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MICROSATELLITE MARKER BASED CHARACTERIZATION AND DIVERSITY ANALYSIS OF WHEAT

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ABSTRACT
Molecular characterization of 18 wheat cultivars and the amplification was successfully carried out 23 SSR primer pairs. A total of 341 allelic variants were detected with an average of 9.2 alleles per locus in which 226 unique alleles were observed at 37 SSR loci, with an average of 6.10 unique alleles per locus. Polymorphism information content (PIC) value ranged from 0.347 for the primer Xgwm369 to 0.858 for Xgwm251 & Xgwm282 with an average of 0.691. A maximum similarity coefficient was found between AKAW 4189-3 and Kauz/AA/Kauz (0.93) while minimum similarity was found between PBW343 and Kauz-dwarf (0.63).The Dendrogram was constructed using Jaccard's similarity coefficients to which entries were classified into six groups on the basis of drawing phenon line at 0.85 coefficient of similarity level. Results of this experiment indicates that hybridization between AKAW4008 with RSP561, AKAW4008 with MB4010, Kauz/AA/Kauz with RSP561, Kauz/AA/Kauz with MB4010 will produce high yielding heat tolerant hybrid of wheat, because these parental lines has wide genetic base . The results of this experiment suggest that hybridization between AKAW4008 with C306 and Raj3765 with C306 will not produce high yielding heat tolerant hybrid of wheat, because these parental lines shows the narrow genetic base.

INTRODUCTION

Wheat *(Triticum aestivum* L.) a self-pollinating annual plant, belonging to the family *Poaceace* (*Gramineae*) is globally one of the three most important crops for human as well as livestock feed (Shewry, 2009; Zala *et al*., 2014). The world's major bread wheat producing areas are in north China, northern India, northern USA and adjoining area of Canada, Europe, Russia, Latin America and Africa. India is the second largest producer of wheat after China with an area of 29.06 million hectares and production of 86.67 million tons in the year 2011 and cover 12 % of the world population (FAOSTAT 2012). It was originated from the southwest Asia and now cultivated worldwide. Wheat is hexaploid with three different genomes (A, B & D) each having basic chromosome number 7(2n = 6X = 42). Wheat is characterized by large genome size approximately 17000 Mb (Hartl and Jones, 2001). Wheat has always been subjected to extensive and ceaseless research so as to maximize grain production and also to improve grain yield per unit area. However, there is still considerable room for improvement, especially to amplify efforts for continued genetic improvement of wheat to meet the growing requirements of an ever increasing population. Genetic manipulation is the best way to boost up wheat production (Yadvad *et al*., 2015). There is wide genetic variability available among varieties of wheat leaving a wide scope for further wheat improvement programs. Understanding the genotyping characteristics and relationships of the germplasm is limited, mainly due to the polyploid nature of wheat (Haudry *et al*., 2007; Zala *et al*., 2014). Unlike high yielding varieties, the landraces are endowed with tremendous genetic variability, as they are not subjected to subtle selection over a long period of time. This variability of complex quantitative traits still remains un exploited. The need to characterize available landraces and varieties has therefore become important in modern crop improvement. Therefore assessment of the extent genetic variation and genetic differentiation among the landraces, locally adopted varieties and elite breeding materials is of fundamental interest to wheat breeders.

Genetic diversity between genotypes is usually estimated by measurements of physiological and morphological differences of quantitative and economically important traits. The disadvantage of this conventional approach are the cost of time and labour during the measurements and the influences of environmental factors (Chakravarthi and Narvaneni, 2006; Seetharam *et al*., 2009. The molecular marker technology provides for one of the most authentic approach to understand the genetic diversity of wheat cultivars within a species. Several molecular marker systems, including restriction fragment length polymorphisms (RFLP) (Sun *et al*., 2001) random amplified polymorphic DNA (RAPD) (Ravi *et al*., 2003) simple sequence repeats (SSRs) (Eizenga *et al*., 2009) inter-simple sequence repeats (ISSRs) (Bao *et al*., 2006) amplified fragment length polymorphism (AFLP) (Saini *et al*., 2004) and single nucleotide polymorphisms (SNPs) (Shirasawa *et al*., 2006) are among a few of the molecular tools available to assess the variability and diversity of germplasm pools and breeding programs at the molecular level. However most of these marker systems show a low level of polymorphism especially in wheat. Among DNA based markers SSR or microsatellites is an

excellent molecular marker in plants and animals for many types of genetic analyses, including linkage mapping, germplasm survey and phylogenetic studies (Blair *et al*., 2007; Sarikamis *et al*., 2009). Microsatellite markers, also known as SSRs have been proposed as suitable markers for assessment of genetic variation and diversity among wheat varieties and lines. SSRs are multiallelic, chromosome-specific, and evenly distributed along the chromosomes (Roder *et al.,* 2002; Powell *et al*., 2006; Chandra *et al*., 2013). SSRs are highly informative markers (unlike RAPDs) because they are easily and economically assessed by PCR, co-dominant and generally have high polymorphic information content (PIC) (Gupta *et al.,* 2000; Powell *et al*., 2006; Chandra *et al*., 2013). The objective of this study was to characterize and genetic divergence analysis of wheat cultivars using microsatellite markers for identification of suitable parental wheat genotype for hybridization to develop heat tolerant wheat cultivar.

