Australian Journal of Crop Science AJCS
AJCS 8(4):515-522 (2014) ISSN:1835-2707

Assessing genetic variation for heat tolerance in synthetic wheat lines using phenotypic data and molecular markers

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Abstract

High temperature is a major environmental stress factor limiting wheat productivity in India. Therefore, improvement for heat tolerance in wheat is an important breeding objective. A number of synthetic wheat hexaploids have shown resistance to major wheat diseases and tolerance to abiotic stresses such as drought, waterlogging and salinity. This study was conduct to assess heat tolerance and genetic variation among twenty-four heat tolerant synthetic wheat lines in order to identify new sources of diversity that could accelerate the development of improved wheat genotypes better suited to meet the challenges posed by changing climate in India. Phenotypic data of nine traits of synthetic wheat lines were evaluated for heat tolerance under non-stress (timely sown) and stress (late sown) field conditions. The heat tolerance and stress susceptibility indices were calculated for Thousand-grain weight (TGW) and genotypes differed significantly for stress indices. A hierarchical cluster analysis was conducted based on phenotypic traits and among 24 synthetics lines, three accessions were classified as highly tolerant, 9 as medium tolerant and 12 as susceptible. Fifteen polymorphic ISSR markers were used to evaluate the diversity profile of synthetic hexaploid wheats (2n=6x=42, AABBDD). In total, 75 alleles were detected (five bands/primer), out of which 36 were polymorphic across the lines and the percentage of polymorphism was 80%. The polymorphic information content value for ISSR markers was calculated in the range of 0.39 to 0.88 with an average of 0.65. Genotypes (SNY 11, SNY 36 SNY 44) characterized as highly heat tolerance were distributed among the ISSR cluster groups. It implies that genetic basis of heat tolerance in synthetic wheat lines was different, therefore enabling wheat breeders to combine these diverse sources of genetic variability to improve heat tolerance in their breeding program.

Keywords: ISSR, genetic variability, synthetic wheats, phenotypic traits, heat tolerance.
Abbreviations: DH_days to heading; DA_Days to anthesis; DM_Days to maturity; GN_Number of grains; GW_Grain weight, TGW_Thousand grain weight; GFD_Grain filling duration; GGR_Grain growth rate; HHT_High heat tolerance; MHT_Medium heat tolerance; LHT_Low heat tolerance; PCA_Principle coordinate analysis.

Introduction

About 20 percent of total area under wheat, sown in the Indo-Gangetic plain is being sown after middle of December. Therefore, production strategy should emphasize the development of short duration genotypes that are tolerant to heat stress at reproductive stage of the plant growth. These genotypes mature when the atmospheric temperature is high. Although, the short duration varieties are available in wheat, yet these are low in productivity and have poor tolerance to heat stress at dough stage of grain growth (Rane et al., 2007). In future the area under wheat in such ecosystem is likely to increase and therefore, breeding efforts should be focused on developing short duration varieties that are capable of germinating and establishing in low temperature, having faster growth rate and tolerant to heat stress at reproductive stage of plant growth. In addition to explore the heat tolerance in hexaploid wheats, the efforts should be made to transfer the late heat tolerance particularly delayed leaf senescence from diploid alien species of wheat. An important factor when considering this new genetic resource is whether it may offer beneficial alleles for increased yield potential. Considering that yield is complex trait, it is unlikely that the best alleles for yield related loci have completely been captured from the wild relatives. For initiating rational breeding programmes, knowledge of genetic diversity of concerned species is necessary, as it affects not only the composition of group variation but also evolutionary potentialities of the group concerned. The availability for genetic diversity in wheat germplasm has been always a pre-requisite for breeding program aiming to improve wheat productivity. For this purpose, a number of genetic diversity studies were undertaken in pedigree analysis morphological traits and biochemical markers (Praker et al., 2002; Labuschagne et al., 2000). However, there were inherent problems with the use of data on biochemical markers and morphological traits, which were limited in number and greatly influenced by the environment. Molecular markers therefore, provided a satisfactory alternative as they could detect higher levels of polymorphism between cultivars and would help to improve the efficiency and accuracy of genetic similarity estimates and wouldn't be influenced by the environment (Divila et al., 1998). Applying molecular markers and recognition of polymorphic nucleotide

sequences dispersed throughout the genome have provided new possibility for evaluating genetic diversity and determining of inter- and intra-species genetic relationships (Gostimsky et al., 2005). Several PCR based molecular markers are available for investigation of genetic diversity such as SSRs (Roder at al., 1995), RAPDs (Williams et al., 1990), AFLP (Vos et al., 1995) and ISSR (Ziekiewicz et al., 1994) are the most important. The major limitations of these methods were low reproducibility of RAPD, high cost of AFLP and need to know the flanking sequences to design specific primers for SSR markers. ISSR markers overcome most of these limitations.

