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Morphological and SSR Marker Based Diversity Analysis of Lentil (*Lens esculenta*) Genotypes using Yield and Yield Contributing Characters

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ABSTRACT

Lentil is an important pulse crop with high nutritional value and high market price worldwide. Molecular markers have emerged as useful tools to assess the genetic diversity across crops. The study was conducted to explore genetic diversity of twenty lentil genotypes considering yield and yield attributing traits. Among all genotypes, BARI Masur-6, BARI Masur-7 followed by genotypes BD-3806 and BD-4090 showed the highest value of yield attributing traits therefore, these genotypes are considered as best performer. The results of cluster analysis based on the Ward's method grouped the genotypes into three clusters and the genotypes of cluster III revealed the maximum value for yield per plants which indicated their importance in the selection for yield improvement program of lentil. Afterwards, 20 genotypes were evaluated through 7 sets of SSR primers to assess genetic diversity among the genotypes. Among them, four sets of primers *viz.,* SSR 19, SSR 33, SSR 90 and SSR 213 showed high polymorphism which suggesting the greater genetic diversity in the genotypes. The unweighted pair group method of arithmetic mean (UPGMA) dendrogram based on Nei's (1973) genetic distance led the genotypes into four major clusters which showed a bit deviation with the morphological cluster. The findings of this study will be very useful for selection of appropriate parents and the genetic understanding for the set up for future systematic lentil breeding programs.

Key words: Genetic diversity, Germplasm, Lentil, Molecular markers, Polymorphism information content.

INTRODUCTION

Lentil (*Lens culinaris* Medik.) is diploid (2n=2x=14) and self-pollinated cool season crop belongs to the family Fabaceae and one of the early domesticated among pulse crops (Arumuganathan and Earle, 1991; Hamweih *et al.,* 2009). Lentil is cultivated in 4.6 million hectares of land worldwide with the production of 4.95 million ton (FAO, 2013). In Bangladesh, lentil is one of the most vital pulse crop and grain legume. It is also known as 'masur dal'. In our country, lentil is the main source of plant protein. In the year 2015-16, lentil was grown in 1,54,449.489 ha of land and the production was 158228 metric tons in Bangladesh (BBS, 2016). But in our country, the production of lentil is not enough to meet up the demand of proper nutrition of increasing population. The reasons behind this are cultivation of lentil in traditional method, due to narrow genetic base, susceptibility to biotic and abiotic stresses, less response to fertilizer and irrigation, inaccessibility of early maturing varieties and genetic erosion.

In modern day plant breeding, choice of appropriate parent is a vital source in detecting variation of genes for hybridization activities and assist as a base for any crop improvement operation (Kushwaha *et al*., 2015). In case of lentil breeding, this process have been found as a proper tool in developing desirable high yielding varieties. Genetic improvement comes from genetic diversity within germplasm. Germplasm delivers facility for broad variability. Thus, it is necessary to assess the genetic diversity of lentil germplasm for the improvement. In case of self-pollinated crop, in hybridization operation genetic diversity is a vital factor for genetic improvement (Joshi and Dhawan, 1996). It can be assessed through advanced biometrical process such as multivariate analysis (Rao, 1952) based on Mahalanobis (1936) D^2 statistics and Ward's non-hierarchical squared Euclidean distance method have become possible to quantify magnitude of diversity among lentil germplasm placing the genotypes in different clusters for their assessment in respect of breeding program which will produce heterotic combinations and wide variability in next generation.

Genetic variation is detected by Molecular markers within

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germplasm and among closely associated species (Nienhuis *et al.,* 1995). Simple Sequence Repeats (SSRs) is an essential implement for diagnose of genetic variation like polymorphism, genetic diversity, molecular mapping, gene mapping, fingerprint construction and genetic purity test (Kumar *et al.,* 2014). Using SSR marker in lentil genotypes, this method can be used as quick and dynamic method for genetic variation, detecting polymorphism at the DNA level and genetic diversity analysis. As superior genotypes are used as parent materials in a hybridization programs, a knowledge of the genetic diversity will be use for further improvement of productivity of lentil genotypes (Kumar *et al.,* 2014). So it is necessary to identify the genetic diversity and characters association with yield and yield attributing traits which is of great importance to the breeders for the development of genotypes with desired qualitative and quantitative characters and wider genetic base which would help the plant breeder in picking the exact parents in breeding operation for desired high yielding varieties. Therefore, the research work was conducted to evaluate the diversity of lentil genotypes at molecular level through SSR markers.