MATERIALS AND METHODS

Plant materials

Plant material (Table.1) for molecular study of eighteen wheat cultivar obtained from RKVY Project titled: "Enhancement of heat tolerance in locally adapted wheat cultivars of Bihar" in department of Agricultural Biotechnology and Molecular Biology, Rajendra Agricultural University, Pusa , Bihar. Seeds were washed in distilled water and sterilized by immersion in mercury dichloride solution (1:1000) for two minutes. The seed were next washed five times in deionizer water and placed in an oven at 28°C for 24 hours. Seeds were germinated in pots containing soil to raise seedlings in green house.

Isolation of genomic DNA

Genomic DNA was isolated from 10 days old seedlings leaves using CTAB method (Doyle and Doyle, 1987) with minor modifications. The concentration and purity of DNA was determined at 260 nm and 280 nm by using UV-Vis-spectrophotometer. The band quality of genomic DNA was observed with the help of electrophoresis on 0.8% agarose gel (Sambrook and Russell, 2001. The DNA samples were diluted to a concentration of 2.0 ng/μl with TE buffer for SSR analysis.

Selection of markers

Twenty three microsatellite markers are selected for the genetic diversity analysis on the basis of different research paper used in analysis of genetic diversity of wheat. The chromosomal position, repeat motifs, primer sequences, annealing temperature for these markers can be found from Roder *et al*. (1994) paper and the Database for *Avena* and *Triticae* (http:// wheat.pw.usda.gov/cgi-bin/ grain genes/browse.cgi). The SSR markers were obtained from Eurofins MWG operon Europe.

Microsatellite marker analysis

The PCR conditions were maintained as described by Emon *et al*. (2010) with minor modifications. PCR amplification reactions were conducted in 15 μl reaction volume containing 1X PCR buffer, 200 μM each dNTPs, 2mM of $MgCl_2$, 0.6 μM of each forward and reverse primer, 1 unit Taq DNA polymerase and 50 ng of template DNA. The PCR amplification was carried out using a Thermo cycler (Biometra). Thermo cycler was programmed to lid temperature 99°C, 1 cycle of 5 min at 94°C as an initial hot start and separation step. This was followed by 41 cycles of 30 Sec at 94°C for denaturation, annealing at 30 Sec at (50-62°C) depending on marker used and 30 Sec at 72°C for primer elongation and final extension 10 min at 72°C. The PCR products were stored at -20°C until electo phoretic separation.

Electrophoresis of amplified products

The 15 μl aliquot of the amplified SSR samples were combined with 3 μl of loading buffer (0.25% bromo-phenol blue, 0.25% Xylene cyanol FF and 30 % glycerol in H_2O) and separated on 2% Agarose gel in 1X TAE buffer containing 0.5 μg per ml of ethidium bromide. A 50 bp DNA ladder was used as size marker to compare the molecular weights of amplified products. Electrophoresis was performed at 95 V using Electrophoresis system for 1 hr and 20 minutes and observed under gel documentation system (Bio-Red).

Allele scoring and data analysis

The size of amplified band of each microsatellite marker was determined based on electrophoretic mobility relative to molecular weight of ladder (50 bp) used. Amplified products from microsatellite analysis were scored qualitatively for presence and absence of each marker allele genotype combination. Binary matrix is used for data analysis 1 for present of band and 0 for absence of band. Anderson *et al*. (1993) formula is used for calculating the polymorphic information content (PIC) value of marker which is used in amplification.

$$PIC_i = 1 - \sum_{j=1}^{k} P^2_{ij}$$

Where, P_{ij} is the frequency of the j^{th} allele for i^{th} marker and summation extends over n alleles.

The genetic association among varieties were analyzed by calculating the similarity coefficient (Jaccard, 1908) for pair-wise comparisons based on the proportions of shared bands produced by markers. The method used for tree building in the analysis involved Sequential Agglomerative Hierarchical nested (SAHN) clustering based on similarity coefficients. The dendrogram based on similarity indices were obtained by Un weighted Pair group Method of Arithmetic Average (UPGMA). The nature and extent of diversity between varieties were assessed by identifying the clusters at appropriate phenon levels. All calculations were performed using the NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System Programme) version 2.10 software (Rohlf, 2000).

RESULTS

Total twenty three SSR markers were used to characterize and assess genetic diversity among eighteen wheat cultivar. The name and sequence of forward and reverse primer is presented in Table 2. Amplification profile of marker WMC175 and Xgwm533 is shown in (figure 1 and 2) across a number of cultivar used. A considerable variability was found among different cultivar of wheat in present study. The polymorphism level among the wheat cultivars was evaluated by calculating number of alleles. Each of the primer pairs differed significantly in their ability to determine variability among cultivars (Table

Table 1: Wheat cultivars used in present study

Sl.No	Cultivars name	Seed Source	Yield performance during heat stress
1.	AKAW4008	IARI ,New Delhi	High
2.	Halna	IARI ,New Delhi	Medium
3.	Pusa gold	RAU, Pusa	Medium
4.	AKAW4189-3	RAU, Pusa	Medium
5.	PBW343	RAU, Pusa	High
6.	HD2733	IARI, New Delhi	Medium
7.	C306	RAU, Pusa	Low
8.	HD2285	IARI, ,New Delhi	Medium
9.	RSP561	IARI, ,New Delhi	Low
10.	Kauz/AA/Kauz	IARI, New Delhi	High
11.	Iepaca rabe	IARI ,New Delhi	High
12.	F5-995	NBPGR, New Delhi	High
13.	HD2888	RAU, Pusa	High
14.	MonsAld's	IARI ,New Delhi	Medium
15.	Kauz-dwarf	RAU, Pusa	Low
16.	MB4010	IARI ,New Delhi	Low
17.	Sonalika	IARI ,New Delhi	Medium
18.	Raj3765	RAU, Pusa	High

