. Estimates for polymorphism information content varied from 0.09 (GWM297) to 0.50 (GWM111 and CFD46), marker index varied from 0.22 (WMC213) to 1.33 (WMC232), and resolving power varied 1.6 (GWM194 and CFD127) to 4.69 (WMC505). A total of 102, including 20 rare and three unique alleles were detected. The number of alleles per marker ranged from 2 to 4 with an average value of 2.2 alleles per marker. Based on SSR polymorphism, the genotypes were grouped into six divergent multi-genotypic clusters. Comparative genotypic grouping using molecular markers and morpho-physiological traits revealed no similarities of genotypic clustering in different crop seasons. However, three out of five genotypes, HD3090, HD3086 and HD3266 identified as terminal heat-tolerant in different clusters based on D^2 values and SSR marker cluster analysis. Therefore, these genetically diverse genotypes could be used as a potential donor for improving the terminal heat tolerance in wheat.

Keywords: Genetic diversity, stress susceptibility index, SSRs, terminal heat stress, wheat

Introduction

Wheat (*Triticum aestivum* L. em Thell), one of the earliest domesticated cereals, is the second most important cereal crop after rice, grown and consumed globally (FAO 2018). It is grown in a wide range of environments ranging from tropical high-rainfall areas to temperate, irrigated areas; and from warm, humid circumstances to cold, arid conditions (Acevedo et al. 2009). In India, wheat is widely cultivated in different agro-climatic conditions covering northern to southern hills and Gujarat to Assam. The wheat crop is adapted to cooler climatic conditions and therefore, its cultivation is being undertaken in cooler or winter seasons in subtropical and tropical environments. Temperature is one of the main natural factors that influence crop development rate by various physiological processes. The heat stress is not confined to any specific regions but rising temperatures reduce global wheat production (Asseng et al. 2015). Wheat crop is highly sensitive to heat stress, particularly at the reproductive stage. Even 1°C increase in mean temperature causes a decrease and may lead to a higher loss in grain yield (Bennett et al. 2012; Yu et al. 2014). In general, heat stress also reduces several plant development phases like chlorophyll biosynthesis, enzyme activation, photosynthesis inflicting the changes in flowering, anthesis, grain filling, and ripening (Vignjevic et al. 2015).

Due to global warming, the prevailing temperature ranges are not perfect during the various crop growth stages.

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How to cite this article: Meena V. K., Sharma R. K., Chand S., Kumar M., Kumar N., Jain N., & Singh A.. 2022. Elucidating molecular diversity in spring wheat (*Triticum aestivum* L. em. Thell.) under terminal heat stress environment using morpho-physiological traits and SSR markers. Indian J. Genet. Plant Breed., **82**(1): 47-55.

Source of support: Nil

Conflict of interest: None.

Received: Nov. 2021 **Revised:** Jan. 2022 **Accepted:** Feb. 2022

In India, late sowing is common practice in regions under the rice-wheat cropping system. It has been estimated that there would be up to 50% yield reduction of wheat in India if the crop is exposed to 32-38^{0}C at a crucial grain formation stage using the prediction of 3-4% yield loss per 1^{0}C above 15-20^{0}C (Gibson et al.1999). Despite its significance, the genetic basis of terminal heat tolerance is poorly implicit in wheat (Sharma et al. 2021). Therefore, identifying genetically diverse terminal heat tolerant genotypes is of paramount importance and becomes crucial requirement in a breeding programme for developing terminal heat-tolerant wheat varieties. Various screening criteria have been proposed to identify terminal heat-tolerant genotypes, including stress susceptibility index (SSI) proposed by Fisher and Maurer (1978). To estimate the genetic diversity, D^2 Statistics (Mahalanobis 1936) based on multiple quantitative traits, which are highly influenced by the environments, is a widely used method in crop improvement. Use of molecular markers to divulge the genetic diversity present in germplasm is also used to assist the breeding. Among the molecular markers, SSR markers have been extensively used for assessing genetic variability in the population due to their desired benefit of locus specificity, multi-allelic, co-dominance, high polymorphism, and wide distribution in the coding and non-coding genomic regions (Sharma et al. 2014; Molla et al. 2015). However, there are limited studies done in India to lay the long-term impact of terminal heat tolerance on wheat yield in times of climate change. Therefore, the present study was undertaken to evaluate a set of genetically diverse bread wheat genotypes for terminal heat tolerance and to study genetic diversity using morpho-physiological characters and molecular markers.

Materials and methods

Plant material, experimental setup and phenotypic characterization

The experimental materials comprised of 36 genotypes, including both released and pre-released advanced breeding lines of bread wheat developed at ICAR-Indian Agricultural Research Institute (IARI), New Delhi (Supplementary Table S1). The field experiment was carried out using randomized complete block design, replicated thrice at Experimental Farm (228MSL, 28^0 40′ N, 77^0 13′ E), Division of Genetics, ICAR-IARI, New Delhi during *rabi* 2016-17 and 2017-18 under timely (2nd week of November) (E1) and very late (1st week of January) sown conditions (E2) to expose genotypes to terminal heat stress. Each plot consisted of six rows of five meter length having a row spacing of 20cm. The experiment material was sown using self-propelled Norwegian Seed Drill in a well-prepared field. Recommended agricultural production practices were followed to raise the healthy crop.

Observations were recorded on 13 morpho-physiological characters at different crop growth stages. Days to 50% heading (DTH), days to maturity (DTM), and canopy temperature depression (CTD) were documented on a plot basis. CTD was recorded at anthesis stage of a non-irrigated plot using a portable infrared thermometer with a view of 2.5^0. Observations on spike length (SL) in cm, number of grains spike^{-1} (GNPS), and grain weight spike^{-1} (GWPS) in gram, were recorded on randomly selected ten spikes from each plot. Observations on number of spikes m^{-2} (SNPMS), grain yield m^{-2} (GYPMS) in gram, biological yield m^{-2} (BYPMS) in gram, and harvest index (HI) in per cent were measured from one square meter area of each plot marked with bamboo pegs. Plant height (PH) in cm was measured on ten randomly selected plants from each plot. Grain filling period (GFP) was calculated as difference in days between DTH and DTM. 1000-kernel weight (TKW) in gram was recorded using randomly selected and counted 1000 kernels after harvesting.

DNA extraction and SSR amplification

The genomic DNA was isolated using the modified CTAB method proposed by Doyle and Doyle (1990). PCR profiling of all 36 wheat genotypes was completed using a set of 82 SSR-reported primers(http://wheat.pw.usda.gov/). PCR analysis was done in a gradient thermal cycler with an initial denaturation at 94°C for 4 min, followed by 35 cycles of 94°C for 30 s, 50- 60°C for 1 min, 72°C for 2 min and a final extension at 72°C for 10 min before cooling at 4°C. The amplified products were resolved on 3.5% metaphor agarose gel in 1.0× TAE buffer at 80V. The ethidium bromide-stained DNA bands were visualized under UV light and were scored.

