

BIOCHEMICAL AND MORPHOLOGICAL VARIATION AMONG BARLEY LANDRACES

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ABSTRACT

Sixty two barley (*Hordeum vulgare* L.) pure lines derived from landraces, mainly farmers' cultivars, were evaluated at two locations in north Shewa, Ethiopia with the aims (i) to know the extent of variability for quantitative characters and seed storage proteins profile, (ii) to determine their genetic relationships, and (iii) to evaluate their potential for improvement through selection. The genetic relationship as revealed by the hierarchical cluster analysis based on data from SDS-PAGE and quantitative characters explicitly showed tightly grouping of lines derived from landraces bearing the same name indicating the predominance of variation among the cultivars rather than within cultivars. Mean genetic distance between lines within cultivars was very low (0.094 in Mage to 0.567 in Kessele). Accordingly, genetic advance through selection for the different agronomic characters within cultivars was lower than the corresponding values observed for all landraces. Hence, progress through pure line selection cannot be warranted. Instead, rouging carefully the inferior biotypes or off types from farmers' cultivars may help exploit their maximum genetic potential than attempting to improve their productivity through pure line selection and evaluation method. Evaluation of landraces from other sources, from within or outside the environment, could be explored to get better yielding landraces than the existing ones. In the long term, crossing may be scheduled between cultivars which appear to be diverse in order to maximise the potential gain from selection in the progeny.

Key Words: Cluster analysis, Ethiopia, genetic distance, *Hordeum vulgare*, pure line selection

RÉSUMÉ

Soixante deux orges (*Hordeum vulgare* L.) lignées pures dérivées des races de terre, essentiellement des variétés des fermiers, étaient évaluées à deux endroits dans le nord de Shewa, Éthiopie avec les visés (i) de connaître l'ampleur de variabilité des caractères quantitatifs et le profil de stockage des protéines des graines, (ii) de déterminer leurs relations génétiques, et (iii) d'évaluer leur potentiel au perfectionnement à travers la sélection. La relation génétique comme révélée par l'analyse hiérarchique des bouquets basée sur les données de SDS-PAGE et les caractères qualificatifs explicitement a montré un groupement serré des lignées dérivées des races de terre portant le même nom, indiquant la prédominance de variation parmi les variétés qu'à l'intérieur des variétés. La distance génétique moyenne entre les lignées à l'intérieur des variétés était très faible (0,09 en Mage à 0,567 en Kessele). Par conséquent, le progrès génétique à travers la sélection pour les différents caractères agronomiques à l'intérieur des variétés était faible que les valeurs correspondantes observées pour toutes les races de terre. Ainsi, le progrès à travers la sélection en lignées pure ne peut être garantie. Plutôt enlever soigneusement les biotypes inférieurs ou les types externes aux variétés des fermiers peut aider à exploiter leur potentiel génétique maximum que tenter d'améliorer leur productivité à travers la sélection en lignées pure et la méthode d'évaluation. L'évaluation des races de terre d'autres sources, de l'intérieur ou en dehors du milieu, pourrait être explorée pour obtenir des meilleures races de terre (en rendement) que celles existantes. En long terme, le croisement entre variétés qui apparaissent être diverses peut être programmé pour maximiser le gain potentiel de sélection dans la progéniture.

Mots Clés: Analyse des bouquets, Éthiopie, distance génétique, *Hordeum vulgare*, sélection en lignées pure

INTRODUCTION

Natural selection accompanied by human selection during centuries of cultivation resulted in landraces that are genetically variable for qualitative and quantitative characters, have good adaptation to specific environmental conditions and give dependable yields (Harlan, 1992). The highly heterogeneous Ethiopian environment, particularly of the "barley belt", owing to its low latitude and high altitude, has been pointed out as a key factor for diversification. Such environment that created favourable grounds for natural selection must have been augmented the breeding system of *H. vulgare* L. (predominantly self-pollinating, with some out-crossing) which facilitated both fixation of types and occasional genetic recombination (Briggs, 1978). Social factors (social values as criteria for selection diversified uses and association between barley types and uses) are also part of the causes for the diversification in Ethiopian barley landraces (Asfaw, 1989).

Farmers select cultivars for the known production system based on merits. In north, northwest and west Shewa, for instance, the cultivation of 7- 15 different cultivars was reported (Yirga *et al.*, 1998). Farmers' barley cultivars are the backbone of agricultural systems in Ethiopia mainly in low input environments because there, the use of varieties bred for favourable growing conditions has become difficult. While we appreciate the importance of local specificity or the value of local adaptation of landraces, farmers' cultivars have not been thoroughly studied and effectively utilised in national or regional breeding programmes. In other words, the genetic diversity, genetic relationships or genetic worth of these materials is not properly understood. Citable work in this regard is that of Asfaw (1989), although the study has more of taxonomic significance. Lakew *et al.* (2000) have studied the variability for quantitative and qualitative morphological characters of farmers' cultivars from different parts of Ethiopia but the materials from specific environments were not included comprehensively. The aims of this study were, therefore, to (i) establish the extent of variability for quantitative characters in farmers' barley cultivars from north Shewa in Ethiopia, (ii)

determine their genetic relationships, and (iii) evaluate their potential for improvement through selection.

