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Short Communication

Characterization of genetic diversity in barley (*Hordeum vulgare* L.) through DNA fingerprinting

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Assessment of the extent of genetic variability within a cultivated crop has important consequences in plant breeding and conservation of genetic resources [1]. Barley crop is no exception and therefore, for conservation of its genetic resources and for sustained barley breeding in India, it is important to have knowledge about the availability of genetic variation [1]. Genetic variability between genotypes can be estimated either by determining their levels of polymorphism for genetic markers or by analyzing pedigree relationships. Traditionally, morphological characters have been used to evaluate distinctness, uniformity and stability and also to establish the description of a genotype [2]. However, this method is often influenced by environment and is labour intensive. To overcome these problems, DNA fingerprinting has been established. DNA markers have the advantage that they are virtually unlimited and they do not disturb the physiology of the organism. The PCR (polymerase chain reaction) based methods such as Random Amplified Polymorphic DNA (RAPD) are considered faster and cheaper than non-PCR-based methods. In RAPD, a synthetic oligonucleotide of 9 to 12 bases is used as starting point for DNA amplification with a thermo stable DNA polymerase. In the past, a few studies have indicated the significance of RAPD marker as a tool in establishing genetic diversity and pedigree relationship in barley [3]. However, such studies in Indian germplasm are lacking. Therefore, 26 barley genotypes, which mostly included Indian released cultivars, were used for their genetic characterization using RAPD markers. Seeds of 26 barley genotypes were procured from Barley Improvement Programme of Banaras Hindu University, Varanasi and Directorate of Wheat Research (DWR), Karnal. DNA was extracted from the leaves of each of the 26 genotypes which were grown in the field, using CTAB method [4]. Twenty one RAPD primers procured from Operon Technologies, USA were used (Table 1).

DNA amplifications were carried out in 25ul reaction mixtures, each containing 50ng template DNA in 2µl TE, Taq Buffer 1X, $Mgcl_2$1.5mM, dNTPs 200µM each, primer 2µM, *Taq* polymerase 1U using following PCR profile in a PTC-200, Thermal Cycler, Techne, UK, initial denaturation at 95°C for 5 min, with 36 cycles of denaturation at 94°C for 30 sec, annealing at 36°C for 45 sec, and amplification at 72°C for 2 min, final extension at 72°C for 10 min. The amplified products were resolved on 1.2% agarose gel following ethidium bromide staining (0.05mg/ml). Fragment sizes were calculated by comparing with fragments of λ DNA *Eco R* I/*Hind* III double digested marker DNA and photographed on a trans-illuminator. Bands on RAPD gels, bands were scored as present (1) or absent (0). Data were analyzed and similarity matrix was constructed from binary data with Jaccard's coefficients [5] and dendogram was generated with unweighted pair-group method arithmetic average (UPGMA) algorithm, using NTSYS-pc Version 2.02 software.

All the 21 primers were found to display reliable amplification profile for obtaining genetic relationship among the 26 barley genotypes (Fig. 1 and 2). The amplification profile generated by the 21 primers is summarized in Table 1. The Jaccard's genetic similarity (GS) values showed substantial variation among the barley genotypes. The similarity coefficients varied from 0.037 to 0.586. In general, barley genotypes were found dispersed across several sub-clusters. However, some of the genotypes displayed high genetic similarity. The genetic similarity values were the highest between Karan 521 and Karan 741 (0.586) while, the lowest GS was observed between Lakhan and C 138 (0.037). Other genotypes that displayed high similarity were NDB 1180 and Jyoti, Karan 280 and RD 2035, and Karan 551 and Azad. The UPGMA cluster analysis to graphically display the grouping between genotypes is presented in Fig. 3. It was interesting to note that the variety DL-88 was distinct from all other 25 genotypes.

In the present study, the percentage of polymorphic bands displayed by 21 primers was 50%. The highest genetic similarity was observed between Karan 521 and Karan 741 (58%) and the lowest genetic similarity between Lakhan and C 138 (3.7%). The wide range of genetic distances observed among 26 barley varities established substantial genetic diversity. This diversity

Table 1. List of 21 primers used in the RAPD analysis and the percent polymorphism displayed among 26 barley genotpyes

