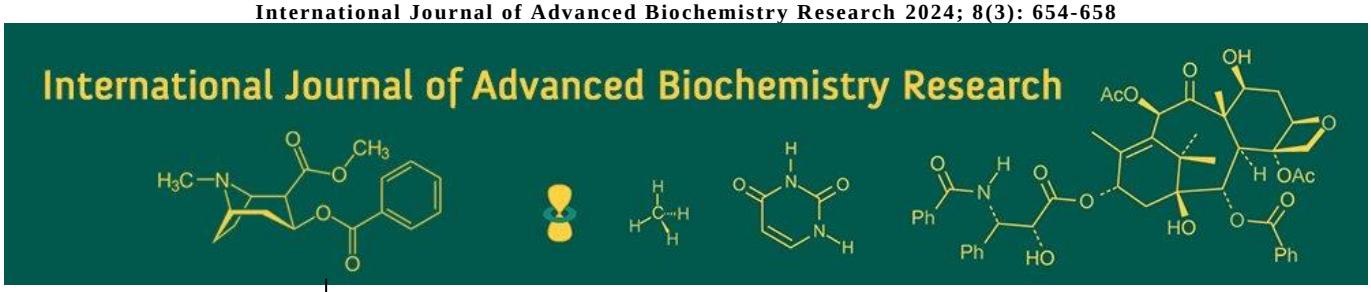

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Exploring genetic divergence in quantitative traits of exotic and indigenous barley (*Hordeum vulgare* L.) genotypes

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Abstract
This study sought to evaluate the genetic variation among forty-nine different genotypes of barley, comprising both exotic and indigenous lines, In addition to incorporating four check varieties (BH 902, RD 2552, RD 2035, and DWRB 92). The analysis of variance unveiled noteworthy discrepancies among the genotypes across a range of traits, except for 1000-seed weight. Block-wise analysis indicated no significant differences, suggesting comprehensive inclusion of factors influencing seed yield. The genetic coefficient of variation was highest for the number of productive tillers per plant (GCV) at 16.62, followed by peduncle length at 14.83, with phenotypic coefficient of variation (PCV) values at 17.14 and 15.33, respectively, suggesting minimal environmental influence on trait expression. The non-hierarchical Euclidean cluster analysis grouped all genotypes into eight clearly defined non-overlapping clusters. Cluster III comprised the highest number of genotypes (17), whereas Cluster VI contained only one genotype (IBYT-HI-4). Intra-cluster distance analysis revealed cluster I as having the highest value, followed by Clusters V, III, VIII, and VII. Notably, inter-cluster distances were notably high between clusters II and VI (59.26) and clusters VI and VIII (49.95), suggesting potential for obtaining superior segregants through mating between genotypes of these clusters.

Keywords: Genetic divergence, barley, *Hordeum vulgare* L., quantitative traits, cluster analysis, phenotypic variation

Introduction
Barley (*Hordeum vulgare* L.) stands as a cornerstone of agricultural history, as a diploid species capable of self-pollination, with a chromosome count of 2n=2x=14. Its cultivation traces back to antiquity, identified as among the earliest crops cultivated by humanity (Salamini *et al*., 2002) [13]. Within the vast array of barley varieties, landraces emerge as genetically diverse populations, comprising both inbreeding lines and hybrid segregates, owing to sporadic outcrossing events (Nevo, 1992) [12]. The Hordeum genus encompasses 28 species characterized by a base chromosome number of x=7, further classified into diploid (2n=2x=14), tetraploid (2n=4x=28), and hexaploid (2n=6x=42) groups. Notable species include H. vulgare (cultivated barley) and several wild counterparts such as *H. chilense*, *H. comosum*, *H. muticum*, and *H. pusillum* within the diploid category, alongside *H. capense* and *H. secalinum* among the tetraploids.
Barley landraces embody a reservoir of genetic diversity, harboring valuable genes pivotal for adaptation to various biotic and abiotic stresses (Brush, 1995) [4]. Despite their potential, these genetic resources remain underutilized, holding promise for enhancing the resilience of modern cultivars (Ceccarelli and Grando, 1989; Hadjichristodoulou, 1995) [6, 8]. Notably, studies have identified nineteen major genes (Rph) governing resistance against Puccinia hordei within barley landraces and their wild relatives (*Hordeum vulgare* ssp. spontaneum) (Weerasena *et al*., 2004) [19]. Motivated by these observations, the present study endeavors to investigate the genetic divergence among forty-nine diverse barley genotypes, encompassing both exotic and indigenous lines. This exploration seeks to unravel potential avenues for crop enhancement and stress resilience in barley breeding programs.

