Electronic Journal of Plant Breeding, 8(2): 558-565 (June 2017)
ISSN 0975-928X
DOI: 10.5958/0975-928X.2017.00084.9

Research Note

Ascertaining narrow genetic base in commercial accessions of wheat commonly grown in Gujarat *via* molecular markers

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(Received: 20 Feb 2016; Revised: 05 May 2017; Accepted: 21 May 2017)

Abstract
Wheat is one of the most important cereal in India; it is under cultivation in the sub-continent for more than 5000 years. With population explosion, development of newer wheat varieties with better yield, high quality seed set, resistance to biotic and abiotic stresses are need of the hour. Screening of available genetic diversity is important to develop new varieties through breeding programs. The SSR markers with its co-dominant nature assist in genetic diversity studies. In present study, 13 commercial wheat accessions comprising of 2 *durum* and 11 *aestivum* varieties were characterized using 25 microsatellite (SSR) molecular markers. The PIC (Polymorphic Information Content) value ranged between 0 (csSr2 marker) to 0.899 (Xgwm146 marker). The current investigation of 13 wheat genotypes also confirms coefficient of similarity ranges from 0.3 to 0.8. More than eight accessions are falling in same cluster with similarity range from 0.45 to 0.80, pointing lack of genetic diversity, which could lead sudden decrease in yield in case of biotic and abiotic stresses. Hence, new breeding programs have to be initiated with marker assisted breeding to create new genotypes of wheat. From the study, 10 SSR molecular markers scoring the highest PIC values could be developed into a multiplexing panel for diversity and varietal identification based analysis of wheat genotypes.

Key words
Wheat, SSR markers, UPGMA, clustering

Wheat is the one of the most popular staple food cereal of Indian subcontinent. It has been cultivated in vast plains of northern India for the past 5000 years (Feldman 2001). The original species of wheat was *Triticum sphaerococcum* which was popularly known as Indian wheat. Three types of wheat are cultivated in Gujarat- *Triticum aestivum* (Tukdi), *Triticum dicoccum* (Popatiya) and *Triticum duram* (Kathiya). *T. duram* can be cultivated in drained as well as non-drained area and the other two varieties *T. dicoccum* and *T. aestivum* can be cultivated only in drained area (ikhedut.gujarat.gov.in). The major wheat producing states are Uttar Pradesh, Punjab, Haryana, Madhya Pradesh, Rajasthan, Bihar, Maharashtra, Gujarat, Karnataka, West Bengal, Uttarakhand, Himachal Pradesh and Jammu & Kashmir. These states contribute about 99.5% of total wheat production in the country. Remaining states such as Jharkhand, Assam, Chhattisgarh, Delhi and other North Eastern States contribute only about 0.5 % of the total wheat production in the country.

All the success in wheat production, so far is achieved by conventional breading. However, breeding and selection also leads to loss of great numbers of alleles, making it more difficult to develop resistant and tolerant wheat cultivars. Hence, diversity analysis of germplasm is mandatory. To estimate genetic diversity in wheat germplasm, various markers can be used along with morphological traits for assessing genetic diversity (Li *et al*. 2009). The SSR markers are based on genomic data and have more polymorphic detection capabilities in comparison to single primer PCR based techniques like RAPD and ISSR. Various studies have used SSR markers to investigate genetic diversity in cultivated hexaploid wheat genotypes of *T. aestivum* L. (Liu *et al*.2005; Schuster *et al*. 2009). The SSR markers have also been successfully used to characterize genetic diversity among wild relatives of wheat. The SSR markers are co-dominant, reproducible and have high polymorphism levels and equal distribution when it compared to other types of molecular markers. The SSRs are more abundant, ubiquitous in presence, hypervariable in nature and have high polymorphic information content. Thus the availability of a large number of molecular markers in wheat suggests their use in intraspecific analysis, comparative analysis and gene introgression studies as well as in wheat breeding (Borner *et al*., 2000 and Hammer 2000). .

