

Genetic divergence analysis of Ethiopian faba bean (*Vicia faba* L.) landraces

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Abstract

*Genetic divergence is important in plant breeding to select parents for crossing. A study was initiated to assess the extent of genetic divergence and group faba bean accessions based on their similarity and dissimilarity. The experiment was conducted at Mecha district in 2019 main cropping season. A 9x9 simple lattice design was used and 14 important agronomic traits were collected. Cluster analysis distinguished the 81 accessions into seven groups. The discrimination of accessions into so many discrete clusters suggested the presence of genetic diversity in the material evaluated. The maximum inter-cluster distance was detected between cluster I and cluster III ($D^2 = 152.28^{**}$) followed by cluster III and cluster IV ($D^2 = 130.22^{**}$). The high values of inter-cluster distances indicate divergence among the accessions and may be used in selection of genetically divergent parents for exploitation in crossing programs for better genetic recombination. The results of principal component (PC) analysis demonstrated that first four PCs explained 73.81% of the total variations among the 81 faba bean accessions. Plant height, number of pods per plant, number of branches per plant, biomass yield, grain yield and disease scores (chocolate spot, ascochyta blight and rust) were important in the first and second PC axes; as a result, they are important in discriminating the faba bean landraces. The study shows the importance of multivariate methods in assessing genetic divergence so as to identify possible parents for crossing considering traits of agronomic importance.*

INTRODUCTION

Faba bean (*Vicia faba* L.), also known as fava bean, broad bean and horse bean, is one of the oldest crops cultivated worldwide (Mínguez & Rubiales, 2021). Mediterranean countries, Ethiopia, Egypt, China, Afghanistan, India, Northern Europe, and Northern Africa are major producers of faba beans (Rahate et al., 2020). Out of more than 50 faba bean-producing countries, about 90% production is concentrated in Asian, European Union (EU) and African region (FAO, 2020). The world production of faba beans was 5.43 million metric tons in 2019, representing a 25% increase compared with 4.35 million metric tons in 1990. Regionally, Asia leads with 33.55% of total faba bean production globally, followed by Europe (EU) and Africa with 29.36% and 27.04% share, respectively (FAO, 2020). China was the leading producer of faba beans, followed by Ethiopia; these two countries represented about 50% of the total global production, whereas among the EU, the United Kingdom and France were among the top five producers. Also, in 2019, Australia was the leading exporter of faba beans with 265,543 metric tons or nearly 30% of total exports, followed by the United Kingdom, Lithuania, Egypt and Latvia (FAO, 2020). Egypt led importers with 309,355 metric tons or 40.48% of total global imports followed by Norway, Germany, Saudi Arabia, and France (FAO, 2020).

Faba bean is an important crop for an ecological, nutritional, and economical point of view (Xiao et al., 2021). It is a versatile crop providing various ecosystem services, that is, cultivated primarily as a food source for the human population residing in Asia and Africa, as animal feed/silage in the European region, and fixation of atmospheric nitrogen in agricultural soils, thereby significantly reducing the application of synthetic fertilizers (Zhou et al., 2018). Nutritionally, mature seeds of faba bean are rich in proteins (26.1%), carbohydrates (58.3%), and dietary fiber (25.0%) (USDA, 2021).

Genetic divergence refers to genetic distance among the genotypes under consideration. It is determined by using cluster analysis. D-square statistics (D^2) is one of statistical technique developed by Mahalanobis (1936) used to classify the different genotypes into different groups. The extent of diversity present between genotypes

determines the extent of improvement gained through selection and hybridization. The more divergent two genotypes are the more will be the probability of improving genotypes through selection and hybridization.

Principal component analysis (PCA) is a multivariate analysis that transforms a number of possibly correlated variables into a smaller number of uncorrelated variables. It is a standard tool in modern data analysis used to extract relevant information from confusing data sets by identifying directions, called principal components (Chatfield and Collin, 1980). Therefore, PCA used to identify and minimize the number of traits for effective selection and improvement of yield and its related trait. The first principal component accounts more of the variability in the data as possible followed by each succeeding component accounts for the remaining variability as possible. Therefore, the present study was initiated to assess the extent of genetic divergence and group faba bean accessions based on their similarity and dissimilarity.

