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Genetic diversity for malting quality in barley (*Hordeum vulgare* L.)

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Abstract

Genetic diversity for malting quality was studied on a set of 131 barley genotypes of indigenous and exotic origin representing both two and six row types. Observations were recorded on seven grain and seven malt traits. The study revealed that lot of variability exists in material for all malting quality traits. It was also observed that in general two-row barleys have better malting quality. Sources for different quality traits were identified for use as donors in breeding programme. The clustering analysis revealed four major clusters amongst the genotypes with varying standards for quality traits. The non hierarchical Euclidean cluster analysis indicated that genotypes with very good malting quality were grouped in one cluster. Clustering pattern based on Ward's minimum variance method also revealed four major discrete clusters among the genotypes studied.

Key words: Barley, cluster analysis, genetic diversity, malting quality

Introduction

Barley (*Hordeum vulgare* L.) is the fourth most important cereal crop after wheat, rice and maize in the world. It is one of the oldest and man's most dependable cereal crop to be domesticated and cultivated since the beginning of civilization [1]. In India barley is grown since ancient times and has been traditionally considered as poor man's crop because of its low input requirement and better adaptability to harsh environments like drought, salinity, alkalinity and marginal lands. It occupies nearly 0.62 m ha area producing nearly 1.21 m tones grain, with productivity of 19.58 q/ha [2].

The produce from irrigated fertile areas is mainly consumed for industrial purposes, which amounts to nearly 20-25% of the total production and the remaining quantity from rainfed and less fertile areas is utilized for feed and food purposes. In recent times the demand of barley for malting increased with increase in number and installed capacity of brewing units and due to increased preference of malted food and drinks in the society especially for kids, the non-brewing type malt requirements also increased many folds. The requirement of better malt type grain (raw material) was not met with the existing six row barley varieties being cultivated in the country as these were not having minimum standard for various grain and malt traits for classification as malting grade barley [3]. In order to combine the good malting quality with high grain yield, the breeding programme was initiated involving two x six row hybridization and selection for grain and malt traits along with the resistance to prevalent diseases and pests. A number of grain and malt traits are considered important by malting and brewing industries and also the requirement of the industry is different for different end products. In order to evaluate and classify the new barley variety as malt or non-malt type, the minimum acceptable standards for various grain and malt traits have been determined [4, 5].

Although there are numerous grain and malt parameters important in barley, however, amongst them hot water extract (HWE), diastatic power (DP), friability and homogeneity are considered to be the more important from brewing point of view [6]. Majority of these traits are not independent and also influenced by the environmental conditions [7-9]. Therefore, it requires a comprehensive breeding strategy to bring all these important traits into a single genetic back ground. No information is available on the genetic diversity for malting quality traits as well as on the sources of important quality traits in Indian barley programme except a study on genetic diversity on a few grain quality traits [10]. Therefore, the present study was undertaken to identify the new sources as well as their genetic diversity for different grain and malt quality traits amongst the available barley collection to strengthen breeding

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efforts for developing better malting quality barley by utilizing the diverse sources.

Materials and methods

A set of 131 barley genotypes, representing both two-row (98) and six-row (33) types were grown at Karnal and Patiala for two consecutive years (2003-04 and 2004-05). The material comprised of released varieties, germplasm accessions and advanced breeding lines of indigenous and exotic origin (Table 1). Out of these 131 genotypes, 42 were indigenous and 89 were of exotic origin. Exotic accessions included introductions from different countries (Canada, Australia, Denmark, USA and Argentina) and selections from international observation nurseries supplied by two CGIAR institutes, CIMMYT, Mexico and ICARDA, Syria.

The sowings were done in 2nd week of November at both places in two crop seasons. Material was grown in two row plots of 2.0 m length for each genotype with out replication, with 30 cm row to row spacing. The standard agronomic practices were followed to raise a good crop. The genotypes were harvested during 2nd week of April in each year at both the locations and the bulk grain samples of two row plots from each location were evaluated for various grain and malt quality traits.

Grain traits

Observations were recorded on grain physical parameters like, thousand grain weight (1000 g w), Test weight (kg/hl), Percent plump (>2.5mm) and Percent thin (<2.2mm) grain (on Sortimat M/s Pfeuffer GmbH, Germany), Germinative energy (GE %) at 72 hrs in 4ml water test [11]. The Grain Protein (GP %) and Husk Content (Hull %) were analysed by Kjel Tec Auto analyzer 1030, and by sodium hypo-chlorite method, respectively [11].