Statistical analysis

The analysis of variance (ANOVA) was carried out following Panse and Sukhatame (1985). The genetic divergence amongst the genotypes was estimated using Mahalanobis D^2 statistics as elaborated by Rao (1952). Stress susceptibility index (SSI) was calculated for grain yield over heat stress (very late sown) and non-stress (normal sown) environments employing the formula suggested by Fischer and Maurer (1978), SSI = [1-(Ys/Yp)]/D. Where-Ys = grain yield of the genotype in E2. Yp = grain yield of the genotype in E1. Whereas D (stress intensity) = 1- [mean grain yield of all genotypes under E2 /mean grain yield of all genotypes in E1]. Pooled SSI values were calculated employing the pooled grain yield data of both years.

The genetic similarities were calculated for each pair of lines using the Jaccard similarity index. The genotypes were grouped using Euclidean cluster analysis. Cluster analysis was performed with the NTSYS-pc 2.02 package based on the unweighted pair-group method with arithmetic average

(UPGMA) (Boppenmaier et al. 1993). A primer's resolving power (RP) and band informativeness (Ib) were calculated using the method suggested by Prevost and Wilkinson (1999). The information regarding effective multiplex ratio (EMR), polymorphism information content (PIC), arithmetic mean heterozygosity/diversity index (DI) of each primer was determined using the method suggested by Powell et al. (1996).

Results

Effect of heat stress on grain yield

The ANOVA revealed highly significant differences for all the characters under study during both the crop seasons suggesting considerable genetic variability and suitability of genotypes chosen for study (Supplementary Table S2). Average genotypic performance varied in E1 and E2 environments during both the crop years. In comparison

Table 1. Effect of heat stress (E2) on grain yield and stress susceptibility index (SSI) over the normal condition (E1 during production year 2016-17 and 2017-18

Genotype	2016-17			2017-18			Pooled		
	Yield (E1)	Yield (E2)	% Loss	Yield (E1)	Yield (E2)	% Loss	Yield (E1)	Yield (E2)	SSI
HD3171	1311	595	54.00	1566	733	53.19	1439	514	1.39
DW1616	791	343	56.64	1355	867	36.01	1073	605	0.94
HD2864	964	405	57.99	1416	864	38.98	1190	534	1.19
HD3086	549	233	57.56	787	704	10.55	668	469	0.64
HD3249	893	290	67.53	1324	780	41.09	1108	535	1.12
DW1627	751	199	73.50	1196	472	60.54	973	336	1.42
DW1628	1064	412	61.28	1351	875	35.23	1207	544	1.19
DW1629	1076	450	58.18	1075	711	33.86	1076	431	1.30
HD3184	1280	436	65.94	1394	898	35.58	1337	567	1.24
HD3252	1168	553	52.65	1343	856	36.26	1255	554	1.21
DW1630	1070	390	63.55	1185	1141	03.71	1127	715	0.79
HD2932	796	337	57.66	1185	799	32.57	991	518	1.03
DW1631	1692	536	68.32	1569	545	65.26	1630	390	1.64
DW1632	1197	532	55.56	955	691	27.64	1076	511	1.13
WR544	854	420	50.82	939	835	11.08	897	528	0.89
HD3255	950	563	40.74	1255	735	41.43	1102	649	0.89
DW1633	794	460	42.07	1437	679	52.75	1116	570	1.06
DW1634	800	622	22.25	1225	649	47.02	1012	636	0.80
DW1635	1116	647	42.03	1465	866	40.89	1290	757	0.89
HD3090	502	365	27.29	1096	1065	02.83	799	715	0.23
HD3262	1534	733	52.22	1445	856	40.76	1490	795	1.01
DW1636	961	751	21.85	1239	1040	16.06	1100	896	0.40
DW1637	1016	434	57.28	1396	627	55.09	1206	531	1.21
DW1638	593	495	16.53	922	750	18.66	757	622	0.39
DW1639	794	343	56.80	1226	943	23.08	1010	593	0.89
DW1640	1109	557	49.77	1331	503	62.21	1220	380	1.49
HD3318	1411	699	50.46	1518	972	35.97	1465	636	1.22
HD3265	1285	506	60.62	1113	1071	03.77	1199	789	0.74
DW1642	1043	553	46.98	1362	1039	23.72	1202	796	0.73
DW1643	738	359	51.36	1212	779	35.73	975	569	0.90
DW1644	768	441	42.58	1326	828	37.56	1047	634	0.85
DW1645	1146	499	56.46	1492	959	35.72	1319	679	1.05
HD3059	1170	516	55.90	837	737	11.95	1004	627	0.81
HD3266	1333	783	41.26	1337	1066	20.27	1335	925	0.66
DW1615	1167	655	43.87	1226	935	23.74	1196	795	0.73
HDCSW18	1061	590	44.39	1461	953	34.77	1261	771	0.84
Mean	1020.76	489.24	50.94	1265	828	34.55	1143	614	0.97

to non-stress environment (E1), an average grain yield reduced by 50.94% (range 16.53 to 73.50%) and 34.55% (range 2.83 to 65.26%) under terminal heat stress conditions (E2) during 2016-17 and 2017-18, respectively (Table 1). In 2016-17, maximum yield reduction was observed in DW1627 (73.50%) followed by DW1631 (68.32%) and HD3249 (67.53%), whereas the minimum was found in DW1638 (16.53%). In 2017-18, genotype DW1631 (65.26%) showed maximum yield reduction followed by DW1637 (55.09%), whereas minimum yield reduction was recorded for HD3090 (2.83%). Pooled SSI values over both the years ranged from 0.14 (HD3090) to 1.00 (DW1631) with overall mean value of 0.45. Based on the desirable mean SSI values, five genotypes *viz.,* HD3090, DW1638, DW1636, HD3086 and HD3266 emerged as terminal heat tolerant genotypes (Table 1).

Genetic diversity based on morpho-physiological traits

Euclidean distance analysis using 13 morpho-physiological traits under E2 environment during 2016-17 grouped 36 genotypes into eight clusters (Table 2). The cluster I included maximum of 26 genotypes with 890.64 intra-cluster Euclidean distances followed by cluster III, with three genotypes with 586.19 intra-cluster distance; and cluster V encompassed two genotypes with 577.27 intra-cluster distance. However, remaining clusters contained only one genotype each. The inter-cluster distance was found highest between cluster III and VIII (9057.56), followed by cluster III and V (5399.00). In contrast, the minimum inter-cluster distance was observed between cluster IV and cluster VII (794.39), followed by cluster II and IV (891.97) (Table 3). During 2017-18, clusters II, I and V were composed of 15, 11 and six genotypes with the intra-cluster Euclidean distance of 608.4, 494.19 and 405.7, respectively, whereas the remaining clusters contained only one genotype each (Table 3). High intra-cluster distance in cluster II revealed genetic dissimilarity between grouped genotypes. The maximum inter-cluster distance was observed between cluster V and VI (3862.95) followed by cluster III and VI (3796.94). The minimum inter-cluster distance was observed in between cluster IV and V (571.83), followed by cluster I and III (880.18) (Table 3).

