FUNCTIONAL PLANT BREEDING JOURNAL
Scientific Journal. ISSN 2595-9433
Volume 4, Number 1, Article n. 5, January/June
D.O.I. http://dx.doi.org/10.35418/2526-4117/v4n1a5
Received: 06/13/2021 - Accepted: 09/14/2021

PHENOTYPIC AND GENETIC VARIABILITY AND GENETIC DIVERGENCE IN LENTIL (*Lens culinaris* Medik.) GERMPLASM

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Abstract: Lentil is a leguminous crop which plays important role in both human health and agriculture. Lack of genotypic and phenotypic variability limits the scope of breeding for developing high yielding lentil varieties. In order to know the genotypic and phenotypic variability and diversity in lentil, research was carried out with 30 lentil genotypes following a randomized complete block design using three replications. Highly significant variability was found for all of the traits among the genotypes under study. The genotype ILL 4127 showed superior performance whereas ILL 2894 showed poor performance. The environment had great impact on the genotypes as the PCV (phenotypic coefficient of variation) was greater than the GCV (genotypic coefficient of variation). High PCV coupled with high GCV, heritability, genetic advance and genetic advance in percentage of mean was found for number of pods plant^{-1} and number of seeds plant^{-1}. Thirty genotypes were divided into 5 clusters where cluster I and cluster V had the maximum inter-cluster distance, specifying the existence of wider genetic diversity among the genotypes of these clusters. Hence, suitable transgressive segregants might be found from crosses between genotypes of those clusters. Genetic diversity at molecular level was found 0.861 to 1.112 which indicated the existence of genetic variation within the studied genotypes. Microsatellite profiling revealed that SSR19 was the highly informative and detectable polymorphic marker followed by SSR48, SSR156, SSR33, SSR13 and SSR130. The results showed the consistency both in molecular and morphological clustering. The genotypes ILL 2894, ILL 3823, ILL 2764, ILL 3201, BM 680, ILL 2083, ILL 5103, ILL 4355, ILL 4707 and ILL 5844 were found as stable considering genetic variability and diversity under study. Therefore, these genotypes might be used as suitable breeding materials for developing advanced lentil varieties.

Keywords: Variability, heritability, genetic advance, genetic diversity, SSR markers.

Introduction

Lentil (*Lens culinaris* Medik.) is a very important legume crop in Bangladesh which has the capacity to sequestrate atmospheric nitrogen (Ganjali et al., 2012). It is a self-pollinated and diploid species (2n=2x=14), belongs to the family Leguminosae, placed under subfamily Faboideae, tribe Fabeae (Soltis et al., 2011). Lentil is originated from Near East and Central Asia (Sandhu and Singh, 2007). In Bangladesh, it is mostly cultivated in the Gangetic floodplain of the western part of the country during *Rabi* season (November-March).

Lentil is known as the "Meat of the poor" an alternative to animal protein for the people of Bangladesh who do have not the ability to buy animal protein (Nath et al., 2014). Seeds of lentil contain high protein (21.2-32.5%) after soybeans (Bhattacharya and Narasimha, 2005). Moreover, it's a very good source of cholesterol lowering fiber (Thavarajah et al., 2011) and antioxidant compounds as well as diverse non-nutritional components such as protease inhibitors, tannins, α-galactoside, oligosaccharides and phytic acid (Urbano et al., 2007). After chickpea (*Cicer arietinum* L.) and pea (*Pisum sativum* L.), cultivated lentil is the third most important cool-season grain legume in the world (FAOSTAT, 2015) but stands first in the consumer's preference in Bangladesh (Uddin et al., 2015). In Bangladesh, demand for diversified food items is a new challenge to agriculture for the overgrowing population as the precedence of agriculture has been shifted towards the nutritional security of the growing population (Das and Kabir, 2016). In 2016-2017 and 2017-2018, the cultivation area and production of lentil were 0.382 and 0.385 million acres and 0.169 and 0.177 million metric tonnes, respectively, with an average yield of 450 kg acre^{-1} (BBS, 2018). In the last year, Bangladesh imported 4.9 metric tonnes lentil which accounts for 0.58 million US$ (BBS, 2017).

In spite of so many advantages, lentil in Bangladesh is generally grown under minimum fertility and management practices. The average yield of lentil in our country is gradually declining mainly due to the use of existing varieties or landraces with low genetic potential and instability of yield. Varieties with high yield potential give higher growth and biological yield (Minhas et al., 2007) and usually have relatively higher number of pods and seeds pod^{-1} (Islam and Islam, 2006). So, to improve yield status of the crop, the development of high potential genotypes with good, stable yield and higher protein content is urgently needed.

For any crop improvement program, genetic variability is a must as the narrow genetic base is a limiting factor in the genetic improvement of a crop (Iddrissi et al., 2013). The lack of information available on the genetic variation of lentil limits cultivar development for higher yield and stability. The knowledge of genetic variability and association of characters with yield is of great importance to the breeder for making an improvement of qualitative characters which generally show little response to direct selection. The nature and magnitude of genetic diversity present in a population are helpful for selecting appropriate parents for hybridization to maximize genetic gain (Gautam et al., 2014). As superior genotypes are used as parent materials in a hybridization program, knowledge of the genetic diversity can be used for additional improvement of lentil genotypes (Kumar and Solanki, 2014). Besides this, the genetic relation among the accessions can be explained by cluster analysis which facilitates in choosing genetically diverse parents for hybridization program.

Simple sequence repeat (SSR) markers have been extensively developed and used in many crops including lentil, is a primary molecular tools for genetic and genomic researches (Bakir and Kahraman, 2019). Molecular markers provide a direct measure of genetic diversity based on morphological traits and facilitate the identification of genomic location linked with the trait of interest (Nandakumar et al., 2004). Although plenty of research studies have been accomplished with lentil, research on genetic diversity and variability study among different lentil genotypes are still limited and fragmentary in Bangladesh. More research is needed for making

a tangible improvement of this crop through appropriate genotyping and phenotyping of the germplasm. Therefore, to determine the phenotypic variability, heritability and genetic advances of yield and yield contributing traits of 30 diverse lentil genotypes, the present study was carried out. The genetic diversity was also estimated through morpho-molecular studies.

Material and methods

Plant materials

The experiment was consisted of 30 lentil genotypes *viz.,* ILL 2083, ILL 2469, ILL 2508, ILL 2530, ILL 2577, ILL 2580, ILL 2589, ILL 2699, ILL 2740, ILL 2741, ILL 2764, ILL 2894, ILL 3130, ILL 3201, ILL 3251, ILL 3662, ILL 3823, ILL 3839, ILL 4187, ILL 4355, ILL 4703, ILL 4707, ILL 5099, ILL 5103, ILL 5127, ILL 5844, ILL 6299, BM 680, BM 513 and P-1463 collected from International Center for Agricultural Research in the Dry Areas (ICARDA), India.