MATERIALS AND METHODS

Plant materials. Emphasis was given to predominantly farmers' cultivars grown in north Shewa. Two accessions collected by the Biodiversity Conservation and Research Institute/Ethiopia were also included. Ten random plants from each landrace were selected and seed multiplication was done during the off-season (February to May, 2001) to get pure lines with sufficient seeds for the field experiment. Because of phenotypic similarity between the lines, only five to six lines from each landrace were included in the experiment, except for the landrace (Baleme) represented by one. Misrach (released regionally for north Shewa) and HB42 (released nationally) were included as checks for comparison. Total entries for evaluation were 62 lines and two checks.

Experimental procedure. The 62 lines and the two checks were planted at two locations, Keyit and Sheno' in north Shewa, Ethiopia in the last week of June, 2001. Each entry was planted on a plot size of two rows of 2.5 m length spaced at 20 cm between rows. A randomised complete block design with four replications was used at each location. Seed rate was 85 kg ha⁻¹ and each plot received urea and Diamonium Phosphate (DAP) fertilisers at the rates of 41 and 46 kg ha⁻¹ of N and P₂O₅, respectively, at planting. Weeds were controlled manually before reaching a critical level for nutrient competition.

Data collection. Days to heading and maturity, biomass, and grain yield were recorded on a plot basis. A plot was judged to be at heading when heads were fully visible in 50% of the plants in a plot. Physiological maturity was recorded when the peduncle turned yellow and plants showed complete leaf senescence in 90% of the plants in a plot. Plant height, spike length, number of seeds per spike, and grain yield per spike, were recorded from five randomly selected spikes in each plot. Productive heads were counted from a one-meter section of each plot and converted to spikes per

square meter. Harvested biomass per plot was air-dried before measuring biomass and 25 g of grain from each plot was oven-dried at 130 °C for 2 hours so that grain yield was adjusted to 12.5% moisture level. Two hundred and fifty seeds were counted from each plot, weighed and converted in to get 1000 seed weight. At Sheno, all the materials were planted on a camber bed to avoid waterlogging and all the data from this site were recorded from a 1.5 m section of each plot by trimming one metre from the end to avoid irregularity in performance of plants within a plot.

Principal component and cluster analyses.

Genetic variability was studied both within and across cultivars bearing different names based on quantitative variables and SDS-PAGE of seed storage proteins. To classify the farmers' cultivars, principal component analysis (PCA) and cluster analyses were performed on the quantitative variables. The quantitative characters were first scaled so that their variances were equal and PCA was done on the standardised variables prior to the cluster analysis to isolate the most important variables. Hierarchical cluster analysis was carried out using UPGMA (Unweighted Pair Group Method Using Arithmetic Means) based on Sneath and Sokal (1973). Genetic relationships were further investigated using data from SDS-PAGE of seed storage proteins. All computations were done using the NCSS 2000 (Hintze, 1998) computer programme.

RESULTS AND DISCUSSION

Principal components. The important principal components, the percentage of the total variance that each represents, and the coefficients used in the weighted sum (loadings) are presented in Table 1. The first four principal components (PC) explained 83.23% of the variation of landraces evaluated at Sheno, 84.44% at Keyit, and 81.28% in combined data. Characters that contributed more to the first principal component (variables with largest coefficients) were days to maturity, 1000-kernel weight, days to heading, grain filling period, grain yield per spike and spike length at Sheno; days to maturity, grain filling period, days to heading and plant height at Keyit; grain filling period, days to maturity and plant height when

data were combined from the two locations. The contribution of these characters was negative except plant height from data across environments. The second component illustrated primarily the variation in number of heads per square meter and grain yield per plot at Sheno; number of seeds per spike, grain yield per spike and number of heads per square meter at Keyit; 1000-kernel weight, days to heading, spike length, days to maturity and grain yield per spike when Sheno and Keyit were combined. The third principal component separated landraces only with number of seeds per spike at Sheno; spike length at Keyit and with grain yield and number of heads per square meter for the combined data. The fourth principal component described the variation in plant height at Sheno, grain yield per plant at Keyit; number of seeds per spike, grain yield per spike and number of heads per square meter for the combined data. Generally, it indicates that the landraces with high PC1 values are low yielding ones characterised by low 1000-kernel weight, relatively short grain filling duration, comparatively short spike length and early in heading and maturity. Those landraces with high PC2 values had high grain yield characterised by high number of heads per square meter across environments, except in combined data. In most of the cases, the contribution of heads per square meter to each PC was in the opposite direction to the contribution made by number of seeds per spike and grain yield per spike (Table 1). This indicated the need to consider the relationships of these yield components in selection programme.

