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Article

Evaluation of morphological and yield traits in the populations of *Vicia* spp.

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Abstract:

The study was focused on estimation of genotypic variation for the morphological and forage yield traits of some vetch genotypes to assess their breeding potential. A small-plot trial was carried out in 2018-2020 at the experimental field of the Research Institute of Forests and Rangelands, Alborz province, Iran. Fifty eight (58) vetch genotypes of *Vicia* spp. from the natural resources gene bank of Iran, were tested. There was significant (P<0.01) genotypic variation among populations, for all the traits measured. *V.monantha* (32845) produced high plant and large pods, while *V. villosa* (322) produced more biomass than other accessions. In the shorter growing seasons, the earliness of *V. sativa* var.angustifollia (4740,7243), *V. sativa* var.stenophylla (1862), *V. villosa* (315, 322) resulted in high seed yield. The principal component analysis showed that the variations observed were mainly caused by traits such as days to flowering and seed ripening and seed traits, that their contribution was important in discriminating the populations. Direct selection can also be made for the populations with high biomass yield based on the recorded performance of these populations during the field experiments. A cluster analysis of the tested vetch populations based on measured traits, at 11.49 genetic distance, created five main groups that showed the similarity of members of each group. Generally, vetch species and their populations had different growth features, phenology, forage and seed productivity. The generated information in this study gives a base for genetics variety of genus *Vicia* L. and could be useful for including in the future breeding programs.

Key words: Biomass yield, Morphological traits, Phenology, Seed yield, *Vicia* spp.

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Introduction

Vicia L. is a genus with around 232 species in the world and 45 species in Iran, from the legume family, Fabaceae, as an annual and perennial herb. These species have been known by the common name vetches. The genus is primarily found in the Mediterranean and Irano-Turanian regions, such as in Iran, Anatoly, Caucasus, Iraq, Afghanistan, Central Asia, Talesh, Syria, Armenia, Turkmenistan, Jordan, North Africa, Greece, Pakistan, and Palestine(1). Vetches are short-lived forage plants that are highly resistant to cold and dehydration conditions and can be grown in rainfed and irrigated climates. They fix nitrogen in the soil by fixation in root nodes, and help to soil erosion by planting in sloping areas(2,3). As a legume crop, it provides nitrogen to the soil and reduces the incidence of diseases in succeeding non-leguminous crops. Their widespread adaptation and excellent capacities to produce biomass make them very attractive to farmers(4). One attraction of vetch is its versatility, which permits diverse utilization as either ruminant feed or green manure. Because of rapid growth in the first year, different species of *Vicia* spp. can be used to improve overall livestock, feed quality, improve soils, agriculture for fodder, green manure, human nutrition, and the pharmaceutical industry(5).

Iran is a genetics resources of the genus *Vicia* and it is widely distributed in different habitats and conditions. Most of the plants in *Vicia* genus show more variety in morphological traits and sometimes it is difficult to distinguish species of this genus(6,7).

Genetic variation among *Vicia* genotypes is imperative for their efficient utilization in plant breeding schemes and effective conservation. Diversity studies available in germplasm, collections have been performed on many plant species for *Vicia* genus from different regions of the world. In comparison to other annual forage legumes, advances in breeding vetches (*Vicia* spp.) are rather modest. It has been one of the morphological characteristics of the plant reported in *V. sativa*(8-14), *V. faba*(15), *V. narbonensis*(8,10,11,15), *V. ervilia*(16), *V. villosa*(10,11), *V. atropurpurea*(11), *V. dasycarpa*(8), *V. hybrid*, *V. pannonica*, *V. lutea*, *V. peregrine*, *V. lathyroides* and *V.* grandiflora(11).

There are 335 accessions of 25 *Vicia* spp. in natural resources gene bank of Iran, that have been collected from different geographical regions of Iran. In this study, it was aimed to determine some morphological characteristics and forage yields of different vetch genotypes by collecting from natural flora of Iran region. The present study was focused on the estimation of genotypic variation for 12 morphological traits within the *V. michauxii*, *V. michauxii* var.stenophylla, *V. monantha, V. narbonensis, V. sativa* with three varieties: *V. sativa* var.angustifollia, *V. sativa* var.cordata, *V. sativa* var.sativa and *V. villosa*, to assess their breeding potential and suitability for developing novel common vetch lines with improved agronomic characteristics related to grain production and quality.

Material and methods

Germplasm

A total of 58 germplasm populations were evaluated in this study. This consisted of 1 *V. michauxii*, 1 *V. michauxii* var.stenophylla, 1 *V. monanta*, 1 *V. narbonensis*, 34 *V. sativa*, 9 *V. sativa* var.angustifollia, 1 *V. sativa* var.cordata, 4 *V. sativa* var.sativa and 6 *V. villosa*. The populations were acquired from the Natural Resources Genebank of Iran (Table 1).

