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Molecular marker-based characterization and genetic diversity of wheat genotypes in relation to Boron use efficiency

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Abstract

Boron deficient soils pose a serious problem to world wheat production including Bangladesh. In the present study, 21 diverse wheat (*Triticum aestivum* L.) genotypes were subjected to SSR analysis to identify and characterize boron efficient varieties. In the DNA profiling of the 21 genotypes, including two control varieties, Fang 60 (efficient) and SW 41 (inefficient), using 32 SSR loci, a total of 234 alleles were detected. Allele number per locus ranged from 3 to 11, with an average of 7.312, and the PIC value ranged from 0.562 to 0.873 with an average of 0.776. Average genetic diversity over all SSR loci for the 21 genotypes was 0.804, ranging from 0.637 to 0.884. All the loci were polymorphic and clearly distinguished the genotypes. Cluster analyse (NJ tree, UPGMA, PCO) identified a similar pattern of variation. The study found that INIA 66 and BAW1086 were the most boron efficient genotypes and thus could be used for developing boron efficient varieties.

Key words: Wheat, genetic diversity, SSR markers, boron deficiency

Introduction

Genetic diversity represents the heritable variation within and between populations of organisms [1]. Knowledge of the genetic diversity and population structure within germplasm collections is an important foundation for crop improvement [2]. Progress in plant breeding requires a broad genetic base with a rich and diverse germplasm collection being the backbone of every successful crop improvement program. Recent advances in molecular biology have created new opportunities for evaluating and characterizing germplasm collections beyond the traditional phenotypic limits within a biological species or genus. The molecular marker technology is one possible approach to understand the diversity within a species. Several molecular marker systems including restriction fragment length polymorphisms (RFLP) [3], random amplified polymorphic DNA (RAPD) [4], simple sequence repeats (SSRs) [5, 6], inter-simple sequence repeats (ISSRs) [7], amplified fragment length polymorphism (AFLP) [7, 8], and single nucleotide polymorphisms (SNPs) [9] 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. Common wheat (*Triticum aestivum* L.) is a self-pollinating polyploids crop that has been bred for a wide array of specific end-use quality traits and various adaptive characteristics, resulting in the development of distinct cultivars tailored to specialized end uses and specific production environments. Boron deficiency in wheat is a critical problem for wheat production in Bangladesh and other regions with high rainfall including many areas of the subtropics [10]. Boron deficiency is common in the light soils of Bangladesh [11]. Boron requirements are well known to vary among plant species and also among genotypes within a species [12]. Wheat varieties are well known to differ widely in their sensitivity to boron uptake as well as to boron deficiency [13]. Jamjod et al. [11] reported two genes, Bo_d1 and Bo_d2 that could account for genotypic variation in response to boron in wheat genotypes. A boron efficient wheat variety (such

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as Fang 60) may have greater ability to accumulate boron from the growing medium than a boron inefficient variety (such as SW 41) thereby contributing to reproductive development e.g. pollen viability [14].

Recently, microsatellites or simple-sequence repeats (SSRs) have become the marker system of choice owing to their abundance in the genomes, hypervariability, co-dominance, and high reproducibility [15]. SSRs are suitable for diversity study particularly in cereals and appear to be more informative in wheat than any other marker system [16-19]. The present investigation was designed to evaluate 21 diverse wheat genotypes by 32 SSR markers to identify the most efficient boron genotypes, and to assess their diversity at the molecular level so that they can be best utilized in developing boron efficient cultivars.

Materials and methods

Plant material

In the current investigation 21 diverse wheat genotypes comprising of both cultivated varieties and advanced lines were used. The genotypes were collected from the Wheat Research Center (WRC), Dinajpur, Bangladesh (Table 1) and were already categorized based on boron use efficiency.

DNA extraction

Fresh leaves from 18-day old seedlings were used for DNA extraction followed by CTAB mini-prep method [20]. The DNA samples were analyzed both qualitatively and quantitatively using a spectrophotometer and 0.8% agarose gel electrophoresis.

Table 1. Wheat genotypes, country of origin and their pedigree classification.

