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Variability, Genetic Advance, Heritability Study of Advanced Breeding Lines of Lentil (*Lens culinaris* Medic.) at Mid Western Terai of Nepal.

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Abstract

A field experiment was conducted at Grain Legumes Research Program (GLRP), Khajura, Banke, Nepal comprising eighteen genotypes of lentil during 2015/16 in randomized complete block design replicated thrice to observe genetic variability, heritability and genetic advance using six quantitative traits and selection and advancement of early maturing, high yielding, disease resistant genotypes for mid western terai of Nepal. Analysis of variance revealed all characters were highly significant differences (<0.01) except plant height observed non significant response. High broad sense heritability coupled with high expected genetic advance as percent of mean were observed in number of pods per plant (78 and 52.59%), thousands grain weight (92 and 37.58%), and days to fifty percent flowering (93 and 34.97%). Grain yield obtained medium heritability (44%) and high expected genetic advance as percent of mean (20.94), similarly high heritability (98%) and medium genetic advance (12.93) was found in the trait days to maturity. Low heritability (18%) and low genetic advance (7.45%) was obtained in plant height. All parameters obtained high phenotypic coefficient of variance than genotypic coefficient of variance indicating role of environment for the expression of the traits. Mean separation and clusturing showed genotypes ILL-3338, ILL-60265 and X 945-48 were high yielder and having high number of pods per plant and separated in same clusture 4. Total four clusters were obtained in eighty percent euclidean similarity clusturing indicating genetic closeness/distances among the genotypes.

Keywords: Genetic Advance, Heritability, Lentil, Variability, Clusturing

Introduction

Lentil (*Lens culinaris* Medik. culinaris) is a diploid (2n = 2x = 14), self pollinated and annual cool season grain legume with genome size of 4,063 Mpb (Arumuganathan and Earle, 1991). It is one of the first agriculture crop grown more than 8500 years ago (Aghili et al., 2012). Worldwide, lentils are cultivated in 5.48 million ha with a production of >6.32 Tg, with a productivity of 1152 kg ha−1 (FAO, 2016). Global production of lentils was 6.3 million tonnes, led by Canada with 51% and India with 17% of the world total in 2016. Canada is the largest producer of lentil and produces 2.5 million tons followed by India, Turkey and Nepal (FAOSTAT, 2017). Canada, India, Turkey, USA, Nepal, Australia, Ethiopia, Bangladesh, Kazakisthan are top ten lentil producing countries of the world sequentially https://www.atlasbig.com/en-us/countries-lentil-production. Approximately 50% of world's lentils are grown in South Asia, and nearly 1.5 billion people in this area consume ~70% of the global lentil supply (Shrestha et al., 2018).

Lentil is the top most legume of Nepal in terms of area and production. Total area covered by lentil in 2016/17 was 206969 ha producing 254308 mt with productivity 1.229 mt/ha in Nepal (MOAD, 2018). It shares 64% area and 67% production of total legumes of the country. Lentil is cultivated from terai to mountain of Nepal, Terai, hill and mountain shares 94.5, 5.08 and 0.58 percentage of area and 95.6, 4.25 and 0.31 percentage of production of nation respectively. Which shows it's main area is terai, mid western terai alone contributes 27.6% area and 29.6% production to the whole nation (MOAD, 2018). Mid western region of Nepal is the highest contributor to production (30.1%) and second to area (29.6%) among the five development region of Nepal. Banke district, our research venue shares 5.4 and 5.7 percentage of area and production of the country.

Nepalese lentil shared by 3% in area and 3 % in production in the total area and production of the world (MOAD, 2018). Quick cooking quality, tasty pink red cotyledons and high micronutrient contents make Nepalese lentil highly preferable to the international consumers and popular in the international market (Deve et al., 2007). Lentil is one of the exportable commodities. Bangladesh, Singapore, Sri Lanka, Germany, Korea, UK, Indonesia are the major export markets for Nepalese lentils (Gharti et al., 2014). Lentil alone accounts for 90% of the total export of pulses and contributes about 2.3% of total national exports and shares about 3.1% of the total lentil export in the world (USAID, 2011).

