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Banoth Vinesh
Department of Genetics and Plant Breeding, Institute of Agricultural Sciences Banaras Hindu University, Varanasi, Uttar Pradesh, India

LC Prasad
Department of Genetics and Plant Breeding, Institute of Agricultural Sciences Banaras Hindu University, Varanasi, Uttar Pradesh, India

Ravindra Prasad
Department of Genetics and Plant Breeding, Institute of Agricultural Sciences Banaras Hindu University, Varanasi, Uttar Pradesh, India

Correspondence
Banoth Vinesh
Department of Genetics and Plant Breeding, Institute of Agricultural Sciences Banaras Hindu University, Varanasi, Uttar Pradesh, India

Genetic and diversity studies in late sown exotic and indigenous barley (*Hordeum vulgare* L.) germplasm

Banoth Vinesh, LC Prasad and Ravindra Prasad

Abstract
The present investigation comprising of 101 barley genotypes was conducted at Genetics and Plant Breeding, Banaras Hindu University, during *rabi* of 2016-17. Variability and diversity analysis was carried out based on data collected on 14 various quantitative traits. Analysis of variance revealed that there was a significant difference among the sixty four genotypes for all the characters studied. The phenotypic coefficients of variation (PCV) values were higher than genotypic coefficients of variation (GCV) values for all the traits studied. Medium phenotypic coefficients of variation (PCV) and genotypic coefficients of variation (GCV) were recorded for days to 50% flowering, effective tillers per plant, spike length with awn, spike length without awn and plant height. Medium phenotypic coefficients of variation (PCV) and low genotypic coefficients of variation (GCV) values were displayed for SPAD value and leaf rolling. Low phenotypic coefficients of variation (PCV) and genotypic coefficients of variation (GCV) values were recorded for days to maturity which suggests the limitation of selection for these traits. High heritability values were observed in all the characters studied except leaf rolling. The expected genetic advance as a percent of mean ranged from 3.45 (Leaf rolling) to grain yield per plant (77.14). Characters with a high genetic advance as a percent of mean allow the improvement of this character through selection Genetic diversity was assessed in 101 genotypes of barley by using D2 analysis. The genotypes were grouped into 11 clusters. Cluster II comprised 40 genotypes which was followed by clusterI. Cluster with small statistical distances considered less diverse than those with large distances. The intra cluster value was maximum in cluster X and minimum in cluster I. Cluster V showed the highest mean values for days to maturity, effective tillers per plant, stomatal conductivity, grain per ear, yield per plant while cluster V revealed the lowest mean value for leaf rolling. The characters responsible for genetic divergence days to maturity, effective tillers per plant, stomatal conductivity, grain per ear, yield per plant.

Keywords: barley, PCV, GCV, heritability and D^2 analysis

Introduction
Barley (*Hordeum vulgare* L.) a member of the grass family, belong to is a major cereal grain grown in temperate climates globally. The diploid chromosome number 2n= 14 It was one of the first cultivated grains, particularly in Eurasia as early as 10,000 years ago. Archaeological evidence suggests that in the past, barley known as Indra Jau and it was more popular in every religious ceremony as sacred grain. Barley flourishes well in low resources of fertilizer and irrigation. Barley is a mosaic crop, developed from several populations in at least in five regions: Mesopotamia, the northern and southern Levant, the Syrian desert and, 1,500-3,000 kilometers (900-1,800 miles) to the east in the vast Tibetan Plateau.

Barley is a rich source of tocols, including tocopherols and tocotrienols, which are known to reduce serum lethal density level cholesterol through their antioxidant action. Whole barley grain consists of about 65-68% starch, 10-17% protein, 4-9% β-glucan, 2-3% free lipids and 1.5- 2.5% minerals. Hulless or de-hulled barley grain contains 11-20% total dietary fiber, 11-14% insoluble dietary fiber and 3-10% soluble dietary fiber. Due to alternate use of barley in field of brewing industry and medicine, it is considered as highly needed crop of present era. Thus barley has potential to alleviate food shortage and malnutrition as well.