Table 2: Details of microsatellite primer pairs used for screening the wheat genotypes to detect polymorphism

Primers sequence Sl. No.	Name of primer	Forward (5′-3′)	Reverse (5′-3′)	Repeat motifs	An (°C)
1	Xbarc32	GCGTGAATCCGGAAACCCAATCTGTG	TGGAGAACCTTCGCATTGTGTCATTA	(ATT)10	52
2	Xbarc119	CACCCGATGATGAAAAT	GATGGCACAAGAAATGAT	(CT)17	55
3	Xbarc1165	GCGCCATCAAGCCTCAAAACTCTGTA	CGCAACGTTTTCCTTTCCCATAATACT	(CT)15+7	55
4	CFD79	TCTGGTTCTTGGGAGGAAGA	CATCCAACAATTTGCCCAT	(GA)26	60
5	Xgwm33	GGAGTCACCACTTGTTTGAGCA	CACTGCACACCTAACTACCTGC	(GA)19	60
6	Xgwm60	TGTCCTACACGGACCACGT	GCATTGACAGATGCACACG	(CA)30	60
7	Xgwm121	TCCTCTACAAACAAACACAC	CTCGCAACTAGAGGTGTATG	(CAAA)2(CA)28	50
8	Xgwm251	CAACTGGTTGCTACACAAGCA	GGGATGTCTGTTCCATCTTAG	(CA)28	55
9	Xgwm264	GAGAAACATGCCGAACAACA	GCATGCATGAGAATAGGAACTG	(CA)9A(CA)24	60
10	Xgwm282	TTGGCCGTGTAAGGCAG	TCTCATTCACACACAACACTAGC	(GA)38	55
11	Xgwm296	AATTCAACCTACCAATCTCTG	GCCTAATAAACTGAAAACGAG	(CT)28	55
12	Xgwm297	ATCGTCACGTATTTTGCAATG	TGCGTAAGTCTAGCATTTTCTG	(GT)12(GA)18	55
13	Xgwm341	TTCAGTGGTAGCGGTCGAG	CCGACATCTCATGGATCCAC	(CT)26	55
14	Xgwm369	CTGCAGGCCATGATGATG	ACCGTGGGTGTTGTGAGC	(CT)11(T)2(CT)21	60
15	Xgwm493	TTCCCATAACTAAAACCGCG	GGAACATCATTTCTGGACTTTG	(CA)43imp	60
16	Xgwm533	AAGGCGAATCAAACGGAATA	GTTGCTTTAGGGGAAAAGCC	(CT)18(CA)20	60
17	Xgwm540	TCTCGCTGTGAAATCCTATTTC	AGGCATGGATAGAGGGGC	(CT)3(CC)(CT)16	55
18	Xgwm577	ATGGCATAATTTGGTGAAATTG	TGTTTCAAGCCCAACTTCTATT	(CA)14(TA)6	55
19	Xgwm630	GTGCCTGTGCCATCGTC	CGAAAGTAACAGCGCAGTGA	(GT)16	60
20	Xgwm635	TTCCTCACTGTAAGGGCGTT	CAGCCTTAGCCTTGGCG	(CA)10(GA)14	60
21	WMC11	TTGTGATCCTGGTTGTGTTGTGA	CACCCAGCCGTTATATATGTTGA	-	61
22	WMC175	GCTCAGTCAAACCGCTACTTCT	CACTACTCCAATCTATCGCCGT	(CA)16_138 to169	61
23	WMC611	GGTTCGCTTTCAAGGTCCACTC	CGGGACACTAGTGCTCGATTCT	-	61

3). Total 341 alleles were detected across 18 varieties using 23 SSR markers. The number of alleles per locus generated by each marker varied from 10 (Xgwm341) to 21 (Xgwm540) with an average 9.2 alleles per locus. The highest number of alleles per locus was observed in the B genome (a total of 212 alleles) as compared to A and D genomes (genome A contained a total of 173 alleles and genome D contained a total of 99 alleles). This confirmed that there was a higher polymorphism level in the B genome. The smallest size range of alleles varies from 57-80 in a marker (Xbarc119) and largest 346-411 in marker (Xbarc1165) and rest of alleles size varies between them which is shown in (Table 3).

A total 226 unique alleles were observed at 37 SSR loci with an average of 6.1 unique alleles per locus. Maximum numbers of unique alleles were observed 18 in the case of marker Xgwm264 followed by Xbarc32, Xgwm296 and Xgwm635 (14 alleles each), Xgwm540 (13 alleles) WMC 611and Xgwm 630 (12allele) Xgwm60 and Xbarc119 (11allele) respectively and minimum 2 in the case of marker Xgwm341. The presence of unique alleles in the set of cultivars may indicate that these materials are useful for plant breeders and geneticists as a rich source of genetic diversity for wheat.