Interestingly, few genotypes were grouped during the both the crop seasons under E2 environment. For example, a group of 12 genotypes *viz*., HD3171, HD2864, HD3249, DW1627, DW1628, HD3252, DW1630, HD2932, DW1631, DW1632, HD3090 and DW1640 occupied cluster I and cluster II in 2016-17 and 2017-18 crop season, respectively (Table 2). Similarly, eight genotypes namely, DW1637, DW1638, HD3265, DW1642, DW1643, DW1644, DW1645 and HDCSW18 came together in cluster I during 2016-17 and 2017-18. Likewise, two genotypes *viz*., HD3184 and HD3318 appeared together in clusters I and VI in 2016-17 and 2017-18, respectively.

SSR polymorphism and polymorphism information content (PIC)

Eighty-two SSR primers were initially used to determine the polymorphic pattern across the 34 out of 36 wheat genotypes, however, HD3249 and DW1640 did not show

Table 2. Clustering pattern of 36 genotypes using D^2 statistic under heat stress environment during 2016-17 and 2017-18

Year 2016-17		
Cluster	Genotypes	Genotypes in cluster
I	26	HD3171, HD2864, HD3249, DW1627, DW1628, DW1629, HD3184, HD3252, DW1630, HD2932, DW1631, DW1632, DW1633, DW1634, HD3090, DW1637, DW1638, DW1639, DW1640, HD3318, HD3265, DW1642, DW1643, DW1644, DW1645, HD3059, HDCSW18
II	1	DW1616
III	3	HD3262, DW1636, HD3266
IV	1	DW1635
V	2	WR544, HD3086
VI	1	DW1615
VII	1	HD3255
VIII	1	DW1614
Year 2017-18		
I	11	HD3262, DW1636, DW1637, DW1638, HD3265, DW1642, DW1643, DW1644, DW1645, HD3266, HDCSW18
II	15	HD3171, DW1616, HD2864, HD3086, HD3249, DW1627, DW1628, DW1629, HD3252, DW1630, HD2932, DW1631, HD3255, DW1633, HD3090, DW1640
III	1	DW1615. DW1614
IV	1	DW1632
V	6	WR544, DW1639, HD3318, HD3059, DW1634, DW1635
VI	1	HD3184

Table 3. Mahalanobis euclidean intra (diagonal) and inter (non-diagonal) cluster distance under heat stress during 2016-17 and 2017-18

Year 2016-17								
Cluster	I	II	III	IV	V	VI	VII	VIII
I	**890.64**	1412.36	3030.55	1390.21	1817.26	1360.1	1986.23	3700.63
II		**0**	4748.65	891.97	1840.21	1725.88	1204.12	2618.37
III			**586.19**	3247.19	5399.00	1867.39	5280.40	9057.56
IV				**0**	1829.21	1279.91	794.39	3334.25
V					**577.27**	3252.1	2352.34	2706.92
VI						**0**	1715.54	3781.03
VII							**0**	3015.82
VIII								0

Year2017-18						
Cluster	I	II	III	IV	V	VI
I	**494.19**	1412.55	880.18	1900.11	1304.82	1988.00
II		**608.4**	2371.29	931.00	1275.91	1690.38
III			0	2078.59	1123.91	3796.94
IV				**0**	571.83	3667.42
V					**405.7**	3862.95
VI						0

Table 4. List of SSR primers and their identified alleles, PIC, RP and MI values

S. N.	Primers	Located on Chr.	No of allele	PIC[a]	RP[b]	MI[c]
1	CFD1	6DS	3	0.3	3.03	0.90
2	CFD13	6DS	3	0.38	2.06	1.14
3	CFD21	1DS	2	0.22	2.46	0.44
4	CFD39	5AL	2	0.28	3.03	0.55
5	CFD46	7DS	2	0.5	1.94	1.00
6	CFD30	4AL	2	0.35	1.83	0.70
7	GWM102	2DS	2	0.19	3.37	0.37
8	GWM106	1DS	2	0.21	2.29	0.43
9	GWM111	7DS	2	0.5	2.06	1.00
10	GWM133	6BL	2	0.49	2.06	0.99
11	GWM169	6AL	2	0.34	1.89	0.69
12	GWM205	5AS	2	0.21	3.31	0.42
13	GWM213	5BL	2	0.14	3.49	0.27
14	GWM533	3BS	2	0.16	1.94	0.32
15	GWM577	7BL	2	0.32	1.83	0.63
16	GWM644	3BL	2	0.2	2	0.40
17	WMC213	1BL,5BL	2	0.11	2	0.22
18	WMC222	1DS	2	0.14	2.06	0.27
19	WMC489	3AL	2	0.25	2.29	0.50
20	GWM113	4BL	2	0.26	2.63	0.53
21	WMC216	1B	2	0.22	3.49	0.44
22	GWM334	6AS	2	0.29	2.69	0.59
23	WMC232	4AL	3	0.44	2.86	1.33
24	GWM126	5AL	3	0.35	2.17	1.04
25	WMC505	3AS	3	0.2	4.69	0.61
26	WMC818	1AS,2DS,4DS	3	0.24	3.77	0.72
27	GWM297	7BS	3	0.09	1.94	0.27
28	WMC420	4AS	2	0.23	3.2	0.46
29	WMC770	2BS	4	0.33	1.89	1.32
30	CFD190	6DL	2	0.33	1.71	0.66
31	CFD127	3DS	2	0.31	1.6	0.61
32	GWM664	4BS	2	0.32	1.77	0.65
33	CFD71	3DL	2	0.49	1.71	0.98
34	GWM194	4DL	2	0.26	1.6	0.52
35	GWM357	1AL	2	0.24	2.17	0.48
36	GWM332	7AL	2	0.27	2.8	0.54
37	GWM157	2DL	2	0.28	2.8	0.55
38	GWM359	2AS	2	0.38	1.66	0.76
39	GWM271	2BL	2	0.27	2.63	0.55
40	GWM182	5DL	2	0.24	3.26	0.47
41	WMC388	7AS	2	0.25	1.71	0.50
42	GWM413	1BS	2	0.14	2.06	0.27
43	WMC133	5DS	2	0.23	3.2	0.46
44	GWM132	6BS	2	0.2	2.23	0.39
45	WMC773	5BS	2	0.26	2.57	0.51

PIC[a] = Polymorphism information content; RP[b] = Resolving power and MI[c] = Marker index

any amplification. Forty five primers were repeatable and produced high-resolution bands for all the genotypes used to assess genetic diversity (Table 4). Based on amplification of SSR markers, a total of 120 alleles were detected including 20 rare alleles (<20% allelic frequency), three unique alleles (< 5% allelic frequency). PIC value varied from 0.09 to 0.50 with an average value of 0.28. The high PIC value was detected due to the presence of rare alleles in high frequency. The maximum and minimum PIC value was recorded for primer GWM111 and CFD46 equally, and GWM297, respectively. Out of 45 markers, 19 markers showed higher PIC values than the overall mean (0.28), suggesting the utility of these markers in wheat. Moderate PIC value for studied primers could be ascribed to the diverse nature of wheat genotypes and highly informative SSR markers. An almost equal contribution to genetic variation was made by A, B and D genomes with 2.19, 2.21 and 2.20 alleles per locus, respectively (Table 5).

