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Phenotypic Diversity among Faba Bean (*Vicia faba* L) Landraces from the Ethiopian Highlands

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Abstract

Knowledge of phenotypic diversity is important for devising the breeding strategy for faba bean (Vicia faba L.) program in Ethiopia. This study was conducted to determine phenotypic diversity among 50 faba bean genotypes collected from the major faba bean growing areas of Ethiopia and International Centre for Agricultural Research in the Dry Areas (ICARDA). The genotypes were evaluated at two locations using a randomized complete block design with three replications. All agronomic data, yield and yield component, chocolate spot (Botrytis fabae) disease incidence disease severity were collected and analyzed using analysis of variance, principal component analysis and the Shanon-Weaver index, using SAS V9.3 and PAST V 3.0 software. There were significant differences ($p< 0.001$) among the genotypes on most of phenotypic traits, chocolate spot disease severity, yield and its component. The genotypes were categorized into three clusters and different sub-groups. Six principal components were identified explaining more than 80% of the total variation. The Shannon-Weaver diversity index, which ranged from 3.82 for pod weight to 7.15 for number of basal branches per plant, revealed high diversity among and within the genotypes. The observed high variation among the faba bean genotypes would be exploited in new faba bean varieties development program.

Introduction

Ethiopia is one of the Vavilovian centers of diversity for several grain legumes (Vavilov, 1926). It is a secondary center of diversity for faba bean (*Vicia faba* L.) (Zong et al., 2009). This makes Ethiopia an ideal place to search for agro-morphological diversity in faba bean. Globally, faba bean is reported as genetically diverse with more than 38,000 accessions with approximately 37 collections (Duc et al., 2010). Natural inter-crossing among the different faba bean races and genetic drift have contributed to increased faba bean diversity and wide variability (Maxted and Kell, 2009).

Previously morphological traits were used as markers in germplasm management (Panthee et al., 2006; Stanton et al., 1994). However, they have limitations of low polymorphism, low heritability and are affected by environmental influences, which may affect the estimation of genetic relationships (Muthusamy et al., 2008). Nevertheless, morphological markers are still one of the choices for diversity studies for traits which are highly heritable and do not require complicated laboratory facilities and procedures for characterizing the germplasm. In order to increase repeatability phenotypic characterization would be effective when done through replicated trials across multiple environments.

Subsistence farmers use Landraces as a key component in their cropping systems and by breeders for developing modern varieties (Hammer and Teklu, 2008). The success of any breeding program depends upon the genetic variability present in the available germplasm of a particular crop. The genetic variation in current Ethiopia faba bean germplasm has not been determined. However, it is expected to be large because the farmers who retain exchange or introduce seeds of different faba bean germplasm have preserved the landraces. If available, genetic diversity among and within landraces makes them a valuable resource for use in new variety development in the breeding program (Soleri and Smith, 1995). Conversely, the genetic diversity could be small if farmers were selecting for a few traits since time immemorial in the Ethiopian highlands. Thus, it is not clear whether farmers who have been selecting for a few common and desired traits for home consumption or the market have not compromised the diversity.

Characterization and quantification of the genetic diversity in germplasm collections for given crop species is essential in rational use of genetic resources for crop improvement programs (Gwak, 2008). Studies have shown that faba bean collections from Wollo, Gonder, North Shewa and Arsi, exhibited variation for most phenotypic traits (Gemechu et al., 2006). However, a comprehensive study of genetic diversity has not been done. As a result, in Ethiopia, , faba bean improvement ,depend on genotypes from other sources, mainly from the International Centre for Agricultural Research in the Dry Areas (ICARDA) (Gemechu et al., 2006). The potential of the local landraces as sources of breeding material has not been exploited, yet they are likely to provide varieties that combine genes of adaptation to the highlands and the alleles for the consumer desired traits such as good test of '*shero*' (faba bean flour for making souse)

making quality (Beyene et al., 2016). Therefore, the aim of this study was to investigate the levels of genetic diversity among the faba bean landrace collections in Ethiopia.

Materials and Methods

Plant materials

The germplasm used in the study were fitty in total of which forty landraces collected from the farmers' fields in major faba bean growing areas in the Ethiopian highlands. Seven improved varieties were obtained from the Holetta Agricultural Research Centre (HARC) comprising three recently released (Dosha, Gebelcho, Moti) and four old varieties (CS-20-DK, Kassa, Bulga-70 and NC58). In addition three faba bean germplasm lines (ILB-47-26, ILB-938 and BPL-710) were obtained from ICARDA (Table 1).

Sampling of germplasm

The landraces were collected from the farmers' fields during the main cropping season in 2013. Ten samples that represented different regions and different altitudes were collected per location. Dejene (2003) described the altitude classes as follows

- the Wurch, the cold highlands, which are >3000 meters above sea level (masl);
- the Dega, which is the cool humid highlands and ranges from 2500 to 3000 masl;
- Wiena Dega, which is the temperate cool and sub-humid highlands and ranges from 1500 to 2500 masl.

The genotypes from the altitudes < 2000 masl were classified as Altitude class I, 2001-2500 m. a. s. l altitude class II, 2501-3000 as altitude class III and from altitude >3000 classified as altitude class IV.

Experimental sites

The field experiments were conducted at Holetta Agricultural Research Centre (HARC) (09º03'N, 38º30'E, and 2390 masl) classified as M2-5(Tepid to cool moist mid highlands) agro ecology and Kulumsa Agricultural Research Centre (KARC) (08º00'N, 39º09'E, and 2730 masl) classified as ME2 (high rainfall environment) agro ecology. The soil type of HARC experimental fields is Eutric Nitosols, and the annual average precipitation is 933.3 mm with average minimum and maximum temperature of 5.3ºC and 22ºC respectively. The soils KARC soils are Xerosols with and the annual average precipitation is 962.7 with average minimum and maximum temperature of 10ºC and 24ºC respectively.