MATERIALS AND METHODS

Experimental Site, Materials and Design

The experiment was conducted at Bahir Dar University research site in Mecha district, Ethiopia in the 2019 main cropping season. Mecha is located at about 525 km Northeast of Addis Ababa and 34 km Southwest of Bahir Dar. It is located at latitude of 10°30' N and longitude of 37°29' E. The mean annual rainfall of the site is 1572 mm. The mean temperature ranges between 24°C and 27 °C; and the altitude is 2009 m.a.s.l. Totally, 81 faba bean genotypes were used in the study. Seventy-eight of the genotypes were accessions obtained from Ethiopian Biodiversity Institute; the two were standard checks obtained from Adet Agricultural Research Center; and one was a local check from local source.

The experimental design used was 9 × 9 simple lattice. Each accession was planted on two-row plot. Spacing between rows and within rows were 40 cm and 10 cm, respectively. The single plot size was 0.8m × 1m (0.8 m²). Spacing between blocks and replication was 1 meter.

Table 1. List of the 81 Faba bean accessions used in the study

Treatment	Accession	Collection Region	District	Treatment	Accession	Collection Region	District
1	212565	Amhara	Siyadebrina Wayu	42	235709	SNNP	Dirashe Special
2	27279	Amhara	Machakel	43	219089	Oromia	Amigna
3	25299	SNNP	Angacha	44	25338	SNNP	Meskanena Mareko
4	245140	SNNP	Dila Zuria	45	215748	Amhara	Dessie Zuria
5	212566	Oromia	Wuchalena Jido	46	213211	Oromia	Tiro Afeta
6	25006	Amhara	Hulet Ej Enese	47	25336	SNNP	Meskanena Mareko
7	212568	Amhara	Siyadebrina Wayu	48	226125	Amhara	Legambo
8	25018	Oromia	Wuchalena Jido	49	240497	SNNP	Decha
9	27052	Oromia	Kofele	50	25346	SNNP	Meskanena Mareko
10	25274	Oromia	Becho	51	25328	SNNP	Selti
11	229303	Amhara	Lay Betna Tach Bet	52	213214	Oromia	Limu Seka
12	212567	Amhara	Siyadebrina Wayu	53	25335	SNNP	Meskanena Mareko
13	25298	SNNP	Angacha	54	25340	SNNP	Meskanena Mareko
14	219355	Oromia	Adolana Wadera	55	25339	SNNP	Meskanena Mareko
15	208085	Amhara	Weremo Wajetuna Mida	56	215129	Amhara	Mama Midrina Lalo
16	212811	Amhara	Wegera	57	215128	Amhara	Mama Midrina Lalo
17	203105	Oromia	Dedesa	58	228607	Amhara	Goncha Siso Enese
18	235955	Amhara	Debark	59	25337	SNNP	Meskanena Mareko
19	25279	SNNP	Cheha	60	25341	SNNP	Meskanena Mareko
20	208114	Amhara	Weremo Wajetuna Mida	61	25331	SNNP	Selti

21	229310	Amhara	Weremo Wajetuna Mida	62	235433	Tigray	Kola Temben
22	212572	Amhara	Weremo Wajetuna Mida	63	Tumsa	-	-
23	25003	Oromia	Kuyu	64	25325	SNNP	Selti
24	25323	SNNP	Selti	65	25329	SNNP	Selti
25	25022	Oromia	Kuyu	66	25309	SNNP	Angacha
26	25280	SNNP	Cheha	67	25304	SNNP	Angacha
27	235956	Amhara	Debark	68	25330	SNNP	Selti
28	220079	Tigray	Adwa	69	25307	SNNP	Angacha
29	212576	Amhara	Lay Betna Tach Bet	70	25334	SNNP	Selti
30	212575	Amhara	Lay Betna Tach Bet	71	25306	SNNP	Angacha
31	25277	SNNP	Cheha	72	25311	SNNP	Angacha
32	220076	Tigray	Adwa	73	25332	SNNP	Sodo
33	27290	Oromia	Jimma Arjo	74	25310	SNNP	Angacha
34	25264	Oromia	Becho	75	25333	SNNP	Selti
35	25292	SNNP	Limo	76	25302	SNNP	Angacha
36	25270	Oromia	Becho	77	25327	SNNP	Selti
37	212580	Amhara	Mama Midrina Lalo	78	Dosha	-	-
38	25017	Amhara	Enarj Enawga	79	25303	SNNP	Angacha
39	212578	Amhara	Geramidirna Keya	80	25301	SNNP	Angacha
40	25010	Oromia	Gerar Jarso	81	Local	-	-
41	25290	SNNP	Limo				