Barley has been introduced in India soon after invasion of the Aryans. Indians utilize about 80% of the produce as staple food, 10% as animals feed and rest 10% as raw material for industrial products. In our country barley cultivation starts from north- western district of Bihar and extends upto Mathura with highest concentration in eastern. All the cultivated forms of barley are thought to have originated from a wild specie *Hordeum spontaneutn*, a species very similar to present day two rowed barley. Archeologist also supports the two-row species as progenitor of six-rowed barley.

Barley is an annual cereal grain crop that is consumed as a major feed for the animals. The rest is used as malt in whiskey or sugar as well as health food. Overall India's barley production was estimated to be 1781.4 MT spread over an area of 6.93 lakh ha for the year 2016-17. The average productivity was estimated to be 25.80 q/ha (Anonymous, 2017) [13].

The use of a high existing variability in the germplasm that considering the existence of a high genetic variability. The nature and amount of genetic variability available in the germplasm indicates the scope of improvement of the character by exploiting the genetic variability selecting superior genotypes for specific environments. The techniques of multivariate analysis are often used in genetic improvement programs to predict the genetic diversity among the accesses. And the dissimilarity measurements previously estimated and, among these averages, the Mahalanobis' generalized distance (D^2) has been evidenced as a successful tool when studying the barley genetic divergence. The Mahalanobis' D^2 statistic also provides a useful statistical tool for measuring the genetic diversity in germplasm collections with respect to the characters considered together

Material and Methods

The present study was carried out at Genetics and Plant Breeding, Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (U.P.) during *rabi* of 2016-17. Geographically, Banaras Hindu University is situated between 25º18' N latitude, 83º 03´E longitudes and at an altitude of 128.93 meters above the mean sea level in the North Gangetic plain of eastern part of Uttar Pradesh. The experimental materials incorporated 101 exotic and indigenous genotypes which were well-kept by BHU under All India Co-ordinated Wheat and Barley Improvement Project. Randomized Block Design with three replications was adopted for laying out the genotypes for the investigation. Each treatment (genotype) was sown in line having 2.75 m length with row to row and plant to plant distance of 25 cm and 10 cm, respectively. The sowing date was delayed by 25 days as against the recommended one. All the recommended agronomic practices for respective experimental conditions were followed to raise a healthy crop. Five competitive plants, in each plot were randomly selected and tagged well in advance for recording the observations. Data was recorded on various yield and yield attributing traits *viz.*,days to 50 per cent flowering, days to maturity, flag leaf length (cm), number of effective tillers/plant, number of grains/ear, spike length with awns (cm), spike length without awns (cm),stomatal conductivity (m Mol M^{-2} S^{-1}), SPAD values, leaf rolling, proline concentration (µ mol g^{-1}),plant height (cm), 1000-grain weight (gm) and grain yield/plant (gm).

Genotypic, phenotypic and environmental components of variance and their coefficient of variances (Phenotypic: PCV and Genotypic: GCV) were estimated as methods suggested by Lush (1940) [15] and Burton (1952) [12] respectively. The PCV and GCV values were classified as Low: Less than 10%; Moderate: 10 – 20%; High: More than 20% as suggested by Sivasubramanian and Madhavamenon (1973) [17]. Heritability in broad sense [$h^2_{(b)}$] was calculated according to the, formulae given by Lush (1940) [15] and categorized as Low: Less than 30%; Medium: 30-60%; High: More than 60% as suggested by Johnson *et al.* (1955) [14].

From the heritability estimates, the genetic advance was estimated by the following formula given by Johnson *et al.* (1955) [14].

$$GA = (K)\ (\sigma p)\ h^2_{(b)}$$

Where, GA = Genetic advance under selection (expected); σp = Phenotypic standard deviation; $h^2_{(b)}$ = Heritability (broad sense); K = Selection differential at 5% selection intensity (2.06)

Genetic advance as per cent of mean was calculated as per the formula.

$$\text{GA as per cent of mean} = \left(\frac{\text{GA}}{\overline{X}}\right) \times 100$$

Where, GA = Genetic advance; $\overline{X}$ = Grand mean of the character

The range of genetic advance as per cent of mean was classified as Low: Less than 10%; Medium: 10-20%; High: More than 20% as suggested by Johnson *et al.* (1955) [14].