Genetic diversity among germplasm with varied degree of stress tolerance has been well documented (Reynolds et al., 2007; Dodig et al., 2010; Sun et al., 2013). ISSR markers were successfully used for estimating of genetic diversity in several crops (Kantety et al., 1995; Nagoaka and Ogihara, 1997; Blair et al., 1999; Brantestam et al. 2004; Hou et al. 2005). Recently, studies on molecular markers based information to evaluate genetic diversity for drought were reported (Sun et al., 2013; Peleg et al., 2005) but markers based classification for heat tolerance is lacking so far.

Previous studies indicate that the genetic basis of cultivated wheat genotypes was found to be restricted on condition on the modern crop cultivation and the ability of enduring biotic and abiotic stress rapid descent (Ginkel and Ogbonnaya, 2007). *Aegilops squarrosa* (syn. *Aegilops tauschii* L. 2n=2x=14, DD), as a donor of the D genome of common wheat, has many desirable genes for wheat improvement. It is of great value to enrich genetic availability and broaden genetic diversity in wheat gene pool by using the synthetic hexaploid wheats, produced crossing *T. durum* (AABB) with *Ae. squarrosa* (DD). Heat stress is one of the major constraints of wheat production in semiarid, tropical, and subtropical regions of the world. Consequently, development of heat tolerant cultivars is of major concern in wheat breeding programs. Reports on genetic diversity assessment among synthetic wheat lines for heat tolerance are scanty. The objective of the present study were to analyze genetic variability of 24 synthetic hexaploid wheats for phenotypic traits associated with heat tolerance and to classify them based on polymorphic ISSR markers underlying the observed phenotypic traits. A detailed understanding of the genetics and physiology of heat tolerance as well as the use of the proper germplasm and selection methods will facilitate the development of heat tolerant cultivars of wheat.

Results

Phenotypic data and genetic correlations

The summary information of phenotyping traits was presented in Table 2. Over the two years, Thousand-grain weight (TGW) varied from 28.7g to 55.1 g under non-stress and 24.98 to 43.1g under stress conditions. The stress intensity during two years was 0.34 and 0.15 for 2008-09 and 2009-10, respectively. Average reduction for TGW under stress environments was 19.5, 15.5, 3.9, 30.6, 46.4 and 25.0% for DH, DM, GFD, GN, GW and TGW, respectively. The reduction in TGW pooled over two years ranged from 6.76% in SYN 11 to 45.44% in SYN 28 (Table 1). During 2008-09 crop seasons, the reduction in TGW ranged from 10.5% (SYN 36) to 55.2% (SYN 8) and during 2009-10 from -3.1% (SYN 18) to 44.5% (SYN 28). The phenotypic coefficient of correlation between TGW under stress and non-stress environments was positive and significant (r = 0.576, P < 0.01). The values of coefficient of correlations among phenotypic traits and TGW under stress and non-stress conditions using data pooled over years are presented in Table 3. Under both conditions, TGW had a negative and significant correlation with DH and DM and positive and significant correlation with GFD, GW and HTI. There was negative correlation between TGW and HSI (r = –0.01 and r = –0.82 under non-stress and stress conditions, respectively), but significant only under stress conditions. TOL had positive correlation with TGW under non-stress conditions and negative under stress conditions (Table 4).

ISSR marker analysis

Out of the 38 ISSR markers screened, only15 primers gave distinguishable bands were selected for further study. Typical band patterns on 2.0% agarose were presented in Fig 1. Most primers (12 out of 15) annealed to the dinucleotide repeats, whereas each of the remaining three annealed to the tri-, tetra and penta-nucleotide repeats, respectively (Table 5). PCR amplification using ISSR sequences as the primer produced 5.00 bands, on average. Amplified DNA fragments varied in size from approximately 150 bp to 1600 bp (Fig 1). The highest and the lowest number of polymorphic bands per assay unit were 5 and 1, respectively. Maximum bands generated by UBC842, UBC820, UBC824 and minimum by UBC 827, UBC 845 and UBC 859 (Table 4). The studied primer sequences were composed of di-, tri-, tetra- and penta-nucleotide repeat sequences. The highest polymorphism was observed in the case of di-nucleotide (AG and GA repeats) primers in this study, while in some other studies of wheat reported that primer sequences with di-nucluotide (GA repeat) showed lower level of polymorphism (Nagata and Ogihara , 1997).