Phenotypic relationships. Clusters of landraces based on data from each location were very similar to that resulting from combined data, except for few lines which did not follow the same pattern (dendrogram not indicated). Hence, discussions were made based on data combined from the two locations.

Summaries of the agronomic characters of landraces within a cluster and deviations of means for grain yield among clusters are presented in Tables 2 and 3, respectively. The genetic relationship, as revealed by the hierarchical cluster analysis, explicitly showed tight grouping of lines derived from landraces bearing the same name (Fig. 1). This indicates the predominance of

TABLE 1. Eigenvalues, proportion of the total variance and contribution of agronomic characters to the first four principal components of barley landraces evaluated at Sheno and Keyit in Ethiopia during 2001

Characters	Sheno				Keyit				Combined			
	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4
Eigenvalue	3.88	1.45	1.77	1.22	2.64	2.35	1.88	1.56	2.01	2.31	1.61	2.19
Proportion of δ^2	38.77	14.50	17.74	12.23	26.39	23.56	18.84	15.64	20.15	23.12	16.10	21.91
Cumulative δ^2	38.77	53.27	71.00	83.23	26.39	49.95	68.80	84.44	20.15	43.27	59.37	81.28
Factor loadings												
Days to heading	-0.781	-0.050	-0.183	0.393	-0.411	0.129	-0.219	-0.032	0.388	-0.663	0.140	0.198
Days to maturity	-0.916	0.013	-0.270	0.039	-0.451	0.083	-0.333	0.031	-0.686	-0.494	0.142	0.317
Plant height	-0.133	0.200	-0.085	0.871	0.025	0.189	0.618	-0.104	0.602	-0.284	0.245	0.284
Spike length	-0.607	0.183	-0.151	-0.007	-0.303	0.377	0.171	-0.071	-0.250	-0.571	0.394	0.218
No. seeds spike ⁻¹	-0.128	-0.035	-0.979	0.030	-0.278	-0.424	0.262	0.452	0.012	0.043	0.027	0.974
Grain yield spike ⁻¹	-0.679	-0.058	-0.661	0.159	-0.405	-0.251	0.348	0.097	-0.159	-0.475	-0.058	0.839
Plants m ⁻²	0.374	0.818	0.353	0.046	0.236	0.521	-0.148	0.324	0.223	0.283	0.744	-0.475
Grain yield plot ⁻¹	-0.366	0.816	-0.197	0.223	-0.143	0.466	0.221	0.538	-0.041	-0.285	0.892	0.129
1000 kernel wt.	-0.879	-0.153	0.037	0.158	-0.306	0.257	0.173	-0.602	-0.016	-0.912	-0.031	0.010
Grain filling period	-0.718	0.093	-0.275	-0.452	-0.349	-0.007	-0.371	0.105	-0.944	0.007	0.034	0.161

TABLE 2. Clusters and mean values of agronomic characters of lines within each cluster from combined data of Sheno and Keyit during 2001

Cluster	DHE	DMA	PLH	SPL	NS/SP	GY/SP	NH/M ²	GY	BM	HI	TKW
I	81+2.6	125+2.5	101+3.8	7.1+1.11	61+5.9	2.7+0.41	289+34	407.7+29.1	1099.1+78.9	0.48+0.02	41
II	88+1.5	136+4.2	99+4.2	7.1+0.26	53+4.9	2.4+0.17	401+6	516.6+8.3	1322.3+131.5	0.45+0.02	40
III	83+1.9	124+1.7	105+3.3	7.3+0.64	54+6.7	2.5+0.27	383+41	527.5+50.9	1426.7+142.4	0.48+0.03	42
IV	79+2.6	123+2.5	96+3.1	5.1+0.65	44+4.0	1.9+0.35	317+63	376.5+20.1	1015.0+67.3	0.49+0.02	35
V	79+1.9	122+1.3	97+3.1	6.4+0.34	54+2.7	2.0+0.10	446+33	464.0+37.2	1159.5+91.8	0.49+0.03	35
VI	82+1.8	123+1.5	100+3.0	4.5+0.15	56+3.7	2.4+0.13	297+21	315.9+22	921.0+94.0	0.39+0.01	38
VII	84+3.2	126+3.8	105+3.5	8.1+1.00	36+6.6	2.0+0.31	429+29	438+15	1305.0+117.6	0.41+0.03	48
VIII	92+0.5	141+2.6	100+3.1	8.8+0.36	67+2.2	3.4+0.18	281+44	511+39	1341.0+122.8	0.47+0.01	44