Table 1 Description of 40 faba bean landraces and 10 genotypes from Ethiopia and ICARDA

Entry no	Genotype code	Geographical location	Population	Population code	Genotype status	Altitude (m)
1	FBColl-001	Arsi/Bekoji	Arsi1	Pop1	Landraces	2784
2	FBColl-002	Arsi/Arsi Robe	Arsi2	Pop1	Landraces	2410
3	FBColl-003	Arsi/Limu Jara	Arsi3	Pop1	Landraces	2853
4	FBColl-004	Arsi/Dawa Bursa	Arsi4	Pop1	Landraces	2908
5	FBColl-005	Arsi/Chole	Arsi5	Pop1	Landraces	3050
6	FBColl-006	West Gojam/Yilmanadensa	Gojam1	Pop2	Landraces	2240
7	FBColl-007	West Gojam/ Awi	Gojam2	Pop2	Landraces	2610
8	FBColl-008	East Gojam/ Hulet-Eju Enebse	Gojam3	Pop2	Landraces	2412
9	FBColl-009	Gonder: North Zone/Wogera	Gonder1	Pop3	Landraces	2961
10	FBColl-010	Gonder: North Zone/Debark	Gonder2	Pop3	Landraces	2739
11	FBColl-011	Gonder: North Zone/Wogera	Gonder3	Pop3	Landraces	2951
12	FBColl-012	Harar/Borda	Harar1	Pop4	Landraces	2240
13	FBColl-013	Harar/Kulubi	Harar2	Pop4	Landraces	2380
14	FBColl-014	Harar/Arberkoti	Harar3	Pop4	Landraces	2266
15	FBColl-015	Harar/Gerawa	Harar4	Pop4	Landraces	2288
16	FBColl-016	North Shewa/Moretna Jiru	North Shewa1	Pop5	Landraces	2663
17	FBColl-017	North Shewa/Basonaworana	North Shewa2	Pop5	Landraces	3012
18	FBColl-018	North Shewa/Mehalmeda	North Shewa3	Pop5	Landraces	2667
19	FBColl-019	North Shewa/ Ankober	North Shewa4	Pop5	Landraces	3200
20	FBColl-020	North Shewa/ Molale	North Shewa5	Pop5	Landraces	3100
21	FBColl-021	North Shewa/ Tarmaber	North Shewa6	Pop5	Landraces	3058
22	FBColl-022	North Shewa/Hageremariam	North Shewa7	Pop5	Landraces	2670
23	FBV-023	HARC	CS-20-DK	Pop6	Inbred lines	2300-3000
24	FBV-024	HARC	NC-58	Pop6	Inbred lines	1900-2000
25	FBV-025	HARC	Moti	Pop6	Inbred lines	1800-3000
26	FBV-026	ICARDA	ILB-938	Pop7	Inbred lines	Unknown
27	FBV-027	HARC	Kasa	Pop6	Inbred lines	1900-2300
28	FBV-028	ICARDA	ILB-4726	Pop7	Inbred lines	Unknown
29	FBV-029	HARC	Gebelcho	Pop6	Inbred lines	1800-3000
30	FBV-030	HARC	Bulga-70	Pop6	Inbred lines	2300-3000
31	FBV-031	ICARDA	BPL-710	Pop7	Inbred lines	Unknown
32	FBV-032	HARC	Dosha	Pop6	Inbred lines	1800-3000
33	FBColl-033	East Shewa/Chefe Donsa	Central highland1	Pop8	Landraces	2263
34	FBColl-034	South West Shewa/Tulu Bolo	Central highland2	Pop8	Landraces	2192
35	FBColl-035	South West Shewa/ Tolle	Central highland3	Pop8	Landraces	2127
36	FBColl-036	West Shewa/ Chalya	Central highland4	Pop8	Landraces	2839
37	FBColl-037	West Shewa/ Dendi	Central highland5	Pop8	Landraces	2270
38	FBColl-038	South West Shewa/ Elu	Central highland6	Pop8	Landraces	2100
39	FBColl-039	Tigray: Central /MayTimeket	Tigray1	Pop9	Landraces	1855
40	FBColl-040	Tigray: Eastern /Saese Tsaedamba	Tigray2	Pop9	Landraces	2547
41	FBColl-041	Tigray: Eastern /Atsiwemberta	Tigray3	Pop9	Landraces	2840
42	FBColl-042	Tigray: East-southern /Degua Tembien	Tigray4	Pop9	Landraces	2770
43	FBColl-043	Wollega: Horo Guduru	Wollega1	Pop10	Landraces	2451
44	FBColl-044	Wollega: Horo-Guduru	Wollega2	Pop10	Landraces	2296
45	FBColl-045	Wollega: East /Jima Arjo	Wollega3	Pop10	Landraces	2424
46	FBColl-046	Wollega: Horo Guduru /Horo	Wollega4	Pop10	Landraces	2435
47	FBColl-047	Wollega: East /Jima Arjo	Wollega5	Pop10	Landraces	2460
48	FBColl-048	Wollo: South / Ambasel	Wollo1	Pop11	Landraces	2972
49	FBColl-049	Wollo: North /Wadla	Wollo2	Pop11	Landraces	2930
50	FBColl-050	Wollo: South /Wereilu	Wollo3	Pop11	Landraces	2640

Where: FBColl- faba bean collection; FBV- faba bean variety; HARC-Holetta agricultural research center; ICARDA-International Center for Agricultural Research in the Dry Areas

Experimental design and management

The experimental plots at the two sites had uniform slope and thus a randomised complete block design with three replication was used at each site. Fifty seeds of each genotype were planted on two rows of 2.5 m long and 0.4 m inter-row spacing. A common border row was planted with the less competent faba bean variety *Mesay*. The trials were managed in accordance with the standard practice for each site. This included regular hand weeding to keep field clean of weeds, application of the recommended fertilizer DAP at the rate of 100 kg/ ha with 18 kg/ ha Urea at planting. Planting was done at the onset of the main season rainfall.

Data collection

Fifteen individual plants were randomly selected for each genotype per each replication from the central part of the row and were marked before flowering. All morphological data were collected from the 15 marked plants. The following data were recorded: days to 50% emergence, days to 50% flowering when 50% of the plant per plot flowered, days to 90% physiological maturity, plant height measured in centimeters from the ground to the top of the main stem at maturity on five random sampled plant per plot, number of basal and secondary branches/plant at grain filling stage, diameter of the main stem at grain filling stage, leaf length, leaf width, height to the first pod at grain filling period, pod length, pod weight, number of pod/node, number of pods/plant, number of seeds/pod at grain filling stage, number of viable nodules/plant at 50% flowering stage, seed width, seed length and seed breadth after harvest. The disease reactions of the genotypes were measured. The chocolate spot (*Botrytis fabae*) disease incidence was recorded at different growth stages (flowering, podding and grain filling growth stage) of the crop. The disease severity based on a modified percentage leaf area infected was estimated at 1%, 3%, 6%, 12%, 25%, and 50% (Bernard et al. 2006). At harvest the total biomass, 100 seed weight and grain yield were measured. When records were taken on an individual plant-basis, the mean of 15 plants was used for data analysis. Above ground biomass production rate calculated as total biomass weight divided by number of days to 90% physiological maturity; economic growth rate was calculated as total grain weight divided by grain fill duration and then multiplied by 100; harvest index (HI) was calculated as proportion of total biomass to that of grain yield and the number of days of vegetative growth derived from days from planting to days to 50% podding stage.