In the set of 18 wheat varieties eighteen loci showed null alleles with 9 markers. Xgwm 493 shows null allele at maximum (5 allele) locus, followed by Xgwm 296 (3 allele), Xgwm 577 (3 allele), WMC 611 (2 allele), Xgwm 251 (1 allele), Xgwm 264 (1 allele), Xgwm 282 (1 allele), Xgwm 630 (1 allele(, Xgwm 635 (1 allele) respectively. Null alleles might decrease

Table 3: Details of allelic information using SSR markers

Markers	Chromosomes	No of locus	Total Alleles	Unique alleles	Size range(bp)	Null alleles	PIC
Xbarc32	5B, 7B	2	18	14	227-295	0	0.799
Xbarc119	1A,1B,1D	1	14	11	57-80	0	0.740
Xbarc1165	6A	1	12	7	346-411	0	0.814
CFD79	3A, 3B	2	20	13	270-398	0	0.543
Xgwm33	1A,1B,1D	2	17	9	62-171	0	0.453
Xgwm60	7A	2	20	11	173-275	0	0.475
Xgwm121	5D, 7D	1	12	8	126-162	0	0.771
Xgwm251	4B	1	12	9	133-174	1	0.858
Xgwm264	1B	2	20	18	163-249	1	0.840
Xgwm282	7A	1	15	14	107-197	1	0.858
Xgwm296	2A,2D	2	14	9	72-246	3	0.759
Xgwm297	7B	1	11	6	163-210	0	0.759
Xgwm341	3D	2	10	2	133-178	0	0.456
Xgwm369	3A	2	14	4	183-275	0	0.395
Xgwm493	3B	1	11	9	177-227	5	0.777
Xgwm533	1B	1	12	9	108-181	0	0.753
Xgwm540	5B	2	21	13	120-191	0	0.543
Xgwm577	7B	1	12	9	175-254	3	0.824
Xgwm630	2B	2	17	12	124-171	1	0.672
Xgwm635	7A,7D	2	18	14	130-250	1	0.799
WMC11	3A, 3D	2	14	7	183-285	0	0.558
WMC175	2B	2	12	6	231-304	0	0.774
WMC611	1A,1B	2	15	12	198-256	2	0.783
Total		37	341	226		18	

Table 4: Estimates of twenty-three SSR primers based on Jaccard's similarity coefficients among eighteen wheat cultivar used in the present study

	AKAW 4008	Halna	Pusagold	AKAW 4189-3	PBW 343	HD-2733	C-306	HD-2285	RSP 561	Kauz/AA/Kauz	Iepacarabe	F5-995	HD2888	Mons Ald's	Kauz-dwarf	MB-4010	Sonalika	Raj-3765
AKAW4008	1.000																	
Halna	0.800	1.000																
Pusagold	0.857	0.733	1.000															
AKAW4189-3	0.781	0.727	0.774	1.000														
PBW343	0.793	0.733	0.852	0.833	1.000													
HD-2733	0.867	0.806	0.800	0.903	0.862	1.000												
C-306	0.897	0.833	0.893	0.871	0.893	0.900	1.000											
HD-2285	0.897	0.774	0.828	0.813	0.767	0.900	0.867	1.000										
RSP561	0.733	0.733	0.724	0.667	0.724	0.742	0.710	0.710	1.000									
Kauz/AA/Kauz	0.781	0.781	0.774	0.935	0.833	0.903	0.871	0.813	0.667	1.000								
Iepacarabe	0.774	0.774	0.710	0.758	0.710	0.781	0.750	0.806	0.767	0.813	1.000							
F5-995	0.800	0.742	0.733	0.727	0.733	0.806	0.774	0.833	0.733	0.727	0.833	1.000						
HD2888	0.765	0.714	0.758	0.909	0.758	0.824	0.848	0.794	0.657	0.853	0.743	0.714	1.000					
MonsAld?s	0.813	0.758	0.750	0.794	0.750	0.818	0.844	0.844	0.697	0.743	0.788	0.813	0.882	1.000				
Kauz-dwarf	0.719	0.667	0.656	0.758	0.656	0.727	0.697	0.750	0.710	0.706	0.867	0.833	0.743	0.788	1.000			
MB-4010	0.647	0.697	0.588	0.735	0.636	0.706	0.676	0.676	0.688	0.686	0.727	0.806	0.722	0.765	0.839	1.000		
Sonalika	0.750	0.806	0.688	0.735	0.688	0.758	0.781	0.727	0.688	0.686	0.676	0.806	0.771	0.818	0.781	0.871	1.000	
Raj-3765	0.719	0.833	0.656	0.706	0.656	0.727	0.750	0.697	0.656	0.657	0.647	0.774	0.743	0.788	0.697	0.781	0.900	1.000

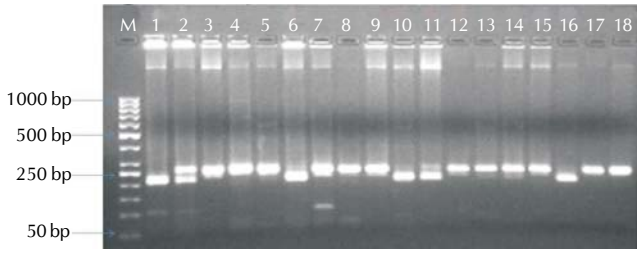

Figure 1: SSR banding pattern of 18 wheat varieties generated by primer pair WMC175.The lanes (M) represent 50 bp molecular size marker; (1) AKAW4008 (2)Halna,(3)Pusa gold (4)AKAW4189-3 (5) PBW343 (6) HD2733,(7) C306,(8) HD2285,(9) RSP561,(10) Kauz/ AA/Kauz,(11) Iepacarabe,(12) F5-995, (13) HD2888,(14) MonsAld's,(15) Kauz-dwarf, (16) MB4010,(17) Sonalika,(18) Raj3765