Materials and Methods

The research took place in the Rabi 2015-16 season at the Genetics and Plant Breeding Research Farm located in Kumarganj, Ayodhya (U.P.), affiliated with Acharya Narendra Deva University of Agriculture & Technology, employing an Augmented Block Design. Narendra Nagar is positioned at approximately 26°47' N latitude and 82°12' E longitude, with an elevation of 113 meters above mean sea level. Ayodhya district experiences a semi-arid climate, characterized by sweltering summers and chilly winters. Rainfall, primarily occurring during the monsoon season (typically up to September), accounts for about 80% of the total precipitation, with occasional winter showers.

The experimental setup comprised forty-nine genotypes, encompassing both exotic and indigenous barley lines, alongside the four check varieties (BH 902, RD 2552, RD 2035, and DWRB 92) that were procured from the Coordinated Unit of Barley within the Department of Genetics and Plant Breeding at the University. The experimental field was partitioned into five blocks, each containing 13 entries, including the four check varieties. Every plot was demarcated by a bed spanning 4 meters in length, with plants spaced 10 cm apart within rows and rows spaced 23 cm apart.

The data collection encompassed a range of traits, including plant height (measured in centimeters), productive tillers per plant, flag leaf area (measured in square centimeters), peduncle length (measured in centimeters), ear length (measured in centimeters), 1000-seed weight (measured in grams), biological yield per plant (measured in grams), harvest index, and grain yield per plant (measured in grams). Measurements were recorded from five randomly selected plants in each row, while data on 50% flowering and days to maturity were noted at the plot level. Following this, the mean values of the recorded data were subjected to statistical scrutiny. This involved the application of an Augmented Block Design analysis (Federer, 1956), along with evaluations of both the Genetic Coefficient of Variation (GCV) and Phenotypic Coefficient of Variation (PCV) (Burton and de Vane, 1953) [5]. Furthermore, Non-hierarchical Euclidean cluster analysis (Beale, 1969; Spark, 1973) [3, 17] was carried out. Divergence analysis was performed using TOCHER's and WARD's methods.

Results and Discussion

Germplasm serves as a reservoir of genetic diversity, pivotal for addressing evolving needs in crop improvement. To ensure profitable exploitation through recombination breeding or selection, germplasm must exhibit considerable variability in economic traits. Traditionally, researchers have relied on the distance of origin as a proxy for genetic diversity, guiding parent selection for hybridization. Notably, Alam *et al*. (2007) [1], Ali *et al*. (2007) [2], Mishra *et al*. (2008) [10], and Singh *et al*. (2014) [15] have emphasized the significance of genetic diversity in plant breeding endeavours.

An optimal level of genetic diversity among parent plants is essential for generating a diverse range of desirable traits in subsequent hybrid generations. Hence, the characterization of genetic divergence for the selection of diverse genotypes should lean on robust statistical techniques, such as D2 statistics and Non-hierarchical Euclidean cluster analysis. These methods evaluate genetic divergence based on the combined effects of various agronomically important traits.

Our Non-Hierarchical Euclidean cluster analysis effectively categorized 49 barley germplasm lines, including both exotic and indigenous varieties, into eight separate clusters that did not overlap. (Table 1), underscoring a significant degree of genetic diversity within the evaluated material. This observation corroborates prior studies conducted by Sharma *et al*. (2005) [14], Singh *et al*. (2006) [16], Alam *et al*. (2007) [1], Ali *et al*. (2007) [2], all of which highlighted considerable genetic variation within barley materials. The substantial genetic diversity detected among the evaluated germplasm lines indicates their promise as valuable resources for identifying diverse parental candidates in hybridization initiatives, intending to isolate desirable segregates for grain yield and other crucial traits.

A critical evaluation of Table 3 reveals that cluster III was the most extensive, comprising 17 genotypes, while cluster I ranked second with 9 genotypes, and cluster VI was the smallest, with only one genotype (IBYT-HI-4).

The average distances within and between clusters for eleven characteristics, as detailed in Table 4, were calculated, indicating minimal genetic divergence among genotypes within clusters, contrasting with substantial diversity observed between exotic and indigenous lines across different clusters. To enhance the likelihood of isolating superior segregates in subsequent generations, it's advisable to cross diverse exotic lines from clusters with higher inter-cluster distances.