Statistical analysis

The data were first subjected to the individual site analysis (ANOVA), then tested for homogeneity of variance for the sites using Bartlett's homogeneity test (Snedecor and Cochran, 1989) in SAS/STAT 9.3 (SAS Institute, 2012).The homogeneity of the error variance allowed the combined analysis of variance to be performed. The Unweighted Pair-Group Method using Arithmetic average (UPGMA) was performed with the program PAST software V 3.0 (Hammer et al., 2001). The PROC CLUSTER macro was used for defining optimum cluster number based on the values of cubic clustering criterion (CCC), Pseudo-F statistics (PSF) and Hotelling's pseudo T^2 value resulted from similarity analysis of the 50 genotypes using the means of 29 traits. The Gower

general similarity coefficient (Gower, 1971) was used to construct the dendrogram. Genstat 12th Edition (Payne et al., 2009) was used for principal components analysis (PCA). The PCs with Eigen values greater than 1.0 were selected as proposed by Jeffers (1967). The diversity index (H') of Shannon and Weaver (1949) was calculated and used as a measure of phenotypic diversity of each trait using the PAST software. The index was estimated for each character over all genotypes. Pearson's phenotypic correlation coefficients were estimated using the PROC CANCORR subprogram of SAS. The step-wise regression was conducted to determine the most important phenotypic traits for grouping the genotypes using SAS/STAT 9.3 (SAS Institute, 2012).

Results and Discussion

Phenotypic Diversity

There were significant ($p < 0.01$) differences among the genotypes for all phenotypic traits except for the number of basal branches per plant, number of days to physiological maturity seed length and seed width (Table 2). Significant ($p< 0.01$) genotype x location interaction effects were recorded for yield and most of its components. The descriptive statistics for each genotype is presented in Table 3. Highly significant ($p<0.001$) differences were recorded among the regions for most of the morphological traits (Table 3.). The genotypes from all regions showed highly significant variation ($p<0.001$) for hundred seed weight and genotypes from Arsi, Gojam, Harar, North Shewa, Tigray and Central highland showed significant variation for yield (Table 4). There was also significant variation ($p<0.001$) for faba bean chocolate spot disease severity in on genotypes from Gojam, Harar and North Shewa.
In general in this study significant variation was exhibited among the genotypes for 22 phenotypic traits including yield and yield components suggesting that there is genetic variability. Moreover, there were strong correlations among different traits which allows for simultaneous selection and use of the related traits interchangeably in parent material selection. These 22 descriptors were considered and would be used for further characterization of faba bean germplasm. The results are consistent with previous investigations in faba bean. Keneni et al. (2009) reported significant differences among Ethiopian faba bean accessions for most of the morphological traits. Al-Barri and Shataya (2013) observed similar phenotypic variation on faba bean landraces from Palestine.

There was significant variation ($p<0.001$) among altitude classes for all traits except basal branches per plant, number of days to physiological maturity and seed length (Table 2). High genetic variation was observed within altitude classes II, III and IV (Table 4). Highly significant variation was observed between altitude class II, III and IV for number of days to 50% flowering, pod length, pod weight, total above ground biomass, hundred seed weight, chocolate spot disease severity and grain yield.

High morphological variation for regions and different altitude classes from where the germplasm was collected based on quantitative characters detected, suggests that the morphological variation in Ethiopia faba bean germplasm is strongly affected by

environmental factors. The results are in agreement with previous studies on other crops. Depending on the elevation at which a species occurs, variability of genetic diversity due to several factors was found along altitudinal gradients (Ohsawa and Ide, 2008). Significant effects of altitude were reported on the genetic differentiation of oat (*Avena sativa* L.) landraces (Boczkowska and Tarczyk, 2013). This study suggested that to exploit the potential of genetic variation breeders can focus within an altitude range of 2000 to 3000 m.

Table 2 Analysis of variance and variance components for phenotypic traits measured on 50 faba bean genotypes

Morphological trait	Mean	CV (%)	δ^2g	δ^2g x l	δ^2e	Region	Altitudes class
Number of basal branches per plant	0.9 NS	40	0.002NS	0.00NS	0.1	NS	NS
Number of days to physiological maturity	135.5 NS	7.1	2.338NS	7.90 NS	93.8	NS	NS
Number of days to 50% flowering	56.6***	4.9	13.46**	3.62NS	8	**	**
Grain filling period (days)	79***	12.1	5.88**	2.6	90.8	**	**
Stem diameter (cm)	8.4**	18	0.017**	0.29NS	2.3	*	*
Number of days of vegetative growth	48.1 ***	5.9	13.46**	3.62**	8	**	**
Leaf length (mm)	7.2*	18.6	0.023*	0.06**	1.8	**	**
Leaf width (mm)	3.4***	20	0.008**	0.01**	0.5	**	**
Plant height (cm)	136.4***	7.4	37.25**	19.14**	102.5	**	**
High to the first pod (cm)	51.4	22	6.091**	3.39**	128.9	**	**
Number of pod per node	1.5**	22	0.014**	0.04**	0.1	**	*
Number of pods per plant	16.9***	30	1.952**	6.68**	26.6	**	**
Number of nodules per plant	104.3***	40	26.198**	263.35NS	1783.1	*	**
Grain production efficacy (kg/ha)	627.5***	40	31393.**	2447.6**	63871	**	**
Pod length (cm)	6.8***	9.5	0.216**	0	0.4	**	**
Number of node with pods	9.6***	24	0.924**	0.38*	5.8	**	**
Number of seeds per pod	2.7***	9.3	0.001**	0.02**	0.1	**	*
Pod weight (g)	439.9	32	12277***	1887.2**	20716	**	*
Number of node per plant	28.7***	10.6	0.206**	3.24**	9.3	**	**
Seed length (mm)	4.8NS	22	0.016NS	0	2.2	NS	NS
Seed width (mm)	4.4 NS	29	0.016NS	0.2	2.1	NS	**
Seed breadth (mm)	4.6***	24	1.251**	0.2	1.7	**	**
Above ground biomass production rate	8.4***	26	2.114**	1.67**	5	**	**
Economic growth rate	438.9***	34	12849.9**	3082.1**	23371.3	**	**
Total above ground biomass (kg/ha)	7077.9***	26	44782.1**	18918**	87629.6	**	**
Grain yield (kg/ha)	2248.9***	33	37103.1**	16353*	56387.8	**	**
Hundred seed weight (g)	64.3***	10	125.89**	54.80**	45.6	**	**
Harvest index	31.12***	26	14968**	248282**	134032.8	**	**
Disease severity (%)	23.5***	28	22.657**	19.40**	54.2	**	**