No.	Genotype	Origin	Pedigree	Status of Boron efficienty(#)
1	Barkat	Bangladesh	BB/GLL//CARP/3/PVN-CM 33483-C-7M-1Y-OM-OJO	MBE^{+}
2	Sonalika	Bangladesh	1154-388/AN/3/YT54/N1OB//LR64-II 18427-4R-1M	BE^{-}, $MBE^{\&}$
3	Sourav	Bangladesh	NAC/VEE-CM 64224-5Y-1M-1Y-2M-0Y	$BE^{\&}$
4	Kanchan	Bangladesh	UP301/C306-1187-1-1P-5P-5JO-OJO	mBE^{-}, $BE^{\&}$
5	Shatabdi	Bangladesh	MRNG/BVC//BLO/PVN/3/PJB-81-CM98472-1JO-0JO-0O-1JO-0JO-0R2DI	BI^{-}, $MBI^{\&}$
6	Prodip	Bangladesh	G. 162/BL1316//NL 297-NC2055-4B-020B-020B-4B-0B	MBE^{-}, $BE^{\&}$
7	Protiva	Bangladesh	UP301/C306-1187-1-1P-5P-5JO-OJO-1	MBI^{-}, $BE^{\&}$
8	Kalyansona	Bangladesh	PJ/GB55-II 8156	MBI^{+}
9	Sufi	Bangladesh	KAN/6/COQ/F61.70//CNDR/3/OLN/4/PHO/5/MRGN/ALDAN//CNO-BD(JE)349-X-0JE-9DI-10HR	MBE^{+}
10	Bijoy	Bangladesh	NL297*2/LR25	MBE^{+}
11	BAW 1027	Bangladesh	NL 297*3/NANZING7840	MBE^{-}, $BE^{\&}$
12	BAW 1047	Bangladesh	RAWAL87//BUC/BJY-BI 94495-2JO-010JO-010JO-010JO-0DI	MBE^{-}, $BE^{\&}$
13	BAW 1045	Bangladesh	FANG60//RL6043/4*NAC-BD(DI) 94556-0DI-10JE-0JE-0JE-0JE-0DI	MBE^{+}
14	BAW 1051	Bangladesh	KLAT/SOREN//PSN/3/BOW/4/VEE#5.10/5/CNO67-NC2142-7B-020B-025B-3B-0B	BE^{+}
15	BAW 1059	Bangladesh	ZSH12/HLB19//2*NL297	MBE^{-}, $BE^{\&}$
16	BAW 1086	Bangladesh	SW89.5214*2/FASAN-CMBW910Y3050F-030TOPM-2Y-010M-010Y-010M-0Y-0M-1PR	BE^{+}
17	HP 1724	India	Unknown	MBE^{+}
18	Seri 82	Mexico	KVZ/BUHO//KAL/BB-CM33027-F-15M-500Y-0M-87B-0Y-0BGD	BE^{+}
19	INIA 66	Mexico	LR 64/SN64-III9008-83M-100Y-100M-100Y-100C-0MEX	MBE^{-}, $BE^{\&}$
20	Fang 60	Thailand	PI/FD/3/PI/MZ//MXP-PK2858-7A-3A-4A-0A	MBE^{-}, $BE^{\&}$
21	SW 41	Thailand	Unknown	MBI^{-}

BI = Boron Inefficient (<70% Boron efficiency); MBI = Moderately Boron Inefficient (71-80% Boron efficiency); MBE = Moderately Boron Efficient (81-90% Boron efficiency); BE = Boron efficient (>90% Boron efficiency); & = Result based on field experiemnt; – = Result based on sand culture experiment; + = Result from both field and sand culture experiment; # = Johiruddin M, 2008.

SSR marker genotyping

Primer pairs were collected from USDA-ARS, University of Missouri, USA and Department of Soil Science, Bangladesh Agricultural University, Mymensingh. Thirty-two selected SSR primers (along with three Barley primers) were used for survey. The total PCR reaction volume was 13 µl, composed of 2.0 µl genomic DNA, 1.5 µl 10X PCR buffer (Tris with 15 mM $MgCl_2$, Conc. 10X), 0.75 µl dNTPs (Contains dCTP, dGTP, dTTP and dATP all in the conc. of 10 mM)), 1.0 µl forward primer, 1.0 µl reverse primer, 0.5 µl Taq DNA polymerase (conc. 5 U/µl) and 8.25 µl sterile deionized water. Samples were subjected to the following thermal profile for amplification in a thermocycler: after the initial 7 min at 95°C, SSR marker amplification comprised 10–15 touchdown cycles of 94°C for 30 s, annealing for 30 s, decreasing the temperature by 0.5°C per cycle until the specified annealing temperature was reached, and 72°C for 30 s. This was then followed by 25–35 cycles of amplification with the specified annealing temperature, and a final extension at 72°C for 10 min. After amplification, the PCR tube was stored at 4°C until electrophoresis. Visualization of amplification products were accomplished on a 3% agarose gel in 0.5 X TBE buffer. The agarose gels were stained with ethidium bromide solution for 20-25 min. The stained agarose gel was illuminated by UV-trans-illuminator and photographed for assessing the DNA profiles. Only two gel pictures have been given in this paper to represent allelic variation at DNA level.

