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MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION IN DIVERSE GENOTYPES OF BARLEY (*HORDEUM VULGARE* L.)

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ABSTRACT
In this study, we used a set of mapped SSRs to survey twenty five barley genotypes obtained from Institute of Agricultural science, BHU. The objectives were to determine (1) to study the potential of microsatellite markers in general and specific SSRs in particular for detection of polymorphism and for genotype identification and (2) to assess the level of microsatellite- based genetic diversity among 25 elite barley genotypes that were potentially useful in barley breeding programmes. A total of 38 alleles were identified at 12 SSR loci in 25 genotypes. The number of alleles per locus ranged from 1 to 21, with an average of 5.92. The number of polymorphic bands per primer ranged from 2 to 4 with an average of 3.16.The PIC of the 12 SSR primers ranged from 0.288 (Ebmac0805) to .998 (Ebmac635) with an average PIC of 0.706. The cluster analysis indicates a high level of diversity between the genotypes. It also shows a lower level of diversity between some genotype. Our results demonstrate that this set of SSRs was highly informative and was useful in generating a meaningful classification of the germplasm that we sampled and therefore making ease in selection of parent for improvement.

INTRODUCTION

Knowledge regarding the amount of genetic variation in germplasm arrays and genetic relationships between genotypes are important considerations for efficient conservation and utilization of germplasm resources (Kresovich *et al*.,1992; Russell *et al*., 1997; Dávila *et al*., 1998). Barley is an economically important cereal, ranking fourth in world crop production. In order of importance, barley is used for animal feed, brewing malts, and human consumption (Hayes *et al*., 2002).

In the context of plant improvement, this information provides a basis for making decisions regarding selection of parental combinations that will maximize gain from selection and maintain genetic diversity. Information on the amount of genetic vari-present, and the location of the genetic determinants of diversity, will help in barley germplasm conservation,would ease in selection of parents for improvement and facilitate to widen the genetic basis of breeding material for selection (Qi, Yang and Zhang, 2008). Among various markers available for genetic analysis in plants, molecular markers are more efficient, precise and reliable in discriminating closely related species and cultivars (Chandra *et al.,* 2013). From the last few decades SSRs had proved a valuable tool in classification and characterizing genotypes in many species including barley (Ferreira *et al.,* 2016).It has been reported previously that SSRs are a valuable tool for characterizing germplasm in various crop species (Chandra *et al*., 2013). This is due to their properties of high reproducibility, co-dominance, abundance, wide dispersal throughout the genome and multi-allelic variation (Chandra *et al.,* 2013). Database derived SSR markers were used for cultivar differentiation in *Hordeum vulgare* L (Wang *et al*., 2010).

In terms of germplasm resources, there are more than 260,000 Hordeum accessions conserved in gene banks around the world .Only a portion of these accessions has been characterized at the phenotypic level, and an even smaller portion has been characterized at the genotypic level. So in the current study our main objectives were to determine (1) the potential of microsatellite markers in general and specific SSRs in particular for detection of polymorphism and for genotype identification and (2) to assess the level of microsatellite- based genetic diversity among 25 elite barley genotypes that were potentially useful in barley breeding programmes. Genetic distances will facilitate in identifying genetically diverse genotypes, which then can be utilized in breeding programme .

MATERIALS AND METHODS

Plant material and DNA-extraction

Twenty-five genotypes of *Hordeum vulgare* was obtained from Institute of agricultural sciences, BHU. The details of the genotypes are given in Table 1.

Isolation of Genomic DNA

7 days leaves from healthy plants of each barley genotype were collected and DNA was extracted by following Saghai Maroof *et al*. (1984) with slight modifications. The DNA was

quantified using 2.5% agarose gel.

PCR

PCR was carried out under the following conditions: 100Ð150 ng of template DNA, 250 nM of each primer, 200 lM of dNTPs, 1 U of Taq Polymerase, 1.5Ð2 mM of MgCl2. The reaction, depending on the primer pair, was run for 35Ð45 cycles (denaturing at 94¡C for 1 min, annealing at 55°C or 60°C for 1 min, with a 2-min extension at 72°C), followed by a single extension at 72°C for 60 min. The amplification products were detected on polyacrylamide gels by 4 llof loading buffer (formamide containing 0.5% w/v of bromo phenol blue) and 1.5 ll of PCR products.