*δ^2g, δ^2g xl, δ^2e genotype, genotype x location and error variance, respectively; CV (%): coefficient of variation; NS: data is not significant at 5% probability level; *, **, *** data Significant at 0.05, 0.01 and 0.001 respectively.*

Table 3 Descriptive statistics for selected traits of 50 faba bean landraces based on different regions

Region	NDTF	PH	HFP	NPPN	NPPP	GPF	NNWP	NSPP	PWt	NNPP	BM	GY	HSW	CHDS
Arsi	53.23ed	140.5ab	49.0bcd	1.55abc	15.75b	845.4a	9.66bc	2.83a	550.5a	30.2a	1251ab	2794.8a	79.1b	25.4ef
Gojam	59.17c	140.1ab	55.4ab	1.49bc	18.59b	554.0ed	9.66bc	2.7abc	430.3bc	29.0a	1139bc	2188cde	65.2c	26.2def
Gonder	53.17ed	140.5ab	50.6bcd	1.62ab	18.16b	683.3cd	11.26a	2.62c	439.6bc	29.0a	1092bcd	2238bcd	61.1ef	28.1cde
Harar	52.29e	134.4bc	46.7cd	1.45bcd	15.53b	604.0ed	9.35c	2.79ab	396.2cd	29.0a	950.0d	1999de	67.9c	30.1cd
N Shewa	54.26d	134.7abc	49.5bcd	1.62ab	17.31b	764.7abc	9.95abc	2.7abc	494.0ab	28.6a	1217abc	2511abc	57.3fg	31.1c
HARC	54.02ed	137.5abc	52.7abc	1.52abc	15.39b	839.3ab	10.32d	2.7abc	543.2a	29.3a	1230abc	2878.7a	78.4b	27.9cde
ICARDA	70.00a	123.1d	59.1a	1.26d	9.45c	234.7g	7.19c	2.4abc	283.9ef	28.5a	1179abc	1217.7f	83.9a	1.1G
Chighl	59.47c	138.2abc	55.7ab	1.59ab	17.20b	690.5bcd	9.39ab	2.67bc	511.0ab	29.1a	1345.3a	2673ab	62.9de	27.9cde
Tigray	52.58ed	133.4c	47.2cd	1.55abc	17.65b	504.3ef	11.20ab	2.58c	3234 ef	26.5b	947.5d	1732e	54.9g	37.1b
Wollega	64.40b	141.1a	54.7ab	1.74a	22.72a	363.4fg	10.9abc	2.60c	368cde	28.7a	1053cd	1819de	46.3h	23.1f
Wollo	53.22ed	132.5c	44.1d	1.33cd	16.93b	371.3fg	10.2abc	2.59c	246.7f	26.2b	694.3e	1254f	46.1h	42.4a
Summary statistics over all genotypes														
Min	51.33	112.2	41.9	1.13	7.1	123	4.35	2.19	129.1	22.8	542	658	38.07	1
Max	71.33	150.2	65.1	1.99	25.3	1115	12.57	3.04	820	31.83	2017	4388	112.71	45.8
Mean	56.58	136.4	51.4	1.54	16.93	627.56	9.96	2.67	439.95	28.68	1132.5	2248.96	64.29	27.7
SE	0.84	1.7	0.9	0.03	0.55	37.7	0.22	0.02	21.63	0.27	40.88	116.94	2.31	1.3
Var	34.86	69.5	37.2	0.03	15.02	71059.3	2.35	0.03	23382.5	3.65	83554.1	683760	266.21	85.8
SD	5.9	8.3	6.1	0.18	3.88	266.57	1.53	0.16	152.91	1.91	289.06	826.9	16.32	9.3

Means with the same letter in each columns are not significantly different ($p<0.05$)

Where: *NDTF=Number of days to 50% flowering; PH= Plant height; HFP=High to the first pod (cm); NPPN= Number of pod per node; NPPP= Number of pods per plan; GPF= Grain production efficacy; NNWP= Number of Node with pod; NSPP= Number of seeds per pod; PW= Pod weight (gm); NNPP= Number of node per plant; BM=Total above ground biomass; GY= Grain yield (kg/ha); HSW=Hundred seed weight (gm); CHDS= Chocolate spot Disease Severity (%); N=number of genotypes; Min=Minimum; Max=Maximum; SE=Standard error; Var=Variance; SD=Standard deviation; NShewa= North Shewa; Chighl= Central highland.*

Table 4: The variance components of faba bean genotypes for selected traits over eleven collection regions/ source and four altitude classes

Region/Altitude class	NDTF	PH	HFP	NPPP	NNWP	NSPP	Pwt	NNPP	SB	BM	GY	HSW	CHDS
Region													
Arsi	6.79	31.21	5.1	6.94*	0.73	0.02**	9119.6	1.11	0.52	43961	41847**	144.91*	5.39
Gojam	72.5**	8.28	70.7	1.68	0	0.01	11436*	0	3.24	5000	312877*	135.5**	26.37*
Gonder	0	50.49	2.36	3.41	0	0	699.8	0	0.31	0	48475.5	76.97**	7.61
Harar	0	74.0**	0	0.93	0.03	4.00E-04	9291.2*	0	0.61	61167**	233713*	90.45**	16.18*
North Shewa	1.72	0	18.3	3.48	0	0	14174**	0.63	0.15	64228**	397339**	117.92**	34.99**
HARC	12.0**	19.68	0	2.04	1.39	0.02	9368.2	0.55	2.43**	39027**	175959	255.33**	11.43
ICARDA	0	17.23	0	1.39	0	0.02	2809.7	0.22	6.17**	20189	12965	631.12**	0
Central highland	29.6**	175.8**	10.5	14.4**	6.0**	0	50962**	2.70**	0.53*	204796**	1651943**	26.34*	17.26
Tigray	0	0	0	0	0	0	4530.7	0	1.30*	0	50595.6*	151.33**	10.07
Wollega	0.6	29.48	12.5	6.91	0.96	0.007	0	0.85	0.05	1916.7	111823	31.03**	1.49
Wollo	0	22.33	0	2.48	0	0.012	0	3.52*	0.35	17500	0	43.78*	0
Altitude class													
I	0	0	0	0	0	0	0	0	0	0	0	0	0
II	25.6**	34.8**	9.78*	5.43**	0.41	0.009*	16079**	1.013	1.59**	80647**	544739**	203.95**	10.75*
III	28.4**	33.7**	14.7	6.78**	1.86**	0	12116**	0	1.50**	29362**	375553**	229.48**	28.25**
IV	7.44**	17.54	9.53	1.45	0.59	0.003	13910**	0.31*	1.97**	50531**	422297**	213.36**	37.99**