Experimental methodology

At the Field Laboratory of the Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh, the experiment was conducted following a Randomized Complete Block Design using three replications. The size of the whole experimental area was 250 m^2. The whole experimental area was divided into 90 plots in total where the size of individual plot was 2.5 m^2.The seeds were sown on 25th November 2018 using seed rate 1 g m^{-2}, line to line distance 25 cm, line length 3 m and depth 4-6 cm. For initial establishment of the plant up to the nodule formation stage, the fertilizers *viz,* Urea, TSP and MoP were applied @ 30, 80 and 30 kg ha^{-1}, respectively. As there was sufficient rainfall during experimentation, no irrigation was applied. Other intercultural operations including harvesting were done manually in time.

Data collection

Data on yield and yield attributing traits *viz.*, seed yield $plant^{-1}$ (g), stover yield $plant^{-1}$ (g), 100-seed weight (g), number of seeds pod^{-1}, number of pods $plant^{-1}$, number of branches $plant^{-1}$, plant height (cm), days to 50% flowering and days to maturity, were recorded from ten randomly plants $replication^{-1}$.

Data analysis

The appropriate software tools were used to record and analyze the data. A one-way analysis of variance was performed using MINITAB®17 statistical software packages following a RCBD design for yield and yield attributing characters. Based on the formula suggested by Johnson et al., 1955, the genotypic variance (GV) and phenotypic variance (PV), heritability (%), genetic advance (GA) were estimated; GCV and PCV values were calculated following the formula suggested by Singh et al., 1997; GA (%) was calculated according to the formula of Comstock and Robinson, 1952. The Mahalanobis distance (D^2) values were enumerated from transformed uncorrelated means of characters according to Singh and Choudhury, 1985. Cluster analysis was done using statistical software R, version 3.3.2.

SSR analysis

DNA fingerprinting was conducted with SSR markers (Table 1) at Molecular Biology Laboratory of Plant Genetic Resource Centre, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur, during August to September, 2019. Lentil DNA extraction was performed by Modified CTAB method (Doyle and Doyle, 1987) using standard protocol followed by documentation of DNA samples, DNA quantification, PCR amplification, gel separation and scoring of gel separated bands. The amplified products were scored as bands. The DNA bands were observed under UV light on a UV trans-illuminator using gel documentation system and scored in a range of 0 to 1.

Power Marker version 3.23 (Liu and Muse, 2005) was used to determine the number of alleles per locus, effective number of alleles per locus, major allele frequency, gene diversity, Nei's genetic identity and genetic distance values. Molecular weights for microsatellite products in base pairs were estimated with Alpha version

software. The individual fragments were assigned as alleles of the appropriate microsatellite loci. The gene diversity was planned by Nei's, 1972 formula. PIC value described by Anderson, 1993 for self-pollinated species was calculated using the formula, PIC = 1 - $\sum Pi^2$ (Pi is the frequency of i^{th} microsatellite present in the genotypes). Power Marker version 3.23 was used to construct UPGMA (Unweighted pair Group Method with Arithmetic Averages) dendrogram showing the distance-based inter-relationships among the genotypes.

Table 1. Details of the markers used for polymorphism survey

Serial No.	Chr.	Locus name	Forward Primer	Reverse Primer
1	1	SSR13	gAAAcAAcAccg AAA Tac Ac	cgAAgTcAgATgAAg TTT g
2	2	SSR19	gAcTcATAc TTT gTTcTTAgc Ag	gAAcggAgcggTcAc ATT Ag
3	3	SSR33	cAAgcATgAcgccTATgA Ag	cTTTcAcTcAcTcAAcTcTc
4	4	SSR48	cATggTggA ATA gTgATggc	cTccATAcAccAcTc ATT cAc
5	5	SSR130	ccAcgTATgTgAcTg TAT g	gAAAgAgAggcTgAAAcTTg
6	6	SSR156	gTAcATTgAAcAgcATcATc	cAAATgggCATg AAA ggA g

Results

Evaluation of performance of the studied lentil genotypes for yield and yield attributing traits

For all the studied traits, highly significant ($p<0.01$) variation was observed among the genotypes (supplementary table 1). The results of mean performance table indicated that the genotype ILL 5099 required maximum days to 50% flowering (70.88 days) whereas the genotype ILL 4187 required minimum days to 50% flowering (59.99 days) (Table2). For days to maturity, the genotype ILL 3201 required maximum days (104.36 days) to mature and the genotype ILL 6299 required minimum days (94.73 days) to mature. Data on plant height expressed that the tallest plant (28.82 cm) was recorded in the genotype ILL 3130 whereas the shortest plant (17.85 cm) was recorded in the genotype ILL 5099. In case of number of branches plant^{-1}, number of pods plant^{-1} and number of seeds plant^{-1}, the number was recorded the highest (12.54, 75.24 and 141.38, respectively) in the genotype ILL 5127 while the lowest (5.69, 34.12 and 50.49, respectively) in the genotype ILL 2894. The maximum number of seeds pod^{-1} (1.88) was recorded in the genotype ILL 5127 followed by the genotype ILL 6299 (1.82) while minimum number of seeds pod^{-1} (1.48) was recorded in the genotypes ILL 2741, ILL 2894 and ILL 3251. Hundred seed weight and seed yield plant^{-1} were also marked the highest (2.05 and 2.13 g, respectively) in the genotype ILL 5127 whereas the lowest (1.53 and 0.93 g, respectively) in the genotype ILL 2894. On the other hand, the maximum stover yield plant^{-1} (3.54g) was obtained from the genotype ILL 6299 whereas the genotype ILL 3662 produced minimum amount of stover yield plant^{-1} (1.49 g) (Table 2).

Table 2. Mean performance of the studied lentil genotypes

Topics	DFF	DM	PH (cm)	NBPP	NPPP	NSPP	NSP	HSW	StYPP (g)	SYPP (g)
Max.	70.88	104.36	28.82	12.54	75.24	141.38	1.88	2.05	3.54	2.13
Min.	59.99	94.73	17.85	5.69	34.12	50.49	1.48	1.53	1.49	0.93
Mean	63.30	100.92	23.65	7.97	47.85	79.02	1.63	1.69	2.23	1.29
SE	1.22	1.47	1.16	0.56	2.42	3.62	0.02	0.02	0.05	SE
SD	3.26	2.87	3.13	2.04	12.04	25.07	0.11	0.12	0.50	SD
LSD $_{(0.05)}$	2.44	2.94	2.32	1.13	4.84	7.24	0.03	0.04	0.10	0.09
CV (%)	2.36	1.78	6.00	8.66	6.19	5.61	1.14	1.49	2.67	4.27

[Here, Max.=Maximum, Min.=Minimum, Gen.=Genotypes, SE=Standard Error, SD=Standard Deviation, LSD $_{(0.05)}$=Least Significant Difference at 5% level, CV (%)=Coefficient of Variation in percentage, DFF=Days to 50% flowering, DM=Days to maturity, PH=Plant height (cm), NBPP=Number of branches plant^{-1}, NPPP=Number of pods plant^{-1}, NSPP=Number of seeds plant^{-1}, NSP=Number of seeds pod^{-1}, HSW=100-seed weight (g), StYPP=Stover yield plant^{-1} (g) and SYPP=Seed yield plant^{-1} (g)]

Estimation of genetic variability, heritability and genetic advance of the studied lentil genotypes for yield and yield contributing traits

In a breeding program, higher proportion of genotypic and phenotypic coefficient of variation is advisable. All the studied traits showed moderate to high genotypic and phenotypic variance (Table 3).