Data Analysis

Analysis of variance

The data for each trait were subjected to analysis of variance (ANOVA) for simple lattice design using SAS software version 9.2 (SAS, 2008).

Clustering of accessions and genetic divergence analysis

Cluster analysis was made using SAS Software version 9.2 (SAS Institute, 2008). Based on the squared distances (D^2) values, clustering of accessions was done using Tocher's method as described by Singh and Chaudhary (1985). Unweighted Pair Group Methods with Arithmetic-average (UPGMA) based on the generalized D^2 distances by average linkage method of agglomerative hierarchical clustering (successive merger) was built to group the faba bean genotypes into genetically distinct classes based on phenotypic traits. The number of clusters was determined by using points where local peaks of Pseudo F-statistics join with small values of the Pseudo t^2 statistics followed by a larger Pseudo t^2 for the next cluster fusion. The dendrogram was constructed using JMP software.

Genetic distance between pair of clusters was calculated using the generalized D^2 statistics (Mahalanobis, 1936). Average intra cluster D^2 values were estimated using the formula $\frac{\sum D^2_i}{n}$ where $\sum D^2_i$ is the sum of distance between all possible combinations (n) of the accessions included in a cluster. The D^2 statistics measures the force of differentiation at intra- and inter-cluster levels. Hence, genetic distance between clusters as standardized D^2 statistics were calculated as follow:

$$D^2_{ij} = (X_i - X_j) S^{-1} (X_i - X_j)$$

Where D^2_{ij} = the square distance between any two genotypes i and j,

X_i and X_j = the vectors for the values for genotype i^{th} and j^{th} genotypes, and

S^{-1} = the inverse of the pooled variance covariance matrix.

The significance level of genetic distance between and within clusters was tested at both 1% and 5% level of probability using Chi-square (χ^2) test. The D^2 values obtained for pairs of clusters using SAS statistical package were considered as the calculated values of χ^2 and tested for P degree of freedom, where P is the number of traits considered (Singh and Chaudhary, 1985).

Principal component analysis

Principal component analysis (PCA) was used to find out the traits, which accounted more to the total variation. The data were standardized to mean zero and variance of one before computing principal component analysis. Principal components were calculated based on correlation matrix using SAS computer software. As suggested by Johnson and Wichern (1988), eigenvector greater than half divided by the standard deviation (square root) of the eigen value of the respective PC was employed as general guideline for weighing the relative significance of traits constituting to the PCs.

RESULTS AND DISCUSSION

Analysis of variance

The result of analysis of variance showed that the accessions vary significantly ($p < 0.01$) for all the traits investigated except days to maturity and number of seeds per pod (Hiywotu et al., 2022).

Clustering among accessions

Cluster analysis distinguished the 81 accessions into seven groups of different sizes (Table 2 and Fig. 1), members within a cluster being assumed to be more closely related in terms of the traits under consideration with each other than those members in different clusters. Similarly, members in clusters with non-significant distance were assumed to have more close relationships with each other than they are with those in significantly distant clusters. The number of accessions in each of the seven clusters ranged from 3 to 24 in the smallest and largest cluster, respectively.