Marker index (MI) value ranged from 0.22 (WMC 213) to 1.33 (WMC 232) with an average value of 0.61 across the loci (Table 6), while the values of resolving power (RP) for all the primers varied from minimum 1.60 (GWM194 and CFD127) to maximum 4.69 (WMC505) with mean value of 2.44 across the loci (Table 4). The PIC, RP, and MI values were found highest in genome D (0.31, 2.49 and 0.67), followed by genome A (0.29, 2.47 and 0.64), while genome B exhibited the minimum values (0.23, 2.24 and 0.52), respectively. The genetic relationship among wheat genotypes was determined using Jaccard similarity matrix based on SSR binary data and UPGMA method (Supplementary Table S3). The 34 accessions were grouped into six divergent multi-genotypic clusters based on SSR polymorphism. Cluster III comprised the maximum number of (11) genotypes followed by cluster IV (8) and cluster II (6), whereas cluster I, V and VI were composed of three genotypes each.

Discussion

In general, the reduction in mean grain yield was recorded under heat stress environments during both the crop seasons as compared to normal environments suggesting the effect of terminal heat stress on genotypes. Comparatively, more reduction in grain yield in 2016-17 was ascribed to relatively higher mean temperature during the reproductive phase ranging from 15.9 to 34.7°C compared to 17.8-31.5°C in

Table 5. Genome wise comparison of used SSR primers and their descriptors

Chromosome	No. of markers used	Number of alleles detected	Average allele per locus	PIC[a]	RP[b]	MI[c]
1A	2	5	2.5	0.24	2.97	0.60
2A	1	2	2	0.38	1.66	0.76
3A	2	5	2.5	0.23	3.49	0.56
4A	1	2	2	0.35	1.83	0.70
5A	3	7	2.3	0.28	2.84	0.67
6A	2	4	2	0.32	2.29	0.64
7A	2	4	2	0.26	2.26	0.52
A genome	13	29	2.19	0.29	2.47	0.64
1B	3	6	2	0.16	2.51	0.31
2B	2	6	3	0.30	2.26	0.93
3B	3	6	2	0.16	1.98	0.32
4B	2	4	2	0.29	2.20	0.59
5B	3	6	2	0.17	2.69	0.34
6B	2	4	2	0.34	2.14	0.69
7B	2	5	2.5	0.20	1.89	0.45
B genome	17	37	2.21	0.23	2.24	0.52
1D	3	6	2	0.19	2.27	0.38
2D	3	7	2.3	0.23	3.31	0.55
3D	2	4	2	0.40	1.66	0.80
4D	2	5	2.5	0.25	2.69	0.62
5D	2	2	2	0.23	3.23	0.46
6D	3	8	2.6	0.34	2.27	0.90
7D	2	4	2	0.50	2.00	1.00
D genome	17	36	2.20	0.31	2.49	0.67

PIC[a] = Polymorphism information content; RP[b] = Resolving power and MI[c] = Marker index

2017-2018. Grain filling rate under optimum temperature conditions, decreased grain filling duration is compensated by increased grain filling rate, but this compensation does not happen under high temperature stress, which results in a significant reduction in individual grain weight thereby reducing productivity (Sofield 1977; Narayanan 2018). Also, under heat stress during the reproductive phase, deactivation of RuBisCO enzyme (Kumar et al. 2016), low photosynthetic capacity, aberrant assimilate translocation rate (Raines 2011; Lukac et al. 2012), premature leaf senescence, and decreased chlorophyll content (Farooq et al. 2011; Haque et al. 2014) are some of the other important physiological activities taking place during this phase. The SSI is a ratio of genotype performance under stress and non-stress conditions. Based on desirable SSI value during two consecutive crop seasons five genotypes namely, HD3090, DW1638, DW1636, HD3086, and HD3266 emerged as terminal heat tolerant genotypes. Terminal heat tolerance was also assessed by Krishna et al. (2020) in a set of 34 wheat genotypes considering 14 traits and reported three genotypes, namely, DBW39, DBW16 and DBW14, which showed lowest heat susceptibility index (0.34-0.36) for plot yield. Thus, these genotypes were considered heat tolerant by both Hierarchical Cluster analysis and Discriminant analysis. Heat-responsive spring wheat genotypes were evaluated for heat tolerance under optimum field conditions, and two heat-stress conditions by Devi et al. (2021) who reported a few heat tolerant genotypes based on estimated values of different heat stress indices. They advocated that the identified genotypes may be utilized in breeding for heat tolerance.

Grouping the genotypes into different clusters based on genetic distances for multiple quantitative characters is a pre-requisite for identifying genetically divergent parental lines for an effective recombination breeding programme. The genotypes grouped in a cluster had a higher degree of similarity among themselves; however, more genetic diversity among the genotypes belongs to different clusters. Therefore, the present study displayed substantial genetic variability among wheat genotypes for their ability to cope with heat stress as observed earlier (Shah et al. 2003).

In the present study, clustering based on Euclidean distance analysis grouped 36 genotypes into eight and six clusters under heat stress conditions during 2016-17 and 2017-18, respectively. Comparison of clustering pattern in both years indicated that some genotypes grouped together in one cluster while some are not, which may be due to change in the environmental conditions. The perusal of the immediate parentage of these genotypes indicated that none possessed the same parentage. However, similarity in parentage at the grand parental level cannot be ruled out. Murty and Arunchalam (1966) reported that various genotypes originating from or developed at one research centre were found to be scattered over different clusters in diverse environments. It could be due to various factors like heterogeneity, the genetic architecture of the populations, history of selection, developmental traits and degree of general combining ability. The genotypes having DW and HD numbers were scattered over different clusters. Such a clustering pattern of the genotypes suggested a lack of relationship between pedigree of different genotypes and genetic divergence between them.

Microsatellite primer pairs are locus-specific and, therefore, generally considered single-locus markers as against other molecular markers like RFLP probes and RAPD primers, which are multi-locus in nature. It has been suggested that three mechanisms for creating a new allele at SSR loci are replication slippage for short tandem repeat sequences (Tachida and Iizuka 1992), unequal crossing-over for very large number of alleles for long tandem repeat arrays (Harding et al. 1992) and genetic recombination. All SSR markers used in the present study were di or tri-nucleotide repeats; hence, replication slippage probably plays a major role in creating new alleles at these SSR loci. In the present study, allelic diversity of 102 alleles revealed the high polymorphic ability of SSR markers. The average PIC, RP and MI values were 0.28, 2.44 and 0.61, respectively. SSR markers are co-dominant, robust, PCR-based, reliable and highly reproducible, with greater discriminatory ability are better suited for molecular diversity analysis than the other markers. The PIC values have been used extensively in previously studied genetic diversity analyses (Bhusal et al. 2017; Al-Ashkar et al. 2020). Najaphy et al. (2011) reported that MI in the range of 0.41 to 3.36 with an average of 1.34 using 10 ISSR primers in 36 wheat accessions.