Analysis of SSR data

Molecular weights for microsatellite products, in base pairs, were estimated with AlphaEaseFC 4 software (Unknown Publisher). The summary statistics including the number of alleles per locus, major allele frequency, genetic diversity and polymorphism information content (PIC) values were determined using POWER MARKER version 3.23, a genetic analysis software [21]. Allele molecular weight data were also used to determine the genetic distance for phylogeny reconstruction based on the neighbor-joining method [22] as implemented in POWER MARKER with the tree viewed using TREEVIEW [23]. The allele frequency data from POWER MARKER was used to export the data in binary format (allele presence = “1” and allele absence = “0”) for analysis with NTSYS-PC version 2.1 [24]. The 0/1 matrix was used to calculate genetic similarity as DICE coefficient [25] using SIMQUAL subprogram and the resultant similarity matrix was employed to construct dendrograms using Sequential Agglomerative Hierarchical Nesting (SAHN) based Unweighted Pair Group Method of Arithmetic Means (UPGMA) as implemented in NTSYS-PC (version 2.1) [24] to infer genetic relationships and phylogeny. For estimating the similarity matrix, null alleles were treated as missing data to reduce the biased genetic or similarity measures [26].

Results and discussion

Overall SSR diversity

The 21 wheat genotypes were evaluated using 32 SSR markers selected on the basis of their known genetic locations to give a uniform coverage for the chromosomes in the three wheat genomes (A, B and D) and chromosome H in barley (*Hordeun vulgare* L.) known to be influencing boron efficiency. A total of 234 alleles were detected at 32 loci (Table 2). A wide range of allelic variants was observed for each locus. The number of alleles per locus ranged from 3 alleles (EBmac0679) to 11 alleles (WMC332 and Xgwm296) across the 32 loci. The PIC values ranged from a minimum of 0.56 (EBmac0679) to a maximum of 0.87 (WMC332), with an average of 0.78 (Table 2).

The number of alleles per locus ranged from 3 to 11, with an average of 7.3 alleles across the 32 loci, which agrees with earlier [29, 30] results. The PIC values recorded in this study are significantly higher than the PIC values reported from other studies [17, 16, 29, 30], but Uddin and Boerner [32] found similar observations.

Markers WMC332 and Xgwm296 produced the highest number of alleles (11), and also showed the maximum PIC values (0.87 and 0.85, respectively).

A genotype was assigned a null allele for an SSR locus whenever an amplification product(s) was not detected for the particular genotype × marker combination. Of the 32 SSR loci used in this study, on an average 4 had null alleles in one to twelve of the 16 accessions. The locus that showed the highest frequency of null alleles was BARC182 (nulls detected in twelve genotypes). In the case of null alleles, PCR amplifications were repeated to exclude the possibility that a failed PCR reaction could be the cause of the null allele. Null alleles can arise from point mutation(s) in one or both of the primer binding sites and thereby inhibiting primer annealing [27]. Multiple alleles (2 or 3 alleles per locus) were detected at one or more loci per accession even in standard varieties such as Fang 60 and SW 41 (Figs. 4&5). Accessions with 2 alleles per locus were identified when two different bands had the

same intensity. Whenever the two bands had different intensities, the stronger band was always considered for analysis. Five different SSR markers produced multiple alleles with 3 bands per locus, ranging from 1 (Xgwm192) to 7 (WMC357, Xgwm533.1 and Xgwm642) accessions with the most intense band was considered as the variety norm. The average percentage of the high frequency alleles was 28.84%. It ranged from 14.29% (WMC296) to 47.62% (EBmac0679). Markers WMC332 and Xgwm296 produced the highest number of alleles (11), and also showed the maximum PIC values (0.87 and 0.85, respectively).

Table 2. Allelic variation of the 32 simple sequence repeat (SSR) loci in the 21 wheat accessions surveyed.