SSR analysis

A total of 12 simple sequence repeat (SSR) markers, selected from Barley SSR linkage map (Liu *et al*., 1996; Ramsay *et al*., 2000; Thiel *et al*., 2003) were used for genetic diversity analysis among 25 barley genotypes. The details of SSR primers used are listed in Table 2

Molecular analyses

SSR markers, generated clear and unambiguous bands of various molecular weight sizes, were scored for the presence (1) and absence (0) of the corresponding band among the 25 genotypes. The marker data was used to generate a data matrix in Microsoft Excel 2007. This data matrix was subjected to further analysis using NTSYS-pc version 2.11. The SIMQUAL program was used to calculate the Jaccard's similarity coefficients. The resulting similarity matrix was used to construct UPGMA (Unweighted Pair Group Method with Arithmetic Mean) based dendrogram. The binary data was subjected to principal component analysis (PCA) using the EIGEN and PROJ modules of NTSYSpc.

RESULTS

Genetic variability within the barley genotypes based on morphological traits

Number of grain per spike

It ranged from 17.50 to 76.33 with a mean value of 51.15. Lowest grain per spike was observed in HORMAL (17.50) and the highest was in PL-708 (51.15).

Test weight

Data for this trait revealed that it varied from 28.40 g to 56.43 g with a mean value of 39.49 g. The genotype BH-546 has the lowest test weight (28.40g) while the genotype HORMAL has the highest test weight (56.43g).

Number of tillers

Number of tillers per plant shown a huge variability illustrated it revealed that the superiority of genotype DL100 (9.33) over other ones and PRESTIGE exhibited lowest tiller number(3.83).

Genetic diversity based on molecular characterization

Allelic variation of SSR markers

The results of PCR amplification of a number of microsatellite loci in 25 barley genotypes using 12 barley microsatellite primer pairs are summarized in Table 2. The amplification profiles obtained were scored for the presence (1) and absence (0) of the corresponding band among the 25 genotypes. While scoring the above amplification profiles, we took due care to exclude the possibility of stuttering effect. Using the above criteria, we detected a total of 38 alleles.

Among these 14 primer pairs initially selected, 12 primers were on chromosome 1H,2H,3H,4H,5H and 6H were identified as polymorphic whereas primers with mono morphic banding patterns were excluded. The number of alleles per locus generated by each marker varied from 2 to 4 alleles, with an average of 3.16 alleles per locus. The highest number of alleles (4.0) was detected on each of locus Bmac0134, Bmag0013, Bmag0125 and HVM36 and the lowest number of alleles (2.0) was detected on each of locus Bmac0113 and Ebmac0806.

Genetic Similarity (GS)

Significant genetic variation was found among all barley accessions with the GS value ranging from 0.00 to 0.92. ATHOULPA, HUBL-99-30 and AMBER showed the highest genetic similarity between them and the rest of them were highly diverse among themselves.

PIC value

SSR markers were highly informative and polymorphic as evident from its PIC value. The polymorphism information content (PIC) value is a measure of polymorphism among varieties for a marker locus used in linkage analysis. The PIC value of each marker, which can be evaluated on the basis of its alleles, varied greatly for all tested SSR loci - from 0.288 to 0.998 (Table 2).

Cluster analysis

The UPGMA-based dendrogram was obtained from the binary data deduced from the DNA profiles of the samples analyzed where the genotypes that are derivatives of genetically similar

Table 1: List of Genotypes

S.No.	Name of Genotypes
1.	DL 70
2.	DL 100
3.	DL 456
4.	DWR UB 52
5.	DWR UB 6
6.	BH 543
7.	BH 546
8.	BH 668
9.	K 409
10.	PL 708
11.	HUB 172
12.	HUBL 09 17
13.	HUBL 99 30
14.	ATHOULPA
15.	AMBER
16.	BEECHER
17.	VMORALES
18.	RATNA
19.	HIMANI
20.	RIHANI
21.	HORMAL
22.	YARDU
23.	BH 664
24.	PRESTIGE
25.	LAKHAN