Table 2
List of faba bean accessions grouped into seven clusters

Cluster	Number of accessions	Name of accessions
C I	8	212565, 212568, 25018, 212567, 208085, 212575, 25010, 215748
C II	9	245140, 212566, 229303, 219355, 212811, 25003, 25323, 213214, 228607
C III	3	Tumsa, Dosha, Local
C IV	24	27279, 25006, 27052, 203105, 25279, 208114, 25022, 25280, 235956, 220079, 25277, 220076, 25264, 212580, 212578, 25338, 226125, 25346, 25340, 215128, 25341, 25329, 25332, 25333
C V	12	25274, 229310, 25270, 25290, 235709, 219089, 240497, 25337, 235433, 25325, 25334, 25310
C VI	8	212572, 212576, 27290, 25336, 25328, 25339, 215129, 25331
C VII	17	25299, 25298, 235955, 25292, 25017, 213211, 25335, 25309, 25304, 25330, 25307, 25306, 25311, 25302, 25327, 25303, 25301

Clusters CI and CVI had 8 accessions for each. Clusters CII and CIII had 9 and 3 accessions, respectively. Cluster CIV was the largest cluster group consisting of 24 accessions followed by CVII which had 17 accessions. CV had 12 accessions. These indicate the presence of adequate variability among the accessions. The discrimination of accessions into so many discrete clusters suggested the presence of genetic diversity in the material evaluated. The presence of substantial genetic diversity among the accessions implied that these accessions may serve as good source for selecting the diverse parents for a hybridization program aimed at isolating desirable segregants for grain yield and other important traits. Similarly, substantial genetic divergence in the faba bean germplasm was reported previously (Keneni et al., 2005; Qi and Xing, 2009; Berma et al., 2016 and Chaudhary et al., 2018).

Genetic distance among clusters

Pair wise generalized distances (D^2) among the seven clusters were presented in Table 3. Out of 21 possible pairs of clusters, differences between 14 pairs were highly significant ($P < 0.01$) and 4 pairs were significant ($P < 0.05$), while those between the rest of the clusters were non-significant. The maximum inter-cluster distance ($D^2 = 152.28^{**}$) was detected between CI and CIII. Cluster CI constituted accessions from two regions (Amhara and

Oromia) except one while cluster CIII constituted standard and local checks as shown in Table 3 of the 81 faba bean accessions used in the study. The high values of inter-cluster distances indicated divergence among the accessions and might be used in breeding programs for better genetic recombination and selection of genetically divergent parents for exploitation in crossing programs. This finding is consistent with Fikreselassie and Seboka (2012) and Keneni et al. (2005) who used 25 and 160 faba bean genotypes respectively and found high D^2 value. The second most divergent clusters were cluster CIII and CIV ($D^2 = 130.22^{**}$). Cluster CIV constituted accessions from all regions (Amhara, Oromia, Tigray and Southern nation nationalities people). This indicates accessions from different regions might have similar genetic background.

The third most divergent clusters were CIII and CVI ($D^2 = 121.99^{**}$). Cluster CVI constituted accessions from three regions (Amhara, Oromia and Southern nation nationalities people). The results implied that crossing of accessions from CIII with accessions from CI, CIV and CVI will result in useful segregants in the F_1 and subsequent generations. Similar findings were reported by Lal *et al.* (2019) who clustered 83 faba bean genotypes in to ten clusters. The minimum distance ($D^2 = 6.93$) was observed between CIV and CVII suggesting that the materials may be very closely related. Cluster CVII constituted accessions from Southern nation nationalities people region except three accessions. In the present investigation, the highest intra-cluster distance was found for CI followed by CIV, CII, CV, CVII, CVI and CIII. This implies that the accessions present in a cluster have little genetic divergence from each other with respect to aggregate effect of twelve traits under study, while more genetic diversity was observed between the accessions belonging to different clusters.