Out of the 75 loci, 36 were polymorphic, with average of 2.50 polymorphic fragments per genotypes. Percentage of polymorphic bands ranged from 80% to a minimum of 20% with an average of 44.25%. Polymorphism information content of each primer ranged from 0.39-0.88 with an average of 0.65%. In general, dinucleotide repeats anchored with A,T,C or G, showed clearer patterns and best polymorphism is in agreement with studies of Wang et al. (2012). The results obtained using AMOVA shows that variation were significant (P<0.001) within group component (91.69%). Dodig et al., (2010) also reported similar trends for larger within group genetic variation in wheat cultivars from different regions of Asia, Australia, North America, Europe and Siberia. This result indicates that the synthetic hexaploid wheat is efficient way to enrich wheat genetic background for heat tolerance, especially to use the genetic variations of the D genome from *Ae. squarrosa* for wheat improvement.

Cluster analysis of phenotypic and marker based data

Cluster analysis was conducted to categorize 24 synthetic wheat lines for genetic variation in heat tolerance based on the TOL and HSI parameters (Table 1). A dendrogram was constructed based on the phenotypic parameters, which classified the synthetics lines into two groups (Fig 2). Cluster I consists of six synthetic lines; Cluster IIa had 12 lines and

Table 1. Phenotypic traits in 24 synthetic lines evaluated under non-stress and stress conditions.

Genotypes	Pedigree	TOL	HSI	% reduction of traits	Tolerance
SYN 11	D67.2/P66.270// *Ae. squarrosa*	2.96	0.27	6.76	HHT
SYN 36	DOY 1/*Ae.squarrosa*	5.94	0.54	13.65	HHT
SYN 44	68.111/RGB-U//WARD/3/ FGO/4/RABI/5/ *Ae. squarrosa*	7.11	0.62	15.48	HHT
SYN 27	GARZA/BOY// *Ae.squarrosa*	7.17	0.68	17.04	MHT
SYN 35	68 .111/RGB-U/iWARD/3/ *Ae. squarrosa*	7.66	0.70	17.44	MHT
SYN 42	YAR/ *Ae.squarrosa*	7.80	0.71	17.72	MHT
SYN 57	LC,K59. 6'1/ *Ae. squarrosa*	8.29	0.74	18.42	MHT
SYN 37	68.111/RGB-U//WARD/3/ FGO/4/RABI/5/*Ae.squarrosa*	8.93	0.76	19.04	MHT
SYN 9	ALTAR 84/ *Ae. squarrosa*	10.04	0.81	20.22	MHT
SYN 52	ALTAR 84/ *Ae. squarrosa*	10.45	0.86	21.44	MHT
SYN 46	CROC_1/ *Ae. squarrosa*	10.83	0.92	23.15	MHT
SYN 16	ALTAR 84/ *Ae. squarrosa*	10.94	0.97	24.21	MHT
SYN 51	PBW114/ *Ae. squarrosa*	11.32	1.09	27.42	LHT
SYN 18	D67.2/P66.270// *Ae. squarrosa*	11.49	1.11	27.88	LHT
SYN 14	YUK/ *Ae. squarrosa*	12.22	1.13	28.34	LHT
SYN 67	SNIPE/YAV79//DACK/TEAL/3/ *Ae. squarrosa*	12.64	1.19	29.75	LHT
SYN 77	RASCON/ *Ae. squarrosa*	13.07	1.25	31.25	LHT
SYN 22	D67.2/P66.270// *Ae. squarrosa*	13.13	1.25	31.26	LHT
SYN 31	68112/WARD//*Ae.squarrosa*	13.19	1.25	31.33	LHT
SYN 34	DOY 1/*Ae. squarrosa* (511)	13.35	1.28	32.00	LHT
SYN 38	FGO/USA2111// *Ae. squarrosa*	15.27	1.33	33.22	LHT
SYN 24	CROC_1/ *Ae. squarrosa*	15.34	1.35	33.69	LHT
SYN 8	CPI/GEDIZ/3/GOO//JO69/CRA/4/*Ae.squarrosa*	15.77	1.39	34.83	LHT
SYN 28	69 .111/RGB-U//WARD/3/ *Ae. squarrosa*	23.24	1.81	45.44	LHT

Note: HHT-high heat tolerance; MHT-medium heat tolerance; LHT- low heat tolerance; TOL-tolerance; HSI- heat susceptibility index; SYN-synthetics.