Where: NDTF=Number of days to 50% flowering; PH= Plant height; HFP=High to the first pod (cm); NPPN= Number of pod per node; NPPP= Number of pods per plan; NNWP= Number of Node with pod; NSPP= Number of seeds per pod; Pwt= Pod weight (gm); NNPP= Number of node per plant; SB= Seed breath (mm); BM=Total above ground biomass; GY= Grain yield (kg/ha); HSW=Hundred seed weight (gm); HI= Harvest index; CHDS= Chocolate spot Disease Severity (%)

Associations between traits

There was significant ($p<0.01$) positive phenotypic correlation between grain yield and pod weight, total biomass, plant height, number of node per plant, number of pod per plant, number of node with pod, number of seed per pod (Table 5). There were significant negative correlation between yield and disease and; yield and height of the first pod. There was also highly significant ($p<0.001$) positive correlation of hundred seed weight with pod length and seed breadth. Similarly highly significant ($p<0.001$) negative correlation was recorded for disease severity with number of pod per plant, seed breadth, pod weight, and grain yield.

The positive correlation of grain yield and hundred seed weight with other traits can be used to determine the yield potential. This was also observed in previous studies (Mohsin et al., 2009). The recorded associations among the phenotypic traits have implications for breeding of faba bean. That is, the positive or negative strongly correlated traits are possibly under the influence of the same genes or pleiotropic effects (Miko, 2008). Therefore, if two strongly correlated traits are desired, they can both be selected simultaneously basing on one of the traits (Kwon and Torrie, 1964).

Contribution of morphological characters towards divergence of the genotypes and Shannon-Weaver diversity index (H) values for the 29 traits studied are presented in Table 6. The first 6 principal component (PC) having Eigen values above 1 with a value of 80.92% cumulative variance among the genotypes for the 29 quantitative traits of which 52.03% was contributed by the first two PCs. The high degree of variation in the first six PCs indicates a high degree of variation in these characters. Of the 6 major components, only 5 were able to demonstrate existence of morphological variability in the faba bean genotypes. The first principal component (PC1) had 9.6 of the original variables and contributed 33.1% to the total observed variability; PC2 had 5.49 and contributed 18.92% of the total variation (Figure 1). In PC1, there were 13 morphological traits that contributed to the variability, 8 morphological traits in PC2, 4 traits in PC3 and only 1 each in PC4 (days to physiological maturity) and PC6 (number of basal branch). The first PC contrasted and averaged 18 out of the 29 traits. The traits; grain yield, harvest index, total above ground biomass, economic growth rate, above ground biomass production rate, pod weight, number of seeds per pod, grain production efficacy, number of pod per node, plant height, leaf width, leaf length and stem diameter gave high weight and were the most important contributing traits for the first PC. Hence the first PC was mostly for yield components. The second PC, accounted for 18.92% variability and related with the most predominant characters of number of pods per plant, disease severity number of node per plant, hundred seed weight, number of nodes with pods, pod length, seed breadth and pod length. The most predominant traits for the third PC were number of days to 50% flowering, grain filling period, number of days of vegetative growth and height to the first pod. The Shannon-Weaver diversity index (H') value for the traits ranged from 3.85 - 7.15, with a mean of 4.18.

Table 5 Phenotypic correlation coefficients (above diagonal) and the level of significance (below diagonal) among faba bean traits (n=50)