Sl.	SSR marker	Chromo-some	Repeat type	Annealing temp. (^{0}C)	Allele no.	Size range (bp)	No. of null	Allele with high frequency Size (bp)	Frequency (%)	PIC value
1	BARC32	5B, 7B	(ATT)10	52	4	170-184	9	179	41.67	0.6218
2	BARC123	6D	(CA)9	52	9	171-211	-	185	23.81	0.8361
3	BARC182	7B	(CT)15	58	4	100-109	12	103	33.33	0.6932
4	Bmac0093	2H	(AC)24	55	8	60-83	-	60, 78	23.81	0.8028
5	Bmag0603	3H	(AG)24	55	7	175-207	-	195, 201	23.81	0.7908
6	EBmac0679	4H	(AC)22	55	3	40-44	-	44	47.62	0.5629
7	WMC31	1B, 1D	(GT)19	61	9	53-81	-	76	23.81	0.8305
8	WMC112	2D	(CT)34	61	8	109-139	-	118	38.10	0.7667
9	WMC116	7A	(CT)12	61	10	79-109	2	81	21.05	0.8629
10	WMC208	5B	(GT)27	61	8	75-103	2	77	36.84	0.7689
11	WMC245	2B, 2D	(CA)10	61	9	105-136	-	129	28.57	0.8268
12	WMC276	7B	(CA)19	51	5	42-53	-	46, 48	33.33	0.6641
13	WMC296	2A	(GT)28	61	9	73-106	-	73,100,103,106	14.29	0.8674
14	WMC331	4D	(CA)13	61	7	103-126	1	123	25.00	0.8018
15	WMC332	2B	(CT)12	61	11	83-140	-	113	19.05	0.8734
16	WMC357	5D	(GT)10	61	6	121-145	4	137, 141	29.41	0.7407
17	WMC537	5B	(CT)10	51	6	72-83	1	76	35.00	0.7297
18	WMC581	7B	(GT)8	61	8	77-97	-	95	28.57	0.7982
19	Xgwm46	7B	(GA)2GC(GA)33	60	5	83-100	-	86	33.33	0.7293
20	Xgwm47	2A, 2B	(CT)7TT(CT)16	60	7	81-137	6	97	33.33	0.7526
21	Xgwm146	7B	(GA)5GG(GA)20	60	8	38-59	-	44	28.57	0.8038
22	Xgwm192	5D	(CT)46	60	7	45-80	1	47, 77, 80	20.00	0.8069
23	Xgwm193	6B	(CT)24imp(CA)8	60	5	37-48	2	40	31.58	0.7261
24	Xgwm264	1B, 3B	(CA)9A(CA)24	60	7	107-130	5	121	31.25	0.7696
25	Xgwm296	2D, 2A	(CT)28	55	11	114-165	1	130	25.00	0.8518
26	Xgwm358	5D	(GA)18(G)2(GA)4	55	7	70-88	-	85	28.57	0.7862
27	Xgwm471	7A	(CA)34	60	6	176-208	6	208	26.67	0.7813
28	Xgwm493	3B	(CA)43imp	60	9	71-97	5	73	25.00	0.8352
29	Xgwm533.1	3B	(CT)18(CA)20	60	7	103-129	1	107	30.00	0.7727
30	Xgwm577	7B	(CA)14(TA)6	55	10	94-139	1	100	30.00	0.8299
31	Xgwm635	7A, 7D	(CA)10(GA)14	60	7	55-74	-	69, 72	23.81	0.7908
32	Xgwm642	1D	(GT)14	60	7	103-135	-	110	28.57	0.7862
Mean					7		4		28.84	0.7769

*PIC, polymorphism information content

According to Nei's [22] the highest level of genetic diversity value (0.88) was observed with loci WMC332, the lowest level of genetic diversity value (0.64) was observed with loci EBmac0679, and a mean diversity of 0.80 was observed. It was noted that a marker detecting a lower number of alleles also showed lower genetic diversity, compared to markers detecting a higher number of alleles, which revealed higher levels of genetic diversity. The maximum number of repeats within the SSRs was also positively correlated with the genetic diversity.

Genetic similarity analysis using UPGMA

This study used UPGMA cluster analysis based on genetic similarity values for SSR alleles from all the wheat accessions to construct a dendrogram. Results from this analysis (Fig. 1) showed two major cluster groups (Group I and II). The cluster analysis showed significant genetic variation among the wheat accessions studied, with a similarity coefficient varying between 0.11 and 0.37. The first group clustered with 62% of accessions, which consisted of boron inefficient and/or moderately inefficient genotypes, while the second group included 38% of the accessions related to the boron efficient and/or moderately efficient genotypes (Table 1). In this study, the SSRs clearly illustrated the well-documented differentiation that was reflected by subspecies-specific alleles at all the loci studied. Cluster I consisted of all Bangladesh-released varieties and few advanced lines along with the accession of SW 41 (boron inefficient variety). The few popular varieties such as Sourav, Shatabdi and Prodip were very close to SW 41, which is also boron inefficient. Other Bangladesh varieties included in cluster I were Sonalika, Kanchan, Bijoy, and Sourav, which perform moderately inefficient in their field performance on boron soils. The Cluster II was composed of the genotypes Fang 60 (known boron efficient variety), INIA 66, Seri 82, HP1724, and Bangladesh advanced lines BAW1045, BAW1059, BAW1051, BAW1086 (Fig. 1; Table 2). In Cluster II, INIA 66 and BAW1086 were close to Fang 60 in the dendrogram and are more boron efficient than the other evaluated germplasm.