Malt traits

Barley grain samples (100 g each) of each genotype from both locations were micro-malted through automatic micro-malting system (M/s Phoenix Systems, Australia), following a 16 hours steeping, 96 hours germination and 30-32 hours kilning cycle. In each batch of micro-malting, Alfa 93 and DWR28, the two commercially released malt barley varieties in India, were taken as control to determine the progress of malting process. After completion of micro malting

Table 1. Names and sources of material used in the study

Source	Name of the genotypes collection	Type of genotypes	Total
CIMMYT	BCU407, BCU424, BCU550, BCU551, BCU553, BCU554, BCU572, 20th IBON3, 20th IBON38, 20th IBON139, 20th IBON52, 20th IBON71, 22nd IBON282, 22nd IBON62, 2nd EMBSN24, 2nd EMBSN25, 2nd EMBSN30, 2nd EMBSN31, 2nd INFBON131, 30th IBON205, 30th IBON307, 30th IBON348, 30th IBON355, 30th IBON290	Exotic	24
ICARDA	BCU1, BCU8, BCU729, BCU775, BCU1605, BCU1636, 3rd IWFBCB94, 4th INWFBCB5, BONMRA(94-95)73, BYTMRA(91-92) 8, ICARDA54	Exotic	11
United Breweries Ltd., R&D unit, Patiala, India (Introductions)	VM61, VJM329. ANDRE, AZURE, BANDULLA, VJM516, CHARIOT, CLARK, DERKADO, VJM510, FAIRFIELD, VJM509, VJM507, VJM514, VJM515, MISCAL-16, VM51, VJM513, OMEGA, PIROLINE, PRISMA, SHABET, TREMOIS, UBE1000, UBE1006, UBE435, UBE441, UBE477, UBE868, UBE990, UBE991, VANGUARD, VJM201, VJM522, VJM524	Exotic	35
DWR , Karnal, INDIA (Introductions)	BCU199, BCU284, BCU390, BCU1264, DWR17, DWR18, DWR30, DWR31, DWRUB53, DWRUB55, EB921, SHEBEC, ALFA93, CANUT, CARUSO, CDC MANLEY, CDC Mc GWIRE, CDC SISLER, HARRINGTON	Exotic	19
DWR , Karnal (varieties and advanced breeding lines)	BCU131, BCU277, BCU2030, BH393, BK9806, BCU6349, BCU6347, BK9813, BK9823, BCU6348, DL100, DL3, DL348, DL456, DL472, DL88, DWR27, DWR32, DWR33, DWR34, DWR36, DWR38, DWR39, DWR41, DWR42, DWR43, DWR44, DWR45, DWR49, DWR50, DWR51, DWRUB54, DWRUB56, VM155, K18, K647, PL172, RD2508, VM151, VM158, VM130, VM152	Indigenous	42

process, the malt (after removal of dried rootlets) was stored at room temperature in plastic interlocking envelops to avoid moisture uptake by the malt.

The malt thus obtained from each genotype was analysed for important malt quality traits. The malt friability was measured on malt friability meter (M/s Pfeuffer GmbH, Germany) on 50 gm sample. Malt homogeneity was measured as percent homogeneous malt (using malt sample retained in malt friability meter mesh during friability analysis and passing this fraction on Sortimat for sieve analysis) and the non-homogeneous fraction is that which is retained on the 2.2 mm sieve and rest is considered as homogeneous malt [11]. Hot water extract (HWE %) was measured in percent on fine grind dry weight basis. The grinding of malt was done in the EBC approved Buhler Malt Grinding Mill and mashing of the grounded malt was performed in mashing bath to extract the soluble components from the malt [11]. Wort filtration rate (FR) was measured in ml/hr as amount of wort passing through standard Whatman No.1 filter papers. Wort viscosity was measured in mpas/ second unit using the Viscomat (M/s Pfeuffer GmbH, Germany) [11]. Kolbach index (KI) was determined as the ratio of soluble nitrogen in wort to the total nitrogen in the malt, both analysed on Kjel Tec auto analyzer 1030 [11]. Malt Diastatic power (DP) was measured in degree linter value (^{o}L) as the total enzymatic activity of the amylases in malt samples as per Institute of Brewing (I0B) Method.

The data recorded on all these traits were analysed by the non hierarchical Euclidean cluster analysis for grain and malt quality traits using statistical software available at computer centre, DWR, Karnal, for clustering of genotypes into different groups and also to indicate the extent of diversity available in our collection for malting quality. Ward's minimum variance clustering method was used to classify genotypes in discrete clusters [12] by using original data and also by converting original data into a binary data matrix (each observation given value of 0= undesirable range and 1= desirable range for each quality trait as decided for malt barley) [4].