The RP index is utilized to identify the markers that recognize genotypes most efficiently and provides a more accurate estimation of the number of genotypes identified by a primer (Prevost and Wilkinson, 1999). Two SSR primers (WMC505 and WMC818) had high RP values (4.69 and 3.37, respectively), and therefore seem to be more enlightening primers to differentiate the genotypes. Najaphy et al. (2011) reported RP value ranged from 7.2 (UBC-815) to 16.5 (UBC-845), with an average RP value of 12 per primer. However, RP cannot provide information on the ability of a primer to reveal the genetic relationship amongst the studied genotypes (Prevost and Wilkinson 1999; Gilbert et al. 1999). The PIC, RP and MI values were recorded by different genomes were almost equal for D and A genome, while B genome showed relatively low performance for these parameters.

The similarity coefficient, evaluated using SSR primers between any two genotypes, ranged from 0.58 to 0.86, demonstrating adequate genetic diversity amongst the genotypes. Most genotypes were grouped into clusters II, III and IV based on molecular markers. In contrast, these

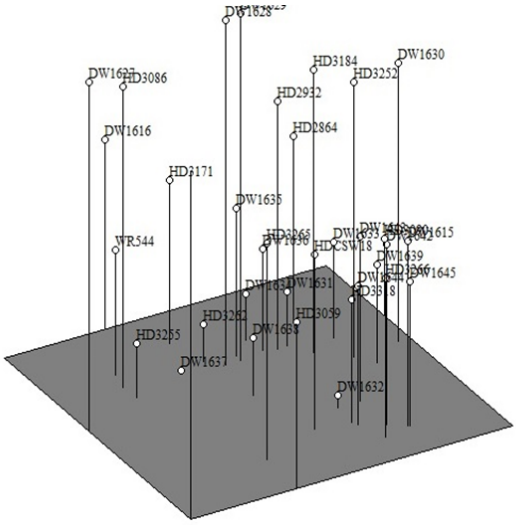

Fig.1. Principal component analysis of 34 wheat genotypes revealed by SSR markers

genotypes came together to a single cluster (cluster I) and different clusters during 2016-17 and 2017-18, respectively, based on morpho-physiological traits. Najaphy et al. (2011) classified 30 wheat genotypes into five clusters based on ISSR binary data using the complete linkage method of Jaccard similarity matrix. Comparative genotypic grouping using molecular markers and morpho-physiological traits revealed no similarities in the genotypic clustering pattern. It might be due to conceptual differences while measuring the genetic distances between genotypes using molecular and morphological distances. The outcome from principal component analysis (Fig.1) was in agreement with the results of hierarchical cluster analysis. It demonstrated that the clustering of wheat genotypes was comparative in the two cases. Genotypes within a typical cluster have fallen closer to each other and the other way around, subsequently speaking to an indistinguishable gathering from in cluster analysis.

Among the five genotypes emerged as terminal heat tolerant, three genotypes, HD3090, HD3086 and HD3266 were grouped into different clusters based on SSR marker cluster analysis and D^2 values in 2016-17, indicating these are genetically quite diverse which can be used in the future breeding programme. However, based on D^2 values, genotypes HD3086 and HD3090 were grouped together in same cluster in 2017-18. Interestingly, genotypes HD3086 and HD3090 involved multi parents in their parentage, HD3086 suited to timely sown conditions and HD3090 suited to late sown conditions, thus differs with each other in a significant way. Grouping of these genotypes into single cluster might be due to environmental variations. The diversified parentage leads to the grouping of genotypes into different clusters, supported by molecular diversity analysis. The genotypes identified in the present study are diverse and heat tolerant and hence, could be used as potential donors in improving the terminal heat tolerance in wheat. However, the important component of breeding for heat stress tolerance is identifying and characterizing high temperature stress-responsive genes in bread wheat (*Triticum aestivum* L.) and their regulation at various stages of development (Chauhan et al. 2011).

Acknowledgments

The first author is grateful to Indian council of Agricultural Research (ICAR), New Delhi for providing Junior Research Fellowship (JRF) for master's degree program.

Authors' contribution

Conceptualization of research (RKS); Designing of the experiments (RKS, VKM); Contribution of experimental materials (RKS); Execution of field/lab experiments and data collection (VKM, AS, NJ); Analysis of data and interpretation (RKS, NK); Preparation of manuscript (VKM, RKS, SC).

Supplementary materials

Supplementary Tables S1 to S3 are presented.

References

Acevedo E., Silva P. and Silva H. 2002. Wheat growth and physiology. Bread wheat, improvement and production (Eds B.C. Curtis, S. Rajaram and H. Gómez Macpherson), FAO, Rome, Italy, 30:39-70.

Al-Ashkar I., Alotaibi M., Refay Y., Ghazy A., Zakri A. and Al-Doss A. 2020. Selection criteria for high-yielding and early-flowering bread wheat hybrids under heat stress. PloS One, **15**:e0236351.https://doi.org/10.1371/journal.pone.0236351.

Asseng S., Ewert F., Martre P. Rötter R.P. Lobell D.B., Cammarano D., Kimball B.A., Ottman M.J., Wall, G.W., White, J.W. et al. 2015. Rising temperatures reduce global wheat production. Nat. Clim. Chang., **5**: 143-147.

Bennett D., Izanloo A., Reynolds M., Kuchel H., Langridge P. and Schnurbusch T. 2012. Genetic dissection of grain yield and physical grain quality in bread wheat (*Triticum aestivum* L.) under water-limited environments. Theor. Appl. Genet., **125**: 255-271.

Bhusal N., Sarial A.K., Sharma P. and Sareen S. 2017. Mapping QTLs for grain yield components in wheat under heat stress. PLoS One, **12**(12): e0189594. https://doi: 10.1371/journal.pone.0189594.

Boppenmaier J., Melchinger A.E., Brunklaus-jung H., Giegler H. and Herrmann R.G. 1993. Genetic diversity for RFLPs in European maize inbreds. I. Relation to performance of flint x dent crosses for forage traits. Crop Sci., **32**: 895-902.

Chauhan H., Khurana N., Tyagi A.K., Khurana J.P. and Khurana P. 2011. Identification and characterization of high temperature stress responsive genes in bread wheat (*Triticum aestivum* L.) and their regulation at various stages of development. Plant Mol. Biol., **75**: 35-51.

Devi K., Chahal S., Singh S., Venkatesh K., Mamrutha H. M., Raghav N., Singh G., Singh G.P. and Tiwari R. 2021. Assessment of wheat genotypes based on various indices under different heat stress conditions. Indian J. Genet. Plant Breed., **81**(3): 376-382. DOI: 10.31742/IJGPB.81.3.4

Doyle J.J. and Doyle J.L. 1990. Isolation of plant DNA from fresh

tissue. Focus, **12**: 13-15.

FAO.2018. FAOSTAT, FAO, Rome, Italy. http://faostat.fao.org.

Farooq M., Bramley H., Palta J. A. and Siddique K. H. 2011. Heat stress in wheat during reproductive and grain-filling phases. Crit. Rev. Plant Sci., **30**(6): 491-507.