It is also a key commodity crop enhancing in crop diversification, intensification and sustainable agriculture in the country. In these days the demands of lentil are sharply increasing in Nepal because of changing the people food habits i.e. lentil dal is the important component of Nepalese and well adapted in the existing farming system. Most importantly, it can grow well in limited rainfall areas of the world which might be the good option to cope with the climate change.

Prior knowledge of genetic variability and characterization of germplasm available at the station has an important implication for future utilization to identify areas of major priority for conservation and improvement programs (Allard, 1960). Quantitative traits provide an estimate of genetic diversity, and various numerical taxonomic techniques have been successfully used to classify and measure the pattern of genetic diversity in germplasm, as in lentil (Erskine et al., 1949 and Ahmad et al., 1997). Knowledge of level of genetic diversity available in germplasm is prerequisite for any breeding programs that genetic gain could not be limited due to the availability of narrow genetic variability between parental lines involved in crossing programs (Kumar et al., 2004). In the past years, breeders used morphological data and pedigree information to assess genetic diversity among the lentil varieties. However, these studies could not make much contribution to our knowledge due to limited phenotypic diversity, high genotype x environment interaction, and paucity of accurate record of ancestry (Darai et al., 2019).

Cluster analysis can be a good source to identify the variation in the germplasm and to classify based on similarity and dissimilarity index. This analysis is also useful for the selection of parents for the breeding program and crop modeling (El-Deeb and Mohamed, 1999; Jaynes et al., 2003).

Main problems of lentil in Nepal are uncertain occurrence of stemphylum blight, low priority for input, lack of drought tolerant varieties, lack of sprinkler and drip irrigation, so farmers are searching for more new varieties. The present study was, undertaken to identify a proper plant type for selection so as to improve the seed yield keeping in view the variability, genetic advance, clusturing and heritability of concerned studied parameter of lentil. Selection and advancement of early maturing, high yielding, disease resistant and drought tolerance lentil genotypes for mid western terai of Nepal.

Materials and Methods

A field experiment was conducted at Grain Legumes Research Program (GLRP), Khajura, Banke, Nepal, during winter season of 2014/15 (November Ist week 2015 to February Ist week 2016). Geographically Grain Legume Research Program (GLRP) is located at Janaki VDC, Banke district. It lies between at 81^0 37" East longitudes and 28^0 06" North lattitude and an altitude of 181 meters above mean sea level. Average annual rainfall of the station is 1000-1500 mm. However, delayed onset and early termination of monsoon rains is a regular feature, causing occasional failure of annual crops in the region. The maximum and minimum temperature at the station is 46^0C and 5.4^0C respectively, with relative humidity (R.H.) ranging between 27 % to 94 %. Humidity remains low in most parts of the year. Soils of the station have sandy to silty loam, poor in organic carbon and available N but medium in available P_2O_5 and K_2O, pH varies from 7.2-7.5. Detail of weather parameters is given in Annex 1.

Annex: 1: Monthly agro-Meteorological Data of the Station during the period of research

Year Month	8:45 NST Temperature (degree celcius)		17:45 NST Temperature (degree celcius)		Rain (mm)	Relative humidity (%)
	Max	Min	Max	Min		
Jul-15	35	27	35	29	27	73
Aug-15	33	26	34	28	18	83
Sep-15	34	25	34	29	5	78
Oct-15	32	20	32	25	15	81
Nov-15	29	14	29	20	2	85
Dec-15	24	9	23	12	-	96
Jan-16	22	7	22	10	2	96
Feb-16	27	11	28	15	2	85
Mar-16	33	15	33	23	3	54
Apr-16	40	18	40	29	-	29
May-16	37	24	37	29	12	47
Jun-16	35	26	35	28	39	81

Eighteen advanced breeding lines of lentil were used including one standard check released variety Sagun for this study. The experiment was carried out in randomized complete block design in three replications. Four rows of each entry were planted having row-row distance of 25 cm with 4 m row length with 4 m2 plot area. All recommended dose of manure and fertilizer (20:40:20 kg $N:P_2O_5:K_2O$ and 6 ton FYM/ha) were applied as basal in lentil. All the recommended cultural practices were followed to raise the healthy crop. Days to 50% flowering and days to 90% maturity were recorded on the plot basis. Ten random plants in each plot were used to record the plant height, number of pods per plant. Grain yield was taken from net harvested area of 4 m2 and 12% moisture percent was maintained while weighing and thousands grain weight were taken from same harvested lot.