Genetic diversity between genotypes was estimated by using D^2 analysis given by Mahalanobis's (1936) [16].

The D^2 value between i^{th} and j^{th} genotypes for P characters was calculated as

$$D_{ij}^2 = P\ \Sigma_{t=1}\ (\overline{Y}_{it} - \overline{Y}_{jt})$$

Where,$\overline{Y}_{it}$ = uncorrected mean value of i^{th} genotype for t character; $\overline{Y}_{jt}$ = Uncorrected mean value of j^{th} genotype for t character; D_{ij}^2 = D^2 value between i^{th} and j^{th} genotype.

Grouping of the genotypes into various clusters was done by using Tocher's method as described by Rao (1952) [10].

Results and Discussion

Analysis of variability: In the present study, ANOVA of traits revealed significant variability for various traits studied in the germplasm. Mean squares of the 14 characters from analysis of variance (ANOVA) are presented in (Table 1). Highly significant differences among genotypes (P<0.01) were observed for seven characters (days to 50 % flowering, number of productive tillers per plant, spike length, spike without awn, 1000 kernel weight, grain yield plant, days to maturity, flag leaf length, proline concentration and plant height), significant at (p<0.05) for the rest one characters; namely leaf rolling. This result indicating that there is variability among the genotypes studied and would respond positively to selection. Several researchers reported significant differences among wheat genotypes studied. Shashikala (2006) [18]. Similar findings were in consonance with earlier reports made by Kumar *et al.* (2009) [19] and showed that significant differences among 30 genotypes of bread wheat and among 21 genotypes of bread wheat respectively.

Table 1: Analysis of variance (ANOVA) for 14 quantitative traits in 101 barley genotypes (Late sown condition)

Source of variation	Df	Mean Sum of Squares													
		DF	DM	FL	ET	SPAD	SC	PC	SL	SLW/O	PH	LR	G/E	GW	GY
Replication	2	5.79	4.44	1.02	0.89	4.23	126.82	87.30	7.68	0.14	15.84	0.04	8.8	0.76	1.28
Treatment	100	194.33**	91.50**	20.73**	3.72**	48.27 **	6204.75**	234.63**	22.46**	3.04**	378.03**	0.10	87.56**	140.7**	10.11**

Error	200	2.14	2.43	3.54	0.53	12.96	180.37	88.29	3.266	0.17	5.78	0.07	5.66	13.47	0.35

**Significant at p< 0.01.DF=Days to 50% flowering, FL=flag leaf length, ET=effective tillers/plant, SPAD, SC=stomatal conductivity, PC=proline concentration, SL=splike length with awn, SLW/O=spike length without awn, PH=plant height,G/E=grain per ear, GW=1000 grain yield, LR=Leaf rolling DM= days to maturity, GY =grain yield

The values of GCV and PCV were very close which strengthens the greater contribution of genotype rather than environment. So the selection can be operated very well based on the phenotypic values for trait interest. The PCV was higher than the corresponding GCV for all the traits which might be due to the interaction of the genotypes with the environment to some degree or other denoting environmental factors influencing the expression of these characters.

High Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) was observed for grain yield per plant followed by grains per ear and proline concentration (Table 2). Similar result obtained by wold *et al.* (2011) [1]. While working with the 36 barley genotype also reported that PCV is higher than GCV for traits grain yield per plant, grains per ear and proline concentration. This finding similar with Birhanu *et al.* (2016) [3] while working with 64 wheat genotype.