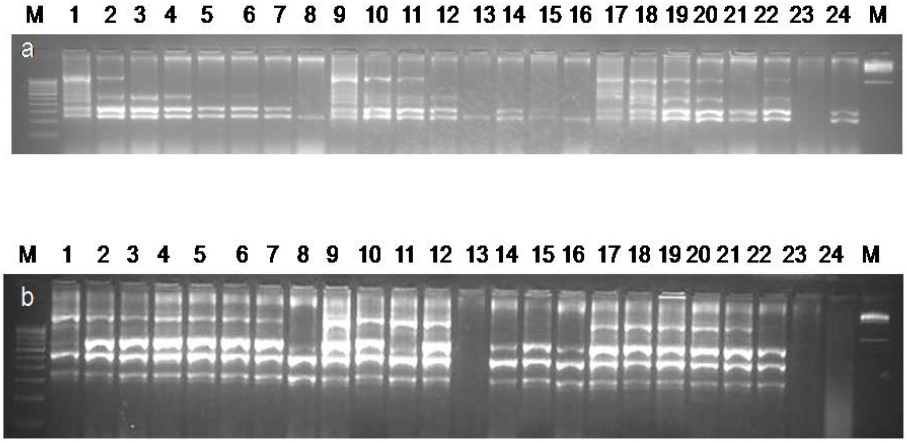

Fig 1. ISSR based profiling of 24 synthetic wheat lines with primer UBC 808 (a) and UBC 857 (b). M-100 bp ladder, Lane 1-SNY8, Lane 2-SNY9, Lane 3-SNY11, Lane 4-SNY14, Lane 5-SNY16, Lane 6-SNY18, Lane 7-SNY22, Lane 8-SNY24, Lane 9-SNY27, Lane 10-SNY28, Lane 11-SNY31, Lane 12-SNY34, Lane 13-SNY35, Lane 14-SNY36, Lane 15-SNY37, Lane 16-SNY38, Lane 17-SNY42, Lane 18-SNY44, Lane 19-SNY46, Lane 20-SNY51, Lane 21-SNY52, Lane 22-SNY57, Lane 23-SNY67, and Lane 24 SYN77.

cluster IIb included six lines. Cluster IIa included lines exhibiting HHT (SNY 11, SNY 36, and SYN 44) and MHT (SYN 27, SYN 35, SYN 42, SYN 57, SYN 37, SYN 9, SYN 52, SYN 46 and SYN 16). The cluster analysis clearly differentiates synthetic wheat lines encompassing LHT (Fig 2). This analysis based on the ISSR data assigned the genotypes into at least four groups (Fig 3). Genotypes from HHT were distributed among all cluster groups, which implied that genetically different genotypes were identified with HHT. It is reasonable to assume that the genetic basis of TOL in these synthetic lines is different, which could enable wheat breeders to combine these different sources of genetic variability to improve TOL in their breeding programmes.

The genetic distance (GD) for all the possible pairs of accessions ranged from 0.12 to 0.61, indicating good level of diversity. In order to determine the ability of ISSR analysis to display genetic relationships among accessions, principle coordinate analysis (PCA) was carried out and accessions were plotted in the coordinate system for the first two co-ordinates, which accounted for 48.72 and 8.91% of the variation, respectively. PCA provided a better graphical illustration and a clear separation of species (Fig 4). The genetic relationships among the 24 genotypes were also revealed by PCA (Fig 4). The overall grouping pattern of PCA corresponded well within the clustering pattern of the ISSR dendrogram. In agreement with dendrogram, HHT

Table 2. Descriptive statistics of phenotypic traits in 24 synthetic lines evaluated under non-stress and stress conditions.

Variable	Mean	Minimum	Maximum	σ	CV (%)	S/NS
Non stress condition						
DH (TS)	106.5	93.3	120.8	5.5	5.2	-
DA (TS)	110.7	100.3	123.3	5.2	4.7	-
DM(TS)	145.6	140.0	148.5	2.2	1.5	-
GFD(TS)	34.8	23.0	43.3	4.3	12.3	-
GN(TS)	34.4	28.7	51.4	5.9	17.2	-
GW(TS)	1.6	1.1	2.2	0.3	17.3	-
TGW(TS)	44.6	36.8	54.1	3.9	8.7	-
Stressed condition						
Variable	Mean	Minimum	Maximum	σ	CV (%)	S/NS
DH(LS)	85.7	71.2	98.0	6.4	7.5	80.5
DA(LS)	88.7	57.8	103.5	8.8	9.9	80.1
DM(LS)	123.0	115.7	129.8	4.1	3.3	84.5
GFD(LS)	33.5	26.3	39.7	2.9	8.7	96.2
GN(LS)	23.9	11.5	52.0	9.7	40.4	69.4
GW(LS)	0.8	0.4	2.0	0.4	46.2	53.6
TGW(LS)	33.5	25.0	43.1	4.8	14.4	75.0

Note: DH- days to heading; DA- days to anthesis, DM- days to maturity; GFD- Grain filling duration (in days), GN- Grain number and GW- grain weight (g); TS-timely sown; LS- late sown; S-stress; NS- non-stress; cv- coefficient of variation; σ- standard deviation. Blank cells: The values are same that of stressed conditions. it is relative performance under stress condition. Hence not required under non-stress condition.