Results and discussion

The results from the analysis of different grain and malt quality traits in the 131 genotypes indicated that most of the genotypes do not possess the desired levels of values for all the traits together. However, very good sources for individual grain and malt traits (Table 2) have been observed. These genotypes can be used in desirable fashion for hybridization to get optimum levels of performance for most of the traits in one back ground.

To optimise the levels of different quality traits for malting is a complex problem for making selection in breeding programme, which is further complicated by various positive and negative correlations within these traits as well as, between quality traits and agronomic characters [7]. Any variety to be recommended for commercial cultivation as malt barley must have high level of grain yield, resistance to prevalent biotic and abiotic stresses and in addition, it must meet the prescribed levels [4] of grain and malt traits. The identification of suitable donors for different quality traits will help in the development of better quality variety. An earlier study [13] identifies sources of only few grain quality traits while in the present study information on almost all traits is covered.

Observations recorded on different grain and malt quality traits were subjected to a non hierarchical Euclidean cluster analysis. Based on clustering results genotypes were grouped into four clusters. The mean values and standard deviations for each trait in different clusters (Table 3) show that cluster 1 have entries with most desirable grain and malt quality traits. Similarly cluster 4 has entries with poorest over all malting quality as well as lowest values for individual traits. In cluster II, genotypes are comparatively better in malting quality than clusters III and IV for majority of the traits.

The cluster 1 consists of 59 genotypes out of which 46 are exotics and 13 are indigenous in origin (Table 4). Out of these 59 genotypes, 50 are of two-row types and nine six-row genotypes in this cluster. All of the cluster 1 genotypes are of good malting quality. In this cluster VM numbers are the exotic six row type cultivars having good malting quality, but lack in disease resistance, hence can not be cultivated directly but can be good source for breeding improved six row types in India.

It was also observed that genotypes of both exotics and indigenous are distributed in all clusters. In cluster 2, there are 24 genotypes of which 7 are indigenous & 17 exotics; cluster 3 consists of 39 genotypes out of which 17 are indigenous and 22 exotics, where as cluster 4 consists of 5 indigenous and 4 exotics out of total 9 genotypes. These observations clearly indicate that enough variability exists in the collection and there is a good proportion of indigenous genotypes with better quality.

Table 2. Sources identified for different grain and malt quality traits

Traits	Range	Genotypes
Hectolitre weight	>67 (kg/hl)	CDC McGWIRE, DWR55, VJM515, VANGUARD, VM61, ANDRE, CLARK, DWR17, DWR51, MISCAL-16, PRISMA, VM152, UBE1006, VJM522
Bold Grain	>98 (%)	20th IBON-139, DWR56, VJM522, BK9823, VJM510, DWR38, K647, MISCAL-16, 2nd EMBSN24, 20th IBON52, 30th IBON355, BCU6347
Thin grain	<0.5 (%)	DWR56, 20th IBON-139, 20th IBON-52, , DWR38, EB921, K647, OMEGA, VANGUARD, CHARIOT, DWR32, DWR51, MISCAL16, UBE435, 2nd EMBSN 25, 30th IBON 348, VJM509, VJM522
Thousand grain weight	>54 gm	30th IBON355, 30th IBON348, DWR17, DWR30, DWR33, DWR36, DWR41, OMEGA, 3rd IWFBCB 94, 20th IBON 38, DWR38, ICARDA54, K647, UBE1006
Germinative energy	100 (%)	20th IBON 3, 20th IBON 38, 20th IBON-52, 30th IBON 205, 30th IBON 355, 30th IBON290, BCU131, BCU1605, BCU424, BCU553, BCU554, BCU729, CDC MANLEY, CDC SISLER, DWR18, DWR30, DWR31, DWR33, DWR34, DWR39, DWR41, DWR42, DWR45, DWR49, DWR50, DWR53, DWR54, DWR56, EB921
Grain Protein	< 10.5 (%)	VM151, BH393, DWR55, K18, VM152, VM130, UBE435, RD2508, SHABET, UBE1006, UBE868
Husk	< 9.5 (%)	ANDRE, PRISMA, TREMOIS, UBE435, MISCAL-16, SHABET, WM861-5, OMEGA, UBE1006, BANDULLA, DWR18, CLARK, FAIRFIELD, DWR53, DWR45, BCU572, CLARK, VJM507, VJM329, CARUSO, 3rd IWFBCB 94, VJM329, PIROLINE, VJM524
HWE	>83 %	PRISMA, VJM515, UBE1000, PRISMA, VJM522, MISCAL-16, VJM507, CLARK, TREMOIS, UBE1006
Friability	>75 %	VM151, DWR56, VM130, CDC SISLER, RD2508, SHEBEC, UBE868, UBE1000, VJM522, VJM524, BCU1264, BCU1264, BCU1264, BCU1264, TREMOIS
Homogeneity	= 98% mpas/s	20th IBON-71, CDC MANLEY, DWR56, VM151, VJM522, 30th IBON290, BCU1264, CDC McGWIRE, CDC SISLER, OMEGA, VM130, SHEBEC, UBE435, UBE441
Viscosity	< 1.400	VJM509, VM51, VJM522, CARUSO, VJM524, CANUT, UBE477, CDC MANLEY, SHEBEC, TREMOIS, DL100, VM158, DWR39, DWR53, UBE441, DWR43, BCU131, VM155, CHARIOT, DL348, DWR55, CDC SISLER, DWR49, DWR44, FAIRFIELD
Diastatic Power	100 ± 5 ^{0}L	DWR30, DWR50, HARRINGTON, RD2508, ICARDA54, UBE1006, BK9823, DWR32, BCU554, BCU572, DWR49, 3rd IWFBCB94, BANDULLA, DL88, DWR17, DWR18, PL172, UBE990, VJM329, ANDRE, DWR55, DWR56, K18, SHABET, UBE435, VANGUARD, DWR34, 30th IBON355, BCU2030, CDC McGWIRE
Filtration Rate	>300 (ml/hr)	2nd EMBSN24, DL100, CANUT, VJM509, DWR43, DWR34, 2nd EMBSN 24, TREMOIS, VM155, DWR39, BCU572, BCU131, DWR30, DWR45, DL100, DWR55, BONMRA(94-95)-73, VJM524, DWR39, BH393