MATERIALS AND METHODS

Plant materials

Twenty lentil genotypes were used in the experiment were collected from Plant Genetic Resources Centre (PGRC), BARI,Gazipur, Bangladesh. The identity of 20 lentil genotypes are presented in Table 1.

Experimental details and estimation of quantitative traits

The experiment was carried out during Rabi season. The land was prepared properly by 5-6 ploughing and cross ploughing. The clods were broken into small pieces and leveled by ladder. All the stubbles and weeds were removed from the plot properly. Urea, triple super phosphate and muriate of potash were used as source of nitrogen, phosphorus, potassium at the rate of 32, 77, 32 kg ha^{-1}, respectively at the time of final land preparation. The experiment was conducted following randomized complete block design (RCBD) with three replications. The seeds of the 20 lentil genotypes were sown in the field on 8th November 2016. The length of line was 1.2 m and line to line distance was 30cm.

Generally different genotypes matured at different times. Therefore, harvesting was done at least 90% of the pods when turned brown and plants turned in yellow or straw color. Five plants were selected from each replication and uprooted to collect data of different traits *viz.,* days to first flowering, days to fifty percent flowering, days to maturity, plant height (cm), total number of primary branches, total number of pods $peduncle^{-1}$, total number of pods $plant^{-1}$, the number of seeds pod^{-1}, the total number of seeds $plant^{-1}$, 100-seed weight (g) yield $plant^{-1}$ (g).

Genomic DNA extraction of plant materials

Genomic DNA has been extracted from young 21 days old fresh leaves of the 20 genotypes using the Cetyl Trimethyl Ammonium Bromide (CTAB) mini-prep method. Total, seven primers namely SSR 19 (forward 5′-GACTCATACTTTGTTC TTAGCAG-3′ and reverse 5′-GAACGGAGCGGTCACAT TAG-3′), SSR 33 (forward 5′- CAAGCATGACGCCTATGAA G-3′ and reverse 5′-CTTTCACTCACTCAACTCTC-3′), SSR 48 (forward 5′-CATGGTGGAATAGTGATGGC-3′ and reverse 5′-CTCCATACACCACTCATTCAC-3′), SSR 90 (forward 5′-CCGTGTACACCCCTAC-3′ and reverse 5′-CGTCTTAA AGAGAGTGACAC-3′), SSR 156 (forward 5′-GTACATT GAACAGCATCATC-3′ and reverse 5′-CAAATGGGCA TGAAAGGAG-3′), SSR 207 (forward 5′-GAGAGATA CGTCAGAGTAG-3′ and reverse 5′-GATTGTGCTTCGGTG GTTC -3′) AND SSR 213 (forward 5′-CACTCGCACCTCTTA TG-3′ and reverse 5′- GAAATTGTCTCTTAGCAAG -3′) were used to estimate genetic diversity among lentil genotypes. PCR reactions were performed on each DNA sample in a 10 µl reaction mixture consists of Taq polymerase buffer (1 µl), primer forward (0.5 µl), primer reverse (0.5 µl), maximo Taq DNA polymerase (4 µl), dNTPs (0.2 µl), MgCl2 (1 µl), genomic DNA (1 µl) and suitable amount of sterile deionized water. DNA amplification was performed in an oil free thermal cycler and PCR amplification process consist denaturation at 94°C for 3 min, 32 cycles of denaturation at 94°C for 45 seconds, annealing (60°C and 62°C) for 45 seconds and extension at 72°C for 6 min at last. The amplified products were scored as bands on visualization on gel on UV illuminator. Only the reliable bands were included in analysis. The pattern of bands obtained after amplification with the primers was scored using Alpha Viewer (Version 3.2.8) to identify the molecular weight of DNA band comparing with known size DNA ladder. The size (in nucleotide base pairs) of the amplified band for each microsatellite marker was determined based on its migration relative to a molecular weight size.