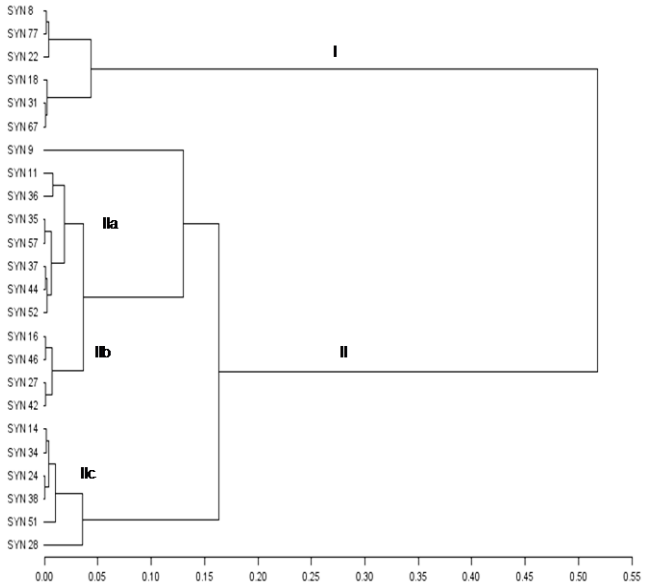

Fig 2. Dendrogram of measured traits mean for 24 synthtic accessions by using UPGMA method in trials conducted during 2008-09 & 2009-2010.

synthetic wheat lines viz. SNY11, SNY 36 and SYN44 falls into distant groups in the PCA also. This clearly confirmed the genetic distinctness among the synthetics.

Discussion

Genetic diversity for traits like heat, salt and drought tolerance is limited in conventional wheat, introgression of genes from wild relatives into elite genotypes has been a major breeding objective within India. *Ae. tauschii* is a rich source of resistance genes to many biotic and abiotic stresses (Mujeeb-Kazi and Rajaram, 2002). Synthetic hexaploids were created by artificially crossing between *T. durum* x *Ae. tauschii*. These act as a good source for creating genetic diversity. The genetic diversity and relationships among heat tolerant synthetic wheat lines were analyzed in this study. These synthetic lines developed at CIMMYT possess many other biotic traits that can be utilized for wheat improvement (Das et al., 2007). Recently, Chinese researchers has released four cultivars developed using CIMMYT synthetic heaxaploid wheat (Yang et al., 2009). Considering the results of this study, it was observed that TGW had significant and positive correlation with grain filling duration, grain number and grain weight and negative with days to heading under stress conditions. It was also correlated with heat tolerance index under both conditions whereas with TOL and HSI, there was negative correlation under stress conditions. Days to heading had positive correlation with HSI but negative with HTI whereas grain-filling duration was positively correlated with HTI and negative with HSI. DH had significant negative correlation with GFD, GW, TGW and HTI under both conditions. The longer days to heading leads to reduction in GFD, which is responsible for reduction in GW and TGW. The three groups of synthetics differed in phenological traits and TGW.

Table 3. Phenological and grain traits and stress indices under non-stress and stress conditions.

Trait	Non-stress						Stress					
	GFD	GW	TGW	TOL	HSI	HTI	GFD	GW	TGW	TOL	HSI	HTI
DH	-0.90**	-0.76**	-0.44*	0.25	0.40	-0.58**	-0.86**	-0.84**	-0.60**	0.35	0.49*	-0.56**
GFD		0.64**	0.38	-0.15	-0.26	0.47*		0.73**	0.53**	-0.37	-0.48*	0.47*
GW			0.40	-0.36	-0.49*	0.59**			0.61**	-0.56**	-0.65**	0.48*
TGW				0.27	-0.01	0.81**				-0.63**	-0.82**	0.94**

Note: DH- days to heading; GFD- Grain filling duration; GW- Grain weight; TGW- thousand grain weight; TOL- Tolerance; HTI-high thermal index; HSI- high susceptibility index. . *- P<0.05; **- P<0.01.