The maximum distance was observed between cluster 1 and 4 (6.51) followed by cluster 2 and 4 (5.43); cluster 3 and 4 (3.98); cluster 1 and 3 (3.60) where as lowest distance was observed between cluster 2 and 3 (2.89), followed by 1 and 2 (3.44). So the contrasting parent from clusters 1 and 2 may be selected for different quality traits and used in the crossing programme to widen the genetic base of the materials as well as incorporating those few traits which are lacking in genotypes of each cluster. Most of the genotypes in cluster 2 and 3 were not meeting the desirable limits for different malting quality traits; however, some of the genotypes in these groups were having better viscosity and KI values.

Table 3. Means and standard deviation of genotypes within clusters for quality traits

Traits	Cluster			
	I	II	III	IV
TW (kg/hl)	64.3± 1.7	61.8± 2.7	59.2± 2.7	57.1 ± 3.6
Plump (%)	89.9± 6.0	86.2± 8.0	77.2 ± 9.6	57.6 ± 12.0
Thin (%)	1.7± 1.0	2.4± 1.2	5.1 ± 3.0	12.0 ± 6.1
GP (%)	13.0± 1.0	13.3± 1.0	13.1± 0.9	12.6 ± 1.3
1000gw (g)	48.6± 3.1	46.2± 4.6	42.7± 4.0	39.5 ± 4.5
GE (%)	96.1± 3.9	91.4± 10.4	95.6± 4.9	73.8± 10.9
Hull (%)	11.1± 1.1	12.0± 1.0	12.4± 1.4	13.4± 1.0
HWE (%)	80.1± 1.6	77.7± 1.6	75.5± 2.1	74.7± 3.0
MF (%)	66.0± 8.9	47.9± 9.9	49.9±14.0	43.8±13.1
MH (%)	91.6± 5.0	85.0± 8.0	82.6± 10.2	81.2± 17.7
DP (^{o}L)	105.0± 13.5	108.0± 18.6	103.0± 18.6	120.3± 20.8
Viscosity (mpas)	1.45± 0.04	1.64± 0.20	1.46± 0.05	1.45± 0.05
FR (ml/hr)	281.3± 20.2	219.2± 29.8	274.3± 24.2	273.22± 39.5
KI	0.54± 0.11	0.42± 0.05	0.41± 0.07	0.42± 0.07
No. of genotypes	59	24	39	9