Table 3
Estimates of average intra- (diagonal) and inter-cluster (above diagonal) distances for 7 clusters of 81 faba bean accessions

Cluster	CI	CII	CIII	CIV	CV	CVI	CVII
CI	206.99	27.25**	152.28**	19.54	58.57**	49.81**	29.65**
CII		170.43	104.81**	13.92	22.94*	37.03**	19.69*
CIII			10.90	130.22**	109.29**	121.99**	93.40**
CIV				195.76	23.29*	24.57*	6.93
CV					91.32	29.22**	25.57**
CVI						27.21	26.11**
CVII							61.58
** = highly significant at probability level $p < 0.01$ ($\chi^2 = 24.72$), * = Significant at probability level of $p < 0.05$ ($\chi^2 = 19.67$)							

Traits mean of clusters

Cluster means showed significant differences among the clusters for most of the traits (Table 4). Cluster CI constituted inferior accessions (susceptible to chocolate spot, rust, the shortest in plant height, low 100 seed weight and harvest index) for most of the traits. Similar results were obtained by Keneni et al. (2005) for chocolate spot, the shortest in plant height, and low 100 seed weight. Clusters CII, CIV and CV comprised intermediate accessions for most of the traits, but CV superior in grain yield. Cluster CVI showed the highest mean for harvest index and lowest mean of biological yield, ascochyta blight score and rust disease score. Cluster CVII was

characterized by the least days to flowering, number of pods per plant and number of branches/plant but superior in ascochyta blight disease. Cluster CIII constituted superior accessions for most of the traits including chocolate spot disease, rust disease, plant height, pod length, number of pods per plant, number of branches per plant, biological yield and 100 seed weight.

Overall, the results showed wide variation from one cluster to another in respect of cluster means for twelve traits, which indicated that accessions having distinctly different mean performance for various traits were separated into different clusters. The crossing between the entries belonging to cluster pairs having large inter-cluster distance and possessing high cluster means for one or other traits to be improved may be recommended for isolating desirable recombinants in the segregating generations in faba bean. However, the results suggested that selection of parents should consider not only the distance between clusters but also the special merits of each cluster and each accession within a cluster depending on the specific objectives of hybridization as suggested by Singh (1990).

Table 4
Cluster means for 12 traits of 81 faba bean accessions

Traits	CI	CII	CIII	CIV	CV	CVI	CVII	Mean
DF	50.88 ^a	49.72	45.17	44.71	44.58	43.94	43.56 ^b	46.08
PH	84.04 ^b	103.52	121.71 ^a	93.01	109.02	91.42	98.63	100.19
PL	3.46	3.84	5.78 ^a	3.44 ^b	3.75	3.54	4.04	3.98
NPP	9.47	10.30	19.37 ^a	9.75	16.53	13.03	8.86 ^b	12.47
NBP	1.78	2.18	2.40 ^a	1.55	2.06	1.45	1.35 ^b	1.82
BY	7283.69	9926.04	13349.67 ^a	7520.44	10402.26	5211.28 ^b	8145.63	8834.14
GY	1438.02 ^b	1979.36	2962.10	1703.18	3231.91 ^a	1958.92	1910.47	2169.14
HSW	33.29 ^b	38.13	82.32 ^a	37.92	42.38	40.06	46.76	45.83
HI	20.22 ^b	20.65	22.52	23.58	32.10	38.98 ^a	23.80	25.98
AB	4.25	3.78	2.33	3.38	2.33	4.50 ^a	2.29 ^b	3.27
CS	5.25 ^a	2.11	1.00 ^b	3.75	1.67	3.00	2.82	2.80
RT	4.63 ^a	1.00 ^b	1.00 ^b	2.17	1.17	4.63 ^a	2.35	2.42

a=Highest value, b=Lowest value. DF= days to flowering, PH= plant height, PL = pod length, NPP= number of pods per plant, NBP= number of branches per plant, BY= biomass yield, GY= grain yield, HSW = 100 seed weight, HI= harvest index, AB= ascochyta blight, CS= chocolate spot, RT= rust disease score.

Principal component analysis

In the present study, the first four principal components exhibited eigen values more than one (4.856, 2.006, 1.672 and 1.063) and explained about 73.81% of the total variations among the 81 faba bean accessions (Table 5). The first four PCs were given due importance for further explanation. Similar findings were reported by Tiwari (2019)

who found the first three principal components exhibiting eigen values more than one and explained about 75.53% of the total variations.