Data analysis

Data was analyzed by two-way analysis of variance (ANOVA) using R studio software. The data were presented as means with different alphabetical letters in the same column indicates significant differences among the treatments and cultivars at $P < 0.05$ according to a least significant difference (LSD) test. The cluster analysis was determined by Ward's Method based on Euclidean distance and hierarchical cluster analysis using R software. The summary statistics including the number of alleles per locus, major allele frequency, gene diversity and Polymorphism Information Content (PIC) and Nei's genetic identity and genetic distance values were determined using Power Marker version 3.23 (Liu and Muse, 2005), a genetic analysis software.

RESULTS AND DISCUSSION

Analysis of variance

Knowledge of genetic diversity in germplasm is essential for active germplasm collection, conservation, utilization and strategies in and crop improvement programs (Alghamdi *et al.,* 2014). In the current investigation, analysis

Table 1: List of 20 lentil genotypes used in this experiment with their characters.

Genotypes	Characters
BARI Masur-6	Bushy plant, medium leaflet size and violet color flower
BARI Masur-7	Bushy plant, large leaflet size and violet color flower
BD-3804	Semi-erect plant, medium leaflet size and white color flower
BD-3806	Erect plant, medium leaflet size and violet color flower
BD-3808	Semi-erect plant, medium leaflet size and white color flower
BD-3810	Semi-erect plant, medium leaflet size and white color flower
BD-3945	Semi-erect plant, medium leaflet size and white color flower
BD-3948	Bushy plant, medium leaflet size and white color flower
BD-3975	Bushy plant, medium leaflet size and white color flower
BD-3985	Semi-erect plant, large leaflet size and white color flower
BD-3986	Bushy plant, large leaflet size and white color flower
BD-3995	Bushy plant, large leaflet size and white color flower
BD-4028	Semi-erect plant, large leaflet size and violet color flower
BD-4088	Bushy plant, medium leaflet size and violet color flower
BD-4090	Semi-erect, medium leaflet size and white color flower
BD-4095	Bushy plant, large leaflet size and white color flower
BD-4134	Bushy plant, medium leaflet size and white color flower
BD-5958	Semi-erect, small leaflet size and white color flower
BD-5959	Semi-erect, medium leaflet size and white color flower
BD-5983	Semi-erect, medium leaflet size and white color flower

Table 2: Analysis of variance for different traits of 20 lentil genotypes.

Traits	Sources of variation		
	Replication (df=2)	Genotype (df=19)	Error (df=38)
Days to first flowering	0.050	29.104***	0.436
Days to fifty percent flowering	0.866	27.139***	0.621
Days to maturity	0.150	30.943***	0.500
Plant height (cm)	0.521	23.360***	1.121
Primary branches plant^{-1} (no.)	0.580	2.7816***	0.425
Pods peduncle^{-1} (no.)	0.043	0.336***	0.037
Pods plant^{-1} (no.)	4.00	1853.06***	10.31
Seeds plant^{-1} (no.)	2.6	6652.5***	34.4
Seeds pod^{-1} (no.)	0.001	0.040***	0.001
100-seed weight (g)	0.003	0.064***	0.002
Seed yield plant^{-1} (g)	0.001	2.334***	0.015

*** indicates significant at 0.001 statistical level.

of variance showed statistically significant difference among the genotypes for all traits under study *viz*., days to first flowering, days to 50% flowering, days to maturity, plant height, number of primary branches plant^{-1}, number of pods peduncle^{-1}, number of pods plant^{-1}, number of seeds plant^{-1}, number of seeds pod^{-1}, 100-seed weight and seed yield plant^{-1} (Table 2) indicating the existence of genetic diversity in the genotypes. Hence, selection could better be employed considering these traits in practical lentil breeding program and the broadening of genetic base (Gupta and Sharma, 2006). This results were in consistent with Gautam *et al.* (2013) and Roy *et al.* (2013).

Trait wise mean performance of the genotypes

The mean performances of the lentil genotypes for different yield and yield attributing traits are presented in Table 3. The genotypes displayed considerable amount of difference in their mean value and this indicating the presence of variability among the genotypes for the characters studied (Table 3). Considering the traits days to first flowering, days to fifty percent flowering and days to maturity, the genotype BD-5983 was earliest followed by the genotype BD-3975, BD-3808 and BD-3810 reflected that this material could be used to develop early maturing variety, which is the vital need for lentil improvement program in Bangladesh context (Roy *et al.,* 2013). Maximum plant height (39.94 cm) was observed in genotype BD-3995 which was followed by BD-4095 (39.89 cm) while minimum (28.27 cm) was found in genotype BARI Masur-6. The maximum number of primary branches plant^{-1}, number of pods plant^{-1}, Number of pods peduncle^{-1} and number of seeds plant^{-1} was observed in the

Table 3: Mean performance of twenty genotypes based on different quantitative traits related to yield.