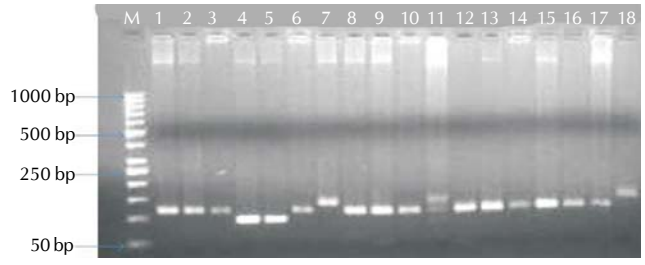

Figure 2: SSR banding pattern of 18 wheat varieties generated by primer pair Xgwm335.The lanes (M) represent 50 bp molecular size marker; (1)AKAW4008 (2)Halna,(3)Pusa gold (4)AKAW4189-3 (5) PBW343 (6) HD2733,(7) C306,(8) HD2285,(9) RSP561,(10) Kauz/ AA/Kauz,(11) Iepacarabe,(12) F5-995, (13) HD2888,(14) MonsAld's, (15) Kauz-dwarf, (16) MB4010,(17) Sonalika,(18) Raj3765

Table 5: Composition of clusters based on similarity coefficients for twenty three primer pairs used for amplification in eighteen wheat cultivar

Cluster	Number of entries	Composition of cluster
A	8	AKAW4008, C306, HD2285, Pusa gold, AKAW4189-3, Kauz/AA/Kauz, HD2733 and PBW343
B	3	Halna, Sonalika and Raj3765
C	3	Iepeca rabe, Kauz–dwarf and F5-995
D	2	HD2888 and Mons Ald's
E	1	MB4010
F	1	RSP561

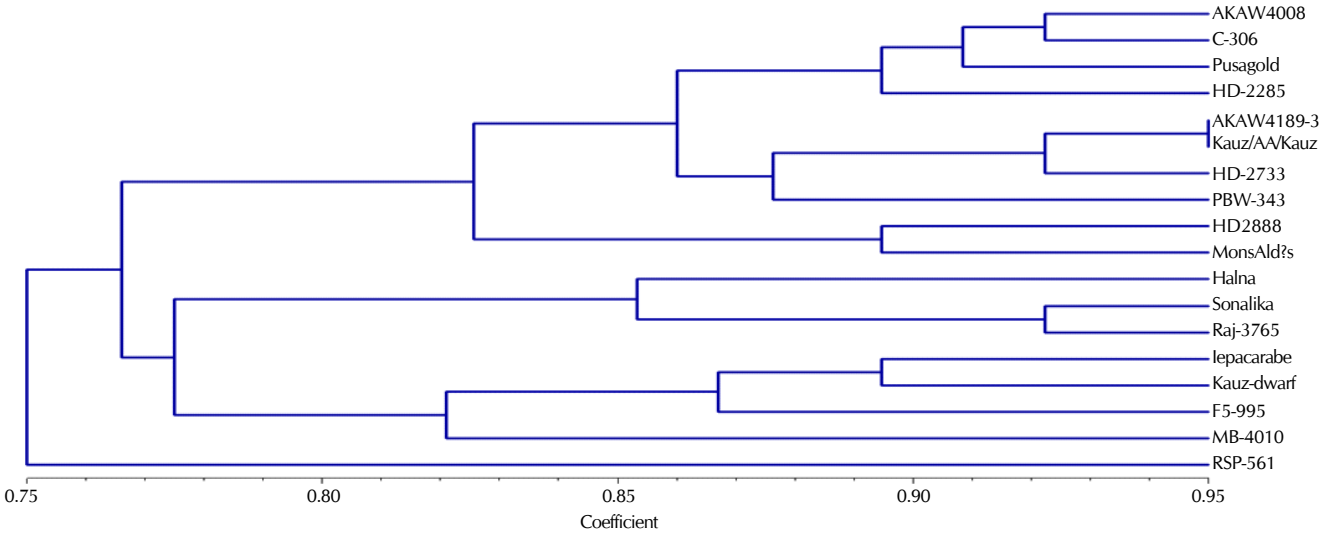

Figure 3: UPGMA cluster analysis showing the diversity and relationship among 18 wheat cultivars based on 237 alleles generated by 23 SSR markers

the apparent hetero zygosity in a population and may result in the deviation of genotypes from Hardy- Weinberg expectation (Lapitan *et al*., 2007; Kalinowaski *et al*., 2006). Although stutter bands was very common and more serious in case of SSR markers. Presence of stutter bands was detected in 8 markers Xbarc32, Xbarc1165, Xgwm282, Xgwm296, Xgwm341, Xgwm369, Xgwm540, and Xgwm630.

Polymorphic information content (PIC) Values

The PIC values a reflection of allele diversity and frequency among the wheat cultivars and also varied from one locus to another locus. The level of polymorphism determined by PIC values was quite high and varied range 0.395 (Xgwm369) to 0.858 (Xgwm251 and Xgwm282) and average value 0.691 among SSR loci. The detail of PIC values of all 23 markers used in study is presented in Table 3.