Table 1: The list of studied 58 vetch (*Vicia* spp.) populations

Taxon	Code	Abbre. code	Origin, province	Longitude		Latitude		Altitude (m asl)
V. michauxii	2944	Vmi	East Azerbaijan, Kaleybar	47°	02´	38°	51´	1500
V. michauxii	37129	Vmis	Qom	50°	56´	34°	11´	2482
V. monantha	32845	Vmo	Kermanshah	47°	14´	34°	8´	1338
V. narbonensis	34878	Vn	Lorestan, Aleshtar	48°	10´	33°	45´	1495
V. sativa	5321	Vs	East Azerbaijan	46°	16´	37°	54´	1750
	6646	Vs	Lorestan, Kohdasht	33°	40´	47°	30´	1200
	6654	Vs	Lorestan, Kohdasht	33°	17´	47°	27´	1130
	6681	Vs	Lorestan, Kohdasht	33°	32´	47°	37´	1260
	11760	Vs	Gilan, Rezvanshahr	37°	31´	49°	13´	280
	11761	Vs	Gilan, Rasht	36°	51´	49°	37´	80
	11762	Vs	Gilan, Rezvanshahr	37°	37´	49°	07´	280
	11763	Vs	Gilan, Rasht	37°	59´	49°	33´	100
	11764	Vs	Gilan, Talesh	37°	32´	45°	55´	280
	11771	Vs	Gilan, Talesh	37°	42´	48°	55´	150
	11772	Vs	Gilan, Rezvanshahr	37°	32´	49°	07´	20
	11774	Vs	Gilan, Rasht	37°	11´	49°	39´	120
	24062	Vs	Gilan, Astaneh Ashrafiyyeh	37°	20´	49°	47´	25
	24069	Vs	Gilan, Chabuksar	36°	56´	50°	32´	170
	24074	Vs	Gilan, Astaneh Ashrafiyyeh	37°	19´	50°	07´	16
	24076	Vs	Gilan, Chabuksar	36°	57´	50°	35´	210
	24084	Vs	Gilan, Rahimabad	37°	02´	50°	18´	40
	24097	Vs	Gilan, Rahimabad	37°	01´	50°	17´	45
	32972	Vs	Kermanshah, Hersin	34°	13´	47°	25´	1367
	33456	Vs	Hamadan	47°	57´	34°	24´	1545
	38517	Vs	Gilan, Siyahkal	49°	57´	36°	59´	342
	38523	Vs	Gilan, Talesh	49°	3´	37°	36´	405
	38526	Vs	Gilan	48°	46´	37°	41´	827
	38527	Vs	Gilan, Astra	48°	58´	38°	24´	21
	38528	Vs	Gilan, Rudsar	50°	12´	36°	48´	608
	38531	Vs	Gilan, Rezvan shahr	49°	20´	37°	30´	315

	38532	Vs	Gilan, Talesh	49°	4´	37°	37´	450
	38533	Vs*	Gilan	38°	10´	48°	20´	600
	38536	Vs*	Gilan	36°	54´	49°	26´	577
	40310	Vs	Kermanshah, Salase babajani	34°	49´	46°	05´	1395
	40315	Vs	Kermanshah, Salase babajani	34°	49´	46°	05´	1395
	40326	Vs	Kermanshah, Javanrud	34°	48´	46°	33´	1525
	40334	Vs	Kermanshah, Salase babajani	34°	51´	46°	01´	1395
	43100	Vs	Khozestan, Masjed soliman	31°	56	49°	18´	870
V. *sativa* var.angustifollia	38524	Vsa	Gilan, Siahkal	50°	14´	36°	53´	670
	38525	Vsa	Gilan, Talesh	48°	51´	37°	41´	281
	38530	Vsa	Gilan, Talesh	48°	52´	37°	41´	215
	38534	Vsa	Gilan, Rasht	49°	35´	37°	0´	137
	38535	Vsa	Gilan, Rodbar	49°	40´	36°	46´	968
	38537	Vsa	Gilan, Gilan	49°	31´	36°	56´	187
	4740	Vsa	Ilam, Ivan	46°		33°	38´	1170
	7243	Vsa	Kohkiloye ve Boyerahmad,	52°		28°	86	1900
	38529	Vsa	Gilan, Rezvan shahr	49°	5´	37°	28´	307
V. *sativa* var.cordata	34295	Vsc	Gilan, Rezvan shahr	49°	4´	37°	36´	310
V. *sativa* var.sativa	1862	Vss	Kermanshah	47°	06´		31´	1350
	24631	Vss	Kermanshah	47°	06´	34°	31´	1400
	29802	Vss	Kohkiloye ve Boyerahmad	30°	59´	51°	07´	2380
	32900	Vss	Kermanshah	34°	16´	46°	09´	1444
V. villosa	315	Vv	Alborz, Karaj	35°	83´	51°	01´	1460
	322	Vv	Karaj	35°	83´	51°	01´	1470
	6268	Vv	Fars, Shiraz, Sepidan, Sheshpir	30°	25´	51°	98´	2350
	14561	Vv	Merkezi, Arak	34°	09´	49°	70´	1730
	28061	Vv	Ardabil	38°	25´	48°	29´	1350
	34212	Vv	Chahar-mahale Bakhtiyari,	31°	46´	50°	59´	2600