Genotypes	DF	DFF	DM	PH	PBP	PPD	PP	SP	SPD	TSW	SY
BARI Masur-6	61 h-j	73.33 ef	112.3 de	28.27 k	6.78b-e	1.89 bc	174 c	271 cd	1.56 fg	1.89 a	4.96 b
BARI Masur-7	63.33 ef	76.67 b	113 a-c	31.5 j	7.67 ab	2.0 b	188.1 a	303.7 a	1.61 d-f	1.76 bc	5.25 a
BD-3804	60.33 j	70.33 i	108.3 gh	33.55 hi	6.7 b-e	2.33 a	145.9 g	241 hi	1.65 d	1.64 de	4.163 d
BD-3806	62 gh	72.67 f-h	109.3 fg	37.2 b-d	7.89 a	2.0 b	180.9 b	278 cd	1.54 g	1.68 cd	4.84 b
BD-3808	60.67 ij	71.67 h	107.3 h	35 e-h	7.33 a-c	1.67 cd	146.2 g	236.4 i	1.62 de	1.58 ef	3.56 hi
BD-3810	61.67 g-i	72.33 f-h	108 h	32.14 ij	6.8 a-d	2.33 a	129.8 h	186.6 j	1.44 h	1.54 f	3.34 jk
BD-3945	63.67 de	76 bc	113b-d	36.4 c-e	5 f	1.56 d	107.9 ij	168.1 k	1.56 g	1.42 hi	2.55 m
BD-3948	65.67 c	78.67 a	114 ab	38.94 ab	6.44 c-e	1.5 d	129.4 h	178.8 j	1.38 ij	1.59 ef	2.84 l
BD-3975	58 k	69.67 i	104.3 i	35.2 e-h	7.67 ab	1.5 d	158 ef	249 gh	1.5 e-g	1.41 hi	3.23 k
BD-3985	61.67 g-i	72 gh	109.3 fg	37.78 bc	5.11 f	2.17 ab	111.7 i	159.9 k	1.43 hi	1.39 hi	2.11 n
BD-3986	64.67 cd	74.67 d	112 c-e	35.61 d-f	7.78 ab	2.33 a	166 d	291.1 b	1.75 ab	1.38 i	3.74 f-h
BD-3995	63.33 ef	74.33 de	112 c-e	39.94 a	7.67 ab	1.56 d	153.4 f	261 ef	1.70 bc	1.63 de	3.95 e
BD-4028	69.33 a	79.33 a	114.7 a	35 e-h	5.78 ef	1.56 d	177 bc	262 ef	1.48 h	1.63 de	4.25 d
BD-4088	68 b	78.67 a	114 ab	35 e-h	7.11 a-c	2.17 ab	157 ef	281 c	1.78 a	1.45 hi	3.66 g-i
BD-4090	62 e-g	73 fg	109.7 f	36.29 c-f	7.67 ab	2.33 a	166.9 d	275 cd	1.65 cd	1.79 b	4.61 c
BD-4095	63.33 ef	74.33 de	111.7 e	39.89 a	5.89 d-f	1.5 d	172.8 c	269 de	1.56 g	1.54 fg	3.93 ef
BD-4134	65.33 c	75.33 cd	113 b-d	34.7 e-h	5.10 f	1.61 cd	105.3 j	143.9 l	1.37 j	1.45 hi	1.96 n
BD-5958	68.33 ab	78.33 a	113 a-c	34.66 f-h	7.55 ab	2.17 ab	162 de	256 fg	1.5 e-g	1.45 hi	3.62 g-i
BD-5959	62.33 fg	72.67 f-h	108.3 gh	33.83 g-i	6.7b-e	1.61 cd	154.4 f	249 gh	1.62 de	1.46 gh	3.51 ij
BD-5983	57.67 k	69.67 i	104.3 i	35.3 e-g	6.33 c-e	1.56 d	177 bc	258.fg	1.44 h	1.41 hi	3.7 e-g