Fischer R. A. and Maurer R. 1978. Drought resistance in spring wheat cultivars. I. Grain yield responses. Aust. J. Agric. Res., **29**(5): 897-912.

Gibson L.R. and Paulsen G.M. 1999. Yield components of wheat grown under high temperature stress during reproductive growth. Crop Sci., **39**(6): 1841-1846.

Gilbert J.E., Lewis R.V., Wilkinson M.J. and Caligari P.D. 1999. Developing an appropriate strategy to assess genetic variability in plant germplasm collections. Theor. Appl. Genet., **98**: 1125-1131.

Haque M.S., Kjaer K.H., Rosenqvist E., Sharma D.K. and Ottosen C.O. 2014. Heat stress and recovery of photosystem II efficiency in wheat (*Triticum aestivum* L.) cultivars acclimated to different growth temperatures. Env. Exp. Bot., **99**: 1-8. https://doi.org/10.1016/j.envexpbot.2013.10.017.

Harding R.M., Boyce A.J. and Clegg J.B. 1992. The evolution of tandemly repetitive DNA: recombination rules. Genetics, **132**(3): 847-859.

Krishna S., Upadhayay P., Mishra V.K., Kujur S. N., Kumar M., Yadav P.S., Mahto P.K. , Singh P., Sharma A.S. and Chand R. 2020. Evaluation of bread wheat (*Triticum aestivum* L.) for terminal heat tolerance. Indian J. Genet. Plant Breed., **80**(4): 468-470. DOI: 10.31742/IJGPB.80.4.13

Kumar R.R., Goswami S., Singh K., Dubey K., Singh S., Sharma R., Verma N., Kala Y.K., Rai G.K., Grover M. and Mishra D.C. 2016. Identification of putative RuBisCoActivase (TaRca1)—the catalytic chaperone regulating carbon assimilatory pathway in wheat (*Triticum aestivum*) under the heat stress. Front. Plant Sci., **7**: 986.https://doi:10.3389/fpls.2016.00986.

Lukac M., Gooding M.J., Griffiths S. and Jones H.E. 2012. Asynchronous flowering and within-plant flowering diversity in wheat and the implications for crop resilience to heat. Ann. Bot., **109**: 843-850.

Mahalanobis P. C. 1936. On the generalized distance in statistics. Proc. National Institute of Science of India, **2**: 49-55

Molla K. A., Debnath A. B., Ganie S. A., and Mondal T. K. 2015. Identification and analysis of novel salt responsive candidate gene based SSRs (cgSSRs) from rice (*Oryza sativa* L.). BMC Plant Bio., **15**(1): 1-11.

Murty B. R. and Arunachalam V. 1966. The nature of divergence in relation to breeding systems in some crop plants. Indian J. Genet. Plant Breed., **26**: 188-198.

Najaphy A., Parchin R.A. and Farshadfar E. 2011. Evaluation of genetic diversity in wheat cultivars and breeding lines using inter simple sequence repeat markers. Biotechnol. Equip., **25**: 2634-2638.

Narayanan S. 2018. Effects of high temperature stress and traits associated with tolerance in wheat. 2018. Open Access J Sci., **2**(3): 177–186. DOI: 10.15406/oajs.2018.02.00067.

Panse V.G. and Sukhatme P.V. 1954. Statistical methods for agricultural workers. Statistical methods for agricultural workers. Indian Council of Agricultural Research, New Delhi, India.

Prevost A. and Wilkinson M. J. 1999. A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. Theor. Appl. Genet., **98**(1): 107-112.

Raines C. A. 2011. Increasing photosynthetic carbon assimilation in C3 plants to improve crop yield: current and future strategies. Plant Physiol., **155**(1): 36-42.

Rao C.R. 1952. Advanced statistical methods in biometric research. John Wiley and Sons, New York.

Shah N.H. and Paulsen G.M. 2003. Interaction of drought and high temperature on photosynthesis and grain-filling of wheat. Plant Soil, **257**: 219-226.

Sharma P., Mehta G., Muthusamy S. K., Singh S. K. and Singh G. P. 2021. Development and validation of heat-responsive candidate gene and miRNA gene based SSR markers to analysis genetic diversity in wheat for heat tolerance breeding. Mol. Biol. Rep., **48**(1): 381-393.

Sharma P., Sareen S., Saini M., Verma A., Tyagi B.S. and Sharma I. 2014. Assessing genetic variation for heat tolerance in synthetic wheat lines using phenotypic data and molecular markers. Aust. J. Crop Sci., **8**(4): 515-522.

Sofield I., Evans L.T., Cook M.G. and Wardlaw I. F. 1977. Factors influencing the rate and duration of grain filling in wheat. Aust J Plant Physiol., **4**: 785-797.

Tachida H. and Iizuka M. 1992. Persistence of repeated sequences that evolve by replication slippage. Genetics, **131**(2): 471-478.

Vignjevic M., Wang X., Olesen J.E. and Wollenweber B. 2015. Traits in Spring Wheat Cultivars Associated with Yield Loss Caused by a Heat Stress Episode after Anthesis. J. Agron. Crop Sci., **201**(1): 32-48.

Yu Q., Li L., Luo Q., Eamus D., Xu S., Chen C., et al. 2014.Year patterns of climate impact on wheat yields. Int. J. Climatol., **34**: 518-528.

Supplementary Table S1. The details of parentage of wheat accessions under study developed at IARI, New Delhi and their production adaptations