	NBB	SD	LL	LW	PH	GFP	HFP	NPPN	NND	GPF	PL	NPPP	NNWP	NSPP	PWt	NNPP	SL	SW	SB	AGBP	EGR	BM	GY	HSW	HI	DS
NBB	1	-0.01	-0.16	-0.17	-0.48	-0.42	-0.39	-0.39	-0.08	-0.39	-0.09	-0.42	-0.39	-0.34	-0.36	-0.43	-0.31	-0.15	-0.17	0.09	0.22	0.00	-0.37	-0.11	0.21	0.46
SD	0.8436	1	0.21	0.22	0.26	0.02	0.16	0.16	-0.02	0.27	0.21	0.19	0.21	0.22	0.28	0.13	0.04	0.11	0.20	0.31	0.14	0.32	0.29	0.21	-0.23	-0.06
LL	0.0032	0.00	1	0.93	0.34	0.21	0.34	0.34	0.05	0.41	0.27	0.25	0.26	0.19	0.40	0.56	0.21	0.10	0.22	0.32	0.08	0.35	0.42	0.28	-0.41	-0.32
LW	0.0032	0.00	<.0001	1	0.33	0.19	0.32	0.32	0.06	0.40	0.27	0.24	0.25	0.19	0.40	0.53	0.17	0.09	0.23	0.33	0.09	0.35	0.42	0.26	-0.37	-0.31
PH	<.0001	<.0001	<.0001	<.0001	1	0.42	0.60	0.60	0.06	0.64	0.21	0.65	0.60	0.50	0.65	0.69	0.38	0.25	0.22	0.33	-0.23	0.41	0.64	0.14	-0.34	-0.40
GFP	<.0001	0.73	0.00	0.00	<.0001	1	0.31	0.31	0.02	0.58	0.23	0.35	0.33	0.33	0.44	0.46	0.22	0.15	0.22	0.06	-0.38	0.15	0.41	0.24	-0.22	-0.29
HFP	0.1724	0.00	0.68	0.60	0.02	0.01	1	-0.17	-0.06	-0.20	-0.10	-0.22	-0.29	-0.30	-0.19	-0.17	-0.14	-0.14	-0.05	0.00	0.14	-0.02	-0.19	0.02	0.04	0.00
NPPN	<.0001	0.01	<.0001	<.0001	<.0001	<.0001	0.0026	1	0.03	0.54	0.14	0.62	0.55	0.40	0.56	0.64	0.50	0.15	0.06	0.28	-0.24	0.36	0.57	0.03	-0.26	-0.35
NND	0.1821	0.68	0.41	0.27	0.32	0.73	0.31	0.61	1	0.07	-0.02	0.06	0.11	0.02	0.08	0.08	0.05	0.01	-0.01	0.08	-0.04	0.09	0.08	-0.02	-0.02	0.06
GPF	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.00	<.0001	0.20	1	0.41	0.57	0.56	0.49	0.94	0.63	0.19	0.22	0.33	0.59	-0.10	0.66	0.98	0.36	-0.31	-0.40
PL	0.1252	0.00	<.0001	<.0001	0.00	<.0001	0.09	0.01	0.75	<.0001	1	0.05	0.13	0.26	0.40	0.38	0.06	0.19	0.48	0.31	0.40	0.33	0.40	0.62	-0.19	-0.23
NPPP	<.0001	0.00	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.32	<.0001	0.43	1	0.78	0.52	0.59	0.57	0.41	0.22	0.12	0.26	-0.42	0.35	0.59	-0.14	-0.25	-0.36
NNWP	<.0001	0.0003	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0674	<.0001	0.02	<.0001	1	0.48	0.56	0.55	0.30	0.15	0.09	0.24	-0.32	0.32	0.57	-0.03	-0.33	-0.29
NSPP	<.0001	0.0001	0.0011	0.001	<.0001	<.0001	<.0001	<.0001	0.7199	<.0001	<.0001	<.0001	<.0001	1	0.49	0.45	0.26	0.23	0.19	0.20	-0.19	0.26	0.49	0.11	-0.41	-0.25
PWt	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0013	<.0001	0.1842	<.0001	<.0001	<.0001	<.0001	<.0001	1	0.63	0.19	0.21	0.32	0.66	-0.04	0.73	0.96	0.34	-0.30	-0.42
NNPP	<.0001	0.0201	<.0001	<.0001	<.0001	<.0001	0.0036	<.0001	0.1704	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	1	0.36	0.17	0.27	0.32	-0.10	0.40	0.63	0.29	-0.43	-0.50
SL	<.0001	0.443	0.0003	0.0024	<.0001	0.0002	0.0167	<.0001	0.3567	0.0013	0.32	<.0001	<.0001	<.0001	0.00	<.0001	1	0.12	-0.10	0.04	-0.23	0.09	0.19	-0.08	-0.12	-0.14
SW	0.0096	0.0657	0.0819	0.1126	<.0001	0.0074	0.0181	0.0099	0.8321	0.0002	0.00	0.00	0.01	<.0001	0.00	0.00	0.04	1	0.24	0.12	-0.03	0.15	0.21	0.10	-0.10	-0.03
SB	0.0033	0.0005	0.0002	<.0001	<.0001	<.0001	0.377	0.2657	0.8252	<.0001	<.0001	0.04	0.12	0.00	<.0001	<.0001	0.09	<.0001	1	0.26	0.48	0.29	0.31	0.67	-0.07	-0.33
AGBP	0.1227	<.0001	<.0001	<.0001	<.0001	0.3328	0.9778	<.0001	0.1606	<.0001	<.0001	<.0001	<.0001	0.00	<.0001	<.0001	0.46	0.04	<.0001	1	0.26	0.99	0.64	0.33	-0.07	-0.17
EGR	<.0001	0.014	0.1722	0.1338	<.0001	<.0001	0.0127	<.0001	0.5015	0.0772	<.0001	<.0001	<.0001	0.00	0.44	0.09	<.0001	0.55	<.0001	<.0001	1	0.19	-0.05	0.77	0.15	-0.04
BM	0.9738	<.0001	<.0001	<.0001	<.0001	0.0087	0.6834	<.0001	0.1342	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.11	0.01	<.0001	<.0001	0.00	1	0.71	0.34	-0.10	-0.24
GY	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0012	<.0001	0.1783	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.00	0.00	<.0001	<.0001	0.40	<.0001	1	0.33	-0.32	-0.40
HSW	0.0477	0.0002	<.0001	<.0001	0.0156	<.0001	0.7065	0.6533	0.7117	<.0001	<.0001	0.0123	0.6252	0.0531	<.0001	<.0001	0.1816	0.0881	<.0001	<.0001	<.0001	<.0001	<.0001	1	-0.09	-0.26
HI	0.0003	<.0001	<.0001	<.0001	<.0001	0.0002	0.4719	<.0001	0.7092	<.0001	0.0008	<.0001	<.0001	<.0001	<.0001	<.0001	0.0344	0.0872	0.2587	0.2232	0.01	0.09	<.0001	0.10	1	0.16
DS	<.0001	0.3219	<.0001	<.0001	<.0001	<.0001	0.9623	<.0001	0.3364	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0166	0.5483	<.0001	0.0039	0.51	<.0001	<.0001	<.0001	0.01	1

Where: *The colour indicates the strength of correlation, Blue=positively correlated Dark blue to light= high to week correlation; Red color=negatively correlated and the red to light red high to weak correlation: NNB= Number of basal branches per plant; NDTM= Number of days to physiological maturity; NDTF=Number of days to 50% flowering; GFP= Grain filling period; SD=Stem diameter (cm), NDTV= Number of days of vegetative growth; LL= Leaf length (mm); LW= Leaf width (mm); PH= Plant height; HFP=High to the first pod (cm); NPPN= Number of pod per node; NPPP= Number of pods per plan; NND= Number of nodules per plant GPF= Grain production efficacy; PL= Pod length; NNWP= Number of Node with pod; NSPP= Number of seeds per pod; PW= Pod weight (gm); NNPP= Number of node per plant; SL=Seed Length; SW=Seed width; SB= Seed breadth (mm);AGBP= Above ground biomass production rate; EGR=Economic growth rate; BM=Total above ground biomass; GY= Grain yield (kg/ha); HSW=Hundred seed weight (gm); HI= Harvest index; DS= Disease Severity (%)*

Table 6 Percentage and cumulative variances and Eigen-vectors of the first six principal components axes (CPC) and Shannon-Weaver diversity index (H') estimates for the 29 morphological character used to classify the faba bean germplasm evaluated