Notes: DF denotes days to first flowering; DFF denotes days to fifty percent flowering; DM denotes days to maturity; PH denotes plant height; PBP denotes primary branches plant^{-1}; PPD denotes pods peduncle^{-1}; PP denotes pods plant^{-1}, SP denotes seeds plant^{-1}; SPD denotes seeds pod^{-1}; TSW denotes 100-seed weight and SY denotes seed yield plant^{-1}.

genotypes namely BD-3806, BD-3804, BD-3810, BD-3986 and BD-4090, and BARI Masur-7. Seed yield was dependent on number of seeds per pod in lentil genotyeps (Sinha and Chowdhury, 1991; Rajput and Sarwar, 1989). The highest 100-seed weight was recorded in BARI Masur-6 where the minimum value of 100-seed weight was found in the genotype BD-3986. Our results were in consistent with Rahman and Ali (2004) who observed wide range of variability in existing lentil cultivars in case of 100-seed weight. The maximum seed yield plant^{-1} was recorded in genotype BARI Masur-7 followed by genotype BARI Masur-6, BD-3806, BD-4090, BD-4028, BD-3804 and BD-3995 whereas BD-3986 had the lowest seed yield plant^{-1}. Considering all the traits, BARI Masur-6, BARI Masur-7 followed by genotypes BD-3806 and BD-4090 were the best performer considering yield and yield attributing traits. So, there is a great scope of genetic improvement of traits studied in the selected best genotypes. Similar result was observed by Ahamed *et al.* (2014) and Singh *et al.* (2014) among the lentil genotypes.

Clustering of the genotypes considering morpho-physiological traits

Cluster analysis is one of the most powerful tool for estimating the extent of genetic diversity which have a practical use in plant breeding (Sultana *et al.,* 2006). Using Euclidean distance following Ward's method, 20 lentil genotypes were grouped into three separate clusters (Fig 1). The largest cluster III consist of maximum number of genotypes *viz.,* BARI Masur-6, BD-3806, BD-4028, BD-4090, BD-4095, BD-5983, BARI Masur-7, BD-3986 and BD-4088. This genotypes contained maximum value mean value for different traits such as number of pods peduncle^{-1}, number of pods plant^{-1}, number of seeds plant^{-1}, 100-seed weight, and seed yield plant^{-1} (Table 4) which indicates that this genotypes could get the major priority for the yield improvement. Earlier Gautam *et al.* (2014) observed moderate to high yield donating traits in cluster III and II in the lentil genoypes. The members of cluster I were BD-3810, BD-3945, BD-3948, BD-3985 and BD-4134, the members of cluster II were BD-3804, BD-3808, BD-3975, BD-3995, BD-5958 and BD-5959. All the short duration genotypes were grouped into cluster II whereas cluster I included long duration genotypes indicating maximum contribution of this character towards the divergence between cluster II and I. Gautam *et al.* (2014) also reported early maturing genotypes in cluster II and late maturing genotypes in cluster III. The genotypes which are grouped into the same cluster probably disperse very little from one to another (Roy *et al.,* 2013). Many researchers exploited that cluster analysis could be a powerful tool to screen a large number of germplasms on the basis of similarity (Chunthaburee et al. 2016; Siddiqui *et al.* 2017).

Diversity analysis through SSR primers

Genetic similarity analysis using UPGMA

UPGMA dendrogram revealed the 20 genotypes were categorized into four major clusters considering their