Analysis of variance and mean separation among the lines was done with statistical software ADEL-R and clusuring of the genotypes was done with statistical software MINITAB. Estimation and categorization of expected genetic advance as percent of mean was done according to (Johnson et al., 1955). The heritability and genetic advance of the traits were calculated by the formula given by (Falconer, 1960). The Genetic advance as percent of mean (%mean GA) were classified as low, moderate, and high as follows: low <10%, moderate 10–20%, and high >20%. and broad sense heritability values were ranked follows: low <30%, moderate 30–60%, and high >60% according to Robinson et al. (1949).

Results and Discussion

Analysis of variance

Analysis of variance revealed that characters days to 50% flowering (DFF), days to maturity (DM), number of pods per plant (NPPP), hundred seed weight (HSW) and grain yield kg/ha (Yld) were significant at (<0.01) indicating sufficient variation among the tested genotypes at genotypic level for concerned traits. Trait plant height (Pht) was found non significant (Table 1). Breeder can utilize the variability for selection and may use in hybridization for trait improvement, gene transfer to the other genotypes. Jeberson et al., (2015), Jawad et al., (2018), and Bicer and Sakar (2004) reported result which was in support to our findings that days to 50% flowering, days to maturity, plant height, number of pods per plant, thousands grain weight, and grain yield kg/ha were significant differences in lentil, our result contradict for non significant effect of plant height, this may be due to plant height may be more influenced by environment. Bicer and Sakar (2010) reported non sinificant genotypic effect of plant height of lentil.

Table 1. Mean squares, CV and F statistic of different agronomic traits of lentil genotypes tested at mid western terai of Nepal in 2015/16

Traits	Rep	Treat	Error	Fcal	CV	P value
DFF	57.17	467.85	11.97	39.08	4.76	**
DM	9.19	182.15	1.15	158.95	0.86	**
Pht	11.80	13.97	8.35	1.67	7.63	ns
NPPP	441.91	1368.23	119.73	11.43	13.69	**
HSW	0.11	0.45	0.01	37.22	5.25	**
Yld	86401.80	77209.85	22477.66	3.44	11.29	**

Note; DFF: Days to 50% flowering, DM: Days to 90% maturity, NPPP: Number of pods per plant, Pht: Plant height (cm), HSW: Hundred seed wt (gm) and Yld: Grain yield (kg/ha)

Number of pods per plant showed highest variability (13.69) followed by grain yield resembled (11.29) followed by plant height (7.63) and followed by hundred seed weight (5.25). Lowest variability was observed by days to maturity, then days to heading (Table 1). The coefficient of variation represents the ratio of the standard deviation to the mean, and it is a useful statistic for comparing the degree of variation from one data series to another, even if the means are drastically different from one another https://www.investopedia.com/terms/c/coefficientofvariation.asp.

Separation of mean

Values of quantitative traits are given in Table 2. It was observed that genotypes RL-45, RL-71 and RL-56 were earlier for days to 50% flowering (56.7 days) and days to maturity 112.3, 110.7 and 117.7 days respectively. while genotype ILL-6458 was late for both days to flowering and days to maturity 89 and 132 days respectively. Obtained mean value of flowering and maturity were 72.61 and 123.76 days respectively. Genotypes ILL-3338, ILL-60265 and X 945-48 were high yielder with more number of pods 1623, 1588 and 1549.7 kg/ha, and 123.3, 107.3 and 112.3 numbers respectively (Table 2). Genotypes RL-67 and RL-83 were with highest hundred seed weight (2.7 gm), breeder can use these genotypes for respective traits for genetic improvement. Obtained mean value for grain yield, number of pods per plant and hundred seed weight were 1328.5 kg/ha, 79.9 and 2.1 gm respectively.