Field trial

Seed of all 58 populations were sown in seedling pots (December 2018). Then planting and maintenance operations were carried out in the field at the research field of Research Institute of Forests and Rangelands, Alborz province, Iran (2018–2020). A week before planting, the soil was prepared as a fine seedbed to enhance good seedling establishment. The field experimental layout was a One-way analysis of variance (ANOVA) designed. The row and plant spacings were 100 and 40 cm, respectively. The trial was managed according to previouse experiences (several hand weeding was practiced, the first hand weeding was made 40 d after crop emergence, and then repeated every forty days until the end of the growing season, to minimize yield reduction due to weed competitions for soil nutrients, water and solar radiation). Irrigation was applied during the trial. The populations were harvested for seed during the period July to November 2020, depending on their maturity.

Morphological traits

Ten plants (normal growth, uniform performance, disease- and insect pest-free) of each 58 *Vicia* populations were evaluated by 12 different quantitative traits including day to spourat (day to germination), days to first flowering, days to total flowering, days to maturity (days to seed ripening), plant height (at 50 % flowering, cm), internode length (second internode at 50 % flowering, cm), stems number, pod length (cm), pod width (cm), pod index (pod length/width), biomass yield (plant fresh weight) (g), and plant dry weight (g)[17].

Data analysis

Data were subjected to analysis of variance (ANOVA) using the SAS software system[18]. Significant differences among the mean values of 12 traits were compared the DMRT Duncan test. Pearson correlation was determined using SPSS v.21. To evaluate the information contained in the collected morphological data, principal component analysis (PCA) was carried out by Minitab software (version 15). PCA was used to identify the most important traits (plant height, internode length, stems number, pod length, pod width, pod index, biomass yield, dry weight, day to sprout, days to first flowering, days to total flowering, days to maturity) in the data set. Mean values populations were used to create a correlation matrix from which the standardized PCA scores were extracted and a Scatter plot on the first two PCA was performed. Cluster analysis was performed using Ward's methods and Euclidean distance and a dendrogram was calculated.

Results

The results of analysis variance revealed significant (P<0.01) variation for eight morphological and yield traits among taxa and populations of *Vicia* spp. except for pod width trait among populations (Table 2). Table 3 shows the comparison of mean morphological and yield traits in nine taxa of *Vicia* spp. The value of plant height, internode length and stems number differ between 24.50-150 cm, 3.29-15 cm and 2.81-9, respectively. The highest value of plant height (150 cm), stems number (9) and internode length (15 cm), were shown in *V. monantha* (Vmo) and *V. michauxii* var. stenophylla (Vmis), respectively. The variation of pod length between taxa was significant and it differs from1.06 cm in *V. sativa* var.cordata (Vsc) to 4 cm in *V. monantha* (Vmo). There was no significant difference in pod width between taxa and they located as two groups (a and b), so two taxa of *V. michauxii* (Vmi and Vmis) had the widest pod (1.14 and 1.1 cm). Despite the significant differences in biomass yield and dry weight traits, *V. villosa* (Vv) showed the most value of these traits (biomass yield=60.12 g and dry weight=15.63 g).