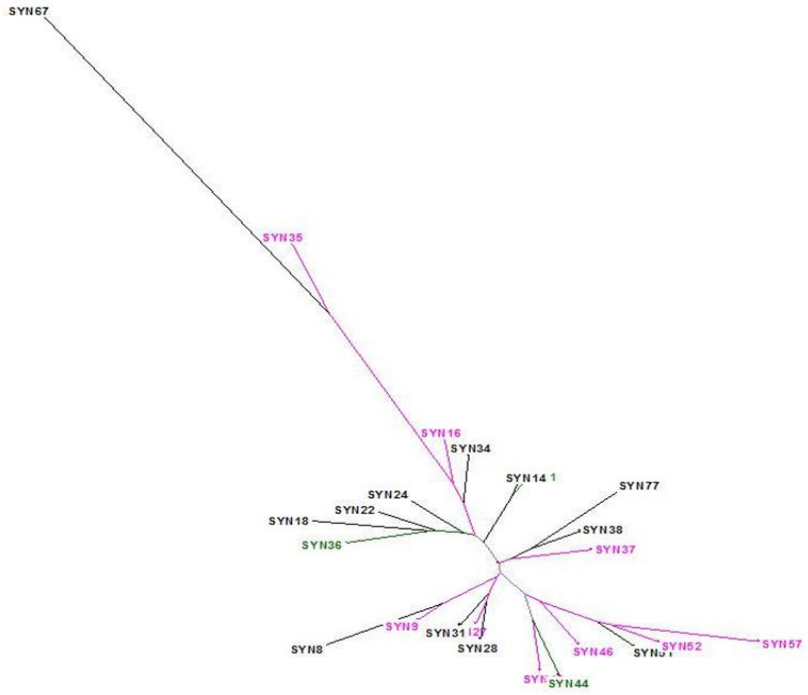

Fig 3. Unweighted Neighbour-Joining (UNJ) dendrogram prepared based on 15 polymorphic ISSR markers using software DARwin showing clustering pattern of 24 heat tolerant synthetic wheats where green colour represents high heat tolerance and magenta colour represent low heat tolerance (magenta coloured-LHT). SNY 11, SNY 36, and SYN 44 representing HHT were distributed among all the cluster, which implied that genetically different genotypes were identified with HD.

The group HHT comprised of early flowering genotypes having longer grain filling duration and higher Thousand-grain weight whereas the group LHT was comprised of late flowering genotypes with shorter grain filling duration and low thousand grain weight. GFD is decreased under high temperature conditions (Altenbach et al., 2003) and is responsible for reduction in grain yield (Bagga and Rawson, 1977; Stone and Nicolas, 1995). Fifteen ISSR markers used in this study revealed high-level genetic diversity among the heat tolerant synthetic lines. When all 24 synthetic lines analyzed together, the average similarity coefficient was 0.88 with the lowest similarity coefficient being 0.39. ISSR markers across the synthetics have revealed a medium level of genetic diversity. Although a higher number of primers need to be analyzed. Our results suggest that di- and tri-nulceotides ISSR occur at high frequencies along wheat genomes. In previous studies, in rice, maize, soybean and common bean and wheat, it was also found that di- and tri-nucleotide. ISSR occur along the genomes at higher frequencies than tetra and penta-nucleotide repeats (Nagoaka and Ohihara, 1997). Diversity detected by penta- and tetra nucleotide repeat primers was found at lower levels. Sofalian et al. (2008) reported that primer sequences with GA repeat showed lower level of polymorphism. However, in our experiments the primers with GA and AG repeats present the high level of polymorphism was in congruence with Fatehi et al. (2011). In this investigation, ISSR markers showed a high level of polymorphism and amplification of bands (Fig 1). Extensive DNA polymorphism has been reported using ISSR markers in several other crops plants (Blair et al.,1999; Sofalian et al., 2008; Kantety et al., 1995; Hou et al., 2005). ISSR markers were good indicators of morphological divergence. ISSR markers are highly polymorphic and repeatable even for intra-specific purposes in wheat varieties and could reflect real genetic relationships among wheat accessions. A decrease in the genetic base of common wheat germplasm in a country is conditioned by both breeder's activities and natural selection. Obviously, only the first of these may be controlled and reduced by breeding genotypes from other countries including landraces and old cultivars from the same country. Local genotypes would be an especially valuable source of alleles and multi locus combinations already suitable for specific environments of the country concerned (Allard, 1996). It is expected that when such diverse lines are involved in breeding programs, because of reshuffling of the alleles due to recombination, there are better chances for the appearance of transgressive segregation with beneficial traits that can be selected to

Table 4. Correlation among reduction in phenological and grain traits under stress conditions and non-stress conditions.

Trait	GFD	GW	TGW	TOL	HSI	HTI
DH	-0.59**	-0.85**	-0.29	-0.24	-0.29	0.22
GFD		0.34	0.06	0.10	0.06	0.16
GW			0.49*	0.43*	0.49*	-0.28
TGW				0.96**	1.00**	-0.58**
TOL					0.96**	-0.34
HSI						-0.58**

Note: DH- days to heading; GFD- Grain filling duration; GW- Grain weight; TGW- thousand grain weight; TOL- Tolerance; HTI-high thermal index; HSI- high susceptibility index. *- P<0.05; **- P<0.01.