DHE = Days to heading; DMA = Days to maturity; PLH = Plant height; SPL = Spike length; NS/SP = Number of seeds/spike; GY/SP = Grain yield/spike; NH/M² = Number of productive heads per square metre; GY = Grain yield; BM = Biomass; HI = Harvest index; TKW = Total kernel weight

variation among landraces rather than within landraces. Cluster I, for instance, comprised of all lines from accession 1153 and four of the five lines from Demoye and Key Ferke each forming distinct subgroups within the Cluster. Three lines from Bukura also appeared in this Cluster. Two of the five lines from Bukura did not appear together with their corresponding lines in this Cluster because they had a significantly lower number of seeds per spike, harvest index and 1000-kernel weight. Two lines from Feleme and one from Feres Gama appeared in Cluster II. Three of the lines from the former landrace were each in different clusters because of significant differences in days to heading, days to maturity, grain yield per spike and plant height. This was also apparent from the analysis of variance in combined data. Lines in this Cluster had high grain yield and biomass such as those in Cluster III but were late in heading and maturity.

All lines from Kessele and all except one from Tikur Gebes, including the check variety Misrach, occurred in Cluster III. These lines were as early as those in Clusters I and IV, but had better grain yield potential than lines in the latter two clusters (Table 2). Cluster IV comprised of those which had less grain yield per plot, grain yield per spike, biomass, number of seeds per spike, and 1000-kernel weight than their corresponding lines from Bukura and Demoye in Cluster I. A high number of plants per unit area was observed from landrace lines in Cluster V and it included all Mage and Key Gebes selections, while Cluster VI had only lines from accession 1182 which had very short and compact spikes. It was not possible to distinguish in the field the lines from Feres Gama and Nech Gebes. However, only two lines from Feres Gama and three lines from Nech Gebes appeared in the same cluster while the rest appeared separately on their own.

The intracluster mean values for grain yield were very high and comparable for Clusters II, III and VIII, whereas the lowest mean was observed in Cluster VI (all derived from accession 1182). Accordingly, mean deviations for grain yield between this cluster and Clusters II, III and VIII were very high (Table 3). Landrace lines with comparable and superior agronomic performance but genetically dissimilar can exhibit heterosis in yield and the hybrids generated would be similar