Among three types of wheat cultivated in Gujarat, in the present study characterization of 13 accessions of *T. aestivum* and two accessions of *T. duram* were done using 25 SSR markers. These accessions were selected based on their market value and popularity among the farming community. Genetic variability can complement the breeding programs for identification of parental combinations effectively to maintain maximum genetic variability in progenies and can be used to create new genotypes of wheat against sudden decrease in yield in case of biotic and abiotic stresses.

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Plant material: In present study, 13 commercial wheat accessions commonly grown in Gujarat were selected for molecular characterization (Table 1).

DNA extraction and molecular marker genotyping: Fresh young leaves from fifteen days old seedlings were collected for DNA extraction. Total genomic DNA was isolated using modified CTAB method (Zala *et al*. 2014). Their qualitative and quantitative analyses were carried out using 0.8% of agarose gel electrophoresis and QIAxpert, Qiagen respectively.

Total by 25 SSR markers were selected on the basis of their polymorphic information content and sequence specific information of markers were retrieved from GrainGenes database (Table 2). The PCR was constituted with 25 µl, containing 1X standard Taq buffer, 10mM dNTPs, 10µM of primer, 1.25U Taq polymerase and 50 ng templates of DNA. The PCR was performed using Veriti 96 well Thermal Cycler, Applied Biosystems where initial denaturation was carried out at 95°C for four mins., followed by 35 cycles of denaturation at 94 °C for 30 sec., annealing ranged between 48 to 65 °C for 30 sec., extension at 72 °C for one min.; and final extension at 72°C for seven mins. PCR products were analysed in 2% agarose gel.

Analysis of molecular data: The cluster analysis and summary statistics including the number of alleles per locus, major allele frequency and polymorphism information content (PIC) values of respective primers were calculated. The PIC values, were calculated according to the formula: PIC = 1 "Σ P2i, where Pi is the frequency of the i^{th} allele (Anderson *et al*. 1993). Presence and absence of each single band was coded as one or zero in a binary matrix and converted to NTS format for analysis in NTSYS 2.02 using DICE coefficient. The cluster analysis was performed by UPGMA method.

Cluster analysis of wheat accessions: In present study, *T. durum* and *T. aestivum* accessions were characterized for their inherent diversity. All 25 SSR markers generated 653 bands with average of 26 bands per marker. The minimum sized fragment (100 bp) was amplified by primers Xgwm 132, Xgwm 497, 265, Xgwm 294, Xgwm 533, Xgwm 155, Xgwm382 and Xwmc 617B, whereas maximum sized fragment(1000 bp) was amplified by primers Xgwm 192, Xgwm219, Xgwm174, Xgwm455 and Xgwm146(Table 4). The PIC value ranged from 0 (csSr2) to 0.8991 (Xgwm146). Xgwm146 marker scored for a total of 42 bands giving it the highest PIC value as 0.899093, making it the most useful single marker to screen the current population in the study (Fig 1). The PIC range was from 0.0 to 0.87 for rest of 24 markers. Out of 24 markers taken in the study, 12 markers were able to differentiate 13 wheat genotypes with more than 80 % efficiency. Five SSR markers were able to differentiate 13 wheat genotypes as different scoring in >70 % efficiency. Remaining six markers ranged in an efficiency of 35 % to 65 %.

Clustering pattern of dendogram generated by pooled SSR data showed 2 major clusters A and B, having similarity coefficient of 0.30 to 0.80. Cluster B was entirely different from cluster A and involved only one genotype (1255). Cluster A was divided into two sub clusters A.1 and A.2. Sub cluster A.2 was major sub cluster as compared to sub cluster A.1. Genotypes HI-8498 and MPO-1215 involved in sub cluster A.1 and GW-11 involved in sub cluster A.2. Cluster A.2 was divided into two groups A.2.1 and A.2.2. Group A.2.1 was divided into two sub groups A.2.1.1 and A.2.1.2. Sub group A.2.1.1 involved the genotypes GW-173 and 496 while sub group A.2.1.2 involved genotype 173-A. Group A.2.2 divided into two sub groups A.2.2.1 and A.2.2.2. Sub group A.2.2.1 divided into two classes A.2.2.1.1 and A.2.2.1.2 where group A.2.2.1.1 involved genotype LOK-1 while genotype 322 and 1018 were involved in sub group A.2.2.1.2. Sub group A.2.2.2 divided into two classes A.2.2.2.1 and A.2.2.2.2 where class A.2.2.2.1 involved GW-1 and A-115 genotypes while genotype 273 was involved in class A.2.2.2.2.