Table 2: Analysis of variance (ANOVA) for 14 quantitative traits in 101 barley genotypes

Trait	DF	DM	FL	ET	SPAD	SC	PC	SL	SLW/O	PH	G/E	GW	GY
Range Min.	62.33	97	6.39	5.96	37.40	313.97	8.61	17.44	5.03	63.11	9.00	25.53	3.53
Max.	97.00	119.33	25.59	13.78	54.33	662.93	27.61	23.16	10.26	117.56	61.00	58.70	24.41
Grand Mean	78.25	113.30	14.75	9.59	45.82	485.35	14.71	20.13	7.35	93.53	39.02	40.24	12.93
SE (±)	0.83	1.03	0.49	0.61	1.84	12.57	0.91	0.83	0.31	1.72	1.80	1.63	0.55
PCV (%)	8.79	4.38	20.71	17.92	10.03	18.78	32.27	8.99	15.90	13.14	30.25	19.49	32.31
GCV (%)	8.60	4.09	19.88	14.12	7.23	18.24	30.43	5.41	14.15	12.75	29.18	18.17	31.45
h^2 % (broad sense)	96	87	92	62	52	94	89	36	79	94	93	87	95
GA as % of mean (5%)	17.32	7.87	39.31	22.93	10.73	36.48	59.11	6.70	25.94	25.48	57.99	34.92	63.05
GA as % of mean (1%)	22.20	10.08	50.38	29.38	13.75	46.75	75.75	8.59	33.24	32.65	74.32	44.75	80.80

DF=Days to 50% flowering, FL=flag leaf length, ET=effective tillers/plant, SPAD, SC=stomatal conductivity, PC=proline concentration, SL=splike length with awn, SLW/O=spike length without awn, PH=plant height/E=grain per ear, GW=1000 grain yielded= days to maturity, GY =grain yield

Heritability (h^2) and Genetic Advance (GA)

Heritability is the heritable portion of phenotypic variance. It is a good index of the transmission of characters from parents to off-spring. The estimates of heritability help the plant breeder in selection of elite genotypes from diverse genetic populations. With the help of GCV alone, it is not possible to determine the amount of variation that is heritable. The GCV together with heritability estimates would give reliable indication of the expected progress in a selection programme (Raikwar *et al*, 2014). High heritability percentage coupled with high genetic variability particularly grain yield per plant under normal situation and emerged as an ideal traits for improvement through simple selection in upcoming generations.

In the current study, high heritability estimates were obtained for all the fourteen quantitative traits studied (Table 2). Broad sense heritability estimate was highest for days to 50% flowering, plant height, days to grain maturity, stomatal conductivity and SPAD value. These findings were in accordance with the finding of Akanksha *et al.* (2012).

However, heritability values alone may not provide clear predictability of the breeding value. Heritability in conjugation with genetic advance over mean is more effective and reliable in predicting the effectiveness of selection. In the present experiment, all the characters studied had exhibited high heritability coupled with high genetic advance as percentage of mean. Estimates of high heritability and high genetic advance together may be ascribed to the conditioning of the characters by additive effect of the polygenes which could be improved upon by adopting selection without progeny testing.

High heritability coupled with high genetic advance was observed for days to 50% flowering, flag leaf length, effective tillers per plant, stomatal conductivity, spike length with awn, spike length without awn, plant height, grains per ear and grain yield. These findings were in corroboration with earlier findings of Sunil *et al.* (2015) [6], Lodhi *et al.* (2015) [7] and Kumar and Shekhawat (2013) [8].

Moderate heritability coupled with moderate genetic advance observed for SPAD value and proline concentration and leaf rolling. This findings were in consonance with earlier reports made Sunil *et al.* (2015) [6]. High heritability coupled with high genetic advance as percentage of mean was found for grain yield per plant followed by grain per ear (Table 2).