Table 2. Mean separation of different agronomic traits of 18 lentil genotypes tested at mid western terai of Nepal in 2015/16

EN	Gentypes	FFD	DM	Pht	NPPP	HSW	Yld
1	RL - 45	56.7 ^{e}	112.3 ^{ij}	36.0	53.7 ^{g}	2.5 ^{b}	1259.0 ^{defg}
2	ILL - 6458	89.0 ^{a}	132.0 ^{a}	38.7	59.0 ^{fg}	2.1 ^{c}	1110.3 ^{g}
3	RL - 71	56.7 ^{e}	110.7 ^{j}	39.0	81.3 ^{e}	2.2 ^{c}	1423.0 ^{abcde}
4	ILL - 1920	83.3 ^{abc}	130.3 ^{abc}	35.7	60.0 ^{fg}	2.0 ^{c}	1164.0 ^{fg}
5	ILL - 9924	84.0 ^{abc}	128.0 ^{e}	37.7	90.7 ^{cde}	1.7 ^{de}	1419.0 ^{abcde}
6	ILL - 6465	77.0	130.0 ^{bcd}	41.0	91.3 ^{cde}	1.7 ^{de}	1284.0 ^{defg}
7	RL - 67	61.0 ^{e}	115.0 ^{gh}	36.0	53.3 ^{g}	2.7 ^{a}	1250.7 ^{defg}
8	LN - 0135	81.0 ^{bcd}	128.3 ^{de}	39.7	75.7 ^{ef}	1.7 ^{de}	1361.7 ^{bcdef}
9	RL - 56	56.7 ^{e}	117.7 ^{f}	33.0	62.7 ^{fg}	2.0 ^{c}	1162.0 ^{fg}
10	RL - 83	58.0 ^{e}	116.0 ^{fg}	37.0	84.3 ^{de}	2.7 ^{a}	1429.7 ^{abcd}
11	ILL - 60265	78.3 ^{cd}	128.0 ^{e}	41.3	107.3 ^{abc}	2.0 ^{c}	1588.0 ^{ab}
12	X 945 - 48	76.3 ^{d}	130.0 ^{bcd}	39.3	112.3 ^{ab}	1.7 ^{de}	1549.7 ^{abc}
13	ILL - 10071	88.3 ^{a}	131.7 ^{ab}	37.7	61.7 ^{fg}	2.4 ^{b}	1094.7 ^{g}
14	RL - 49	58.0 ^{e}	113.7 ^{hi}	36.3	101.7 ^{bcd}	2.7 ^{a}	1431.3 ^{abcd}
15	ILL - 3338	78.7 ^{cd}	129.7 ^{cde}	41.0	123.3 ^{a}	1.8 ^{d}	1623.0 ^{a}

16	L 280 (ILL -970)	85.0 ab	128.3 de	36.7	85.0 de	1.6 e	1271.3 defg
17	RL - 68	59.0 e	116.3 fg	37.7	75.0 ef	2.4 b	1312.3 cdefg
18	Sagun	80.0 bcd	129.7 cde	37.7	60.0 fg	1.6 de	1180.0 efg
StdMSE		**3.5**	**1.1**	**2.9**	**10.9**	**0.1**	**149.9**
LSD(5%)		**5.7**	**1.8**	**4.8**	**18.2**	**0.2**	**248.8**
Mean		**72.6**	**123.8**	**37.9**	**79.9**	**2.1**	**1328.5**

Note; DFF: Days to 50% flowering, DM: Days to 90% maturity, NPPP: Number of pods per plant, Pht: Plant height (cm), HSW: Hundred seed wt (gm) and Yld: Grain yield (kg/ha)

Heritability, coefficients of variation and genetic advance

The genotypic, phenotypic variances, broad sense heritability and genetic advance for all the traits are given in Table 3. The observed phenotypic coefficient varience (PCV) was higher than the genotypic coefficient of varience (GCV) for all six quantitative traits indicating role of environment for the expression of characters. Higher the differences between the GCV and PCV higher the role of environment for character expression. Our result resembled higher role of environment for plant height and grain yield.