The genetic distance-based results observed in the unrooted neighbor-joining tree revealed three major germplasm groups and agreed with genetic similarity analysis using UPGMA (Fig. 2). By using predefined international accessions to assign identities to each group, the first group included the known boron inefficient variety SW 41 along with some boron inefficient advanced lines such as BAW1027, BAW1047. The second group corresponded to the varieties INIA 66, Seri 82, HP1724 and advanced lines BAW1045, BAW1051, BAW1059, BAW1086 along with the known boron efficient variety Fang 60. The third

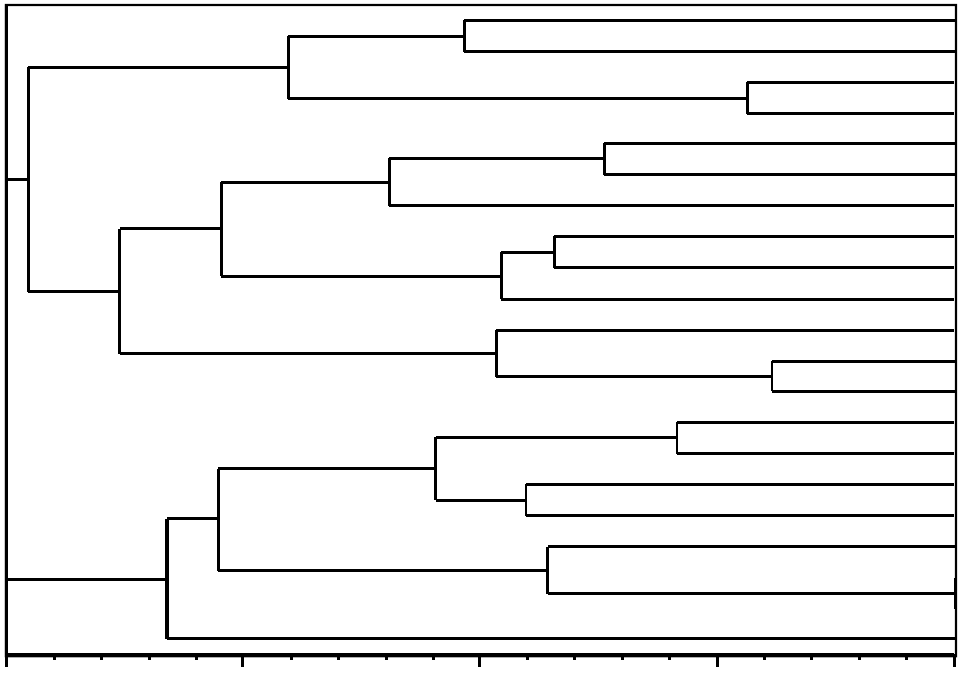

Fig. 1. Dendrogram of 21 wheat genotypes showing the genetic similarity based on 234 alleles detected by 32 SSRs using UPGMA cluster analysis

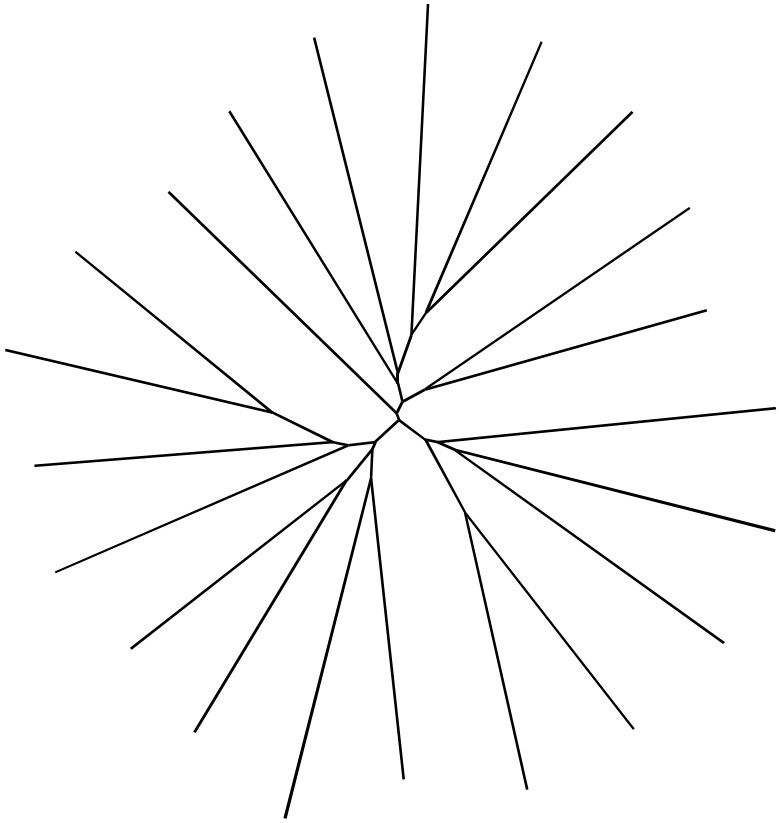

Fig. 2. An unrooted neighbor-joining tree showing the genetic relationship between the 21 wheat genotypes based on 32 SSRs