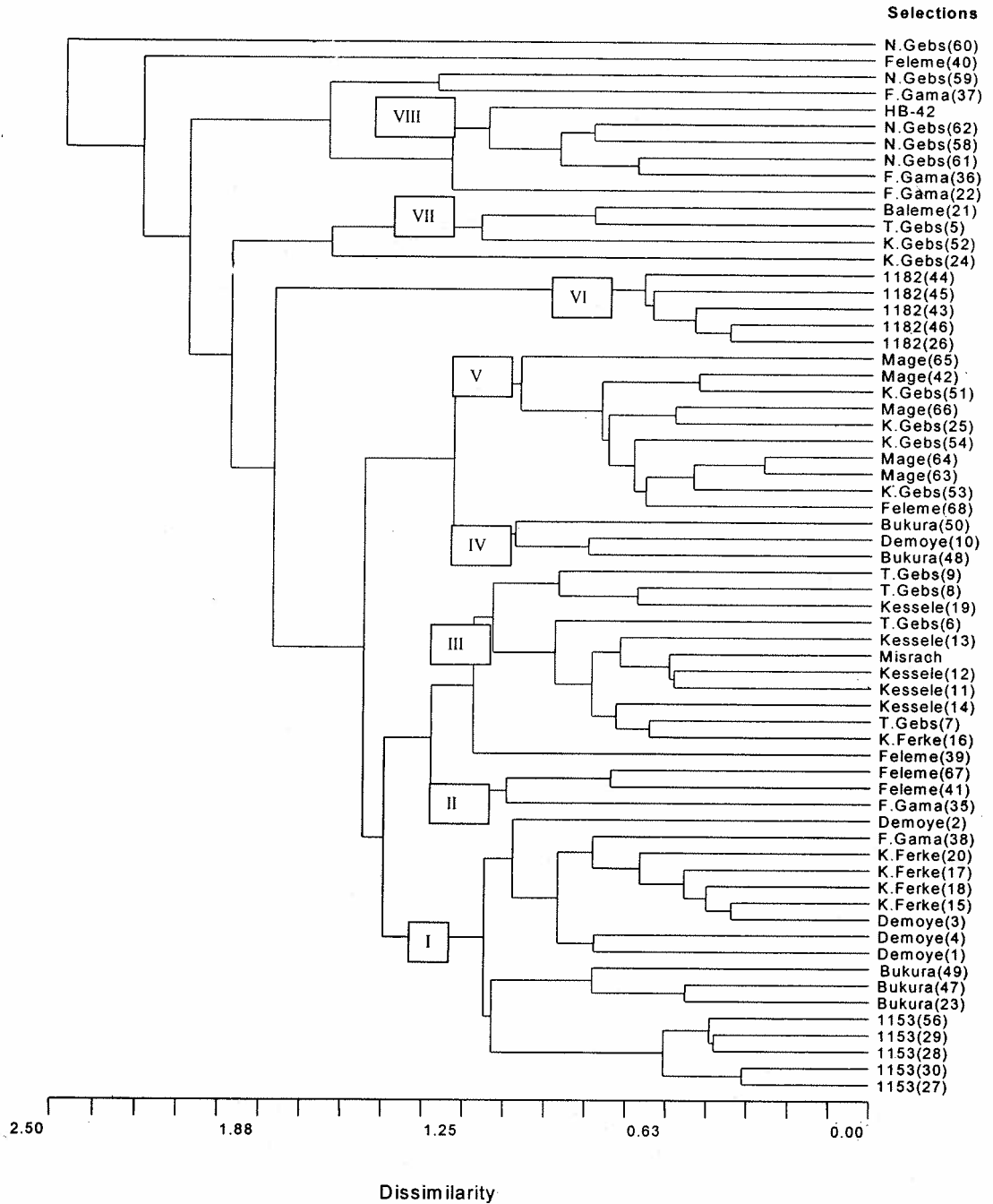


Figure 1. Dendrogram illustrating the genetic relationships between barley landrace lines from north Shewa in Ethiopia evaluated for quantitative agronomic characters at Sheno and Keyit in 2001.

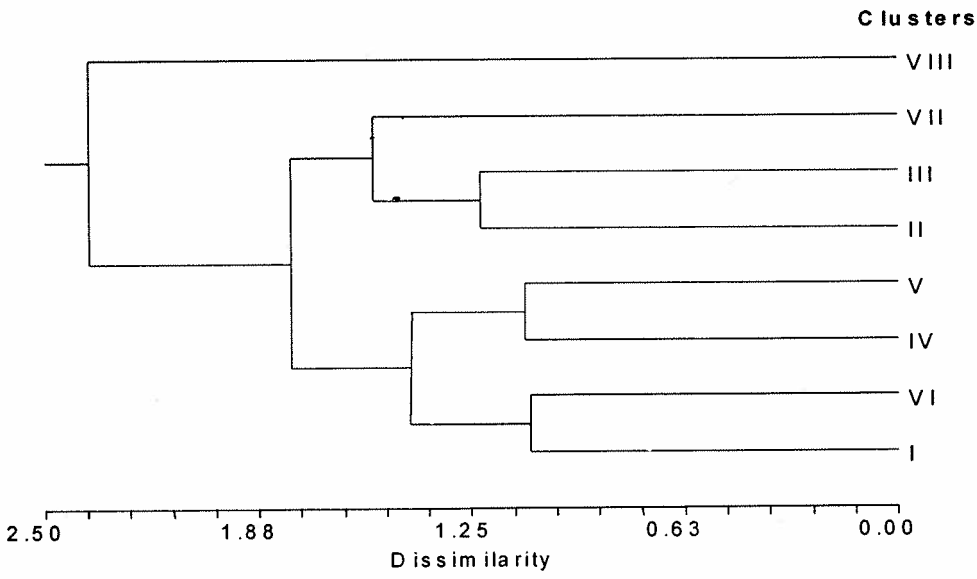


Figure 2. Genetic distance among clusters based on mean of quantitative agronomic characters of landraces within each cluster.