Table 4. Genotypes in different clusters with their origin

Clusters	Origin	Genotypes
I	Exotic	2nd EMBSN24, 3rd IWFBCB94, ALFA93, BCU1605, BCU1636, CDC McGWIRE, CDC SISLER, CDC MANLEY, DWR17, DWR18, DWR30, DWR53, DWR55, HARRINGTON, ICARDA54, UBE435, UBE441, UBE477, UBE1000, UBE1006, VJM201, BANDULLA, CLARK, TREMOIS, FAIRFIELD, VANGUARD, MISCAL-16, DERKADO, PRISMA, CHARIOT, ANDRE, SHABET, OMEGA, VJM507, VJM509, VJM510, VJM513, VJM515, VJM516, VJM522, VJM524, VM51, VM60, VM61, SHEBEC
	Indigenous	DWR33, DWR34, DWR36, DWR41, DWR50, DWR51, DWR27, DWR56, VM130, VM151, VM152, VM155, VM158
II	Exotic	BYTMRA(91-92)-8, 2nd EMBSN25, 2nd EMBSN31, 20th IBON3, 20th IBON38, 20th IBON52, 20th IBON71, 20th IBON139, 22nd IBON62, 30th IBON205, 30th IBON307, 30th IBON348, 2nd INFBON131, BCU1, BCU284, BCU390, VJM329
	Indigenous	BK9823, DWR32, DWR38, DWR54, K647, PL172, RD2508
III	Exotic	BONMRA(94-95)-73, 2nd EMBSN30, 22nd IBON 282, 30th IBON290, 4th INWFBCB5, BCU8, BCU407, BCU424, BCU550, BCU551, BCU553, BCU554, BCU572, BCU1264, CARUSO, DWR31, EB921, UBE868, UBE990, UBE991, PIROLINE, VJM514
	Indigenous	BCU131, BCU2030, BK9813, DL88, DL100, DL348, DL456, DL472, DWR39, DWR42, DWR43, DWR44, DWR45, DWR49, BCU6347, BCU6349, K18
IV	Exotic	BCU199, BCU729, BCU775, CANUT
	Indigenous	BCU277, BH393, BK9806, DL3, BCU6348