The high broad sense heritability coupled with high expected genetic advance as percent of mean was observed for the traits, number of pods per plant (78 and 52.59), hundred seed weight (92 and 37.58) and days to flowering (93 and 34.97) percentage respectively (Table 3). These characters can be considered as favorable attributes for the improvement through selection and this may be due to additive gene action could be improved upon by adapting selection without progeny testing. Low heritability and low expected genetic advance as percent of mean was obtained in plant height 18 and 7.45 percentage respectively. This trait may be governed by non additive gene action and selection for improvement may be difficult. High heritability and medium genetic advance and medium heritability and high genetic advance were observed in days to maturity (98 and 12.93) and grain yield (45 and 20.94) percentage respectively. Hussan et al., 2018, high heritability and %GA for thousand grain weight 93% and 53.02 and 95% heritability and 41.4%GA for number of pods per plant which supports our findings but that contradict to days to flowering and grain yield. Alemayheu Duggasa et al., 2014 and Jawad et al., 2018 reported medium heritability for grain yield in lentil, similar to our finding. Similarly, Bicer and Sakar, 2010 reported high heritability for DFF (70%), DM (80%), NPPP (65%) GW (98%) and low heritability for plant height (16%) and Seed yield per plant (19%) which is in full supports to our findings. Mekonnen et al 2014 also supported our findings as low genetic advance as percent of mean for plant height and high for grain yield and medium heritability for grain yield.Tyagi and Khan (2010) also reported high heritability and high GAM% for FFD, NPPP, and Grain Weight and high heritability and low GAM% for DM and high GAM% for Yld supported our findings.

Table 3. Range, standard deviation, variance, broad sence heritability, PCV, GCV and percent mean genetic advance for quantitative traits of lentil genotypes

Traits	Range	Mean	Std	Vg	Vp	H	GCV	PCV	GA	%mean GA
DFF	56.6-89	72.61	12.49	151.96	163.93	0.93	16.98	17.63	25.39	34.97
DM	110.6-132	123.76	7.79	60.33	61.48	0.98	6.28	6.34	16.00	12.93
Pht	33-41.3	37.85	2.16	1.87	10.22	0.18	3.62	8.45	2.82	7.45
NPPP	53.3-123.3	79.91	22.06	416.17	535.90	0.78	25.53	28.97	42.02	52.59
HSW	1.6-2.7	2.09	0.39	0.14	0.16	0.92	18.24	18.98	0.78	37.58
Yld	1094.6-1623	1328.54	160.43	18244.06	40721.72	0.45	10.17	15.19	278.25	20.94

Note; DFF: Days to 50% flowering, DM: Days to 90% maturity, NPPP: Number of pods per plant, Pht: Plant height (cm), HSW: Hundred seed wt (gm) and Yld: Grain yield (kg/ha)

Clusturing

Dendrogram generated based on Unwaited Pair Group Method with Arithmetic Mean (UPGMA) clustering method and Euclidean similarity coefficient among eighteen lentil genotypes is given in table 4 and figure 1. Clusturing was done with UPGMA clustering method with 80% eucdian similarity coefficient and four clustures were generated. Clusture 1 contains 5 genotypes (27.7%) out of total 18 genotypes. This clusture carries lower values for all the traits than the grand centroid except hundred seed weight. Breeber can use earliness and seed weight from the genotypes of this clusture. Clusture II also carries 5 genotypes which are late and and inferior for yield, HSW and NPPP than grand centroid. Clusture III also represents 5 genotypes which are superior than grand centroid for grain yield, HSW and NPPP. Similarly, clusture IV carries three genotypes they are outstanding for Yld and NPPP namely ILL-60265, ILL-3338 and X 945-48. This clusture is slightly superior for thousand grain weight, number of pods per plant and grain these will be selected for advance trial like CVT (coordinated varietal trial) and large plot demonstration.