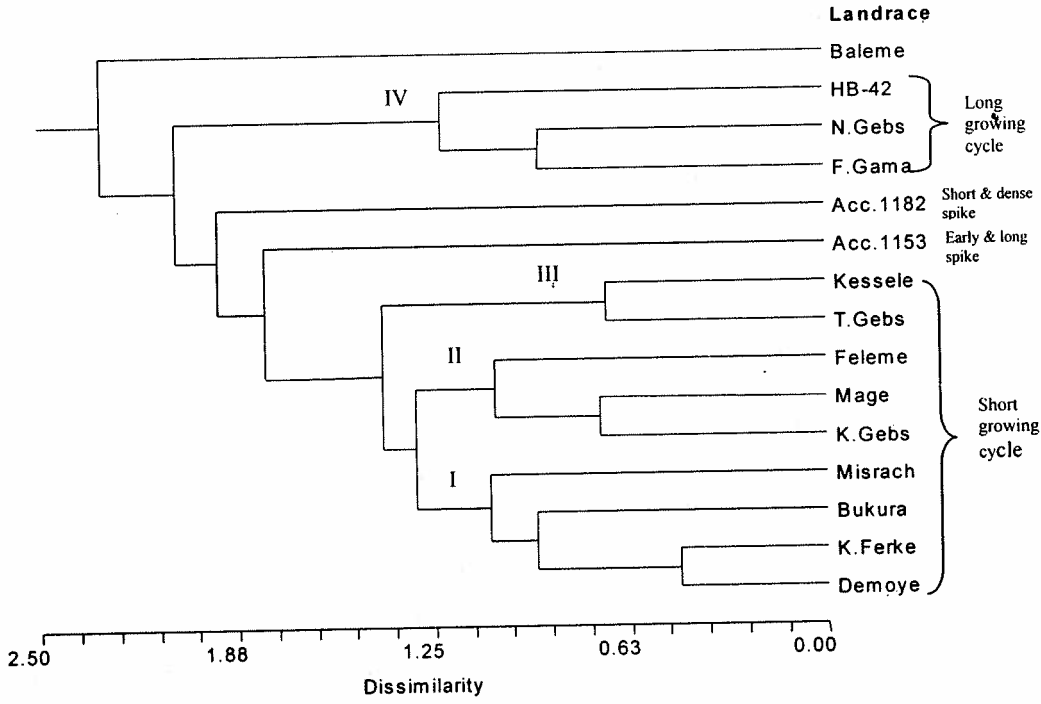


Figure 3. Genetic relationship between farmers' barley cultivars predominantly grown in North Shewa, Ethiopia based on quantitative morphological characters. Acc.1182 and Acc.1553 are landrace collections.