PV values were higher than the GV values in this study for all the studied traits. The inspected data revealed that PCV (%) was higher than the corresponding GCV (%) for all the studied traits. The maximum value of PCV and GCV were recorded for number of seeds plant^{-1} (32.00 and 31.50%, respectively). The lowest value was obtained in days to maturity for both PCV and GCV (2.72 and 2.06%, respectively).

Table 3. Estimation of genetic parameters for morphological traits related to yield

Traits	PV (σ^2_p)	GV (σ^2_g)	PCV (%)	GCV (%)	h^2b (%)	GA	GA (%)
Days to 50% flowering	8.20	5.96	4.52	3.86	72.74	4.29	6.78
Days to maturity	7.52	4.30	2.72	2.06	57.14	3.23	3.20
Plant height (cm)	9.69	7.68	13.16	11.72	79.22	5.08	21.48
Number of branches plant^{-1}	4.26	3.78	25.90	24.41	88.82	3.78	47.39
Number of pods plant^{-1}	147.00	138.24	25.34	24.57	94.04	23.49	49.08
Number of seeds plant^{-1}	619.73	639.37	32.00	31.50	96.93	50.49	63.89
Number of seeds pod^{-1}	0.01	0.01	7.093	6.82	92.50	0.22	13.52
100-seed weight (g)	0.02	0.01	7.310	6.82	86.96	0.22	13.09
Stover yield plant^{-1} (g)	0.26	0.25	22.648	22.47	98.44	1.03	45.93
Seed yield plant^{-1} (g)	0.09	0.09	23.073	22.68	96.63	0.59	45.93

[Here, σ^2_p = Phenotypic variance, σ^2_g = Genotypic variance, PCV = Phenotypic coefficient of variation, GCV = Genotypic coefficient of variation, h^2b= Heritability, GA= Genetic advance and GA (%) = Genetic advance in percentage of mean]

In the present study, all the traits exhibited moderate to high heritability. Estimates of heritability in broad sense indicated that stover yield plant^{-1}, number of seeds plant^{-1}, seed yield plant^{-1}, number of pods plant^{-1}, number of seeds pod^{-1}, number of branches plant^{-1} and 100-seed weight were highly heritable (>80%) and the values for the respective traits were 98.44, 96.93, 96.63, 94.04, 92.50, 88.82 and 86.96%, respectively (Table 3). On the other hand, plant height, days to 50% flowering and days to maturity showed moderate heritability with values of 79.22, 72.74 and 57.14%, respectively. The highest GA (%) was estimated for number of seeds plant^{-1} (63.89%) while the value was recorded the lowest (3.20%) for days to maturity. For number of pods plant^{-1} and number of seeds plant^{-1}, high heritability coupled with high GA were found (Table 3).

In case of number of branches plant^{-1}, number of pods plant^{-1}, number of seeds plant^{-1}, seed yield plant^{-1} and stover yield plant^{-1}, high heritability coupled with high GA in percentage of mean was recorded. On the other hand, high heritability with moderate GA was found in plant height, number of seeds pod^{-1} and 100-seed weight while days to 50% flowering showed low GA among the studied lentil genotypes (Table 3). In case of plant height, days to 50% flowering and days to maturity, the heritability was moderate coupled with low GA.

Nature and magnitude of genetic diversity for yield and yield contributing traits

The contribution of yield and yield contributing traits towards the total genetic divergence was estimated by cluster analysis. The result of cluster analysis of yield and yield contributing traits and their relative contribution towards the total genetic divergence is shown in Table 4, 5, 6 and Figure 1. Thirty lentil genotypes were divided into 5 clusters depending upon the range of diversity (Table 4 and Figure 1).

Table 4. Number, percent and name of the genotypes in different cluster for yield and yield contributing traits

Cluster number	Number of genotypes	Percent (%)	Name of genotypes
I	13	43.33	ILL 2083, ILL 2469, ILL 2577, ILL 2580, ILL 2741, ILL 2764, ILL 2894, ILL 3201, ILL 3251, ILL 3662, ILL 3823, BM 680, BM 513
II	5	16.67	ILL 2508, ILL 2589, ILL 2740, ILL 3130, ILL 4703
III	8	26.67	ILL 2530, ILL 2699, ILL 3839, ILL 4187, ILL 4355, ILL 4707, ILL 5103, P 1463
IV	2	6.67	ILL 5099, ILL 5844
V	2	6.67	ILL 5127, ILL 6299

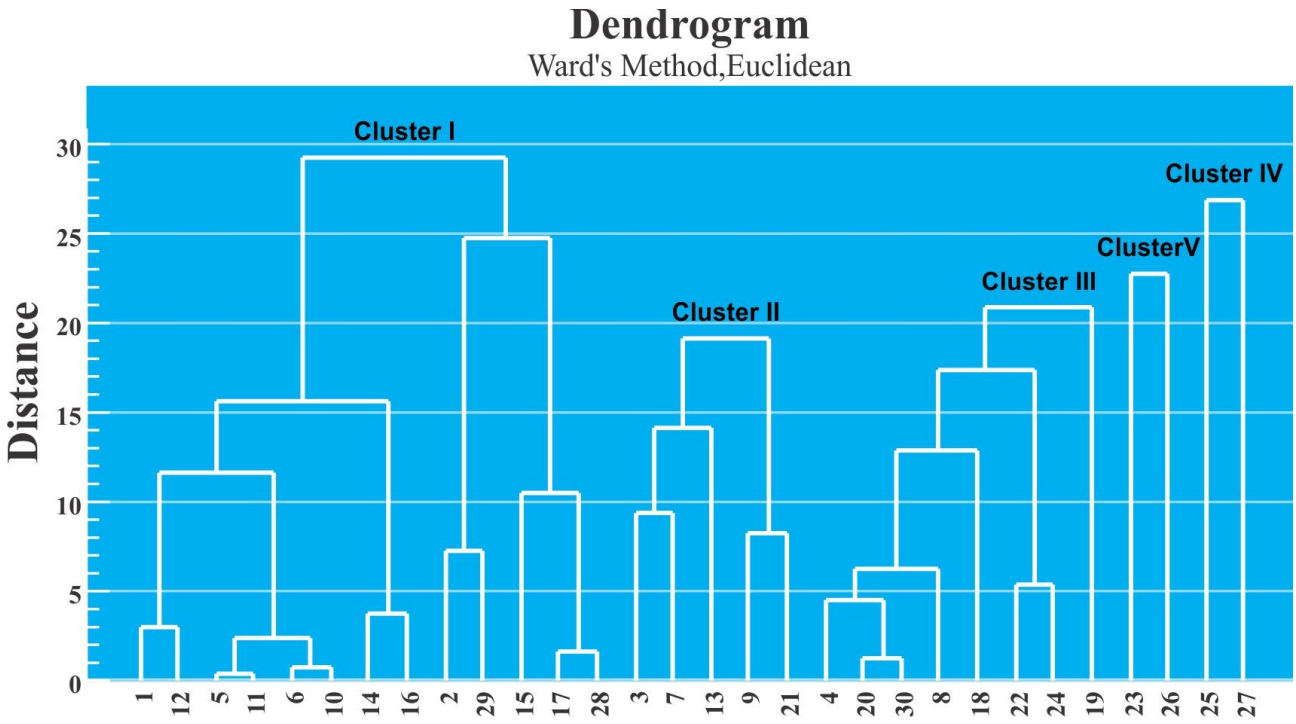

Figure 1. Dendrogram based on summarized data on differentiation among 30 lentil genotypes according to Ward's method