and smaller group contained Bangladesh varieties such as Kanchan, Protiva, Sourav etc., which are moderately boron efficient and fall between efficient and inefficient group (Fig. 2).

Principle coordinate analysis of the 21 genotypes also produced clear distinctions between the boron efficient and inefficient group. In PCO analysis, the genotypes clustered into three distinct groups: first, the inefficient group which included SW 41, which is a known inefficient variety with the Bangladeshi varieties Shatabdi, Prodip, Kalyansona and Bijoy and the second group included Fang 60 (known boron efficient variety) along with INIA 66, BAW1051, BAW1086 and Seri 82. The cultivars Sourav, Sonalika, Protiva, Kanchan, Sufi and BAW1027 were located between efficient and inefficient groups (Fig. 3). The grouping thus agreed with the Bangladesh field performance results [34-36].

The values of pair-wise comparisons of Nei's [28] genetic distance (D) between the genotypes analyzed were computed from combined data for 32 primers. The pairwise distance ranged from 0.61 to 1.00 (Table 3). A comparatively higher genetic distance (1.00) was observed between Fang 60 and SW 41. This indicated that genetically they are diverse compared to those having a lower genetic distance value. Basically this value is an indication of their genetic dissimilarity, since Fang 60 is a known boron efficient variety and SW 41 is a known inefficient variety. On the other hand, the lowest genetic distance (0.061) was found between BAW1086 and INIA 66 indicating that they are much closer in their genetic make-up. Though INIA 66 is an Indian variety (originally from CIMMYT in Mexico), and BAW1086 is a Bangladesh advanced line, their genetic distance revealed that there is a close genetic relationship between them.

The results of this study may be finally tuned through utilization of more informative markers and more diverse set of genotypes. By cluster analysis, Ahmad [37] showed that it is possible to both assess genetic diversity of elite genotypes in wheat and select genotypes with higher genetic diversity using SSRs.