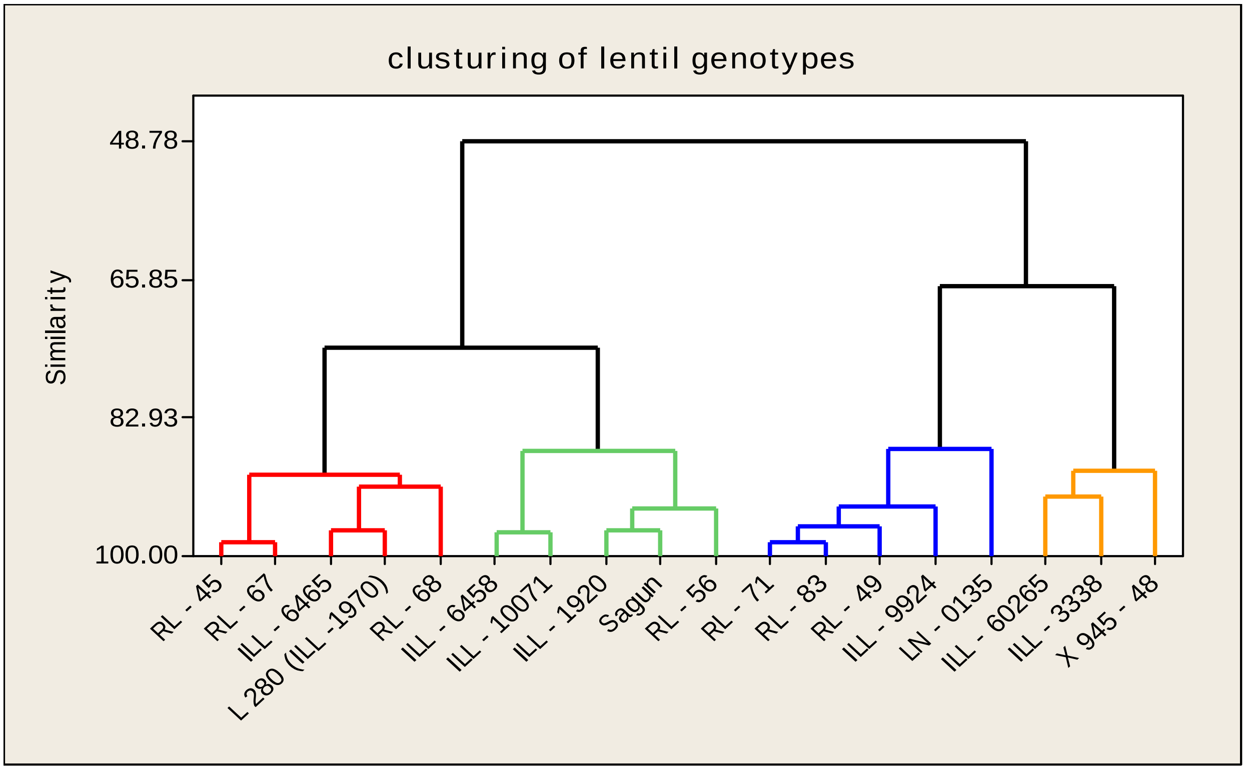

Fig 1. Unwaited pair group method with arithmetic mean (UPGMA) clusturing of cold tolerance lentil genotypes

Table 4. The average of traits for each cluster obtained from UPGMA cluster analysis

Variable	Cluster1	Cluster2	Cluster3	Cluster4	Grand centroid
FFD	67.73	79.47	67.53	77.78	72.61
DM	120.4	128.27	119.33	129.22	123.76
Pht	37.47	36.53	37.93	40.56	37.85
NPPP	71.67	60.67	86.73	114.33	79.91
HSW	2.17	2.03	2.2	1.83	2.09
Yld	1275.47	1142.2	1412.93	1586.89	1328.54

Note; DFF: Days to 50% flowering, DM: Days to 90% maturity, NPPP: Number of pods per plant, Pht: Plant height (cm), HSW: Hundred seed wt (gm) and Yld: Grain yield (kg/ha)

Conclusion

Obtained results indicates presence of sufficient genetic variability for the studied traits and genotypes are suitable for breeding purpose. Result resembled high heritability coupled with high genetic advance for number pods per plan, thousands grain weight and days to flowering shows these traits maybe governed by additive gene effects and selection of these traits would be more effective for genetic improvement. Mean separation and clusturing showed genotypes ILL-60265, ILL-3338 and X 945-48 are superior among the tested genotypes will be selected for further evaluation in advance trial for mid western terai of Nepal. Selection for grain yield improvement should be conducted by simultaneous selection of many traits such as number of pods per plant and higher grain weight.

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Conflict of Interest

The authors declare that there is no conflicts of interest regarding publication of this manuscript.

Authors contribution

N. H. Ghimire: Designed and performed experiment, analyzed data and wrote the paper.
R. A. Shah: Performed experiment, data recorded.

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