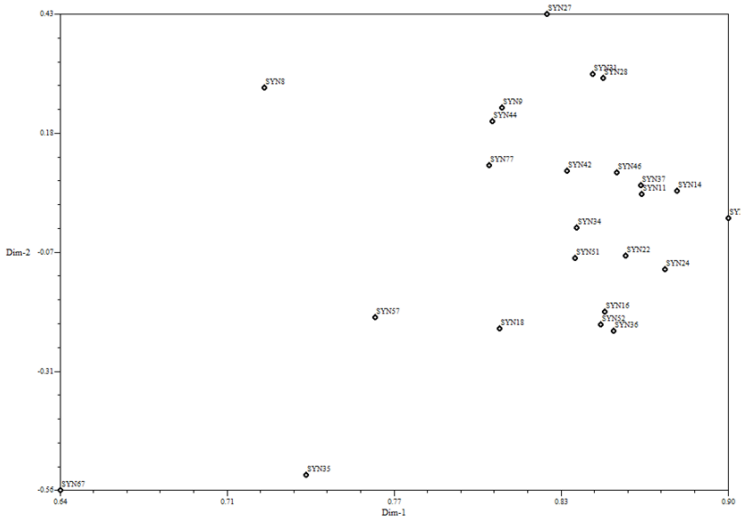

Fig 4. Principal component analyses of marker allele frequencies for 24 synthetic wheats with respective to heat tolerance.

extract high yielding lines with desirable trait combination (Sofalian et al., 2008). In the crop plants such as bread wheat, the polymorphism rate according to other molecular markers was low. The existence of polymorphic markers was an excellent choice in order to use in different breeding aims. Further, large amount of genetic variation, which exists between wheat genotypes, can be used efficiently for genome mapping and gene tagging of crosses to introgress the favourable traits such as high yield potential, insect and disease resistance into the cultivated genotypes (Fatehi et al., 2011). Phenotype based clustering was noticeable for its differences from the ISSR clusters indicating that it measured a different aspect of genetic diversity. This is in agreement with the studies of Dodig et al., (2010) that DNA markers and morphological traits will not necessarily give closely matching results as grouping according to morphology may result in separating genotypes according to a single major gene in heat tolerance. The full proof association between the genotypic and phenotypic data that was observed in this study and other studies will not hinder the usefulness of these data to wheat breeders as the information provided by markers should be considered as complementary rather than as an alternate to that obtained by the phenotype.

Material and Methods

Plant material

Twenty-four synthetic lines were used this study and designated as SYN8-SYN77 (Table 1). These synthetics were selected, out of the ninety synthetic wheat lines procured from International Maize and Wheat Improvement Center, Mexico (CIMMYT), on the basis of preliminary screening for terminal heat tolerance under controlled temperature conditions for two consecutive years (Tyagi et al., 2009). These synthetic wheat lines proved a great source of unknown genetic variability and possessed superior traits like high yield, abiotic and biotic stress tolerance.

Phenotypic analysis

Phenotypic data of these synthetic wheat lines were collected throughout the growing season in the field trails carried out in 2008-09 and 2009-10 under non-stress (timely November sowing) and stress (late December sowing) conditions using RCBD with three replications. The plot size was 2.4m^{-2} with 4 rows of 2m length and 0.3m spacing. Fertilizer and irrigation was applied as per recommendations to grow a good crop. Data were recorded for days to heading (DH), days to anthesis (DA), Days to maturity (DM), number of grains per spike (GN), grain weight per spike (GW), thousand grain weight (TGW) (g), grain filling duration (GFD) and grain growth rate (GGR). Phenological traits were recorded at 75% condition. Grain filling duration was calculated as the period from days to anthesis to days to physiological maturity. The five main shoot spikes sampled from each plot were hand threshed to obtain grain number and grain weight/spike. A measure of heat tolerance (HT) was obtained using the relationship TOL = xp-xs (Rosielle and Hamblin, 1981) and heat susceptibility using the relationship [1-(xs/xp)]/[1-(Xs/Xp)] (Fischer and Maurere, 1978); Where, xs is the trait value (thousand grain weight) of the genotype under stress, xp is the trait value of the genotype under non- stress conditions. Xs and Xp are mean values of the trait of all the genotypes under stress and non-stress conditions , respectively. Genotypes ranked according

Table 5. Amplification result and polymorphism of the 15 ISSR primers used in this study.