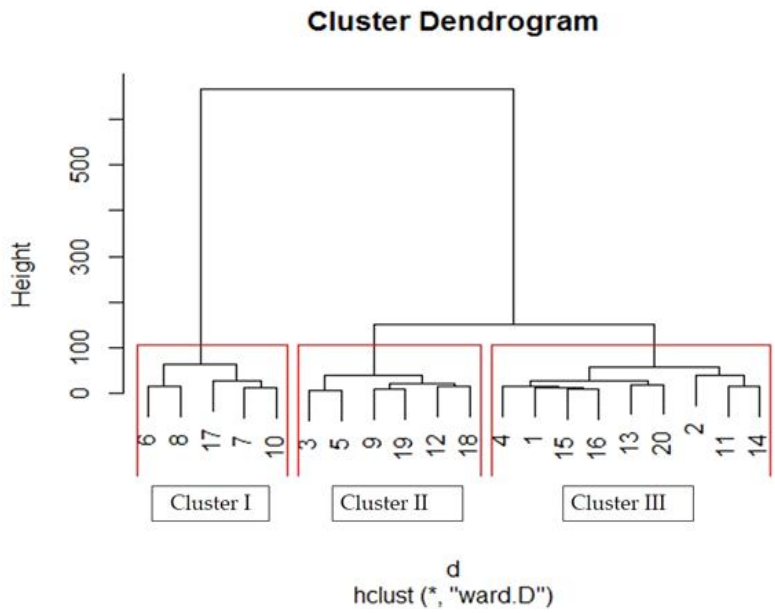

Fig 1: Dendrogram based on Euclidean distance using R software, summarizing the data on differentiation among 20 genotypes of lentil according to Ward's method.

1=BARI Masur-6	4=BD-3806	7=BD-3945	10=BD-3985	13=BD-4028	15=BD-4090	17=BD-4134	19=BD-5959
2=BARI Masur-7	5=BD-3808	8=BD-3948	11=BD-3986	14=BD-4088	16=BD-4095	18=BD-5958	20=BD-5983
3=BD-3804	6=BD-3810	9=BD-3975	12=BD-3995				

Table 4: Cluster mean for yield and yield related characters of 20 genotypes.

Characters	Cluster Mean		
	I	II	III
Days to first flowering	63.60 (H)	62.17 (L)	63.56 (I)
Days to fifty percent flowering	74.87 (H)	72.83 (L)	74.70 (I)
Days to maturity	111.58 (H)	109.1 (L)	111.38 (I)
Plant height (cm)	36.01 (H)	35.37 (I)	34.92 (L)
Primary branches plant^{-1} (no.)	5.69 (L)	7.30 (H)	6.99 (I)
Pods peduncle^{-1} (no.)	1.83 (I)	1.81 (L)	1.93 (H)
Pods plant^{-1} (no.)	116.82 (L)	153.43 (I)	173.46 (H)
Seeds plant^{-1} (no.)	167.46 (L)	249.05 (I)	276.87 (H)
Seeds pod^{-1} (no.)	1.43 (L)	1.62 (H)	1.60 (I)
100-seed weight (g)	1.48 (L)	1.53 (I)	1.61 (H)
Seed yield plant^{-1} (g)	2.56 (L)	3.67 (I)	4.34 (H)

similarity (Fig 2) which somewhat failed to match the earlier dendrogram (Fig 1) based on the data for yield traits. BD-3948 and BD-5983 genotypes of cluster I showed low yield and late maturity in mean performance where cluster II genotypes BD-3995, BD-4088, BD-4090 and BARI masur-6 were moderate yielding in mean performance. Maximum genotypes of cluster III *viz.,* BD-3806, BD-3975, BD-3985, BD-3986, BD-4028, BD-4134, BD-4095 and BARI- masur-7 were the best performer and they were moderate to high yielding and early maturity in mean performance. The genotypes *viz.,* BD-3804, BD-3808, BD-3810, BD-3945, BD-5958 and BD-5959 of cluster IV were also reported as moderate yielding variety. Genetic distance of the genotypes of cluster I and II was higher whereas genetic distance of the genotypes between cluster III and IV was lower. Previous research work of Singh *et al.* (2016) showed the genetic distance of the cluster ranged from fifty to seventy percent with an average of fifty four percent. Genotypic variations based on molecular characterization indicated that genotypes fit in different clusters due to their genetic components itself. Therefore, it will be used for further lentil breeding program, especially for hybridization and genotype that selected from different clusters will provide maximum heterosis as favors yield.

Overall allelic diversity and polymorphic information content (PIC) value

The seven SSR primer sets were employed in the present study, among this four SSR primer sets were polymorphic and produced varying number of alleles with different size.