The similarity matrix based on the proportion of shared SSR alleles was used to establish the relationship between the various wheat cultivars. Pair wise estimates of similarity varied from 0.63 to 0.93 (Table 4). Two varieties AKAW 4189-3 and Kauz/AA/Kauz were found maximum close to each other (0.93). This was followed by HD2888 and AKAW 4189-3; HD2733 and AKAW 4189-3; HD2733 and C-306; HD2733 and HD 2285; HD 2733 and kauz/AA/Kauz; Sonalika and Raj 3765 (0.90). While minimum similarity was found between PBW343 and Kauz-dwarf (0.63). The Jaccard's similarity coefficients ranged from 0.63 to 0.93 indicating the presence of wide range of genetic diversity at molecular level among the eighteen cultivars of wheat.

Clustering of wheat genotypes based on Jaccard's similarity coefficient

The dendrogram obtained from similarity Jaccard's coefficients based on UPGMA method for pair-wise combinations amongst the entries has been shown in Fig 3. Considering broad classification of entries, as indicated by dendrogram, basically the entries were divided into six groups on the basis of drawing phenon line at 0.85 cofficient of similarity level (Table 5). Group A consists of eight genotypes namely AKAW4008, C306, HD2285, Pusa gold, AKAW4189-3, Kauz/AA/Kauz, HD2733, and PBW343 considered as multi-genotypic group. Genotypic group B consist of three genotypes namely Halna, Sonalika and Raj3765. Group C is also considered as multi-genotypic which consists of three genotypes namely, Iepeca rabe, Kauz–dwarf and F5-995. DI-genotypic group D consist of genotypes HD2888 and Mon's Alds. Group E and group F consist of one genotype MB4010 and RSP561 respectively. Results indicate that wheat genotypes used in this experiment has wide genetic base.

DISCUSSION

The polymorphism of wheat cultivars was calculating allele number and PIC values of the 23 SSR loci. Total 341 alleles were detected among 18 cultivar using 23 SSR markers. Number of alleles per locus varied from 10 (Xgwm341) to 21 (Xgwm540) of each marker with an average 9.2 alleles per locus. The number of alleles observed in the present study

crosspond well to some earlier reports (Schuster *et al*., 2009; Emon *et al*., 2010; Zhang *et al*., 2011). Contrarily the number of alleles detected in the present study was significantly higher than the average number of alleles in previous reports (Schuster *et al*., 2009: Emon *et al*., 2010) which has reported 3.2 and 7.3 alleles per locus. Highest number of alleles per locus was observed in the B genome (a total of 212 alleles) as compared to A and D genomes (genome A contained a total of 173 alleles and genome D contained a total of 99 alleles).This confirmed that there was a higher polymorphism level in the B genome. Similar observation for higher polymorphism level of genome B was also reported by Wang *et al*. (2007). The present observations also agree with the results of studies by Cho *et al*. (2000) in rice Scott *et al*. (2000) in grapes and Eujayl *et al*. (2001) in wheat. Total 226 unique alleles were observed at 37 SSR loci, with an average of 6.1 unique alleles per locus. Maximum numbers of unique alleles were observed 18 in the case of Xgwm264 and minimum 2 in the case of primer Xgwm341. The presence of unique alleles in the set of cultivars may indicate that these materials are useful for plant breeders and geneticists as a rich source of genetic diversity for wheat. A variety was assigned null alleles for a microsatellite locus whenever an amplification product could not be detected for a particular genotype marker combination. In the set of 18 wheat varieties eighteen loci showed null alleles in 9 primers. The SSR locus associated with primer pairs Xgwm251, Xgwm264, Xgwm282, Xgwm296, Xgwm493, Xgwm577, Xgwm630, Xgwm635, and WMC611 showed null alleles in some of the varieties under evaluation and maximum nine varieties i.e., AKAW4008, Halna, Pusa gold ,PBW343, RSP561, Kauz-dwarf, MB4010, Sonalika, Raj3765 shows null alleles. Maximum five null alleles present in primer pair, Xgwm493 for which varieties (Halna, RSP561, MB4010, Sonalika, Raj3765) shows null alleles. Whereas Xgwm296 (Pusa gold, PBW343, RSP561) and Xgwm577 (Kauz-dwarf, MB4010, Sonalika), shows three null alleles each. Similar result are also reported many researchers Stachel *et al*. (2000) and Teklu *et al*. (2007). Presences of stutter bands were detected in this study. Stutter band are minor products amplified in PCR that have lower intensity than the main allele and normally lacks or extra repeat units (Walsh *et al*., 1996). Although stutter bands were very common and more serious in SSR such as primer pairs Xbarc32, Xbarc1165, Xgwm282, Xgwm296, Xgwm341, Xgwm369, Xgwm540, and Xgwm630. Stutter bands were produced by the slippage of the polymerase amplification (Roder *et al*., 1994) and the factors that influences the proportion of stutter bands to the main allele were the repeat number, number of PCR cycles, length and the characteristics of the repeat sequence. Similar result also reported by some researchers (Cho *et al*., 2000; Wong *et al*., 2007). The polymorphism level determined by PIC values was quite high and varied range 0.395 (Xgwm369) to 0.858 (Xgwm251 and Xgwm282) and average value (0.691) among SSR loci. The PIC values observed in our steady is comparable to previous estimates of microsatellite marker analysis in wheat (Schuster *et al*., 2009; Zhang *et al*., 2011). They reported it as 0.490 and 0.483 respectively. The PIC value was higher than the earlier observations (Emon *et al*., 2010) who reported 0.776. The Jaccard's similarity coefficients ranged from 0.63 to 0.93 indicating the presence of wide range of genetic diversity at molecular level among the eighteen varieties of wheat. Our result about Jaccard's similarity coefficients is comparable with previous findings of Chandra *et al*. (2013), reported similarity coefficient among genotypes ranged from 0.609 to 0.975. The dendrogram obtained from similarity Jaccard's coefficients based on UPGMA method for pair-wise combinations amongst the entries has been shown in Fig. 3 Considering broad classification of entries, as indicated by dendrogram, basically the entries were divided into six groups on the basis of drawing phenon line at 0.85 coefficient of similarity level. Our experimental findings indicates that hybridization between AKAW4008 with RSP561, AKAW4008 with MB4010, Kauz/AA/Kauz with RSP561, Kauz/AA/Kauz with MB4010 will produce high yielding heat tolerant hybrid of wheat, because these parental lines has wide genetic base . The results of this experiment suggest that hybridization between AKAW4008 with C306 and Raj3765 with C306 will not produce high yielding heat tolerant hybrid of wheat, because these parental lines shows the narrow genetic base. A comparison of values about allelic diversity among the cultivars clearly emphasize the scope for introgression of genes through hybridization of different cluster cultivars will be useful to develop high yielding and abiotic stress tolerant varieties to tolerate the changing climate conditions. The high yielding abiotic stress tolerant varieties will be much useful to solve the food problem.