Analysis of genetic diversity

The multivariate analysis using Mahalanobis D^2 statistics is a valuable tool for obtaining quantitative estimates of divergence between biological populations. For an effective and informative breeding programme, information concerning the extent and nature of genetic diversity within a crop species is essential to researchers. Assessment of genetic diversity was made based on the data recorded for thirteen traits on hundred and one barley genotype using Tocher's D^2 analysis. Using this method a set of 101 barley genotypes were grouped into 11 clusters based on relative magnitude of the D^2 value. Cluster I had 30, cluster II 40 and cluster X 23 genotypes. Other clusters i.e. cluster III, IV, V, VII, VIII, IX, XI and XII consists of one germplasm each (Table 3). Singh and Singh *et al.* (1980) stated that the genetic diversity may not be straightway related to geographical diversity and the similar trend was observed in barley.

Table 3: Cluster pattern of 101 barley genotypes under late sown condition

Clusters	Germplasm lines/Genotypes	Number
I	CIHO-3510, IBRWAGP-04-66, CIHO-7603, CIHO-8355, 26th IBYT-11-1, CIHO-5924, NBPGR-07-08,VIJAY,MARRIA, 11th EMBSN-54, AMBEER, HIMANI, 11th EMBSN-21, HUB-180, SONU, PL-825, CIHO-5923, 29th IBON-6, WfBCB-88, 13th EMBSN-46, RD-2552, RATNA, RD-2715, HUB-113, BH-976, ISBCB-02-9, K-603, GEETANJALI, V-MORALES, 25th IBON-03-6.	30
II	22nd IBYT-7, 22nd IBYT-9-2, 22nd IBYT-99-11, BCB-W-03-91, 12th EMBSN-2, BCB-73, CANUT, IBSCGP-05-16, 24th IBON-1, 14th HBSN-05-6, 26th IBYT-16, 22nd IBYT-5-1, 22nd IBYT-04-86, 22nd IBYT-7-2, CIHO-6260, 14th HBSN-05-8, HANLEY,BEECHER, ATHOULPA,11th HBSN-175, ISBCB-02-10, BCB-W-03-92, IBGP-03-65,25th IBYT-10-3, 22nd IBYT-99-14-1, 25th IBON-11, INBON-05-72, INBON-07-08-71, 11th EMBSN-37-1, 22nd IBYT-04-85, INBON-05-50, WfBCB-91, ISBCB-02-13, 22nd IBYT-01-2-2-4, 11th HBSN-127, HUB-113, 11th EMBSN-40, INBON-07-08-8, LAKHAN, MOROC-9-75	40
III	JYOTI	1
IV	K-551,	1
V	13th EMBSN-71	1
VI	JAGRATI	1
VII	11th EMBSN-26	1
VIII	KARAN-16	1
IX	25th IBON-45-1	1
X	7th HMBSN-15-2, PL-751, 11th EMBSN-34,11th HBSN-1, INBON-05-79, YARDU, 11th EMBSN-22, 11th EMBSN-20, 26th IBYT-49, 7th HMBSN-1-2-1-1, 26th IBON-54-1, HARMAL,11th HBSN-91, AZAD, 11th EMBSN-47-03, HORMAL, 12th HBSN-7, 25th IBON-39-1, ALFA-93, 25th IBON-03-11, 11th EMBSN-23, 24th IBON-40-1, 25th IBON-45	23
XI	IBGP-03-49	1

DF=Days to 50% flowering, FL=flag leaf length, ET=effective tillers/plant, SPAD, SC=stomatal conductivity, PC=proline concentration, SL=splike length with awn, SLW/O=spike length without awn, PH=plant height/E=grain per ear, GW=1000 grain yielded= days to maturity, GY =grain yield

Inter and Intra cluster D^2values

The intra cluster distance was found maximum for cluster X and minimum distance in cluster I while it was zero for cluster III, IV, V, VI, VII, VIII, IX, XI as these cluster consisted of only single genotype (Table 4). The inter cluster distance was maximum between cluster V and XI clusters followed by clusters V and VIII and cluster II and X. it signifying highest genetic divergence existing between the genotypes of these clusters. The minimum inter cluster distance was recorded between cluster XI clusters followed by clusters V indicating close relationship and similarity for most of the characters of barley genotypes come in these cluster.