The first principal component accounted for 37.35% of the total variations. Traits such as plant height, pod length, number of pods per plant, biological yield, grain yield and 100 seed weight had high contribution to the first PC. These indicated that these traits had higher relative contribution to the total diversity and they were the ones that most differentiated the accessions. Keneni et al. (2005) reported traits with relatively greater weight in PC1 like grain yield and plant height had higher relative contribution to the total diversity and they were the ones that most differentiated the populations. The second component accounted for 15.43% of the total variation and predominantly illustrates variation in days to flowering and number of branches per plant. The third principal component accounted for 12.86% of the total variation and it was chiefly accounted by variation in number of pods per plant, grain yield and harvest index. The fourth principal component accounted for 8.17% of the total variation and indicated with high variation in pod length, number of branches per plant, 100 seed weight and rust disease score.

Table 5
Eigenvectors, eigenvalues and variance explained by the first four principal components

Traits	PC-1	PC-2	PC-3	PC-4
Days to flowering	-0.090	0.537	-0.152	0.285
Plant height	0.363	0.113	0.102	0.061
Pod length	0.325	-0.099	-0.156	0.542
Number of pods per plant	0.322	0.221	0.307	-0.264
Number of branches per plant	0.184	0.481	0.162	0.302
Biomass yield	0.333	0.171	-0.324	-0.247
Grain yield	0.373	0.055	0.325	-0.123
Hundred-seed weight	0.303	-0.285	-0.213	0.463
Harvest index	0.046	-0.139	0.706	0.180
Ascochyta blight	-0.208	0.087	0.138	0.071
Chocolate spot	-0.313	0.050	0.081	0.098
Rust disease	-0.304	-0.094	0.202	0.344
Eigen value	4.856	2.006	1.672	1.063
Proportion (%)	37.35	15.43	12.86	8.17
Cumulative (%)	37.35	52.78	65.64	73.82

As it is visualized in Fig. 2, grain yield was highly correlated with number of pods per plant, biomass yield and plant height, whereas weaker but positive correlated with number of branches per plant while negatively correlated with ascochyta blight, chocolate spot and rust disease score. Treatment 8 and 16 were plotted far apart from others. Treatment 8, 16, 7, 45, 15 and 29 highly contributed to PC1 in negative direction and treatment 62, 63, 78, and 81

contributed highly in positive direction for PC1. The biplot gave more opportunity to assess which accessions were good for which traits that would help as excellent baseline information for faba bean improvement. Accordingly, treatment 4, 10, 14, 21, 23, 24, 25, 36, 41, 42, 43, 52, 58, 62, 63 and 64 can be selected for grain yield improvement, whereas treatment 21 and 58 were better for biomass yield. This implies a substantial amount of diversity in the faba bean accessions and possibility of its improvement by selection.

Conclusion

Cluster analysis grouped the accessions into seven clusters based on their similarity and dissimilarity. The maximum inter-cluster distance ($D^2 = 152.28^{**}$) was detected between CI and CIII followed by CIII-CIV and CIII-CVI. The high values of inter-cluster distances indicate divergence among the accessions and might be used in breeding programs for better genetic recombination and selection of genetically divergent parents for exploitation in crossing programs. The principal component analysis shows that the first four principal components exhibited eigen values more than one and explained about 73.81% of the total variations.

Declarations

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Author contributions

Andualem Muche Hiywotu: Conceived and designed the experiments; performed the experiments and wrote the paper.

Fisseha Worede: Conceived and designed the experiments; edited the paper.

Alemu Abate: Analyzed and interpreted the data; materials, analysis tools, or data. All authors have read and agreed to the definitive version of the manuscript.

Temesgen Asmare Molla: Performed the experiments, wrote the paper, and prepared the manuscript.

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Data availability

All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate

Ethical approval: This article contains no studies with human participants or animals performed by any of the authors, "not applicable".