In conclusion the SSR markers are powerful tools to assess the genetic variability of the clusters under study. The information about the genetic diversity of these wheat cultivars, will be much useful for proper identification and selection of appropriate parents for use in the breeding programs, including gene mapping for wheat improvement not only in Bihar but also other states of India.

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REFERENCES

Anderson, J. A., Churchill, G. A., Autrique, J. E., Tanksley, S. D. and Sorrells, M. E. 1993. Optimizing parental selection for genetic linkage maps. *Genome*. **36:** 181-186.

Bao, J., Corke, H. and Sun, M. 2006. Analysis of genetic diversity and relationships in waxy rice (*Oryza sativa* L.) using AFLP and ISSR markers. *Genet Resour. Crop Evol.* **53:** 323-330.

Blair, M. W., Diaz, J. M., Hidalgo, R. and Diaz, L. M. 2007. Microsatellite characterization of Andean races of common bean (*Phaseolus vulgaris* L.). *Theor. Appl. Genet*. **116:** 29-43.

Chakravarthi, B. K. and Naravaneni, R. 2006. SSR marker based DNA fingerprinting and diversity study in rice (*Oryza sativa*. L). *Afri. J. Biotech*. **5(9):** 684-688.

Chandra, V., Pant, V., Bhajan, R. and Singh, A. K. 2013. Studies on genetic diversity among alternaria blight tolerant Indian mustard genotypes using SSR markers. *The Bioscan*. **8(4):** 1431-1435.

Cho, Y. G. M., Ishii, T., Temnyakh, S., Chen, X., Lipovich, L., McCouch, S. R. Park, W. D. and Ayeres, N. 2000. Diversity of microsatellite derived from genomic libraries and gene bank sequence

in rice (*Oryza Sativa L.*). *Theor. Appl. Genet*. **100:** 713-722.

Database for *Avena* and *Triticae* (http://wheat.pw.usda.gov/cgi-bin/graingenes/browse.cgi)

Doyle, J. J. and Doyle, J. L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf material. *Phytochem. Bull*. **119:** 11-15.

Eizenga, G. C., Agrama, H. A., Lee, F. N. and Jia, A. 2009. Exploring genetic diversity and potential novel disease resistance genes in a collection of rice (*Oryza spp*.) wild relatives. *Genet Resour. Crop Evol*. **56:** 65-76.

Emonn, R. M., Gustafson, J. P., Nguyen, H., Musket, T., Jahiruddin, M., Islam, M. A., Haque, M. S., Islam, M. M., Begum, S. N. and Hassan, M. M. 2010. Molecular marker-based characterization and genetic diversity of wheat genotypes in relation to Boron use efficiency. *Indian J. Genet*. **70(4):** 339-348.

Eujayl, I., Sorrells, M. E., Baum, M., Wolters, P., Powell, W. 2001. Isolation of EST-derived microsatellite markers for genotyping the A and B genomes of wheat. *Theor. Appl. Genet*. **104:** 399-407.

FAOSTAT. 2012. Food and Agriculture Organization of the United Nations. Food Outlook. 2.

Gupta, P. K. and Varsheny, R. K. 2000. The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. *Euphytica*. **113:** 163-185.

Hartl, D. L. and Jones, E. W. 2001. Genome evolution in the grass family, In genetics and analysis of genes and genomes. *Fifth edition, Jones and Bartlett publishers Sudbury, Massachusetts U S A.*

Haudry, A., Cenci, A., Ravel, C., Bataillon, T., Brunel, D., Poncet, C., Hochu, I., Poirier, S., Santoni, S. and Gle´min, S. 2007. Grinding up wheat: a massive loss of nucleotide diversity since domestication. *Mol. Biol. Evol*. **24(7):** 1506-1517.