Cluster A involved 12 (HI-8498, MPO-1215, GW-11, GW-173, 406, 173-A, LOK-1, 322, 1018, GW-1, A-115 and 273) genotypes of wheat and cluster B involved 1255 genotype. Dendogram showed 0.30 coefficient similarity of cluster A and B (Fig 2).

Genetic variability: Genetic variability is important for the improvement of many crop species, including wheat, and nearly all crop improvement programs depend on genetic diversity in the available germplasm. Various studies have used SSR markers to investigate genetic diversity in cultivated hexaploid wheat genotypes of *T. aestivum* L. (Liu *et al*.2005; Schuster *et al*. 2009). The SSR markers have been successfully used to characterize genetic diversity to improve wheat germplasm (Borner *et al*. 2000) and wild relatives (Hammer 2000).

Amongst the three types of wheat cultivated in Gujarat i.e. *T. aestivum* (Tukdi), *T. dicoccum* (Popatiya) and *T. durum* (Kathiya), in the present study, characterization of 11 common accessions of *T. aestivum* and two common accessions of *Triticum durum* was carried out. These accessions were selected based on their market value and popularity among the farming community. Our study focused on a combination of hexaploid and tetraploid varieties of wheat. Similar studies were carried out by Abou-Deif *et al*. (2013) for characterization of twenty wheat varieties using

DOI: 10.5958/0975-928X.2017.00084.9

ISSR markers. In our study selection of SSR markers was based on genomic data and which had more polymorphic detection capabilities as compared to single primer PCR based techniques like RAPD and ISSR carried out to analyze the genetic diversity and relationships of wheat genotypes.

The csSr2 marker, even though registered low PIC value gave significant single band patterns in six of 13 accessions namely GW-11, GW-173, 173-A, 496, 322 and 1018. All the above accessions belong to the hexaploid wheat which among all are popularly cultivated in Gujarat. csSr2 is designed to amplify locus Sr2, giving resistance to stem rust and has been used in breeding for around 60 years as a source of durable and broad-spectrum adult plant resistance, which includes resistance to Ug99 and its related isolates. Sr2 is located on the short arm of chromosome 3B and confers partial resistance only in the homozygous state (recessive resistance gene). It was originally transferred from Yaroslav emmer wheat into hexaploid wheat (http://maswheat.ucdavis.edu/protocols/Sr2/).

Spielmeyer *et al*. (2003) determined that a 120-bp allele of the microsatellite locus Xgwm533 was tightly linked to Sr2 in several lines. In present study, the same marker gave 23 polymorphic bands; ranging in 8 allelic variables with 100 to 300 bp. Accessions MPO 1215, 173A, GW173 and 1255 gave characteristic 120 bp band making them more resistant to rust than other aestivum accessions.

In genetic diversity study conducted by Sarkar *et al*. (2014) among 35 Indian bread wheat cultivars using 30 microsatellite (SSR) markers revealed moderate levels of polymorphism in the set of genotypes studied. Ninety alleles with an average of three alleles per locus were detected. PIC value ranged from 0.06 to 0.76 with an average of 0.45. The primers like Xgwm294, Xgwm146, Xgwm455, Xgwm497, Xgwm136 and Xgwm132 could be considered particularly informative, as they revealed four or more alleles per locus and displayed high PIC values. In the present study, primers Xgwm294, Xgwm146, Xgwm455, Xgwm497 and Xgwm132 scored PIC values 0.77, 0.89, 0.87, 0.76 and 0.66 respectively confirming the findings of Sarkar *et al*. (2014).