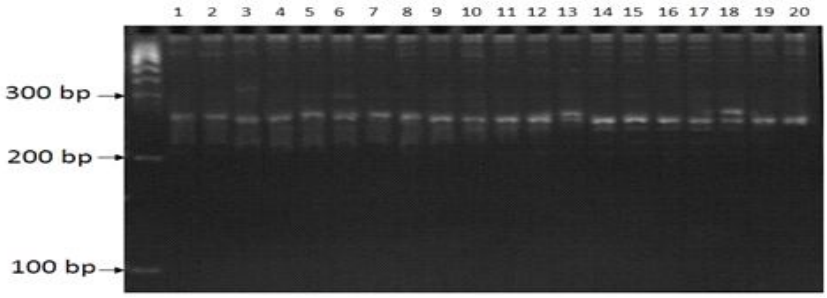

Fig 2 (A): SSR profiles of 20 lentil genotypes using primer SSR 19.

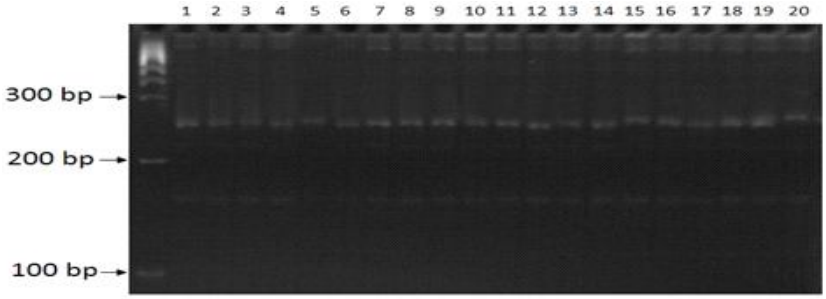

Fig 2 (B): SSR profiles of 20 lentil genotypes using primer SSR 33.

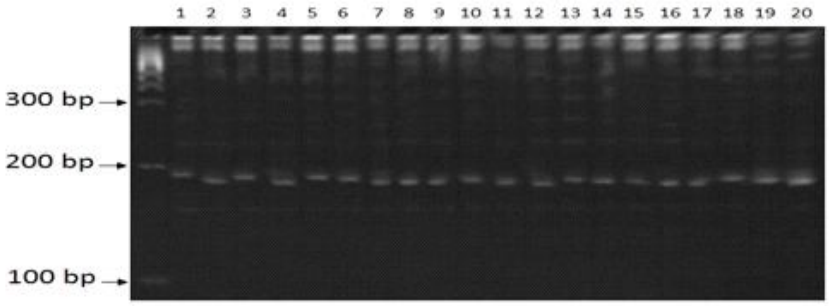

Fig 2 (C): SSR profiles of 20 lentil genotypes using primer SSR 90.

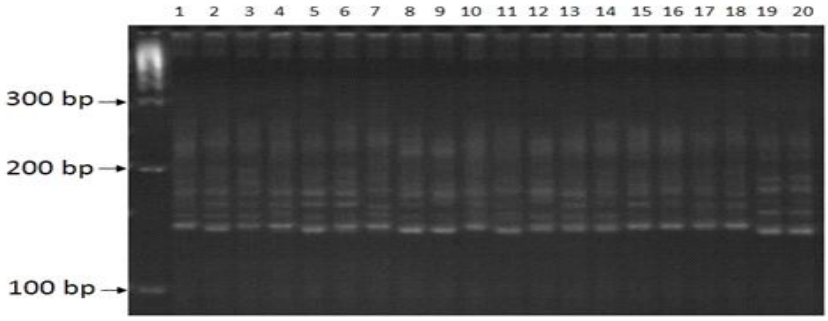

Fig 2(D): SSR profiles of 20 lentil genotypes using primer SSR 213.

1=BD-3804	4=BD-3810	7=BD-3975	10=BD-3995	13=BD-4090	15=BD-4134	17=BD-5959	19=BARI Masur-6
2=BD-3806	5=BD-3945	8=BD-3985	11=BD-4028	14=BD-4095	16=BD-5958	18=BD-5983	20=BARI Masur-7
3=BD-3808	6=BD-3948	9=BD-3986	12=BD-4088				

Among the 20 lentil genotypes, entirely 33 alleles were identified with an average of 8.25 alleles per locus. The maximum number of alleles per locus produced in SSR 19 (10) whereas minimum number of alleles per locus was displayed by SSR 90 (7) (Table 5). The similar result was recorded by Kushwaha *et al.* (2015) who observed the minimum SSR loci in SSR 130 and maximum in SSR 191 markers. Major allele frequency was highest in SSR 90 and lowest frequency was found in two marker namely, SSR 19 and SSR 33. Yadav *et al.* (2016) also found maximum allelic frequency in SSR 99, SSR 113 and SSR 124 and lowest in SSR 90.