[Here, 1.ILL 2083, 2.ILL 2469, 3.ILL2508, 4.ILL 2530, 5.ILL 2577, 6.ILL 2580, 7. ILL 2589, 8.ILL 2699, 9.ILL 2740, 10.ILL2741, 11.ILL2764, 12.ILL 2894, 13.ILL 3130, 14.ILL 3201, 15.ILL 3251, 16.ILL 3662, 17.ILL 3823, 18.ILL 3839, 19.ILL 4187, 20.ILL 4355, 21.ILL 4703, 22.ILL 4707, 23.ILL5099, 24.ILL 5103, 25.ILL 5127, 26.ILL 5844, 27.ILL 6299, 28.BM 680, 29.BM 513 and 30.P-1463.]

The distribution pattern divulged that cluster I had the maximum number of genotypes (13 genotypes) covering 43.33% of the total studied genotypes while cluster IV and cluster V comprised the minimum number of genotypes (2 genotypes each). Cluster II and cluster III possessed 5 and 8 genotypes, respectively which covered 16.67 and 26.67% of the total studied genotypes, respectively. The inter-cluster distances were greater than the intra-cluster distances in most of the cases (Table 5).

The intra-cluster average D^2 values ranged from 9.20 to 27.78. The intra-cluster degree of diversity was observed as the highest in cluster V and the lowest in cluster IV. Regarding the inter-cluster distances, the average D^2 value ranged from 13.67 to 58.36. Cluster I and cluster V had the maximum inter-cluster distance whereas cluster III and cluster IV had the minimum distance.

The results of cluster mean reflected that the short height genotypes (21.34 cm) were grouped into cluster V while the tall height genotypes (24.84 cm) were presented in cluster II (Table 6). Cluster V had the genotypes with high abundance of branches plant^{-1} (12.04), pods plant^{-1} (72.21), seeds plant^{-1} (133.65) and stover plant^{-1} (3.47 g) while genotypes included in

cluster II had low abundance of these traits (6.24, 37.43, 60.23 and 1.79 g, respectively). The highest number of seeds pod^{-1} (1.85), 100-seed weight (1.87 g) and seed yield plant^{-1} (1.96 g) were recorded for the genotypes in cluster V while in cluster I, the lowest value for these traits (1.53, 1.62 g and 1.06 g, respectively) were found. All the early flowering (61.08 days) genotypes were grouped into cluster II whereas cluster IV contained the late flowering (69.45 days) ones. Cluster V included the short duration (96.99 days) genotypes whereas long duration (102.29 days) genotypes were present in cluster IV (Table 6).

Table 5. Average inter and intra-cluster distance among the studied lentil genotypes for yield and yield contributing traits

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
Cluster I	**92.48 (9.62)**	247.78 (15.74)	721.32 (26.86)	436.62 (20.90)	3405.71 (58.36)
Cluster II		**188.42 (13.73)**	658.90 (25.67)	390.16 (19.75)	3344.03 (57.83)
Cluster III			**134.97 (11.62)**	186.87 (13.67)	1467.45 (38.31)
Cluster IV				**84.62 (9.20)**	2084.17 (45.65)
Cluster V					**771.61 (27.78)**

Note: Bold values in the table indicate intra-cluster distances

Table 6. Cluster means for ten traits related to yield of the studied lentil genotypes

Traits	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
Days to 50% flowering	63.20	61.08	62.43	69.45	66.91
Days to maturity	102.07	98.79	101.01	102.29	96.99
Plant height (cm)	24.81	24.84	21.92	22.42	21.34
Number of branches plant^{-1}	6.56	6.24	9.93	9.50	12.04
Number of pods plant^{-1}	39.49	37.43	59.58	56.96	72.21
Number of seeds plant^{-1}	60.46	60.23	102.72	97.18	133.65
Number of seeds pod^{-1}	1.53	1.61	1.72	1.71	1.85
100-seed weight (g)	1.62	1.81	1.71	1.68	1.87
Stover yieldplant^{-1} (g)	1.96	1.79	2.61	2.35	3.47
Seed yieldplant^{-1} (g)	1.06	1.14	1.56	1.44	1.96

Genetic diversity study in the studied lentil genotypes using SSR marker

A total of 20 alleles were detected among the studied lentil genotypes using six primers (Table 7 and Figure 2a and Figure 2b). The number of alleles per locus ranged from 3 to 4 where the average value was 3.333 alleles per locus. SSR130 had the lowest (1.991) and the SSR19 had the highest (2.528) effective number of alleles with a mean value of 2.314. The overall size of the amplified products ranged from 142 to 283 bp (Table 7 and Figure 2a and Figure 2b). Major allele frequency ranged from 0.250 to 0.334 at each locus with being average 0.306. SSR19 had the highest genetic diversity (1.122) while SSR130 had the lowest genetic diversity (0.861) with a mean diversity of 0.979 (Table 7).

The PIC (Polymorphism Information Content) value of each marker among the studied lentil genotypes was estimated on the basis of its allele and it varied greatly for all SSR loci tested. The polymorphism level among the lentil genotypes was evaluated by calculating PIC value for each of the 6 SSR loci. The range of PIC values was 0.498 (SSR130) to 0.604 (SSR19) with an average value of 0.567 (Table 7).

Table 7. Data on major allele frequency, allele number, gene diversity and PIC found among the studied lentil genotypes for six SSR markers

Locus	No. of observation	No. of alleles	Effective number of alleles	Allele sizes (bp)	Major allele frequency	Gene diversity	PIC
SSR13	30	3	2.174	142-167	0.334	0.870	0.549
SSR19	30	4	2.528	190-262	0.250	1.122	0.604
SSR33	30	4	2.381	235-283	0.250	1.089	0.580
SSR48	30	3	2.406	181-200	0.333	0.983	0.584
SSR130	30	3	1.991	188-210	0.333	0.861	0.498
SSR156	30	3	2.406	186-208	0.333	0.948	0.584
Mean	30	3.333	2.314	....	0.306	0.979	0.567
SD	....	0.516	0.196	....	0.043	0.109	0.038

Note:* Major allele frequency means alleles frequency greater than 0.05 (5%).