The analysis of diversity was also carried out using markers Xgwm 389, GWM 160, Xgwm 155, Xgwm 46 registering 0.57 (3 alleles), 0.35 (2 alleles), 0.86 (9 alleles), 0.83(10 alleles) PIC values respectively. The observations were coherent to the findings of Maccaferri *et al*. (2003). The microsatellite analysis revealed a progressive widening of the genetic basis in the elite durum wheat germplasm among 58 accessions covering a wide spectrum of genetic diversity of the cultivated durum wheat gene pool, which was characterized with 70 microsatellite loci. The results reflect the ability of markers used to differentiate between two durum accessions HI 8498 and GW 1 respectively; placing them in two separate clusters proving the inherent diversity in Genetic base.

Foessel *et al*. (2008) used SSR markers Xgwm146, Xgwm344, Xwmc276, and Xwmc273 for identification and molecular characterization of leaf rust resistance gene Lr14a in Durum Wheat. In the present study, markers Xgwm146 and GWM 273 registered PIC values of 0.89 and 0.81. However, maximum numbers of polymorphic bands were observed in GW1 and 273 with five different alleles falling in the same cluster during NTysis analysis.

Wide genetic base of duram accessions: The pooled data analysis of all the markers allowed us to conclude that most varieties are having a common genetic base and the results are coherent to the finding of Zala *et al*. (2014). The exceptions are two duram varieties placed in two different nodes signifying their inherent wide genetic base. The current investigation of 13 wheat genotypes also confirms the narrowing in genetic base among all accessions as coefficient of similarity ranges from 0.3 to 0.8. Durum accessions are still very dissimilar due to less amount of breeding programs focusing on these genotypes. However, more than eight aestivum accessions are falling in same cluster A.2 with similarity range 0.45 to 0.80. Lack in genetic diversity could lead to sudden decrease in yield in case of a pest or pathogen attack. Hence, new breeding programs have to be initiated with marker assisted breeding to create new ideotypes of wheat giving high resistance to pest and pathogens and high yield.

Acknowledgements
This work was supported by the Department of Science & Technology, Government of Gujarat, under Gujarat Genomics Initiative project.

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DOI: 10.5958/0975-928X.2017.00084.9

Table 1. List of wheat genotypes

S. No.	Wheat	Variety	*Genus*	*Species*
1	Durum	HI-8498	*Triticum*	*duram*
2	Bread Wheat	GW-11	*Triticum*	*aestivum*
3	Bread Wheat	GW-173	*Triticum*	*aestivum*
4	Bread Wheat	MPO1215	*Triticum*	*aestivum*
5	Bread Wheat	173-A	*Triticum*	*aestivum*
6	Bread Wheat	496	*Triticum*	*aestivum*
7	Durum	GW-1	*Triticum*	*duram*
8	Bread Wheat	A-115	*Triticum*	*aestivum*
9	Bread Wheat	LOK-1	*Triticum*	*aestivum*
10	Bread Wheat	273	*Triticum*	*aestivum*
11	Bread Wheat	1255	*Triticum*	*destivum*
12	Bread Wheat	322	*Triticum*	*aestivum*
13	Bread Wheat	1018	*Triticum*	*aestivum*