Table 2: List of SSR primers used for detecting parental polymorphism

S.No.	Primer		Sequence 5'----------3'	No. of alleles	PIC
1.	Bmac0134	Forward	CCAACTGAGTCGATCTCG	4	0.6352
		Reverse	CTTCGTTGCTTCTCTACCTT		
2.	Bmag0013	Forward	AAGGGGAATCAAAATGGGAG	4	0.7312
		Reverse	TCGAATAGGTCTCCGAAGAAA		
3.	EBmac635	Forward	TGCTGCGATGATGAGAACT	3	0.9984
		Reverse	TAGGGTAGATCCGTCCCTATG		
4.	Bmag613	Forward	AAGAACACCATATGATCCAAC	3	0.536
		Reverse	CTCCATGACTATGAGGAGAAG		
5.	Bmag0125	Forward	AATTAGCGAGAACAAAATCAC	4	0.6992
		Reverse	AGATAACGATGCACCACC		
6.	HVM36	Forward	TCCAGCCGAACAATTTCTTG	4	0.8016
		Reverse	AGTACTCCGACACCACGTCC		
7.	Bmag0223	Forward	TTAGTCACCCTCAACGGT	3	0.7648
		Reverse	CCCCTAACTGCTGTGATG		
8.	Bmac0113	Forward	TCAAAAGCCGGTCTAATGCT	2	0.8400
		Reverse	GTGCAAAGAAAATGCACAGATAG		
9.	Bmag0353	Forward	ACTAGTACCCACTATGCACGA	3	0.7392
		Reverse	ACGTTCATTAAAATCACAACTG		
10.	EBmac0806	Forward	ACTAAGTCCTTTCACGAGGA	2	0.2880
		Reverse	GTGTGTAGTAGGTGGGTACTTG		
11.	GMS27	Forward	CTTTTTCTTTGACGATGCACC	3	0.8384
		Reverse	TGAGTTTGTGAGAACTGGATGG		
12.	EBmac0824	Forward	GCAAGCTTCCTAAATCCTTA	3	0.6016
		Reverse	TGCAGACAGTTTTTCATATACA		

types clustered together. All 25 barley genotypes were discriminated by SSR markers. The genotypes were classified into two groups (Groups 1 and 2) at the level of GS = 0.14. Group 1 included 2 genotypes, while Group 2 consisted of 23 genotypes. At the level of GS = 0.16, Group 2 was further divided into two subgroups (Subgroups 2a and 2b) containing 7 and 16 genotypes, respectively. SSR markers could not differentiate the genotype ATHOULPA and AMBER.

Principal component analysis

The second approach we used to assess the utilty of SSRs for germplasm classification was PCoA. The eigen values obtained from the SSRs markers that the first four principal components accounted for 57.37% of the total variation, in which 21.59% was accounted by the component 1 and 15.24% by the component 2. In PCoA analysis , broadly three clusters could be observed.

DISCUSSION

The aim of the present investigation was to explore the genetic variability in Indian germplasm of cultivated barley using morpho-physiological traits and SSR markers.We selected 25 cultivated barley genotypes, estimated the extent of variability in morphological traits and surveyed microsatellite DNA polymorphisms to study the genetic divergence. In this study, 38 alleles were detected with 14 SSR loci, and allelic variations existed at 12 SSR loci. The number of alleles per locus generated by each marker varied from 2 to 4 alleles, with an average of 3.16 alleles per locus Wang 2010 also reported average of 2.4 alleles per locus. This relatively small number is probably due to the limited geneotypes and relatively high GS within the investigated group of barley germplasm. However, since the SSR loci selected were evenly distributed along the barley genome, the genetic relationships revealed by this study within the investigated group of barley varieties are representative and meaningful. Outcomes of the assessments provide a general guide for choosing parental lines to make suitable cross combinations for particular breeding purposes. As one of the genetic diversity centers of barley, India is rich in both landrace and cultivated barley .Several studies were conducted to evaluate the genetic relationships among different barley populations (Sing *et al.*,2014; Jaiswal *et al*., 2010). Genetic relationships were found to be very close among the Indian barley varieties analyzed in this study. The fact that barley varieties developed in India were clustered into the same subgroup gave the strong indications of the narrow genetic background in Indian barley germplasm. The introduced foreign genotypes investigated in this study (eg Atahualpa), on the other hand, showed a broader genetic diversity. Barley breeding organizations should stress the necessity for the collection, conservation, and utilization of the cultivated varieties and the landraces. Genotypes HUB172 and Ratna gave unique fragments of size 175 and 160bp respectively at Bmac0134 locus and genotype BH546 gave unique fragment of 170bp at Bmag 0125.The occurrence of the unique allele is an indication of the diversity present in a germplasm and its potential as a reservoir of novel alleles for crop improvement. Similar results reported by Matus and Hayes (2002) and Pillen *et al*. (2000), the highest PIC value (0.998) was displayed by SSR marker (Ebamc635). However, high PIC value (0.947) was also reported by several workers for SSR markers varying in number of alleles (Wang *et al.,* 2016*;* Matus and Hayes 2002). All varieties represented mainly two major clusters. It was reported that the SSR marker EBmac0806 on chromosome 6H was associated with scald resistance (Feriani *et al*.,2012). In our study, the results generated from genotyping of EBmac0806 indicated one barley variety 'DL70'showed the same allele. Thus, it appears that the SSR marker EBmac0806

is also very likely to be associated with scald resistance. However, further validation is needed to confirm the candidate regions for the resistance. So we can conclude that SSR markers classified the 25 barley genotypes and the results were also consistent based on morphological data. The diversity analysis can further be utilized for the development of diverse gene pool. The hybridization among the diverse gene pool depicted here would certainly lead to higher amount of transgressive segregants.

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