No.	Primer	Sequence (5'-3')	Total band	Polymorphic bands	% Polymorphism
1.	OPG-09	CTGACGTCAC	8	5	62.50
2.	OPG-10	AGGGCCGTCT	7	6	85.71
3.	OPG-11	TGCCCGTCGT	6	3	50.00
4.	OPG-12	CAGCTCACGA	4	2	50.00
5.	OPG-13	CTCTCCGCCA	8	6	75.00
6.	OPG-14	GGATGAGACC	9	8	88.89
7.	OPG-15	ACTGGGACTC	6	5	83.33
8.	OPG-16	AGCGTCCTCC	4	2	50.00
9.	OPG-17	ACGACCGACA	11	6	54.54
10.	OPG-20	TCTCCCTCAG	10	7	70.00
11.	OPG-01	CTACGGAGGA	7	4	57.14
12.	OPG-02	GGCACTGAGG	6	5	83.33
13.	OPG-03	GAGCCCTCCA	9	6	66.66
14.	OPG-04	AGCGTGTCTG	6	4	66.66
15.	OPC-01360.00	TTCGAGCCAG	4	2	50.00
16.	OP5D-01	ACCGCGAAGG	5	3	60.00
17.	OPE-05	TCAGGGAGGT	7	5	71.42
18.	OFF-01	ACGGATCCTG	6	4	66.67
19.	OFF-10	GGAAGCTTGG	7	5	71.42
20.	OPG-07	GAACCTGCGG	6	2	33.33
21.	OPG-08	TCACGTCCAC	4	3	75.00
Total		21	140	93	66.42

can be used to plan appropriate crossing programmes for obtaining desirable transgressive segregants. The scope of such advantage in the ongoing barley breeding programmes of different barley centers of our country can further be enhanced by exposing more barley genotypes/cultivars to molecular fingerprinting and using diverse types to avoid inadvertent inbreeding.

The typical banding pattern observed for most of the barley genotypes, that was found repeatable in the present study, can be used as a fingerprint for identification and conservation of barley genotypes. For instance, the banding pattern using primer OPG-13 was quite distinct in the barley cultivars Azad, Jyoti, RS 6, Lakhan and K 560. The banding pattern can also be used for determining the genetic purity of a seed lot or even for identifying the pedigree of an unknown line. The results obtained in the present study further confirm the usefulness of RAPD as a potent tool in the assessment of genetic diversity and creating suitable fingerprints of Indian barley cultivars.

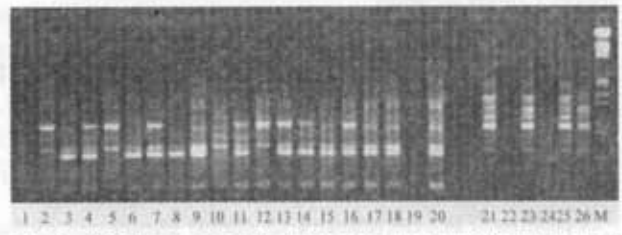

Fig. 1. RAPD pattern differentiating among 26 varieties of Barley as amplified with arbitrary primer OPG-13. M is the molecular weight marker (DNA/*EcoRI +Hind III* digest) lane

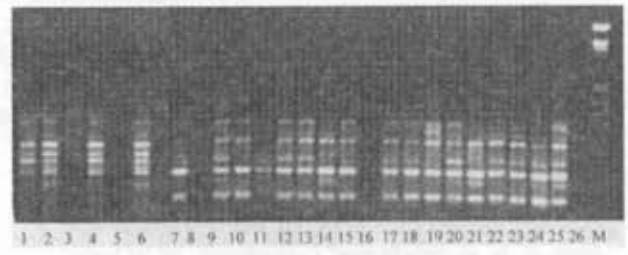

Fig. 2. RAPD pattern differentiating among 26 varieties of Barley as amplified with arbitrary primer OPG-14. M is the molecular weight marker (DNA/*EcoRI +Hind III* digest) lane. 1 = CLIPPER, 2 = RD-2552, 3 = RD-2508, 4 = KARAN-19, 5 = KARAN-741, 6 = KARAN-280, 7 = K-551, 8 = RD-2035, 9 = AZAD, 10 = NDB-1180, 11 = KARAN-4, 12 = JYOTI, 13 = KARAN-521, 14 = KARAN-16, 15 = K-603, 16 = RD-2624, 17 = KARAN-92, 18 = MANJULA, 19 = C-138, 20 = RS-6, 21 = BCU-73, 22 = DL-88, 23 = RD-2503, 24 = LAKHAN, 15 = KARAN-15, 26 = K-560.

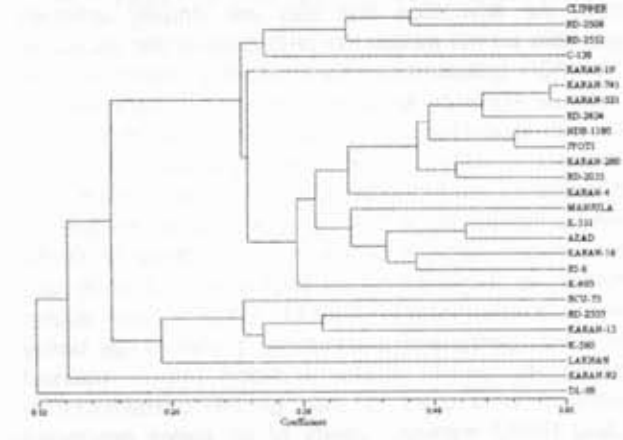

Fig. 3. Dendrogram of 26 genotypes of barley using UPGMA cluster analysis of genetic similarities based on RAPD data with six arbitrary primers

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