Parameter	PC1	PC2	PC3	PC4	PC5	PC6	H'
Number of basal branches per plant	0.04	-0.09	-0.27	0	0.6	-0.66	7.15
Number of days to physiological maturity	-0.26	0.34	-0.02	-0.73	-0.26	-0.11	3.92
Number of days to 50% flowering	0.33	0.49	-0.76	-0.05	-0.02	0.06	3.93
Grain filling period	-0.46	-0.24	0.69	-0.4	-0.14	-0.12	3.92
Stem diameter	-0.68	0.01	-0.18	0.36	0.04	0.04	4.08
Number of days of vegetative growth	0.33	0.49	-0.76	-0.05	-0.02	0.06	3.93
Leaf length	-0.64	0.35	-0.32	0.02	-0.32	-0.4	4.15
Leaf width	-0.65	0.3	-0.36	0.04	-0.27	-0.41	4.44
Plant height	-0.71	-0.37	-0.25	0.19	-0.11	0.07	3.92
High to the first pod	-0.03	0.39	-0.56	-0.25	-0.1	-0.14	3.93
Number of pod per node	-0.5	-0.43	-0.48	-0.13	0.07	0.13	5.34
Number of pods per plant	-0.23	-0.77	-0.52	0.11	0.01	-0.05	3.96
Number of nodules per plant	-0.26	0.3	-0.11	-0.54	0.12	0.22	3.9
Grain production efficacy	-0.92	-0.09	0.28	-0.19	0.06	0	3.82
Pod length	-0.56	0.51	0.38	0.33	0.08	-0.03	4.15
Number of Node with pod	-0.44	-0.67	-0.15	0.06	0.03	0.08	4.04
Number of seeds per pod	-0.57	-0.39	0.24	0.2	0	-0.09	4.8
Pod weight	-0.97	0	-0.01	-0.1	0.1	0.09	3.85
Number of node per plant	-0.24	-0.75	-0.53	0.09	0	-0.07	3.96
Seed Length	0.29	-0.51	-0.28	0.09	-0.48	0.08	4.23
Seed width	-0.35	-0.2	0.11	0.37	-0.38	-0.05	4.23
Seed breath	-0.32	0.72	0.23	0.44	0.03	-0.03	4.17
Above ground biomass production rate	-0.84	0.18	-0.31	0.01	0.18	0.2	4.06
Economic growth rate	-0.95	0	-0.12	-0.05	0.08	0.12	3.85
Total above ground biomass	-0.84	0.19	-0.32	-0.04	0.16	0.18	3.88
Grain yield	-0.97	-0.03	0.02	-0.14	0.08	0.08	3.85
Hundred seed weight	-0.47	0.75	0.31	0.22	-0.02	0.03	3.9
Harvest index	-0.63	-0.19	0.43	-0.24	-0.17	-0.16	3.93
Disease Severity	0.19	-0.77	-0.37	0.05	0.12	-0.04	3.93
Eigen value	9.6	5.49	4.18	1.94	1.19	1.07	
%Variance	33.1	18.92	14.41	6.7	4.1	3.68	
Cumulative%	33.1	52.02	66.43	73.13	77.23	80.92	
Mean diversity index (H)							4.18

Cluster Analysis

All the genotypes were grouped into three major clusters (I, II, and III). The first major cluster had two sub clusters (I-1 and I-2) and the second major cluster had 4 sub-clusters (Figure 1 and 2). The number of genotypes per cluster varied from 35 in cluster II to 4 in cluster III. All improved faba bean varieties released from HARC were grouped in cluster II. The third cluster comprised all exotic faba bean germplasm from ICARDA and one faba bean collection from central highlands of Ethiopia (FBColl-36). The 10 traits which explained the relationships among the genotypes are presented in Table 7.

Principal component analysis (PCA) results illustrated the pattern of genetic diversity of the faba bean germplasm based on morphology. The principal component analysis confirmed the existence of diversity in faba bean germplasm since the entire variation cannot be explained in terms of few PCs. Moreover, PCs indicate the involvement of number of traits in contributing towards the overall observed diversity. In this study the PCA separated variability among the genotypes according to grain yield and ten associated traits in Table 7. This suggests that the ten traits are the most important for use in future faba bean characterization and conservation studies.

The distribution of the genotypes by region of origin and altitude class is presented in Table 8. The genotypes with similar traits were grouped together irrespective of the collection region and all collections from Wollega were grouped in cluster II but separated in sub-cluster II-1. Similarly, faba bean collections from Wollo were grouped in cluster I. The majority (75%) of the landrace collections from Tigray were grouped in the first cluster. Cluster II included 100% of the genotypes collected from Arsi, Gojam, Gonder, North Shewa, Wollega, 75% from Harar, 42.86% from Central highland, and 25% from Tigray. The Shannon-Weaver diversity index (H') for the characters showed high levels of diversity among the faba bean genotypes. The overall mean of H' value 4.18 confirmed the existence of high level of phenotypic diversity among faba bean genotypes (Hennink and Zeven, 1991). Clustering of the genotypes based on the morphological traits evaluated revealed some of the genotypes from the regions; Central highland, North shewa, Tigray and Harar, appeared in different clusters suggesting genotypes from these regions were relatively diverse than from the other regions. This could be partly attributed to gene flow through farmer-to-farmer seed exchange, which was previously reported (Alemu et al., 2010).