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Clinical trial number: not applicable

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Figures

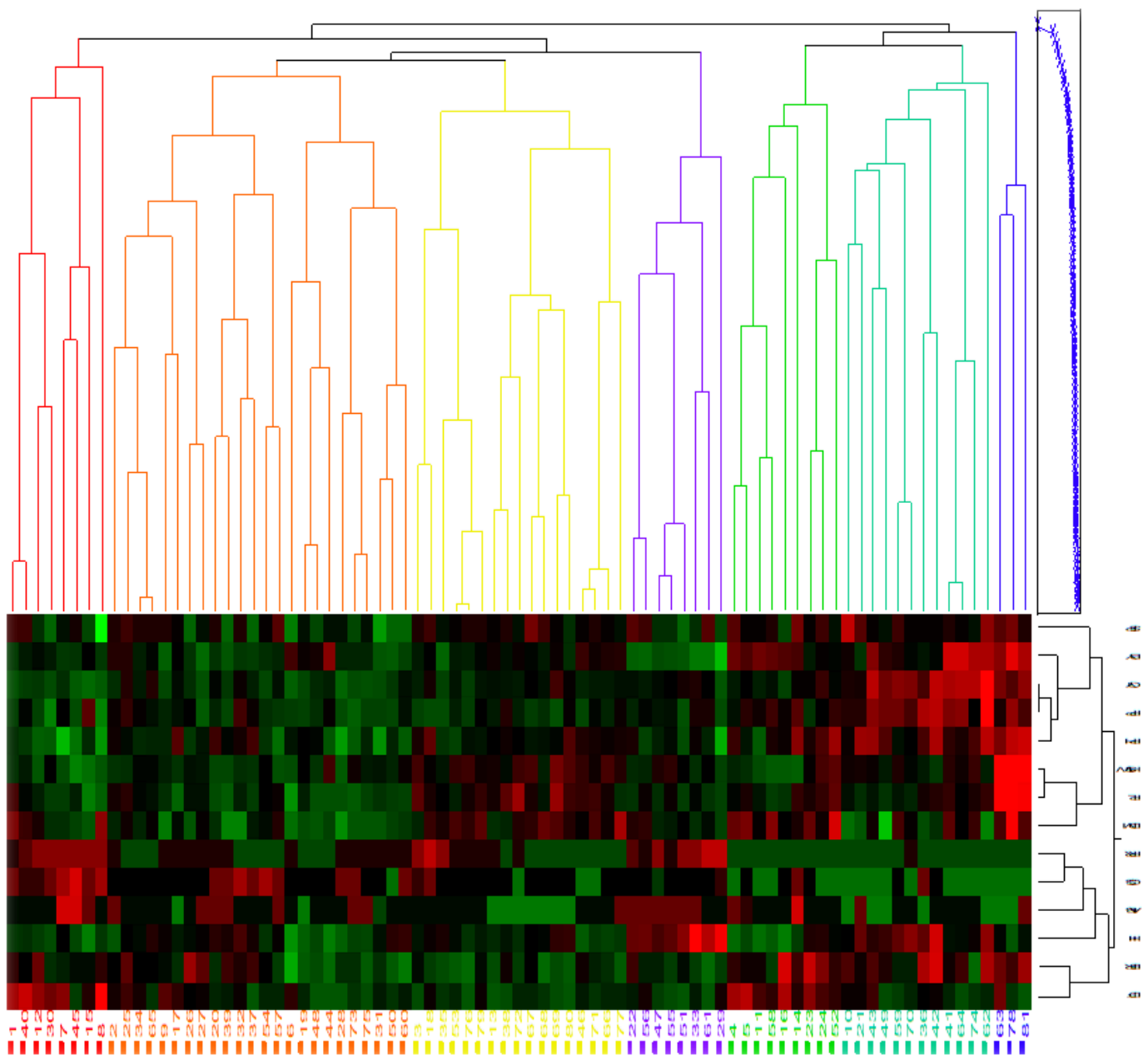


Figure 1

Dendrogram showing relationships among 81 Ethiopian faba bean accessions constructed by using 12 traits