Jaccard, P. 1908. Nouvells researchers sur la distribution florale. *Bull. Soc. Vaund. Sci. Nat*. **44**: 223-270.

Kalinowaski, S. T and Taper, M. L. 2006. Maximum likelihood estimation of the frequency of null alleles at microsatellite loci. *Conser. Genet*. **7:** 991-995.

Lapitan, V. C., Brar, D. S., Abe, T. and Redona, E. D. 2007. Assessment of genetic diversity of Philippine rice cultivars carrying good quality traits using SSR markers. *Breeding Science*. **57:** 263-270.

Powell, W., Morgante, M. and Andre, C. 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Molecular Breeding*. **2:** 225-228.

Ravi, M., Geethanjali, S., Sameeyafarheen, F. and Maheswaran, M. 2003. Molecular marker based genetic diversity analysis in rice (*Oryza sativa* L.) using RAPD and SSR markers. *Euphytica*. **133:** 243-252.

Roder, M. S., Plashke, J., Konig, S. U., Borner, A., Sorrells, M. E., Tanksley, S. D. and Ganal, M. W. 1994. Abundance, variability and chromosomal location of microsatellites in wheat. *Mol. Gen Genet*. **246:** 327-333.

Roder, M. S., Wendehake, K., Korzun, V., Bredemeijer, G., Laborie, D., Bertrand, L., Isaac, P., Rendell, S., Jackson, J., Cooke, R.J., Vosman, B. and Ganal, M. W. 2002. Construction and analysis of a microsatellite-based database of European wheat varieties. *Theor. Appl. Genet*. **106:** 67-73.

Rohlf, F. J. 2000. NTSYSpc (Numerical Taxonomy and Multivariate Analysis System). Version 2.1 Exeter Software. *Appl Biostat. Inc.,* New York, USA.

Saini, N., Jain, N., Jain, S. and Jain, J. R. 2004. Assessment of genetic diversity within and among Basmati and non-Basmati rice varieties using AFLP, ISSR and SSR markers. *Euphytica*. **140:** 133-146.

Sambrook, J. J. and Russell, D.W. 2001. Molecular Cloning A Laboratory Manual Vol **(1)** 3rd Edn CSHL Press Cold *Spring Harbor* New York.

Sarikamis, G., Yasar, F., Bakir, M. and Kazan, K. 2009. Genetic characterization of green bean (*Phaseolus vulgaris*) genotypes from eastern Turkey. *Genet. Mol Res*. **8:** 880-887.

Schuster, I., Vieira, E. S. N., Silva, G. J., Franco, F. A. and Marchioro, V. S. 2009. Genetic variability in Brazilian wheat cultivars assessed by microsatellite markers. *Genet. Mol Biol*. **32(3):** 557-563.

Scott, K. D., Eggler, P., Seaton, G. and Rossetto, M. 2000. Analysis of SSRs derived from grape ESTs. *Theor. Appl Genet*. **100:** 723-726.

Seetharam, K., Thirumeni, S. and Paramasivam, K. 2009. Estimation of genetic diversity in rice (*Oryza sativa* L.) genotypes using SSR markers and morphological characters. *Afr. J Biotech*. **8(10):** 2050-2059.

Shewry, R.P. 2009. Wheat. J.Exp.Bot. **60(6):** 1537-1553.

Shirasawa, K., Shiokai, S., Yamaguchi, M., Kishitani, S. and Nishio, T. 2006. Dot-blot-SNP analysis for practical plant breeding and cultivar identification inrice. *Theor. Appl Genet*. **113:** 147-155.

Stachel, M., Lelley, T., Grausgruber, H. and Vollmann, J. 2000. Application of microsatellites in wheat (*Triticum aestivum* L.) for studying genetic differentiation caused by selection for adaptation and use. *Theor. Appl Genet*. **100(2):** 242-248.

Sun, C. Q., Wang, X. K., Li, Z. C., Yoshimura, A.N. and Iwata, N. 2001. Comparison of the genetic diversity of common wild rice (*Oryza rufipogon Griff.*) and cultivated rice (*O. sativa* L.) using RFLP markers. *Theor. Appl Genet*. **102:** 157-162.

Teklu, Y., Hammer, K. and Roder, M. S. 2007. Simple sequence repeats marker polymorphism in emmer wheat (*Triticum dicoccum*) analysis of genetic diversity and differentiation. *Genet Resour. Crop Evol*. **54(3):** 543-554.

Walsh, P. S., Fildes, N. J. and Reynolds, R. 1996. Sequence analysis and characterization of stutter products at the tetranucleotide repeat locus. *Nucleic Acids Res*. **24:** 2807-2812.

Wang, H. Y., Wei, Y. M., Yan, Z. H. and Zheng, Y. L. 2007. EST-SSR DNA polymorphism in durum wheat (*Triticum durum* L.) collections. *J. Appl. Genet*. **48:** 35-42.

Zala, H., Bosamia, T., Kulkarni, K. and Shukla, Y. 2014. Assessment of molecular diversity in wheat (Triticum aestivum L. and Triticum durum L.) genotypes cultivated in semi-arid region of Gujarat. *The Bioscan*. **9(2):** 731-737.

Zhang, P., Li, J., Li, X., Liu, X., Zhao, X. and Lu, Y. 2011. Population structure and genetic diversity in a rice core collection (*Oryza sativa*L.) investigated with SSR markers. *Pols One*. **6(12):** 27565.