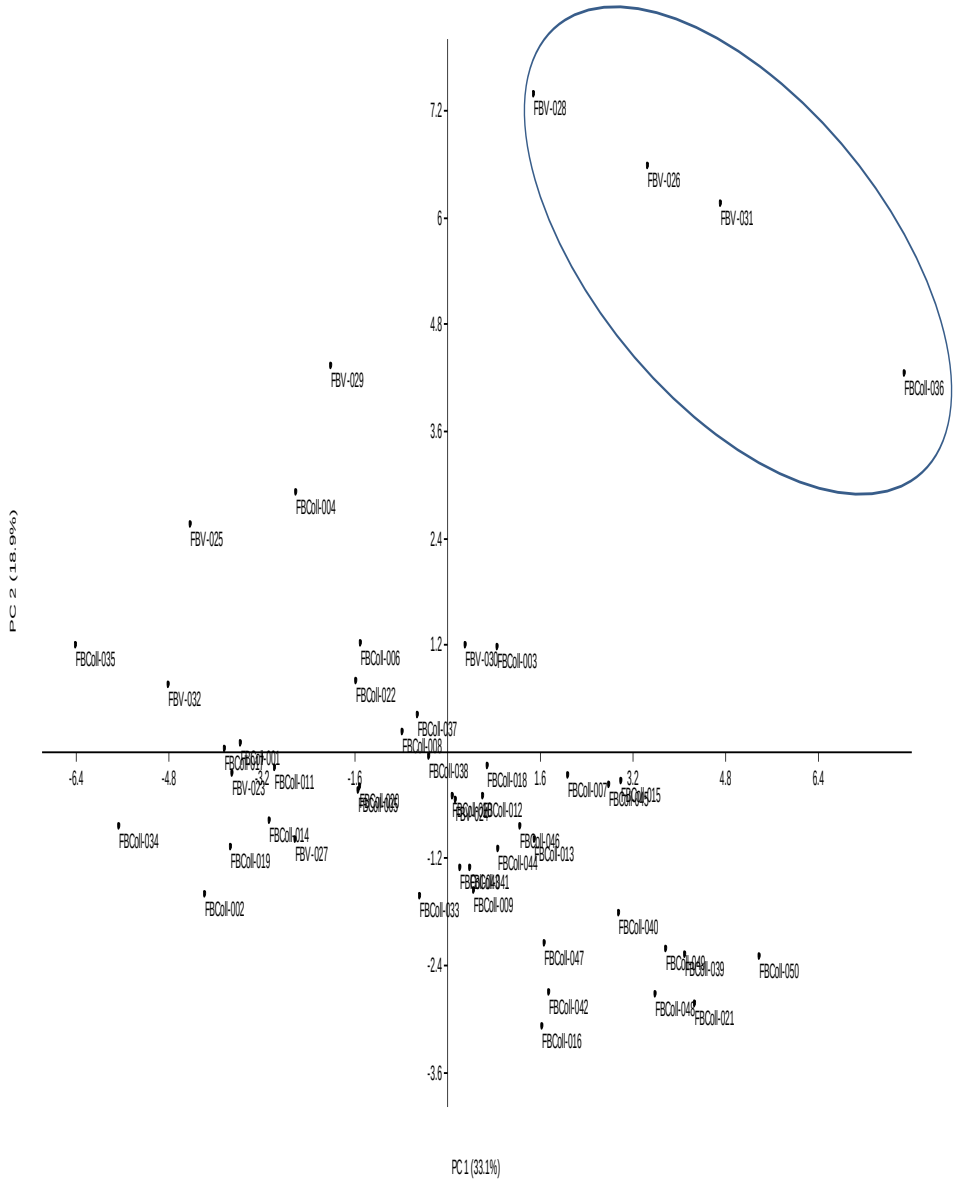

Figure 1 Scatter distribution of 50 faba bean genotypes based on 29 morphological characters on the first and second principal components.

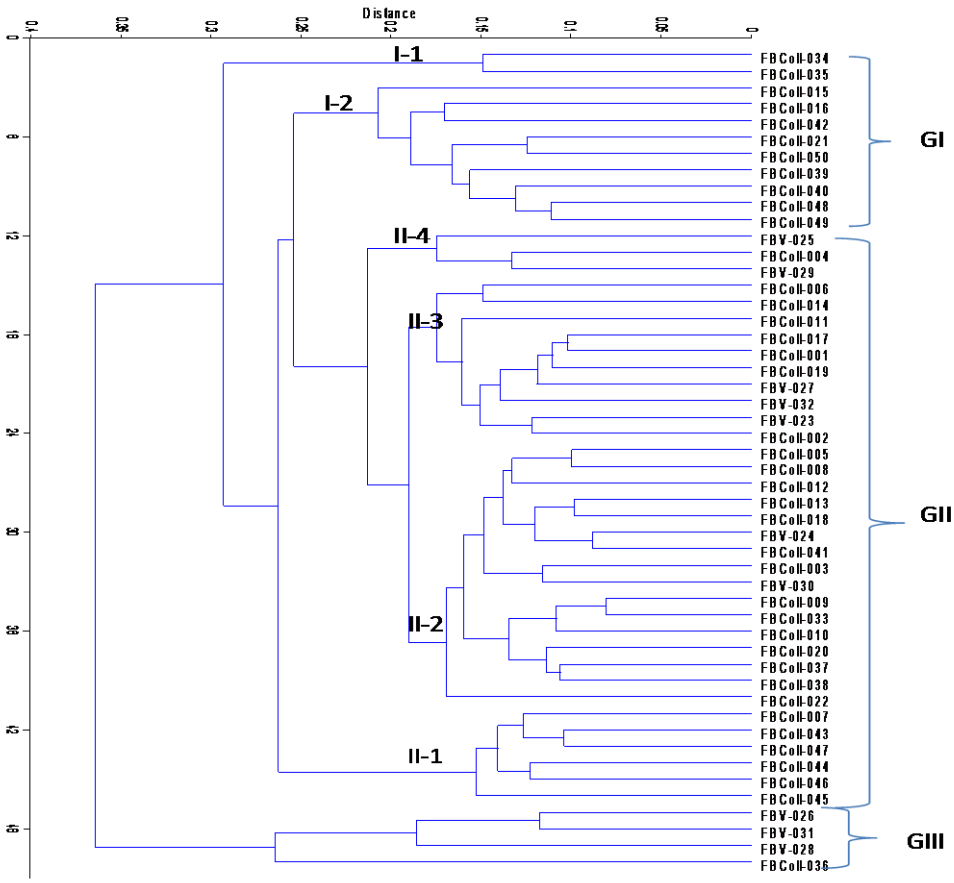

Figure 2 Dendrogram based on 29 phenotypic traits of 50 faba bean genotypes evaluated at HARC and KARC.

Table 7 Step-wise regression for important morphological traits for clustering 50 faba bean lines

Step	Trait	Partial R^2	F-value	Pr>F
1	Number of days to 50% flowering	0.744	11.06	<0.0001
2	Disease severity (%)	0.585	5.22	<0.0001
3	Grain yield (kg/ha)	0.565	4.67	0.0003
4	Number of pods per plant	0.523	3.83	0.0015
5	Number of basal branches per plant	0.409	2.35	0.0311
6	Harvest index	0.496	3.25	0.0051
7	Number of seeds per pod	0.397	2.10	0.0542
8	Pod length	0.399	2.05	0.0612
9	Total above ground biomass	0.464	2.60	0.0212
10	Seed width	0.392	1.87	0.0922

Table 8 Distribution of 50 faba bean genotypes into three clusters and six sub-cluster by eleven regions and four altitude classes

Region/Altitude class	No of genotypes	Number of genotype by cluster			Number of genotypes by sub-cluster for Cluster I and II					
Region		I	II	III	I-1	I-2	II-1	II-2	II-3	II-4
Arsi	5	-	5	-	-	-	-	2	2	1
Gojam	3	-	3	-	-	-	1	1	1	-
Gonder	3	-	3	-	-	-	-	2	1	-
Harar	5	1	4	-	-	1	-	2	1	-
North Shewa	6	2	4	-	-	2	-	3	2	-
HARC	7	-	7	-	-	-	-	2	3	2
ICARDA	3	-	-	3	2	-	-	-	-	-
Central highland	6	2	3	1	-	-	-	3	-	-
Tigray	4	3	1	-	-	3	-	1	-	-
Wollega	5	-	5	-	-	-	5	-	-	-
Wollo	3	3	-	-	-	3	-	-	-	-
Total	50	11	35	4	2	9	6	16	10	3
Altitude class										
I		1	-	-	-	1	-	-	-	-
II		3	17	-	2	1	5	7	4	1
III		6	10	1	-	6	1	6	2	1
IV		4	8	-	-	4	-	3	4	1
Total		14	35	1	2	12	6	16	10	3

Conclusion

There is a broad genetic diversity of faba bean germplasm in Ethiopia. The germplasm clustered into three major groups that can be exploited by the breeding programs that aim to improve varieties for the highland ecologies. Moreover, there were strong correlations among different traits that allows for simultaneous selection and use of the related traits interchangeably in parent material selection. This highly diversified germplasm provide opportunity for identification of parental sources in future breeding programs to develop new faba bean varieties. The ten traits, which should be used for faba bean variety characterization, were identified.

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