S.N.	*Genotype*	*Pedigree*	*Production Conditions*
1	HD3171	PBW343/HD2879	Rainfed, Timely sown
2	DW1616	HW5028/HD2643//UP2565	Irrigated, Timely sown
3	HD2864	DL509-2/DL377-8	Irrigated, Late sown
4	HD3086	DBW14HD2733/HUW468	Irrigated, Timely Sown
5	HD3249	PBW343*2/KUKUNA//SRTU/3/PBW343*2/KHVKAI	Irrigated, Timely Sown
6	DW1627	DW1422/WR1441//HRLSN-7	Irrigated, Timely Sown
7	DW1628	DW1411/HP1744//DW1440	Irrigated, Timely sown
8	DW1629	HD2967/LBE2003-1//LBRL-11	Irrigated, Late sown
9	HD3184	HD2844/F81513//MILAN-2/3/WH730	Irrigated, Timely sown
10	HD3252	BABX/LR43//BABAX/6/MOR/VEE#5DUCULA/3/DUCULA/4/MILAN/5/BAU/NILAN/7/SKAUZ/BAV92	Irrigated, Timely sown
11	DW1630	CL2762/HP1744//CHIRYA3	Irrigated, Late sown
12	HD2932	KAUZ/STAR//HD2643	Irrigated, Late sown
13	DW1631	HD2967/ CHIRYA3//DW1451	Irrigated, Late sown
14	DW1632	HD2998/HD2733//DW1432	Irrigated, Late sown
15	WR544	NW 2078/CL1739//HD2960	Irrigated, Very Late sown
16	HD3255	SOKOLL//PBW343*R/KUKUNA/3/ATTAILA/PASTOR	Irrigated, Timely sown
17	DW1633	VL796/HD2009//HD3003	Irrigated, Late sown
18	DW1634	HD2682/CL3156//WH542	Irrigated, Late sown
19	DW1635	UP2338/WR1909//NING8319//HD2967	Irrigated, Late sown
20	HD3090	SFW/VAISHALI//UP2405	Irrigated, Late sown
21	HD 3262	VL796/HD2009//HD3003	Irrigated, Timely sown
22	DW1636	HD2998/HD3160//PDW621-50	Irrigated, Late sown
23	DW1637	HD2967/HD3027	Irrigated, Late sown
24	DW1638	HD2967/HP1744//LBRL1	Irrigated, Late sown
25	DW1639	HD2998/HD2894//PS940	Irrigated, Late Sown
26	DW1640	HD2967/LBR1724//VHW4668	Irrigated, Late sown
27	HD3118	ATILLA*2/PBW65/WBLL1*2/TUKURU	Irrigated, Late sown
28	HD3265	DW1311/HD2894/HW5028	Irrigated, Timely sown
29	DW1642	HD2967/HD2844/F81-513//MAILAN2/3/WH730	Irrigated, Very Late sown
30	DW1643	HD 2967/E-4870	Irrigated, Very Late sown
31	DW1644	HD2998/DW1403	Irrigated, Very Late sown
32	DW1645	HD2921/HPW277//PBW 621-50	Irrigated, Very Late sown
33	HD3059	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES	Irrigated, Late sown
34	HD3266	TRCH/5/REH/HARE//2*BCN/CROC-1AE.SQUARROSA(213)//PGO/4/HUITES	Irrigated, Late sown
35	DW1615	NW 2078/CL1739//HD2960	Irrigated, Timely sown
36	HDCSW18	PBW343/CL1538	Irrigated, Early sown

Supplementary Table S2. Analysis of variance for different traits under non-heat stress (E1) and terminal heat stress environment (E2) in *Rabi* 2016-17 and 2017-18

2016-17 (E1)														
Source of variation	*D.F.*	*DTH*	*DTM*	*GFP*	*PH*	*SL*	*GWPS*	*GNPS*	*SPMS*	*GYPMS*	*BYPMS*	*HI*	*TGW*	*CTD*
Replication	2	1.361	8.028	16	0.903	0.1225	0.0055	2.528	278.528	4935.121	182.4815	17.3165	7.105	0.01
Genotype	35	61.219**	39.79**	26.850**	78.858**	2.734**	0.331**	83.550**	21982.962**	216628.418**	179246.897**	546.374**	41.391**	6.819**
Error	70	0.133	3.499	3.3	0.701	0.0009	0.004	0.956	39.013	1301.78	544.739	4.4	2.642	0.018
SE(d)		0.297	1.527	1.483	0.684	0.078	0.052	0.798	5.1	29.459	19.057	1.713	1.327	0.11
CD (1%)		0.594	3.053	2.965	1.367	0.156	0.105	1.596	10.193	58.881	38.089	3.423	2.653	0.22
CV (%)		0.401	1.433	4.57	0.904	1.006	3.291	2.034	1.201	3.535	1.326	3.605	4.023	3.791
2017-18 (E1)														
Replication	2	0.111	1	0.4445	0.3405	0.3495	0.0515	21.6225	128.4445	23784.293	4032.25	23.535	0.4565	0.047
Genotype	35	43.950**	113.571**	62.464**	91.974**	5.002**	0.440**	207.973**	13041.726**	125691.674**	514687.648**	155.116**	60.408**	6.609**
Error	70	0.454	1.714	1.244	0.088	0.048	0.007	1.063	75.973	2,912.64	663.393	3.861	2.207	0.003
SE(d)		0.55	1.069	0.911	0.242	0.178	0.069	0.842	7.117	44.065	21.03	1.604	1.213	0.041
CD (1%)		1.1	2.137	1.821	0.483	0.356	0.138	1.683	14.225	88.075	42.033	3.207	2.425	0.082
CV (%)		0.758	1.014	2.772	0.311	2.184	3.658	1.741	1.585	4.275	0.893	4.452	3.792	1.969
2016-17 (E2)														
Source of variation	*D.F.*	*DTH*	*DTM*	*GFP*	*PH*	*SL*	*GWPS*	*GNPS*	*SPMS*	*GYPMS*	*BYPMS*	*HI*	*TGW*	*CTD*
Replication	2	0.84	6.25	2.51	4.04	0.07	0.001	4.84	100.75	0.12	1144.73	8.69	7.15	0.26
Genotype	35	117.66**	68.23**	54.43**	140.83**	2.10**	0.32**	58.56**	17,952.59**	96,195.01**	153,277.78**	1,207.75**	167.70**	2.74**
Error	70	0.38	0.84	0.49	0.24	0.03	0.001	0.70	37.46	211.75	389.79	5.17	1.51	0.01
SE(d)		0.51	0.75	0.57	0.40	0.14	0.03	0.68	5.00	11.88	16.12	1.86	1.00	0.08
CD (1%)		1.01	1.49	1.15	0.80	0.30	0.06	1.37	9.99	23.75	32.22	3.71	2.00	0.16
CV (%)		0.89	0.96	2.76	0.71	1.94	3.29	2.27	1.75	3.64	2.12	5.08	4.11	3.38
2017-18 (E2)														
Replication	2	3.53	3.03	2.29	2.48	0.01	0.002	1.95	40.26	75.34	1973.03	0.21	5.00	0.01
Genotype	35	336.31**	320.10**	56.05**	171.74**	2.91**	0.20**	202.66**	15,829.67**	81,075.51**	280,902.60**	144.02**	262.16**	2.75**
Error	70	0.30	0.43	0.26	1.05	0.04	0.01	1.08	93.95	2,150.53	617.14	7.75	6.49	0.01
SE(d)		0.45	0.53	0.42	0.84	0.17	0.08	0.85	7.91	37.86	20.28	2.27	2.08	0.08
CD (1%)		0.89	1.07	0.83	1.68	0.34	0.16	1.69	15.82	75.68	40.54	4.54	4.16	0.16
CV (%)		1.06	0.82	1.81	1.43	2.08	5.52	2.29	2.00	5.55	1.38	5.96	6.48	2.75

*, ** significant at 5% and 1% level, D.F.= Degree of freedom, SE (d)=Standard error of difference, CD=Critical difference, CV=Coefficient of variation, DTH-=Days to 50% heading, DTM=Days to maturity, GFP=Grain filling period, PH=Plant height (cm), SL=Spike length (cm), GWPS=Grain weight spike^{-1}(g), GNPS=Number of grains spike^{-1}, SPMS=Number of spike m^{-2}, GYPMS=Grain yield m^{-2} (g), BYPMS=Biological yield m^{-2} (g), HI=Harvest index, TGW=Thousand grain weight (g) and CTD=Canopy temperature depression

Supplementary Table S3. Jaccard's similarity coefficient matrix of 34 genotypes using SSR markers