Consistently, the distances between clusters exceeded those within clusters. Particularly noteworthy was the high intra-cluster distance of 13.74 in cluster I, followed closely by cluster V with 13.67. Remarkable inter-cluster distances were observed between clusters II and VI (59.26), followed by VI and VIII (49.95), suggesting the potential for obtaining desirable recombinants through crosses between genotypes from these clusters. Conversely, the clusters showed the smallest inter-cluster distances between clusters I and II (16.87), and subsequently between clusters III and V (17.81), suggesting a closer genetic affinity among members of these cluster pairs.

Comparable results were documented in studies conducted by Singh *et al*. (2014) [15], and Mekonnon *et al*. (2015) [9]. Analysis of cluster means for eleven traits (refer to Table 5) unveiled considerable divergence among clusters, suggesting notable differences in mean trait performances. These clusters offer promising avenues for creating new segregates through targeted crosses.

Moreover, the analysis of each trait's contribution to overall genetic variance (as illustrated in Table 6) underscored the considerable impact of plant height (44.39%). Subsequently, peduncle length (11.90%), flag leaf area (11.82%), days to 50% heading (11.14%), days to maturity (10.97%), and 1000-seed weight (8.16%) followed, while other traits made relatively minor contributions.

In summary, our study underscores the importance of genetic divergence analysis in identifying diverse genotypes for hybridization programs, offering insights into trait variations and potential avenues for crop improvement.

Table 1: Analysis of variance of Augmented Block Design for 11 characters in barley (*Hordeum vulgare* L.)

S.N.	Characters	Sources of variation		
		Blocks	Checks	Error
	D.F.	4	3	12
1	Days to 50% flowering	38.177	16.850 **	2.100
2	Days to Maturity	52.100	19.517 *	4.268
3	Plant Height (cm)	70.095	219.478 **	3.635
4	Flag Leaf Area (cm²)	65.033	19.921 **	2.325
5	Peduncle Length (cm)	27.524	41.187 **	1.097
6	Ear Length (cm)	2.130	3.328 **	0.469
7	Productive Tillers/ Plant	1.642	5.659 **	0.062
8	1000 Seed Weight (g)	33.827	25.056	7.204
9	Biological Yield/ Plant	1.911	14.190 **	0.683
10	Grain Yield/ Plant	0.326	2.477 **	0.061
11	Harvest Index (%)	3.554	26.409 **	1.799

Table 2: Range, mean, coefficient of variation and least significant differences for 11 character of barley genotypes

Characters	Range (Min-Max)	Mean Value	Coefficient of variation (%)			Range of parameters			
			PCV (%)	GCV (%)	Coefficient of variation (%)	LSD_1 5%	LSD_2 5%	LSD_3 5%	LSD_4 5%
Days to 50% flowering	60.75-85.75	73.26	6.05	5.72	7.11	1.99	4.46	4.99	3.86
Days to maturity	107.45-129.45	117.22	3.86	3.43	4.38	2.84	6.36	7.11	5.51
Plant height (cm)	51.85-89.85	73.21	10.51	10.18	11.93	2.62	5.87	6.56	5.08
Flag leaf area (cm^2)	18.31-37.99	24..49	15.87	14.62	17.50	2.10	4.69	5.25	4.06
Peduncle length (cm)	20.16-38.06	26.74	15.33	14.83	17.09	1.44	3.22	3.60	2.79
Ear length (cm)	6.45-11.45	8.04	12.89	9.69	13.32	0.94	2.11	2.35	1.82
Productive tiller plant $^{-1}$	4.03-8.45	6.016	17.14	16.62	20.91	0.34	0.76	0.85	0.66
1000-seed weight (g)	32.13-48.80	41.16	8.38	5.21	9.71	3.69	8.27	9.24	7.16
Biological yield plant^{-1} (g)	26.49-36.32	32.48	6.16	5.60	7.17	1.13	2.54	2.84	2.20
Grain yield plant $^{-1}$ (g)	7.74-11.54	10.03	6.58	6.10	7.83	0.34	0.76	0.85	0.66
Harvest index (%)	27.28-37.41	31.23	5.22	2.90	6.85	1.84	4.13	4.62	3.57

Table 3: Clustering pattern of 49 barley genotypes on the basis of non-hierarchical Euclidean cluster analysis for 11 characters