Primer Name	Sequence (5'-3')	Fragment range (bp)	Number of bands	Number of polymorphic bands	Polymorphic %	PIC %
842	GAGAGAGAGAGAGAGA	150-1000	8	5	62.5	0.568
820	GTGTGTGTGTGTGTGTC	200-1200	8	3	37.5	0.778
847	CACACACACACACACARC	350-1200	5	2	40.00	0.663
844	CTCTCTCTCTCTCTCTRC	150-900	5	3	60.00	0.390
815	CTCTCTCTCTCTCTCTG	350-1000	7	2	28.57	0.650
826	ACACACACACACACACC	450-1000	6	3	50.00	0.779
808	AGAGAGAGAGAGAGAGC	250-1200	6	4	80.00	0.636
810	GAGAGAGAGAGAGAGAT	300-1200	5	2	40.00	0.599
824	TCTCTCTCTCTCTCTCG	200-1200	8	4	50.00	0.880
825	ACACACACACACACACT	300-1000	5	3	60.00	0.442
827	ACACACACACACACACG	300-800	5	1	20.00	0.584
845	CTCTCTCTCTCTCTCTRG	400-800	2	1	50.00	0.746
848	CACACACACACACACAGR	400-1500	4	2	50.00	0.647
859	TGTGTGTGTGTGTGTGRC	600-1500	3	1	33.33	0.676
881	GGGGTGGGGTGGGGTG	500-1600	4	2	50.00	0.623

to TOL, HSI and reduction percent and were afterwards divide into three groups (Table 1). The high heat tolerance (HHT) group includes genotypes ranked in the 1–3 position, medium heat tolerance (MHT) group includes genotypes ranked in the 4–12 position and low heat tolerance (LHT) group includes genotypes ranked in the 13–24 position. TOL and SSI ranked 2.96-5.94 and 0.27 – 0.54 for HHT genotypes, 7.1 – 10.9 and 0.62 – 0.97 for MHT genotypes and 11.3 – 23.2 and 1.11 – 1.81 for LHT genotypes, respectively. Data for all the traits averaged across non-stress and stress treatments were used to develop dendrograms of phenotypic variation for the 24 genotypes.

Molecular marker characterization

For ISSR marker analysis, leaf tissues (~1 g fresh weight) were sampled. Genomic DNA was extracted from each accession using a modified CTAB method (Saghai-Maroof et al., 1994). A set of 38 ISSR primers was synthesized according to the sequences obtained from the University of British Columbia, Canada (Zietkewicz et al., 1994). All the primers were screened for their amplification efficiency using high quality DNA samples. According to the amplification efficiency and reproducibility, 15 ISSR primers were chosen to test the 24 synthetic wheat lines. ISSR-PCR amplifications were performed in 20 µl reaction volumes of 20-50 ng of genomic DNA, 2 µl of 10x Taq buffer (Takara, Japan), 1.5mM MgCl2, 0.2 mM of each dNTP, 0.4 µl primer, and 0.5U of *Taq* DNA polymerase. PCR reactions were performed on a BioRed Thermal Cycler (Bio-Rad, USA), under the following conditions: an initial step of denaturizing for 1 min at 94°C followed by 45 cycles each consisting of a denaturizing step of 1 min at 94°C, an annealing step of 1 min at 34°C and an extension step of 2 min at 72°C. Fragments were separated on 2% low EEO gels in 10 x TBE buffer and visualized by Gel documentation system (Alpha Innotech, USA).

Statistical analysis

Analysis of variance, mean comparisons, correlations and dendrogram were performed using Statically Analysis System (SAS 9.0, SAS Institute, Cary, NC). For ISSR marker classification of genetic diversity and relationships the amplified bands were recorded as either present (1) or absent (0) in each sample. Genetic distance was estimated according to Nei and Li (1979). Polymorphic information content (PIC) for each ISSR estimated following Botstein et al. (1980). DARwin version 5.0 was used for calculating pair-wise genetic distances and for constructing the dissimilarity matrix (Perrier et al., 2003).

Conclusion

Our study identified significant genetic diversity with regard to heat tolerance among 24 synthetic wheat lines based on phenotypic traits and marker analysis. However, ISSR were useful for characterizing genetic relatedness but could not distinguish the level of heat tolerance. Synthetic hexaploid wheat is extremely useful for exploiting genetic diversity originating in *T. turgidum* and *Ae. tauschii*. Our results demonstrate that combining ISSR markers and phenotypic analysis could be powerful approach to describe genetic variation in stress tolerance. Based on stress tolerance indices, synthetic lines SYN11, SYN 36 and SYN 44 were identified as heat tolerant genotypes and these can be employing in breeding programs for stress environments.

Acknowledgements

This work was supported by the Indian Council of Agricultural Research of New Delhi, India (Grant No. DWR/RP/10-5.3). We thank PI, Crop Improvement, Directorate of Wheat Research, Karnal for providing kind support. This is DWR publication No. 28.

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