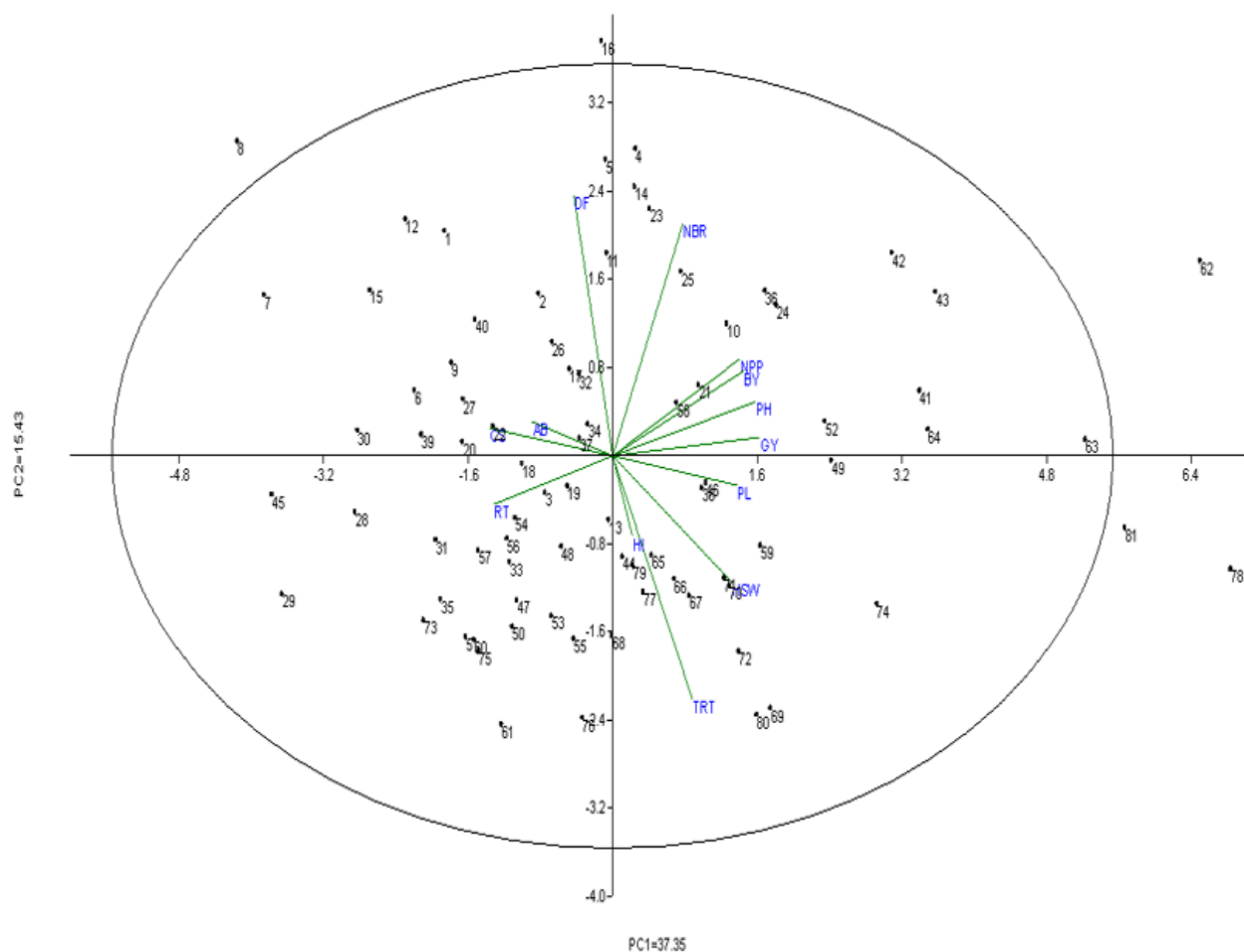


Figure 2

Principal component analysis biplot describing the relative position of 81 faba bean accessions and 12 traits. Identification of treatments is as listed in Table 1.