Cluster No.	Number of genotypes	Genotypes
I	9	IBYT-HI-6, INBYT-HI-21, INBYT-HI-19, IBON-59, INBON-2, GSBSN-49, IBYT-HI-18, GSBSN-8 and INBON-77
II	4	GSBSN-50, GSBSN-146, GSBYT-2 and INBYT-HI-20
III	17	GSBYT-23, GSBSN-132, INBON-78, IBON-75, INBON-34, INBON-37, IBON-44, GSBSN-78, INBON-67, RD-2552[C], INBON-21, INBON-22, IBON-22, IBON-49, IBON-45, IBON-72 and INBYT-HI-9
IV	3	INBON-26, IBON-4 and INBON-25
V	6	INBON-16, INBON-19, IBON-40, INBON-63, IBON-27 and GSBSN-35
VI	1	IBYT-HI-4
VII	4	INBYT-HI-4, INBYT-HI-6, INBYT-HI-3 and INBYT-HI-1
VIII	5	INBON-44, DWRB-92[C], BH-902[C], RD-2035[C] and INBON-76

Table 4: Estimates of average intra- and inter-cluster distances for 8 clusters in barley

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII
Cluster I	13.743	16.872	17.968	22.718	23.583	43.688	28.897	24.591
Cluster II		6.281	22.406	30.008	33.336	59.260	31.426	20.274
Cluster III			11.847	20.981	17.814	41.276	26.353	24.026
Cluster IV				11.072	28.621	36.972	21.123	28.818
Cluster V					13.671	34.133	36.856	36.243
Cluster VI						0.000	40.997	49.952
Cluster VII							11.249	18.352
Cluster VIII								11.824

Table 5: Clusters means for 11 characters in barley

Characters	Days to 50% Heading	Days to Maturity	Plant Height (cm)	Flag Leaf Area (cm^2)	Peduncle length (cm)	Ear length (cm)	Productive Tillers/ Plant	1000 Seed Weight (g)	Biological Yield/ Plant (g)	Grain Yield/ Plant (g)	Harvest Index (%)
1 Cluster	77.444	122.311	71.878	24.646	22.266	7.724	6.397	42.002	32.435	9.863	30.443
2 Cluster	77.250	123.450	71.175	20.490	24.879	7.955	8.318	36.580	34.458	10.945	31.459
3 Cluster	73.618	116.635	76.245	24.141	30.316	7.714	5.284	39.620	32.148	9.794	30.530
4 Cluster	65.917	110.783	62.033	23.265	21.027	7.605	6.197	39.722	30.509	9.304	30.621
5 Cluster	75.250	117.367	80.354	24.640	30.023	9.951	5.193	42.651	30.261	9.195	30.296
6 Cluster	67.750	115.950	72.825	37.990	21.660	10.280	4.730	40.080	26.498	9.590	36.726
7 Cluster	64.250	110.450	62.725	29.365	24.885	8.005	6.305	44.955	35.023	10.965	31.786
8 Cluster	71.700	114.420	73.596	22.642	26.128	7.326	6.892	44.894	35.142	11.208	34.793

Table 6: Contribution of characters towards genetic divergence

Source	Times Ranked I^{st}	Contribution %
1. Days to heading (50%)	131.000	11.14
2. Days to maturity	129.000	10.97
3. Plant height (cm)	522.000	44.39
4. Flag leaf area =LxBx0.75	139.000	11.82
5. Peduncle length (cm)	140.000	11.90
6. Ear length (cm)	0.000	0.01
7. Productive tiller/plant	0.000	0.01
8. 1000 Seeds weight(g)	96.000	8.16
9. Biological yield/plant (g)	12.000	1.02
10. Grain yield/plant (g)	0.000	0.01
11. Harvest index (%)	7.000	0.60

Conclusion

Based on our study's analysis, several genotypes demonstrate superior performance and significant genetic diversity, making them promising candidates for future breeding programs. Clusters with higher inter-cluster distances, like Cluster II and Cluster VI, offer opportunities for obtaining superior segregants through crosses. Additionally, genotypes within Cluster III and Cluster I, which contain a diverse array of genetic material, are also noteworthy. Characteristics that significantly contribute to genetic diversity warrant prioritization, such as plant height, peduncle length, flag leaf area, days to heading, days to maturity, and 1000-seed weight. By harnessing the genetic variation within these clusters, the advancement of barley varieties with increased yield potential and resilience to stressors can be accelerated. This study highlights the importance of genetic divergence analysis in guiding breeding efforts towards selecting diverse and high-performing genotypes for sustainable crop improvement.

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