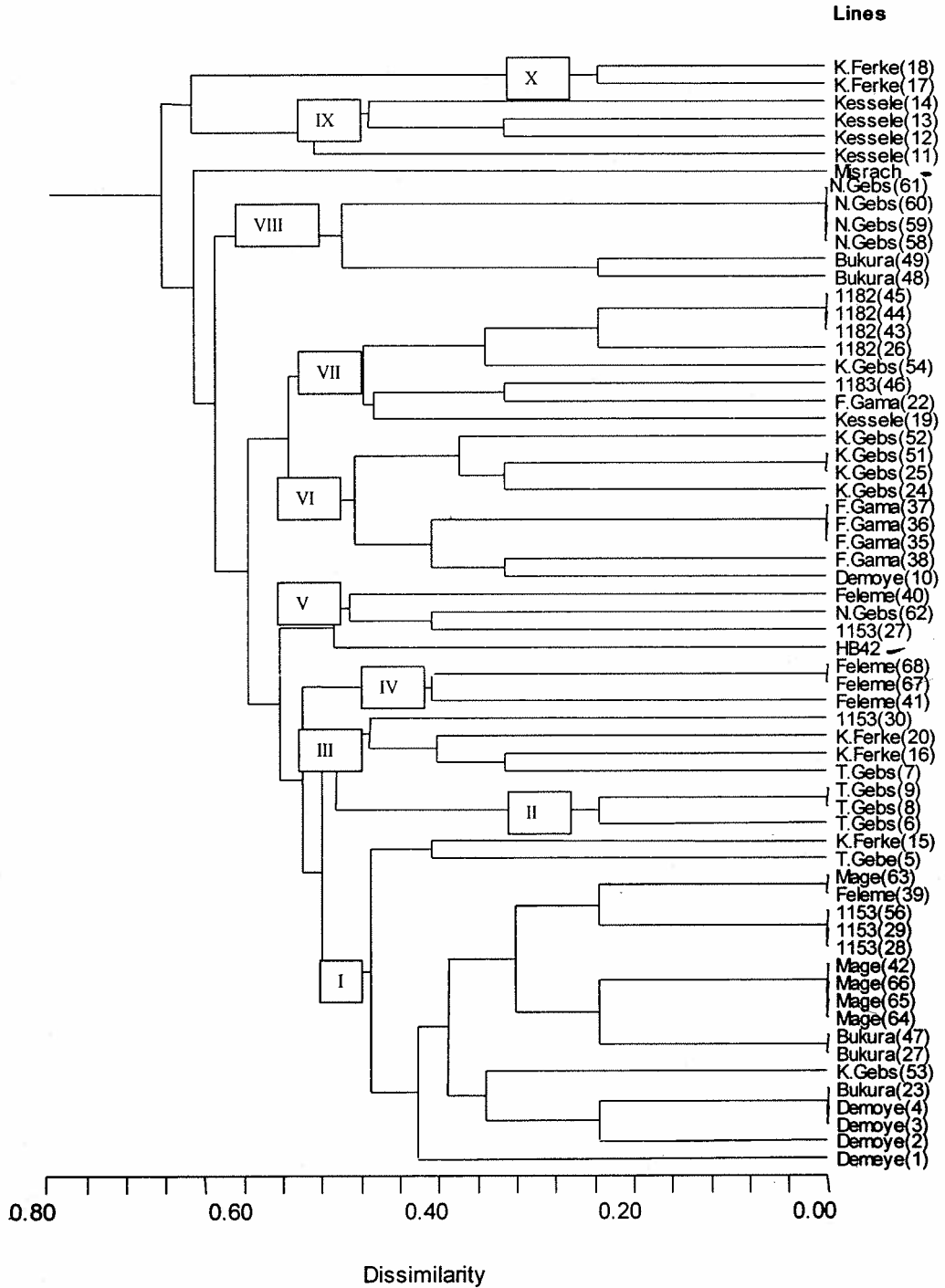


Figure 4. Dendrogram illustrating genetic relationships among barley landrace lines based on SDS-PAGE seed storage protein.

lines is presented in Figure 4. Of the 1953 pair-wise comparisons involving the pure lines and the standard checks (HB42 and Misrach), 29 (i.e. 1.5%) were genetically identical. Eighteen percent of the overall pair-wise comparisons had a genetic distance ≥ 0.700 . The overall genetic distance among the lines ranged from 0 to 0.943, with a mean of 0.574 ± 0.13 ; while genetic distance from pair-wise comparisons of lines within most of the landraces did not exceed 0.666 except between some of the lines within Kessele that revealed dissimilarity as high as 0.781 (Table 6). Moreover, within landraces, mean genetic distance was very low ranging from 0.094 to 0.444, except for Kessele and Key Ferke that had a mean genetic distance of 0.587 and 0.519, respectively. Lines from Mage and Nech Gebes showed genetic similarity in 60% of the pair-wise comparisons, indicating their relative uniformity compared to

the others. This comparison displayed more variation among landraces than within landraces. Correlation between genetic distance values resulting from the two measures of genetic relationship indicated a significant association, although the value is so small ($r=0.199$). Hence, cluster analysis based on quantitative characters (standardised values) and data from SDS-PAGE of seed storage proteins provided fairly identical grouping of landrace lines (Figs. 1 and 4).

Based on this observation, it is plausible to conclude that it requires careful evaluation and selection from very large samples within some of the farmers' cultivars to make slight improvement for agronomic characters. However, apparent progress through selection for yield and yield components within most of the farmers' cultivars from this particular environment is unlikely. Farmers often do not clean their seeds and are

TABLE 4. Estimates of genetic variances and expected genetic advance of lines within clusters

Cluster	Trait	Mean	δ^2g	δ^2gl	δ^2p	GCV	PCV	H	GA	GA (%)
I	DHE	81	9.49	5.65	22.66	3.85	5.95	0.42	4.11	5.14
	DMA	125	3.92	-30.10	45.85	1.59	5.46	0.09	1.19	0.96
	PLH	101	13.28	-8.89	30.27	3.61	5.45	0.44	4.97	4.92
	SPL	7.1	0.95	-0.31	1.17	13.75	15.25	0.81	1.81	25.52
	NS/SP	61	14.77	-26.11	29.87	6.40	9.11	0.49	5.57	9.28
	GY/SP	2.7	0.11	0.00	0.16	12.30	14.94	0.68	0.56	20.87
	NP/M2	289	333.98	-772.3	1061.4	6.30	11.23	0.31	21.12	7.28
	GY	407.7	-40.42	-2465	450.11	-1.558	5.20	-0.09	-3.93	-0.96
	TKW	40	4.14	1.38	8.47	5.09	7.27	0.49	2.93	7.33
III	DHE	83	-1.04	-7.05	29.26	-1.27	6.76	-0.04	-0.39	-0.49
	DMA	124	-6.20	302.73	229.05	-2.09	12.72	-0.03	-0.84	-0.71
	PLH	105	-1.39	-39.42	36.95	-1.16	5.96	-0.04	-0.47	-0.47
	SPL	7.3	-0.38	1.64	1.02	-8.56	14.02	-0.62	-0.77	-10.75
	NS/SP	54	12.49	60.27	72.78	6.67	16.07	0.17	3.02	5.69
	GY/SP	2.5	0.03	0.08	0.15	7.05	15.61	0.20	0.16	6.56
	NP/M2	383	987.67	2915.1	4759.4	8.45	18.55	0.21	29.49	7.93
	GY	527.5	-78.95	1054.6	3028.9	-1.68	10.44	-0.03	-2.96	-0.56
	TKW	41	-2.57	-8.08	4.37	-3.90	5.09	-0.59	-2.53	-6.18
V	DHE	79	2.55	1.49	3.75	2.02	2.45	0.68	2.71	3.43
	DMA	122	2.19	0.69	3.11	1.21	1.45	0.71	2.56	2.10
	PLH	97	8.19	-2.87	10.62	2.95	3.36	0.77	5.18	5.34
	SPL	6.4	0.09	-0.04	0.12	4.68	5.51	0.72	0.53	8.20
	NS/SP	54	7.77	-2.91	9.17	5.26	5.71	0.85	5.29	9.97
	GY/SP	2.0	0.00	0.00	0.01	2.98	5.01	0.35	0.07	3.66
	NP/M2	446	809.81	-1237	1491.1	6.31	8.56	0.54	43.20	9.58
	GY	464	974.45	-995.3	1516.9	6.71	8.37	0.64	51.54	11.08
	TKW	36	9.73	-2.50	13.87	8.66	10.35	0.70	5.38	14.95