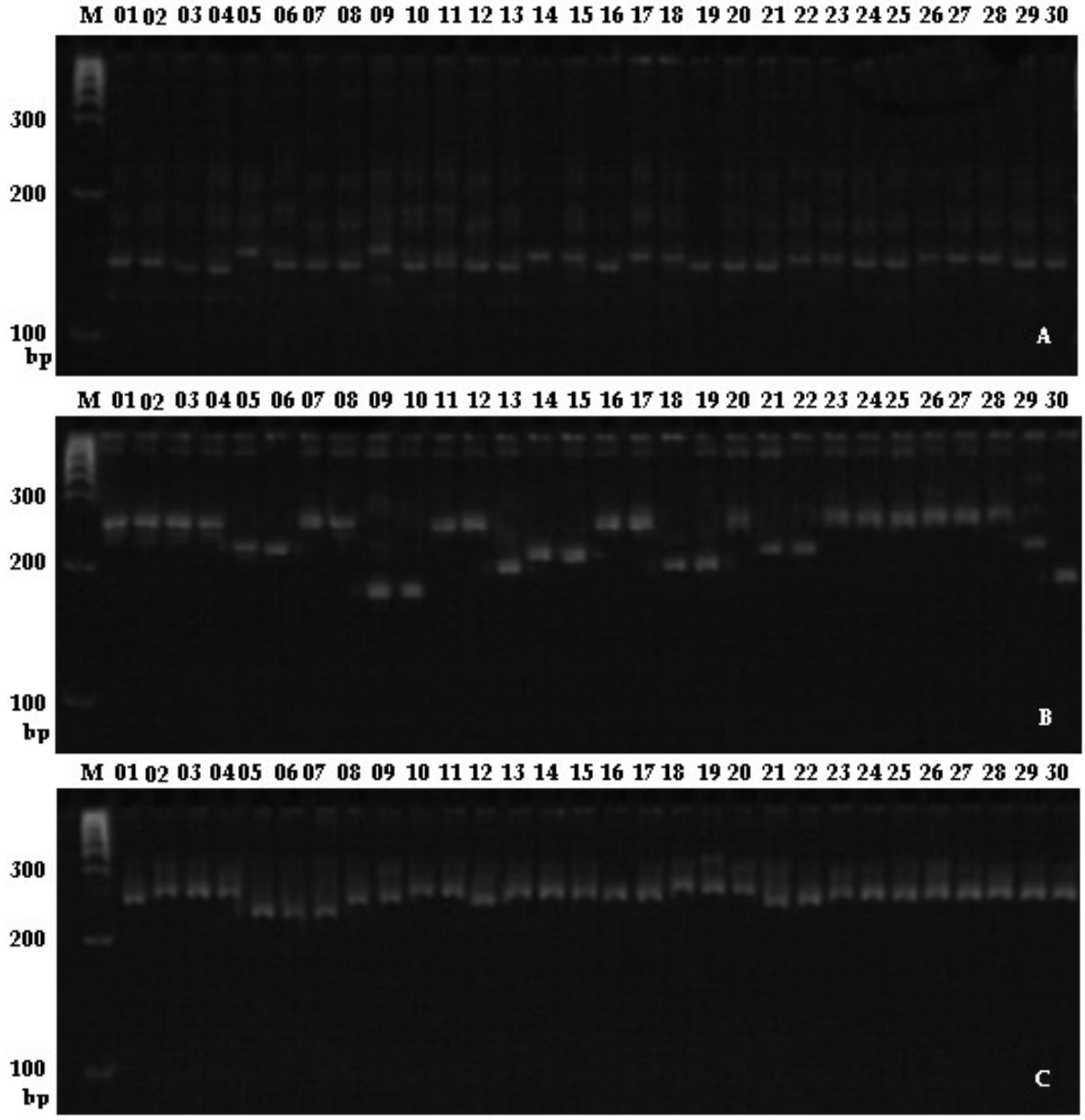

Figure 2a. Microsatellite profiles of 30 lentil genotypes at locus **SSR13 (A), SSR19 (B) and SSR33 (C)**; M: molecular weight marker (100 bp DNA ladder), Lane 01: ILL 2083, Lane 02: ILL 2577, Lane 03: ILL 2589, Lane 04: ILL 2699, Lane 05: ILL 2740, Lane 06: ILL 2741, Lane 07: ILL 2764, Lane 08: ILL 2894, Lane 09: P 1463, Lane 10: BM 517, Lane 11: ILL 5844, Lane 12: BM 680, Lane 13: ILL 3130, Lane 14: ILL 3201, Lane 15: ILL 3251, Lane 16: ILL 3652, Lane 17: ILL 3829, Lane 18: ILL 3823, Lane 19: ILL 4187, Lane 20: ILL 2580, Lane 21: ILL 2508, Lane 22: ILL 2530, Lane 23: ILL 4707, Lane 24: ILL 5099, Lane 25: ILL 5127, Lane 26: ILL 2469, Lane 27: ILL 3355, Lane 28: ILL 4703, Lane 29: ILL 5103, Lane 30: ILL 6299.