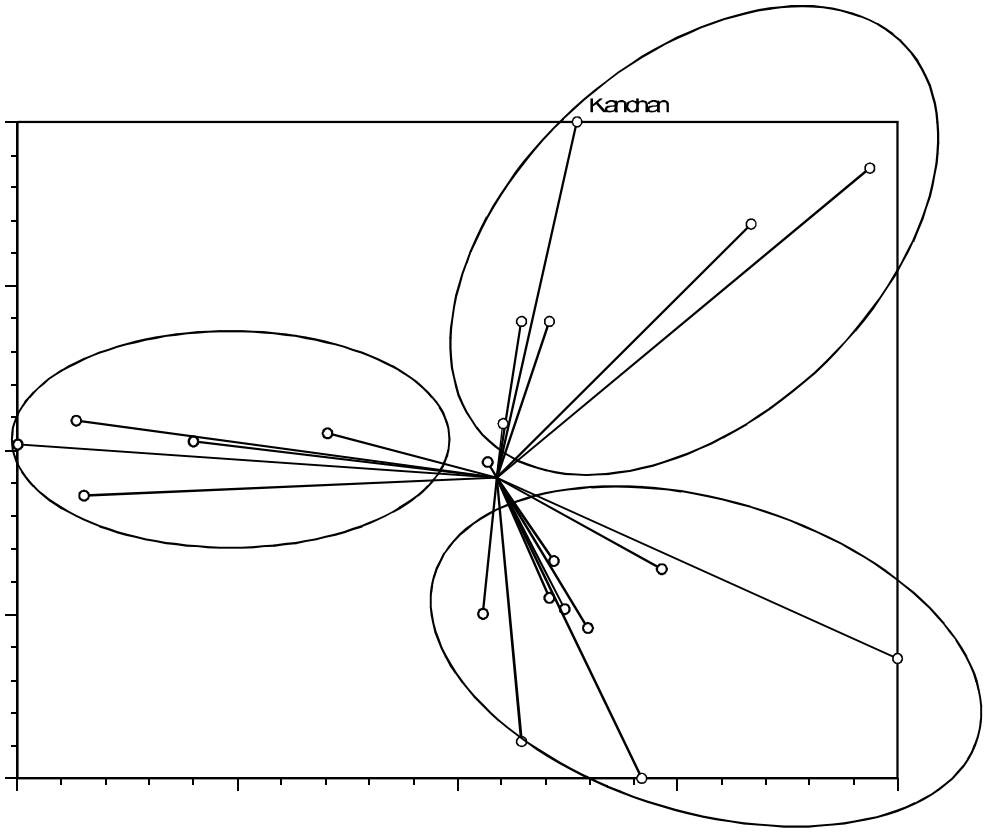

Fig. 3. PCO analysis of 21 wheat genotypes based on SSR data of the 32 loci

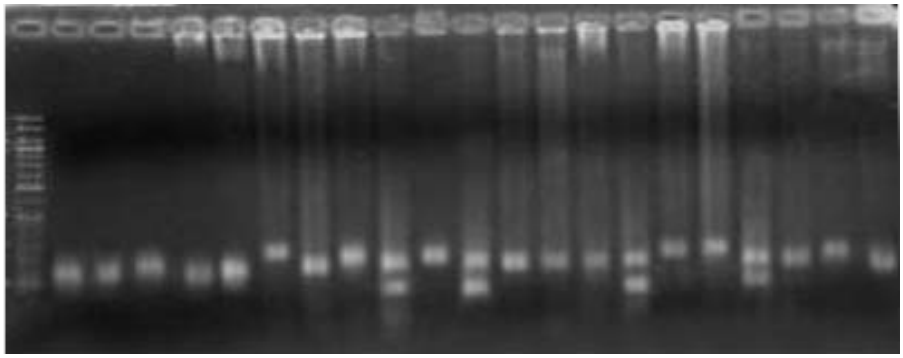

Fig. 4. Banding pattern of WMC 112 primer in 21 wheat genotypes

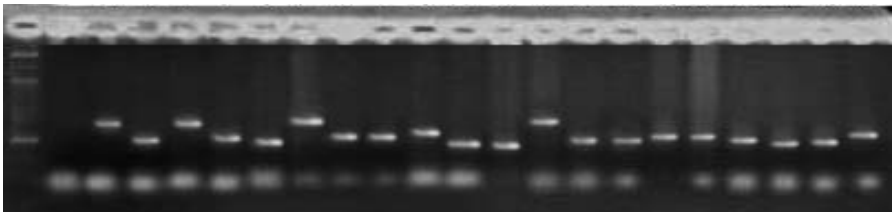

Fig. 5. Banding pattern of Xgwm 577 primer in 21 wheat genotypes

Table 3. Genetic distance of wheat genotypes based on 32 microsatellite alleles.