Table 4: Average Inter & Intra D^2 value among 11 Cluster Distances: Tocher Method

Cluster	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
I	28.850	67.424	111.666	58.178	49.737	108.325	43.792	129.006	37.064	72.517	121.783
II		44.312	75.636	72.253	136.670	87.713	87.140	93.363	71.586	119.848	87.639
III			0.000	50.342	188.928	9.290	71.960	12.527	127.595	115.974	128.684
IV				0.000	111.341	35.006	26.475	49.071	36.791	74.166	83.737
V					0.000	185.730	83.549	205.528	75.649	96.200	211.960
VI						0.000	55.403	12.768	112.090	103.491	128.826
VII							0.000	74.023	45.082	51.006	132.319
VIII								0.000	128.381	124.464	138.102
IX									0.000	83.716	87.337
X										70.643	174.497
XI											0.000

DF=Days to 50% flowering, FL=flag leaf length, ET=effective tillers/plant, SPAD, SC=stomatal conductivity, PC=proline concentration, SL=splike length with awn, SLW/O=spike length without awn, PH=plant height/E=grain per ear, GW=1000 grain yielded= days to maturity, GY =grain yield

Cluster means of various characters studied

The cluster mean values for different characters indicated differences between the clusters for all the traits studied (Table 5). The highest mean value for days to maturity, effective tillers per plant, stomatal conductivity, grain per ear, yield per plant in cluster V. Cluster XI had high mean value for days to 50% flowering, SPAD value, spike length with awn; highest mean values for spike length without awn, plant height and leaf rolling. Cluster VIII had maximum mean value for proline concentration and leaf rolling; cluster VII had high mean value for flag leaf length and leaf rolling and cluster I had high mean value for 1000 grain weight. The result indicates that selection of genotypes having high values for particular trait could be made and used in the hybridization programme for improvement of that character. The trait days to 50% flowering followed by plant height and stomatal conductivity had highest relative contribution towards divergence.

Table 5: Cluster Means for various characters: Tocher Method

Clusters	DF	DM	FL	ET	SPAD	SC	PC	SL	SL W/o	PH	LR	G/E	GW	GY
I	70.222	101.100	11.513	6.604	48.136	191.773	38.161	18.654	7.172	79.015	1.754	18.978	37.902	5.548
II	77.533	103.308	12.135	6.106	49.252	138.512	40.495	18.211	7.097	66.179	1.824	14.533	32.822	3.729
III	67.000	95.667	6.390	5.890	46.700	124.500	27.647	16.200	6.337	45.337	1.603	7.333	31.967	2.273
IV	65.000	97.333	8.367	4.333	46.433	133.633	33.130	21.723	8.443	69.557	1.590	7.333	38.600	2.780

V	65.000	104.333	9.443	8.557	42.900	266.867	36.390	21.000	8.277	83.223	1.273	22.667	37.267	9.157
VI	63.000	94.667	7.690	6.333	52.800	110.933	30.833	16.000	6.943	50.443	1.990	6.333	38.500	2.130
VII	61.667	94.333	13.667	5.223	48.500	172.000	46.593	17.387	6.500	73.443	1.990	14.333	31.667	3.407
VIII	64.000	96.000	8.733	4.890	50.833	98.133	59.463	16.557	7.500	45.890	1.713	8.000	24.920	2.490
IX	70.000	103.333	9.543	5.557	49.500	145.320	41.427	20.913	8.610	86.443	1.713	18.333	31.033	3.257
X	62.667	95.609	11.373	6.715	46.874	169.298	39.532	17.930	6.646	72.873	1.763	18.768	37.334	5.189
XI	79.667	103.333	11.433	7.220	54.867	101.967	43.890	40.000	7.700	70.667	1.990	10.000	33.633	1.833

DF=Days to 50% flowering, FL=flag leaf length, ET=effective tillers/plant, SPAD, SC=stomatal conductivity, PC=proline concentration, SL=splike length with awn, SLW/O=spike length without awn, PH=plant height/E=grain per ear, GW=1000 grain yielded= days to maturity, GY =grain yield

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