Table 2. List of primers used for SSR amplification

SSR marker	Primer Sequence 5' to 3'	No. of bases	Chromosomal position
Xgwm497 F	GTAGTGAAGACAAGGGCATT	20	1A
Xgwm497 R	CCGAAAGTTGGGTGATATAC	20	
Xgwm389 F	ATCATGTCGATCTCCTTGACG	21	3B
Xgwm389 R	TGCCATGCACATTAGCAGAT	20	
Xwmc617B F	CCACTAGGAAGAAGGGGAAACT	22	4B
Xwmc617B R	ATCTGGATTACTGGCCAACTGT	22	
GWM 273 F	ATTGGACGGACAGATGCTTT	20	1B
GWM 273 R	AGCAGTGAGGAAGGGGATC	19	
GWM 160 F	TTCAATTCAGTCTTGGCTTGG	21	4A
GWM 160 R	CTGCAGGAAAAAAAGTACACCC	22	
Xgwm294 F	GGATTGGAGTTAAGAGAGAACCG	23	2A,4A
Xgwm294 R	GCAGAGTGATCAATGCCAGA	20	
Xgwm455 F	ATTCGGTTCGCTAGCTACCA	20	2D
Xgwm455 R	ACGGAGAGCAACCTGCC	17	
Xgwm192 F	GGTTTTCTTTCAGATTGCGC	20	5D
Xgwm192 R	CGTTGTCTAATCTTGCCTTGC	21	
Xgwm146 F	CCAAAAAAACTGCCTGCATG	20	7B
Xgwm146 R	CTCTGGCATTGCTCCTTGG	19	
Xgwm155 F	CAATCATTTCCCCCTCCC	18	3A
Xgwm155 R	AATCATTGGAAATCCATATGCC	22	
Xgwm132 F	TACCAAATCGAAACACATCAGG	22	6B,6D
Xgwm132 R	CATATCAAGGTCTCCTTCCCC	21	

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Table 2. Contd.,

SSR marker	Primer Sequence 5' to 3'	No. of bases	Chromosomal position
Xgwm533 F	AAGGCGAATCAAACGGAATA	20	3B,3D
Xgwm533 R	GTTGCTTTAGGGGAAAAGCC	20	
Xgwm174 F	GGGTTCCTATCTGGTAAATCCC	22	5D
Xgwm174 R	GACACACATGTTCCTGCCAC	20	
Xgwm46 F	GCACGTGAATGGATTGGAC	19	7B
Xgwm46 R	TGACCCAATAGTGGTGGTCA	20	
csSr2 F	CAAGGGTTGCTAGGATTGGAAAAC	24	3B
csSr2 R	AGATAACTCTTATGATCTTACATTTTTCTG	30	
Xwmc580 F	AAGGCGCACAACACAATGAC	20	6A
Xwmc580 R	GGTCTTTTGTGCAGTGAACTGAAG	24	
Xbarc4 F	GCGTGTTTGTGTCTGCGTTCTA	22	5D
Xbarc4 R	CACCACACATGCCACCTTCTTT	22	
Xgwm111 F	TCTGTAGGCTCTCTCCGACTG	21	7B
Xgwm111 R	ACCTGATCAGATCCCACTCG	20	
Xgwm382 F	GTCAGATAACGCCGTCCAAT	20	2D
Xgwm382 R	CTACGTGCACCACCATTTTG	20	
Xgwm371 F	GACCAAGATATTCAAACTGGCC	22	5B
Xgwm371 F	AGCTCAGCTTGCTTGGTACC	20	
Xbarc267 F	GCGTGCTTTTTATTTTTGTGGACATCTT	28	7B
Xbarc267 R	GCGAATAATTGGTGGGTGAAACA	23	
Xbarc77 F	GCGTATTCTCCCTCGTTTCCAAGTCTG	27	3B
Xbarc77 R	GTGGGAATTTCTTGGGAGTCTGTA	24	
Xgwm265 F	TGTTGCGGATGGTCACTATT	20	2A,4A
Xgwm265 R	GAGTACACATTTGGCCTCTGC	21	
Xgwm191 F	AGACTGTTGTTTGCGGGC	18	2B
Xgwm191 R	TAGCACGACAGTTGTATGCATG	22	
Xgwm219 F	GATGAGCGACACCTAGCCTC	20	6B
Xgwm219 R	GGGGTCCGAGTCCACAAC	18	

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Table 3. Integrity analysis of genomic DNA

S. No	Genotype	Ratio 260/230	Ratio 260/280	Concentration (ng/µl)
1	HI-8498	1.79	1.84	1878.6
2	GW-11	2.24	1.63	763.8
3	MPO-1215	1.58	1.85	1010.6
4	GW-173	1.58	1.77	1282.5
5	173-A	2.05	2.02	388.9
6	496	1.97	2.01	240
7	GW-1	2.04	1.88	96.9
8	A-115	1.54	1.85	1052.9
9	LOK-1	2.28	2	617.3
10	273	2.25	2	1933.5
11	1255	2.09	1.95	2157.2
12	322	2.26	1.92	2157.2
13	1018	1.87	1.89	1502.7