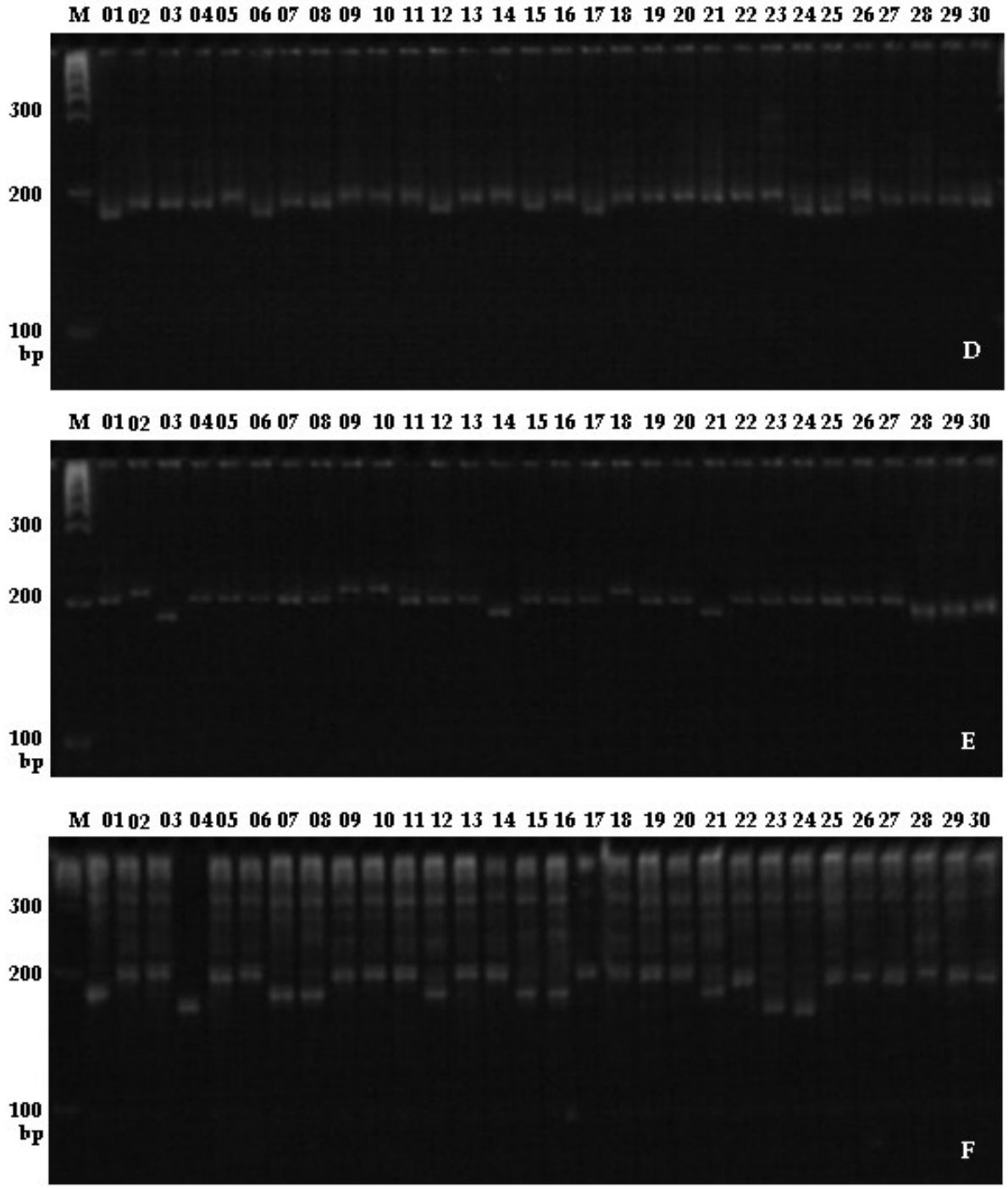

Figure 2b. Microsatellite profiles of 30 lentil genotypes at locus **SSR48 (D), SSR130 (E) and SSR156 (F)**; M: molecular weight marker (100 bp DNA ladder), Lane 01: ILL 2083, Lane 02: ILL 2577, Lane 03: ILL 2589, Lane 04: ILL 2699, Lane 05: ILL 2740, Lane 06: ILL 2741, Lane 07: ILL 2764, Lane 08: ILL 2894, Lane 09: P 1463, Lane 10: BM 517, Lane 11: ILL 5844, Lane 12: BM 680, Lane 13: ILL 3130, Lane 14: ILL 3201, Lane 15: ILL 3251, Lane 16: ILL 3652, Lane 17: ILL 3829, Lane 18: ILL 3823, Lane 19: ILL 4187, Lane 20: ILL 2580, Lane 21: ILL 2508, Lane 22: ILL 2530, Lane 23: ILL 4707, Lane 24: ILL 5099, Lane 25: ILL 5127, Lane 26: ILL 2469, Lane 27: ILL 3355, Lane 28: ILL 4707, Lane 29: ILL 5103, Lane 30: ILL 6299**.**

Pair-wise estimates of similarity ranged from 0.000 to 1.00 among the studied genotypes (Figure 3). The pair-wise genetic dissimilarity indices showed that the highest genetic dissimilarity was observed in the genotype ILL 2083 with the genotypes ILL2589, ILL 2740, P 1463 and BM 513 among the studied lentil genotypes (Figure 3). Thirty lentil genotypes were categorized into 6 clusters (Figure 3) based on UPGMA cluster analysis.

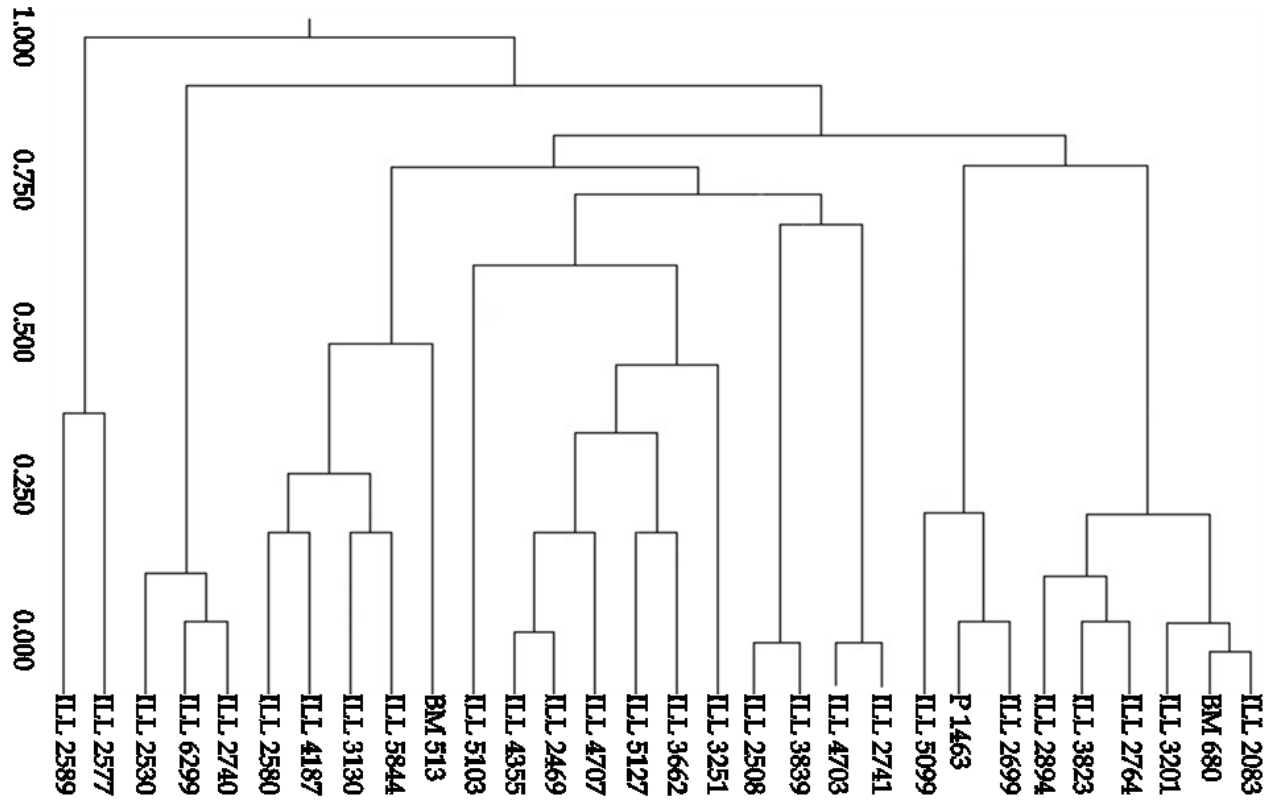

Figure 3. UPGMA dendrogram based on Nei's (1972) genetic distance, summarizing the data on differentiation among 30 genotypes according to microsatellite analysis

Maximum number of genotypes (9 genotypes) were observed in cluster I (30.00%) and minimum (2 genotypes) in cluster VI (6.67%). Cluster II, III, IV and V contained 4, 7, 5 and 3 genotypes, respectively; the frequencies for the respective clusters were 13.33, 23.33, 16.67 and 10.00%, respectively (Table 8).