OTU	Barkat	BAW 1027	BAW 1045	BAW 1047	BAW 1051	BAW 1059	BAW 1086	Bijoy	Fang 60	HP 1724	INIA 66	Kalyan-sona	Kan-chan	Prodip	Protiva	Seri 82	Shata-bdi	Sona-lika	Sourav	Sufi	SW41
Barkat	*	0.8889	0.9167	0.8214	0.8400	0.8333	0.8462	0.8621	0.8929	0.9200	0.9286	0.8929	0.7931	0.8571	0.8333	0.9259	0.8621	0.7586	0.8214	0.7692	0.8929
BAW1027	0.8889	*	0.7083	0.7037	0.8148	0.8462	0.8519	0.7931	0.8571	0.8462	0.8966	0.7500	0.7931	0.9286	0.7826	0.7857	0.7586	0.8214	0.8966	0.6923	0.8966
BAW1045	0.9167	0.7083	*	0.8750	0.9167	0.6522	0.8400	0.8519	0.8333	0.6818	0.8400	0.7407	0.9231	1.0000	0.7826	0.7200	0.8889	0.8800	0.8800	0.6923	0.8800
BAW1047	0.8214	0.7037	0.8750	*	0.7600	0.7917	0.7692	0.7586	0.8571	0.8000	0.7857	0.7857	0.9655	0.8571	0.9583	0.8519	0.8276	0.8966	0.8929	0.8462	0.8571
BAW1051	0.8400	0.8148	0.9167	0.7600	*	0.8519	0.7407	0.8571	0.8462	0.7083	0.7037	0.9259	0.9259	0.9259	0.9167	0.7407	0.8214	0.8462	0.8519	0.8462	0.8889
BAW1059	0.8333	0.8462	0.6522	0.7917	0.8519	*	0.8077	0.9259	0.8000	0.7826	0.8462	0.9615	0.9231	0.8846	0.8696	0.7692	0.8889	0.9200	0.8077	0.8000	0.8462
BAW1086	0.8462	0.8519	0.8400	0.7692	0.7407	0.8077	*	0.8276	0.8519	0.7200	0.6071	0.8571	1.0000	0.8571	0.8750	0.8519	0.9310	0.9259	0.9630	0.9630	0.8929
Bijoy	0.8621	0.7931	0.8519	0.7586	0.8571	0.9259	0.8276	*	0.9310	0.8846	0.9000	0.7742	0.8387	0.8333	0.9615	0.8276	0.7813	0.9000	0.7333	0.7241	0.7333
Fang60	0.8929	0.8571	0.8333	0.8571	0.8462	0.8000	0.8519	0.9310	*	0.8846	0.8621	0.9286	0.9310	0.9643	0.8333	0.7500	1.0000	0.8276	0.8929	0.8846	1.0000
HP1724	0.9200	0.8462	0.6818	0.8000	0.7083	0.7826	0.7200	0.8846	0.8846	*	0.8462	0.9200	0.8846	0.8400	0.9091	0.7200	0.8846	0.8846	0.9615	0.7917	0.9231
INIA 66	0.9286	0.8966	0.8400	0.7857	0.7037	0.8462	0.6071	0.9000	0.8621	0.8462	*	0.9310	0.9333	0.9655	0.9583	0.8276	0.9667	0.9310	0.8966	0.8889	0.9000
Kalyansona	0.8929	0.7500	0.7407	0.7857	0.9259	0.9615	0.8571	0.7742	0.9286	0.9200	0.9310	*	0.9000	0.8276	0.8000	0.9643	0.7097	1.0000	0.8621	0.8571	0.8966
Kanchan	0.7931	0.7931	0.9231	0.9655	0.9259	0.9231	1.0000	0.8387	0.9310	0.8846	0.9333	0.9000	*	0.8333	0.6400	0.8276	0.9032	0.7667	0.8667	0.8571	0.9000
Prodip	0.8571	0.9286	1.0000	0.8571	0.9259	0.8846	0.8571	0.8333	0.9643	0.8400	0.9655	0.8276	0.8333	*	0.8800	0.9286	0.7667	0.9655	0.9655	0.8148	0.7241
Protiva	0.8333	0.7826	0.7826	0.9583	0.9167	0.8696	0.8750	0.9615	0.8333	0.9091	0.9583	0.8000	0.6400	0.8800	*	0.8333	0.7692	0.8000	0.9167	0.8750	0.9583
Seri82	0.9259	0.7857	0.7200	0.8519	0.7407	0.7692	0.8519	0.8276	0.7500	0.7200	0.8276	0.9643	0.8276	0.9286	0.8333	*	0.8966	0.8214	0.8571	0.7778	0.9310
Shatabdi	0.8621	0.7586	0.8889	0.8276	0.8214	0.8889	0.9310	0.7813	1.0000	0.8846	0.9667	0.7097	0.9032	0.7667	0.7692	0.8966	*	1.0000	1.0000	0.9310	0.6667
Sonalika	0.7586	0.8214	0.8800	0.8966	0.8462	0.9200	0.9259	0.9000	0.8276	0.8846	0.9310	1.0000	0.7667	0.9655	0.8000	0.8214	1.0000	*	0.7586	0.8148	0.9310
Sourav	0.8214	0.8966	0.8800	0.8929	0.8519	0.8077	0.9630	0.7333	0.8929	0.9615	0.8966	0.8621	0.8667	0.9655	0.9167	0.8571	1.0000	0.7586	*	0.7407	0.9310
Sufi	0.7692	0.6923	0.6923	0.8462	0.8462	0.8000	0.9630	0.7241	0.8846	0.7917	0.8889	0.8571	0.8571	0.8148	0.8750	0.7778	0.9310	0.8148	0.7407	*	0.7778
SW41	0.8929	0.8966	0.8800	0.8571	0.8889	0.8462	0.8929	0.7333	1.0000	0.9231	0.9000	0.8966	0.9000	0.7241	0.9583	0.9310	0.6667	0.9310	0.9310	0.7778	*

This finding clearly demonstrated the reliability, usefulness, and efficiency of SSRs in analyzing genomic diversity. In previous studies, wheat genotypes from the same origin were analyzed using different DNA marker systems, which produced genetic diversity or similarity levels within a specific group of genotypes [38-40]. Thus, it should be possible to establish a collection of highly polymorphic SSRs for genetic diversity studies, cultivar identification, and plant variety protection in Bangladesh wheat improvement programs.

A previous study [36] showed that the variety Sourav is boron efficient. The results of the present study suggested that Sourav is only moderately efficient, while INIA 66 and BAW1086 are the most efficient cultivars and should be used as parental breeding material for developing boron efficient varieties for production around the world as well as in Bangladesh.

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