Table 4. List of primers used for SSR amplification, total number of loci, the level of polymorphism and PIC value

Primers	Range of band size	Total no. of loci	No of polymorphic loci	PIC value
csSr2	300	6	1	0
GWM160	190 – 280	17	2	0.36
Xgwm192	150 – 1000	8	3	0.406
Xbarc267	130 – 200	13	2	0.473
Xgwm389	120 – 150	11	3	0.579
Xgwm371	130 - -200	11	3	0.628
Xgwm191	110 – 200	19	3	0.654
Xgwm132	100 – 200	18	4	0.667
Xbarc4	120 – 600	22	6	0.756
Xgwm497	100 – 170	19	5	0.765
Xgwm265	100 – 280	42	6	0.772
Xwmc617B	100 – 800	41	5	0.772
Xgwm294	100 – 130	25	7	0.778
GWM273	150 – 600	41	8	0.817
Xwmc580	110 – 500	30	8	0.822
Xgwm46	130 – 400	29	10	0.839
Xgwm219	120 – 1000	37	10	0.843
Xgwm533	100 – 300	23	8	0.847
Xgwm111	120 – 500	37	8	0.855
Xgwm174	130 – 1000	30	9	0.86
Xbarc77	110 – 600	39	10	0.861
Xgwm155	100 – 300	15	9	0.862
Xgwm382	100 – 200	13	9	0.864
Xgwm455	120 – 1000	65	11	0.873
Xgwm146	150 – 1000	42	11	0.899

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Fig. 1. Gel image representing polymorphism of Xgwm146 marker in 13 commercial accessions of wheat

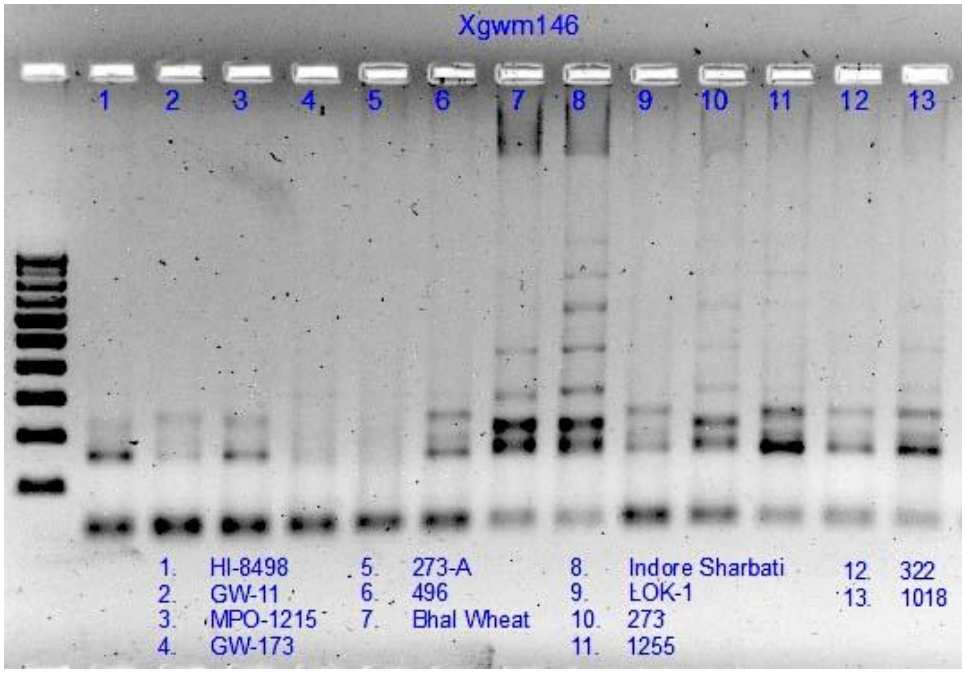

Fig. 2. Dendogram depicting clustering of 13 Wheat genotypes based on SSR profiles of 25 Markers