Table 8. Number, frequency and name of genotypes in different cluster for molecular analysis of the studied lentil genotypes

Cluster Group	Number of genotypes	Frequency (%)	Name of genotypes
I	9	30.00	ILL 2083, BM 680, ILL 3201, ILL 2764, ILL 3823, ILL 2894, ILL 2699, P 1463 and ILL 5099
II	4	13.33	ILL 2741, ILL 4703, ILL 3839 and ILL 2508
III	7	23.33	ILL 3251, ILL 3662, ILL 5127, ILL 4707, ILL 2469, ILL 4355 and ILL 5103
IV	5	16.67	BM 513, ILL 5844, ILL 3130, ILL 4187 and ILL 2580
V	3	10.00	ILL 2740, ILL 6299 and ILL 2530
VI	2	6.67	ILL 2577 and ILL 2589

Discussion

Evaluation of performance of the studied lentil genotypes for yield and yield attributing traits

Thirty lentil genotypes were evaluated for ten quantitative traits to assess phenotypic and genetic diversity. Analysis of variance for all the studied traits was highly significant ($p<0.01$) indicating the presence of adequate genetic variability in the experimental material (Hussain et al., 2014; Singh et al., 2014; Sakthivel et al., 2019).

Significant variations ($p<0.01$) of the studied traits among the studied lentil genotypes (Table 2) might be due to their difference in genetic makeup among genotypes. Days to 50%

flowering varied from 59.99 to 70.88 days with mean 63.30 days which is due to the difference in genetic makeup of the studied genotypes and environmental factors (Ahamed et al., 2014; Rahman et al., 2015). Cristobal et al., 2014 and Hussain et al., 2014 found dissimilar results for this study. Days to maturity ranged from 94.73 to 104.36 days and the mean value was 100.92 days. The result was in line with the findings of Debbarma et al., 2018 who also observed that days to maturity varied from 94.67 to 104.00 days and also with Gupta et al., 2012 but Abdipur et al., 2011 found comparatively higher variation in days to maturity than the present study due to the genetic dissimilarity among the lentil genotypes. The differences in plant height among the studied genotypes were mostly due to the genetic variation among the genotypes. Plant height was significantly ($p<0.01$) varied among the studied lentil genotypes (Table 2) (Gupta et al., 2012; Babayeva et al., 2014; Adhikari et al., 2018).

In case of yield components, significant variation ($p<0.01$) was found. The presence of such variation performs selection to be used as parents in programs of breeding. Number of branches plant^{-1} exhibited high significant ($p<0.01$) variability among the studied genotypes (Table 2) (Hussain et al., 2014; Reja et al., 2017). Relating the number of pods plant^{-1}, the range differed from 34.12 to 75.24 with a mean 47.85. The variation observed for this trait was highly significant ($p<0.01$) which might be due to genotypic and climatic factors (Cristobal et al., 2014; Mekonnen et al., 2014; Adhikari et al., 2018). The results expressed that seed number was ranged from 50.49 to 141.38 with mean value 79.02 among the studied genotypes which might be due to the genotypic factor (Abdipur et al., 2011; Gupta et al., 2012; Babayeva et al., 2014). In case of number of seeds pod^{-1}, the value was recorded from 1.48 to 1.88 with an average of 1.63 (Table 2). Hence, the variation was also significant ($p<0.01$) for this trait. Results of this study were corroborated with some previous findings (Mekonnen et al., 2014) for seed number pod^{-1}. The result was closed with Gupta et al., 2012 and Adhikari et al., 2018.

Higher seed weight might be due to the production of bigger sized seeds resulting in higher yield. Increased demand for assimilates for higher pod numbers plant^{-1} results in higher 100-seed weight (Gupta et al., 2012). The range for this trait was 1.53 to 2.05 g among the studied genotypes with a mean of 1.69 g (Table 2). The result was closed with the findings of Depar et al., 2016 and Debbarma et al., 2018 who found comparatively higher value for 100-seed weight than the present study. The reason for different 100-seed weight might be due to genetic make-up of the genotypes which was primarily influenced by heredity.

Stover yield plant^{-1} ranged from 1.49 to 3.54 g. The mean value for the respective trait was 2.23 g. The result indicated that stover yield plant^{-1} varied significantly ($p<0.01$) among the studied genotypes (Table 2). The results showed consistent with previous finding by Kundu et al., 2014 and Biswas et al., 2018 in lentil.

In this experiment, seed yield plant^{-1} differed significantly ($p<0.01$) among the studied genotypes. The range varied from 0.93 to 2.13 g with being average 1.29 g (Table 2); the result was parallel with Bicer and Sarkar, 2010 who found seed yield varied from 0.5 to 2.37 g. Significant differences for seed yield plant^{-1} were also reported by many researchers earlier (Tyagi and Khan, 2010;Gupta et al., 2012). The probable reason for different seed yields might be due to the different yield parameters which were influenced by the genetic makeup of the variety.

Estimation of genetic variability, heritability and genetic advance of the studied lentil genotypes for yield related traits

A range of variability was found for yield and yield components (Table 3) among the studied lentil genotypes. Similar results were reported in lentil by Dugassa et al., 2014;Paliya et al., 2015; Chowdhury et al., 2019; Akter et al., 2021. In this experiment, phenotypic variance was always higher than the genotypic variance (Table 3) for all the studied traits which might be due to the effect of environmental factors on these traits (Hossen, 2011).

Higher PCV and GCV in traits might allow selection to improve these traits. In the present investigation, the values of PCV were higher than the GCV for all the studied traits (Table 3) which indicated the impact of environment on the traits (Al-Aysh, 2014; Chowdhury et al., 2019; Akter et al., 2020). Both PCV and GCV were high in number of branches plant^{-1}, number of seeds plant^{-1}, number of pods plant^{-1}, seed yield plant^{-1} and stover yield plant^{-1}; moderate in plant height whereas the lowest in days to maturity. Edossa et al., 2010 also reported high PCV for these traits.

Heritability among the genotypes for different traits varied from 57.14 to 98.44% (Table 3). Similar result was also reported by Chowdhury et al., 2019. High heritability was estimated for all the traits under study except days to maturity, days to 50% flowering and plant height. Hence, these traits might get priority during selection for the improvement of seed yield of lentil. High heritability coupled with high GA was expressed by number of pods plant^{-1} and number of seeds plant^{-1} (Table 3). In contrast, high heritability in addition with high GA (%) was observed number of pods plant^{-1} number of branches plant^{-1}, seed yield plant^{-1}, number of seeds plant^{-1}, and stover yield plant^{-1}. The results reflected the accumulation of additive genes and phenotypic selection for these traits will be effective for seed yield improvement (Tyagi and Khan, 2010; Dugassa et al., 2014; Chowdhury et al., 2019).

Nature and magnitude of genetic diversity for yield and yield contributing traits

The genotypes were classified into 5 clusters based on Euclidean distance following Ward's method (Table 4 and Figure 1). Hossen, 2011; Gupta et al., 2012 and Nath et al., 2014 and also found similar result. The distribution pattern of cluster analysis revealed that cluster I contained 43.33% of the total genotypes which was the largest one. On the other hand, both cluster IV and V was the smallest one where each of them contained 6.67% of the total studied genotypes (Table 4 and Figure 1). The genotypes accumulated in the same cluster implying that they are not strongly diversified.