	HD3171	DW1616	HD2864	HD3086	DW1627	DW1628	DW1629	HD3184	HD3252	DW1630	HD2932	DW1631	DW1632	WR544	HD3255	DW1633	DW1634
HD3171	1.00																
DW1616	0.85	1.00															
HD2864	0.81	0.77	1.00														
HD3086	0.63	0.67	0.68	1.00													
DW1627	0.55	0.56	0.60	0.62	1.00												
DW1628	0.58	0.61	0.68	0.61	0.69	1.00											
DW1629	0.57	0.64	0.62	0.67	0.64	0.79	1.00										
HD3184	0.53	0.60	0.65	0.63	0.58	0.75	0.74	1.00									
HD3252	0.55	0.59	0.64	0.62	0.61	0.74	0.73	0.77	1.00								
DW1630	0.58	0.61	0.71	0.64	0.63	0.78	0.75	0.84	0.86	1.00							
HD2932	0.62	0.62	0.65	0.58	0.62	0.72	0.69	0.70	0.76	0.80	1.00						
DW1631	0.67	0.63	0.75	0.57	0.52	0.63	0.56	0.68	0.62	0.70	0.67	1.00					
DW1632	0.59	0.56	0.66	0.56	0.55	0.60	0.53	0.58	0.61	0.62	0.62	0.73	1.00				
WR544	0.54	0.60	0.58	0.59	0.56	0.63	0.64	0.56	0.62	0.59	0.67	0.61	0.66	1.00			
HD3255	0.59	0.61	0.57	0.58	0.51	0.52	0.53	0.55	0.58	0.58	0.63	0.62	0.69	0.77	1.00		
DW1633	0.59	0.62	0.69	0.62	0.59	0.64	0.64	0.64	0.70	0.72	0.68	0.69	0.77	0.78	0.79	1.00	
DW1634	0.57	0.60	0.64	0.56	0.59	0.60	0.59	0.64	0.68	0.67	0.64	0.74	0.70	0.71	0.67	0.77	1.00
DW1635	0.63	0.64	0.64	0.66	0.63	0.62	0.61	0.62	0.59	0.62	0.64	0.66	0.65	0.59	0.56	0.65	0.68
HD3090	0.63	0.65	0.73	0.61	0.53	0.66	0.63	0.73	0.67	0.74	0.68	0.81	0.74	0.65	0.64	0.79	0.77
HD3262	0.58	0.63	0.61	0.57	0.54	0.57	0.60	0.59	0.58	0.64	0.58	0.68	0.66	0.65	0.62	0.69	0.74
DW1636	0.62	0.66	0.69	0.58	0.59	0.58	0.64	0.64	0.66	0.67	0.62	0.67	0.65	0.58	0.59	0.73	0.73
DW1637	0.59	0.62	0.62	0.54	0.48	0.58	0.55	0.56	0.61	0.61	0.62	0.74	0.68	0.60	0.61	0.66	0.70
DW1638	0.59	0.59	0.60	0.54	0.46	0.56	0.59	0.62	0.61	0.63	0.55	0.64	0.70	0.54	0.51	0.59	0.64
DW1639	0.56	0.58	0.67	0.55	0.54	0.69	0.62	0.72	0.73	0.80	0.74	0.74	0.72	0.65	0.63	0.73	0.75
HD3118	0.59	0.57	0.61	0.54	0.57	0.64	0.58	0.64	0.67	0.68	0.65	0.68	0.69	0.55	0.56	0.63	0.63
HD3265	0.49	0.50	0.56	0.69	0.58	0.60	0.59	0.60	0.61	0.65	0.58	0.56	0.68	0.64	0.61	0.66	0.54
DW1642	0.59	0.57	0.62	0.64	0.57	0.64	0.61	0.64	0.65	0.67	0.59	0.62	0.72	0.56	0.58	0.65	0.57
DW1643	0.59	0.61	0.59	0.54	0.53	0.62	0.68	0.69	0.68	0.69	0.64	0.60	0.67	0.62	0.67	0.68	0.61
DW1644	0.52	0.53	0.61	0.55	0.58	0.59	0.60	0.65	0.69	0.68	0.63	0.65	0.75	0.61	0.64	0.69	0.64
DW1645	0.55	0.52	0.65	0.54	0.58	0.63	0.59	0.65	0.66	0.72	0.62	0.69	0.73	0.52	0.57	0.64	0.64
HD3059	0.51	0.48	0.55	0.54	0.59	0.54	0.57	0.53	0.61	0.62	0.57	0.60	0.69	0.52	0.54	0.61	0.55
HD3266	0.58	0.54	0.63	0.57	0.62	0.59	0.60	0.63	0.67	0.68	0.63	0.63	0.75	0.59	0.62	0.69	0.62
DW1615	0.55	0.52	0.65	0.63	0.60	0.61	0.62	0.65	0.66	0.70	0.62	0.65	0.68	0.54	0.59	0.66	0.64
HDCSW18	0.63	0.59	0.66	0.58	0.59	0.58	0.59	0.55	0.63	0.62	0.64	0.60	0.67	0.59	0.60	0.63	0.57

	DW1635	HD3090	HD3262	DW1636	DW1637	DW1638	DW1639	HD3318	HD3265	DW1642	DW1643	DW1644	DW1645	HD3059	HD3266	DW1615	HDCSW18
DW1635	1.00																
HD3090	0.79	1.00															
HD3262	0.66	0.70	1.00														
DW1636	0.75	0.77	0.74	1.00													
DW1637	0.63	0.69	0.71	0.68	1.00												
DW1638	0.61	0.67	0.67	0.61	0.73	1.00											
DW1639	0.66	0.80	0.69	0.68	0.71	0.71	1.00										
HD3318	0.60	0.68	0.64	0.65	0.67	0.69	0.82	1.00									
HD3265	0.59	0.61	0.58	0.54	0.56	0.57	0.64	0.65	1.00								
DW1642	0.67	0.69	0.58	0.63	0.61	0.65	0.68	0.79	0.75	1.00							
DW1643	0.65	0.71	0.62	0.70	0.57	0.63	0.68	0.69	0.65	0.77	1.00						
DW1644	0.62	0.68	0.57	0.64	0.60	0.62	0.69	0.68	0.71	0.71	0.76	1.00					
DW1645	0.61	0.70	0.61	0.62	0.60	0.66	0.76	0.77	0.66	0.75	0.70	0.74	1.00				
HD3059	0.56	0.58	0.58	0.61	0.53	0.55	0.62	0.71	0.65	0.67	0.65	0.66	0.73	1.00			
HD3266	0.56	0.61	0.63	0.64	0.58	0.64	0.67	0.70	0.67	0.73	0.71	0.70	0.74	0.81	1.00		
DW1615	0.66	0.68	0.58	0.64	0.60	0.59	0.71	0.70	0.68	0.75	0.70	0.69	0.79	0.78	0.79	1.00	
HDCSW18	0.61	0.62	0.58	0.63	0.59	0.59	0.64	0.64	0.68	0.69	0.69	0.68	0.68	0.69	0.76	0.75	1