Polymorphism information content (PIC) value is a reflection of allele diversity and frequency among the

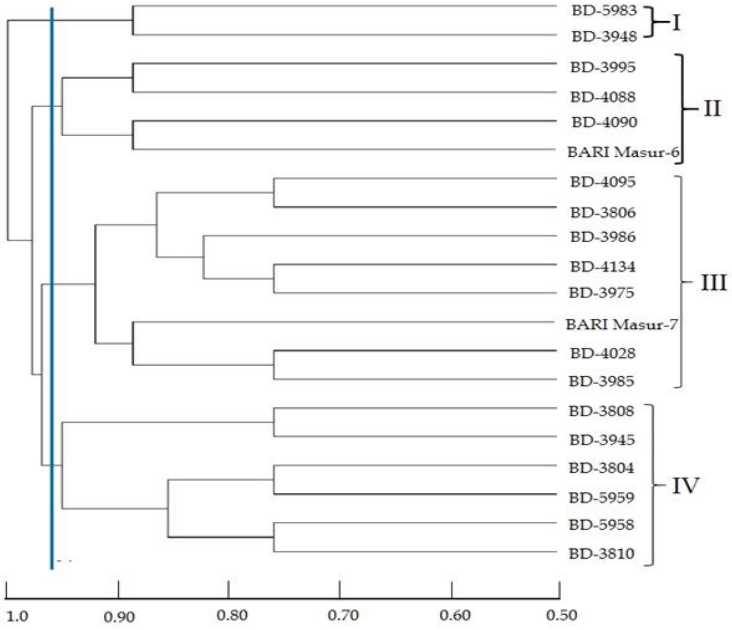

Fig 3: Unweighted pair group method of arithmetic mean (UPGMA) dendrogram based on Nei's (1973) genetic distance, summarizing data on relationship 20 lentil genotype according to SSR data analysis.

Table 5: Details of SSR markers showed polymorphism, number of alleles detected, major allele frequency, allele size range, gene diversity and polymorphism information content (PIC).

Primer	No. of allele	Allele size range (bp)	Major allele frequency	Gene diversity	Polymorphism infor--mation content (PIC)
SSR19	10.00	235-254	0.20	0.88	0.86
SSR33	8.00	257-270	0.20	0.84	0.82
SSR90	7.00	184-191	0.30	0.82	0.79
SSR213	8.00	180-188	0.25	0.84	0.82
Mean	8.25		0.24	0.84	0.82

genotypes. PIC value of each marker can be evaluated on the basis of its alleles and it varied greatly for all the tested SSR loci. In the present investigation, the highest genetic diversity was observed in primer SSR 19 whereas SSR 90 showed the lowest genetic diversity among all the markers for different yield attributing traits (Table 5). Singh *et al.* (2016) also conducted an experiment and he reported maximum genetic diversity and polymorphism information content values in primer PBA_LC_1288 and the lowest value was found in PBA_LC_1423 with mean values, respectively. These result revealed that markers SSR 19 could be best in screening 20 lentil genotypes for yield and yield attributing traits followed by marker SSR 213, SSR 33 and SSR 90. Previously many other researchers, Rao *et al.* (2007), Datta *et al.* (2010), Datta and Lal (2011) and Ruwali *et al.* (2013) recommended that SSR markers can be successfully used in the identification of suitable genotypes.

CONCLUSION

The performance of 20 lentil genotypes for eleven yield and different yield contributing traits were revealed significant variations among the genotypes for all the traits. Considering the traits pods plant^{-1}, seeds plant^{-1}, seeds pod^{-1},100-seed weight and seed yield plant^{-1} BARI Masur-6, BARI Masur-7 followed by genotypes BD-3806 and BD-4090 were best performer. The genotypes with high yielding traits were placed in cluster III. The SSR technique revealed that markers SSR 19 would be best in screening lentil genotypes. The results of this study also showed that microsatellite markers are linked to genes which is suitable tools for genetic understanding for the setup of future systematic lentil breeding programs.

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