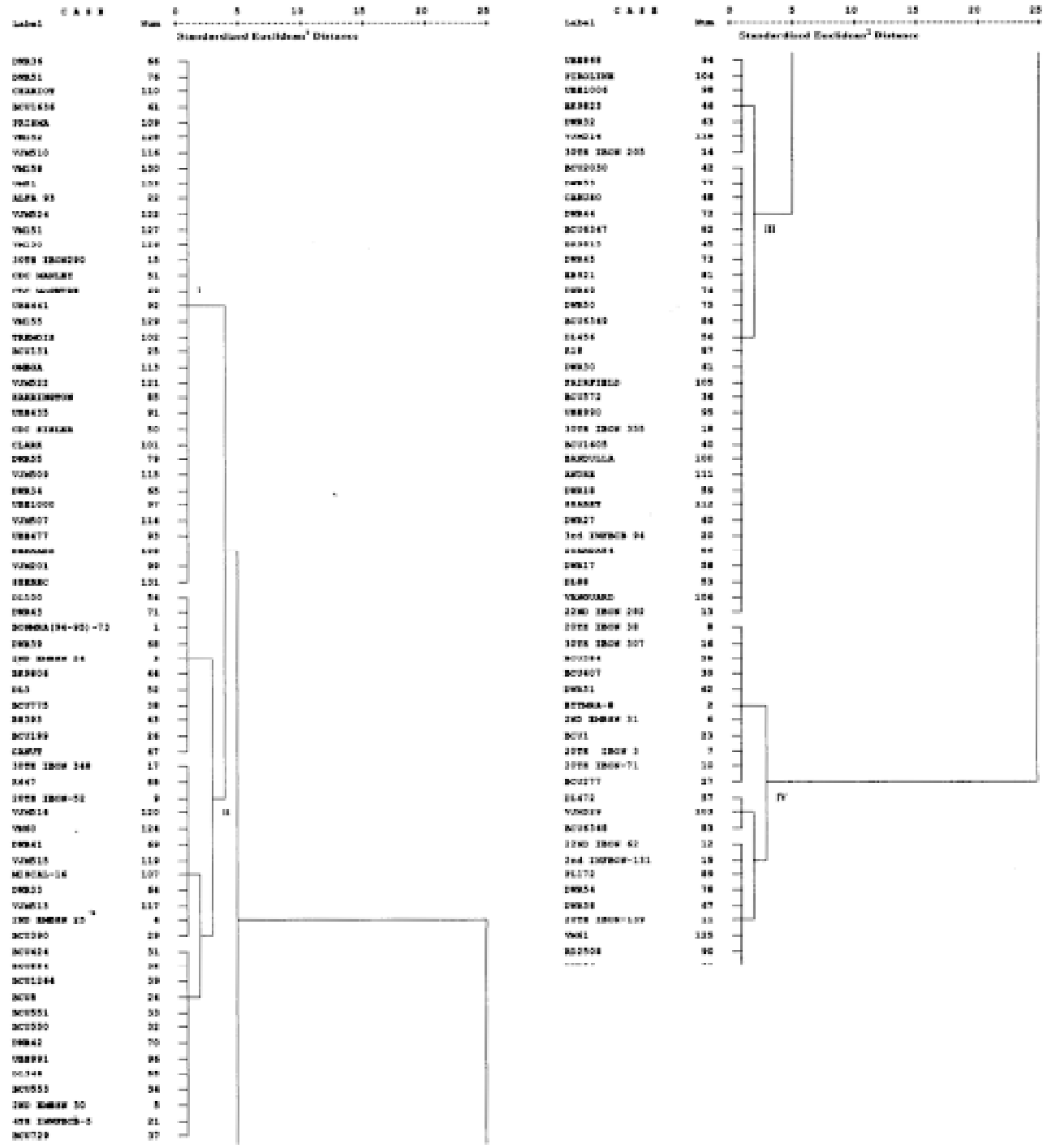

Fig. 1. Ward's Minimum Variance Dendrogram of 131 barley genotyes on quality traits

The clustering pattern of original data was also analysed using Ward's minimum variance method by measuring squared Euclidean distance and clustering pattern based on original data shows that again there are four major clusters (Fig. 1). The trend of genotypes falling in different clusters is similar in both the cases. Genotypes are distributed in different clusters according to their malting quality and genotypes within each cluster are more or less of similar for different quality attributes. Since the unit of measurement and range for different

quality traits are different and also the desirable standards for malting quality traits are also different for two-row and six-row type barley. As a result, clustering based on original data does not take care of lower limits for six-row type genotypes while classifying them into different clusters. Therefore, the absolute values of different genotypes were converted to a binary code (0 = undesirable and 1 = desirable) for clustering analysis based on Ward's minimum variance method. The clustering pattern based on binary data also revealed four major clusters in the similar pattern of original data. In both ways there are bye and large similarities in genotypes falling in different clusters.

Some of the existing released cultivars may lack one or a few traits in malting quality despite having very good performance for grain yield, adaptability and disease /pest resistance and these sources can prove useful in such cases for specific improvement. Two-row barley has an inherent advantage over six-row types for malting quality, where as six-row types are higher yielder and better adapted as compared to two-row types. The present study revealed that, sufficient variability exists in the material for all malting quality traits. The study revealed very high variability for traits like percent thin grain, friability and Kolbach Index, indicating greater environmental influence for these traits. While traits like HWE, test weight and wort viscosity showed very low variation over the environments/climatic condition. It was also observed that the range for germinative energy was quite wide in the genotypes, possibly the dormancy of seeds present in some of the genotype may be the reason for low GE. Although, two-row barleys are generally better in quality; however, numbers of six-row genotypes with equally good quality were identified.

The information on association of various grain and malt traits [7, 14-17] can provide useful information about the improvement in associated traits followed by selection for a particular trait in the breeding programme. Designing a crossing programme by identifying genotypes of interest from different clusters will make the process more directional and effective. Studies on genetic diversity for malting quality traits are limited in India and only few grain quality traits have been studied by [10]. Another study [18] was conducted on the genetic diversity in barley landraces from Uttranchal Himalaya of India for different morphological traits as well as on yield components. Cluster analysis in the present study clearly helped in differentiating genotypes with better malting quality traits. It also revealed that, there are four major clusters identified among the genotypes, with different levels of quality standard. Based on distance between genotypes of different clusters, contrasting parents may be selected and used in the hybridization programme for generation of wider variability for selection in the breeding programme. Since the malting quality is the optimum combination of several grain and malt traits and one may not like to disturb it, in such cases the parents from cluster 1 and 2 may be utilized for improvement of malt barley variety in other agronomic traits as both the cluster have better genotypes for malting quality. The present investigation provided useful information about the level genetic diversity present in the materials studied. More detailed study on genetic diversity for malting quality traits on germplasm available (about 5500 accessions) in active collection at DWR, Karnal may help in identifying more genotypes of interest in future.

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