unable to maintain "true to type" of the different cultivars. Mixing of seeds of different cultivars is possible on a threshing ground not cleaned carefully and often difficult to avoid them when planting. Rouging after heading is also difficult, especially if they have the same seed colour and phenology. Therefore, it is possible that all these factors can contribute to variability within landraces without neglecting the existence of biotypes within farmers' cultivars. Overall, the assessments of genetic variability of the farmers' cultivars, both from morphological and protein profiles perspectives, illustrated less variability within farmers cultivars. Hence, progress through pure line selection cannot be warranted. Instead, it can be suggested that rouging carefully the inferior biotypes or off types from farmers' cultivars may help exploit their maximum genetic potential than attempting to improve their productivity through pure line selection and evaluation method.

CONCLUSION

Estimates of genetic advance and cluster analysis based on data of quantitative characters illustrated less variability within than among farmers' cultivars that was further verified by SDS-PAGE of seed storage proteins. Therefore, genetic gain through selection for yield and yield components within the cultivars from this particular environment is expected to be minimal. Selection may even end up with negative response unless carefully carried out to isolate those that might be useful to bring slight improvement in yield. Careful rouging the inferior types to upgrade their yield potential is suggested rather than attempting to improve them through extensive pure line selection and evaluation methods. Evaluation of landraces from other sources, from within or outside the environment, is suggested to get better yielding landraces than the existing ones. In the long term, crossing may be scheduled between cultivars,

TABLE 5. Phenotypic (PCV) and genotypic (GCV) coefficient of variation, components of variances, broad sense heritability (H) and expected genetic advance from selection (GA) from combined data of 11 characters of barley landrace lines evaluated at Keyit and Sheno, Ethiopia 2001

Characters	PCV (%)	GCV(%)	δ^2_p	δ^2_g	H (%)	GA	GA f (% mean)
Days to heading	8.63	-0.91	49.50	-0.55	-1	-0.15	-0.18
Days to maturity	5.22	4.41	42.30	30.51	72	9.64	7.74
Plant height	5.41	3.77	29.26	14.21	49	5.46	5.47
Spike length	17.13	15.79	1.41	1.20	85	2.08	30.02
Number of seeds/spike	14.63	12.43	64.60	46.66	72	11.92	21.69
Grain yield/spike	20.47	17.04	0.26	0.18	69	0.73	29.48
Plants/M ²	23.58	16.93	6463.65	3332.29	52	86.12	25.26
Grain yield/plot	18.57	12.98	6727.16	3284.95	49	82.79	18.75
1000 kernel weight	10.27	7.94	16.95	10.14	59	5.00	12.47

TABLE 6. Range and mean genetic distances between lines within each landrace or accession (evaluated at Keyit and Sheno) based on SDS-PAGE of seed storage proteins

Landrace	Range	Mean	Landrace	range	Mean
Acc. 1153	0.000-0.577	0.338	Key Ferke	0.235-0.666	0.519
Acc. 1182	0.000-0.527	0.275	Kessele	0.333-0.781	0.587
Key Gebes	0.000-0.623	0.443	Mage	0.000-0.235	0.094
Demoye	0.000-0.577	0.375	Nech Gebes	0.000-0.623	0.249
Feres Gama	0.000-0.577	0.302	Tikur Gebes	0.000-0.471	0.364
Feleme	0.000-0.577	0.444	Bukura	0.000-0.623	0.386

which based on morphological characters and protein profiles, appear to be diverse in order to maximize the potential gain from selection in the progeny.

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