The magnitude of inter-cluster distance was higher than intra-cluster distance in most of the cases indicating wider divergence among the studied genotypes of this group (Pandey and Bhatore, 2018; Kumar, 2019). Maximum intra and inter-cluster divergence were observed in group V which indicated that efficient breeding program could be formulated to improve yield potential by hybridization and selection of superior genotypes in segregating generations. The highest intra-cluster distance was found in cluster V indicating that the genotypes belonged to cluster V were more heterogeneous (Hossen, 2011). In cluster IV, genotypes were comparatively more closely related as intra-cluster distance among the genotypes was minimum. In case of inter-cluster distance, cluster I and cluster V had the maximum inter-cluster distance (Table 5). Hence, genotypes included in these clusters were genetically diverse and might give rise to high heterotic response in segregating generation (Gupta et al., 2012; Paliya et al., 2015). Cluster III and cluster IV showed the minimum inter-cluster distance which specified the least genetic diversity and almost similar genetic architecture among the genotypes of these clusters. Genotypes with minimum inter-cluster distances might also be used for bi-parental crosses between the most diverse and the closest groups in breeding programs to break the awful linkages between yield and its related traits (Hossen, 2011).

The results of cluster means (Table 6) concluded that cluster V was the highest divergent group and the traits number of pods plant^{-1},number of branches plant^{-1}, number of seeds pod^{-1}, number of seeds plant^{-1}, 100-seed weight, stover yield plant^{-1} and seed yield plant^{-1} were the highly significant divergent traits providing maximum to the total divergence that had positive correlation with seed yield plant^{-1} signifying the predominance of additive gene action for these traits among the studied genotypes of lentil. Therefore, these traits should be given more emphasis for determining the type of cluster for the further selection and choice of

parents for hybridization. This finding was in close harmony with the results obtained by many researchers (Babayeva et al., 2014; Kurshee, 2014; Nath et al., 2014).

The results of the present study suggested that the material involved in this study had sufficient amount of diversity for important agronomic traits and may be exploited with great extent by resorting to hybridization that consequently would result into the development of better lentil varieties.

Diversity analysis through SSR markers

Overall allelic diversity

In the present study, diversity of studied lentil genotypes was analyzed using six SSR primers (Figure 2a and Figure 2b). The results of diversity analysis revealed that all the primers detected the polymorphism among the studied lentil genotypes and produced a varying number of alleles. The microsatellite loci were multi-allelic varied from 3 to 4 alleles per locus with a mean value of 3.333 alleles per locus (Table 7) (Dikshit et al., 2015; Pandey et al., 2018) in lentil. The primers SSR19 and SSR33 produced maximum number of alleles per locus those were highly polymorphic whereas the allele number per locus was minimum in the primers SSR13, SSR48, SSR130 and SSR156 (Table 7). Asimilar result for the primer SSR19 was also reported in different studies (Yadav et al., 2016; Pandey et al., 2018). Singh et al., 2016 reported a contrary finding who noticed higher number of alleles per locus than the current study with an average of 5.9 alleles per locus for lentil genotypes.

The effective number of alleles was found the lowest in SSR130 and the highest in SSR19 with a mean value of 2.315. Major allele frequency ranged from 0.250 to 0.334 (Table 7). But Yadav et al., 2016 found higher allele frequency which was contradictory with this study. Genetic diversity ranged from 0.861 (SSR19) to 1.112 (SSR130) with a mean value of 0.979 (Table 7), indicated the presence of high genetic variation within the genotypes. Mekonnen et al., 2016 and Singh et al., 2016 found comparatively lower genetic diversity for lentil than the current study. It was observed that markers those detected higher number of alleles showed higher genetic diversity than those detected lower number of alleles. The wide genetic variability of these genotypes could help for better adaptation to biotic and abiotic stresses.

In the current study, the primer SSR19 showed the maximum PIC value while primer SSR130 showed the minimum PIC value. The range of PIC values was 0.498 to 0.604 (Table 7). The average PIC value of these markers was 0.567 (Mekonnen et al., 2016; Singh et al., 2016). The result of present study suggested that SSR19 was the best marker based on PIC values to determine the diversity of the lentil genotypes followed by SSR48, SSR156, SSR33, SSR13 and SSR130 which indicates the command and advanced resolution of those marker systems in identifying molecular diversity

The range of genetic distance or coefficient of similarity among the studied lentil genotypes differed from 0 to 1.00 with a mean of 0.500 among various genotype combinations (Figure 3). Mekonnen et al., 2016 and Datta et al., 2011 also found almost similar values for similarity indices among lentil genotypes using SSR markers. The highest genetic dissimilarity was observed in the genotype ILL 2083 with the genotypes ILL 2589, ILL 2740, P 1463 and BM 513 among the studied lentil genotypes (Figure 3). Higher genetic distance indicates lower inter-germplasm similarity index and vice-versa. Higher genetic variability could be found in genotypes that are collected from the same location and homozygous in nature (Popi et al., 2000).

UPGMA reveals different degrees of genetic relationships. All the studied genotypes were divided into six clusters using a UPGMA dendrogram with 0.500 cut-off similarity coefficient, below which the similarity values narrowed conspicuously (Figure 3). Genetically similar genotypes clustered together and showed comparatively same banding pattern. Thirty percent of total genotypes were grouped together in cluster I, 13.33% in cluster II, 23.33% in cluster III, 16.67% in cluster IV, 10.00% in cluster V and

the rest 6.67% in cluster VI (Table 8). Genotypes selected from different clusters will provide maximum heterosis which favors yield.

The key findings of genetic variations based on molecular characterization revealed that the genotypes were grouped into different clusters because of their genetic components themselves. Therefore, these findings can be used for further lentil breeding programs, especially in selecting parents in hybridization programs and eventually in the application of marker-assisted selection programs.

Conclusion

From the above study, it may be concluded that the presence of significant variation among the studied traits resembling the existence of genetic diversity among the studied lentil genotypes and the selection of the traits like number of seeds plant^{-1},number of pods plant^{-1}, number of branches plant^{-1}, 100-seed weight and stover yield plant^{-1} might be effective to increase seed yield in lentil as these traits showed high GA (%) together with high heritability and positively significant association with seed yield plant^{-1}. On the other hand, genotypes having wider genetic diversity presenting in cluster I and cluster V might produce high heterotic response in the segregating generation. Finally, the genotypes ILL 2894, ILL 3823, ILL 2764, ILL 3201, BM 680, ILL 2083, ILL 5103, ILL 4355, ILL 4707 and ILL 5844 were found stable which could be used as suitable breeding materials for further genetic improvement of lentil based on their agronomic performance.

Acknowledgements

The authors acknowledge the Ministry of education, Bangladesh for providing financial support to complete the research work.

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Supplementary Table: Analysis of variance (ANOVA) for yield and yield contributing traits of the studied lentil genotypes

Sources of variation	Replication	Genotypes	Error
Degrees of freedom	2	28	56
Days to 50% flowering	113.670	20.215**	2.235
Days to maturity	38.631	16.123**	3.225
Plant height (cm)	13.270	25.053**	2.015
Number of branches plant^{-1}	0.072	11.818**	0.476
Number of pods plant^{-1}	58.876	423.472**	8.764
Number of seeds plant^{-1}	154.170	1878.830**	19.640
Number of seeds pod^{-1}	0.001	0.038**	0.001
100-seed weight (g)	0.001	0.043**	0.001
Stover yield plant^{-1} (g)	0.033	0.760**	0.004
Seed yield plant^{-1} (g)	0.023	0.261**	0.003

Note: ** indicates significant at 1% level of probability.