Fifty eight (58) populations of *Vicia* spp. were compared for vegetative and phenology traits (Table 4). There was a wide range of value in plant height from 19 cm in *V. sativa* var.angustifollia (38534) to 150 cm in *V. monantha* (32845), also the most value of plant height between populations of species were shown in *V. villosa* (322) (100.33 cm). *V.michuxii* var.stenophylla (37129) (100 cm), *V. sativa* (38527) (90 cm) and *V. sativa* var.cordata (34295) (85.13 cm). The length of the internode was a very differet from 1.83 cm in *V. sativa* (24062, 40334, 43100) to 15 cm in *V. michauxii* var.stenophylla (37129). Also, 9.83, 8.69, and 8.28 cm of internode length were shown in *V. sativa* (38527), *V. sativa* var.cordata (34295), and *V. sativa* var.angustifollia (38525), respectively. The highest and lowest number of stems were 2 and 15, which were shown in two different taxa of *V. sativa* species (Vsa38530 and Vs11774). This trait in populations of *V. villosa* was no significant different. Four populations of *V. sativa* (38527, 33456, 24074 and 32972), *V. sativa* var.angustifollia(38525) and *V. monantha* (32845), had the largest pod in terms of length (4-4.53 cm) and populations *V. michauxii* var.stenophylla (37129), and *V. sativa* (5321) had the largest pod in terms of width (1.1 and 1.06 cm). In compare of yield traits (biomass yield and dry weight), three populations of *V. sativa*: Vs11761, Vs24062, Vs40326, and two populations of *V.villosa*:Vv322, Vv6268, had the most values of these traits. The values of these traits in these populations were Vs11761 (83 and 26 g.), Vs24062 (83 and 26 g), Vs40326 (103.67 and 36.33 g), Vv322 (108.33 and 38.60) and Vv6268 (83.50 and 19.73 g).

The results of phenology traits showed that all of the populations based on day to sprout and days to first flowering traits were divided into two groups (a and b). *V. narbonesis* (34878), *V. monantha* (32845) and two taxa of *V. michauxii* (Vmi2944 and Vmis37129) had the same value in day to sprout and days to first flowering traits, but populations *V. sativa* var.angustifollia (Vsa4740, Vsa7243), *V. sativa* var.stenophylla (Vss1862, Vss24631) and two populations of *V. villosa* (Vv315, Vv6268) with 21 and 90 d for day to sprout and first flowering were separated from the rest of populations by earlier germination and flowering. In days to total flowering and maturity traits, populations were divided as four groups (a, b, c and d). Days to total flowering as four groups:125a,120b,115c,107d and seed maturity:167a,162b,158c,150d. Populations in group d (107 and 150 d of flowering and seed maturation) had the shortest time required for full flowering and seed maturation. That is, they reached full flowering and seed maturity earlier than other populations. Populations of *V. sativa* var.angustifollia (Vsa4740, Vsa7243), *V. sativa* var.stenophylla (Vss1862, Vss29802, Vss32900) and *V. villosa* (Vv315, Vv6268), having the shortest day for full flowering and seed maturation (Table 4).

Analysis of the genetic correlations among the mentioned traits in the tested vetch populations revealed the existence of several significant positive coefficients (Table 5), namely between plant height with internode length (r_{gxy}=0.43; P<0.01), stems number (r_{gxy}=0.38; P<0.01) and pod length with internode length (r_{gxy}=0.24; P<0.05), pod width (r_{gxy} =0.23; P<0.05), day to sprout (r_{gxy}=0.28; P<0.05), days to first flowering (r_{gxy}=0.28; P<0.05) and days to maturity (r_{gxy}=0.26; P<0.05), pod index with day to sprout (r_{gxy} =0.23; P<0.05) and days to first flowering (r_{gxy}=0.23; P<0.05). On the other hand, the relationship between pod width with pod index (r_{gxy} =−0.26; P<0.05), biomass yield (r_{gxy} =−0.35; P<0.01), and dry weigth (r_{gxy} =−0.28; P<0.05), internode length with dry weigth (r_{gxy}=−0.38; P<0.01) were negatively and significant.

Two-dimensional principal component analysis showing the relationship among quantitative traits of studied populations is presented in Figure 1. Populations *V. sativa* var.angustifollia (4770, 7243), *V. sativa* var.sativa (1862), *V. villosa* (315, 6268) were separated partially by PC1; traits related to this separation are mainly phenology traits (day to spourat, days to first flowering, days to total flowering, days to maturity).

A cluster analysis of the tested *Vicia* spp. populations showed five main groups (Table 6 and Figure 2). Cluster G_1 contained five populations, belonging to *V.sativa* var.angustifollia with two populations (7243, 4740), *V. sativa* var.sativa one population (1862) and *V. villosa* with two populations (315, 6268).They are characterized by the lowest values of phenology traits (day to spourat, days to first flowering, total flowering, and seed maturity). Cluster G_2 contained 13 populations: 11 populations belonging to *V. sativa* (6646, 6681, 11761, 24062, 24069, 24074, 32972, 40310, 40315, 40326, 40334), population 38530 of *V. sativa* var.angustifollia and 322 of *V. villosa*. They are also characterized by the highest amount of vegetative, seed and yield traits compared to other populations. Cluster G_3 included 16 popullations belonging to *V. sativa* (6654, 11760, 11762, 11771, 11772, 24076, 24084, 24097, 43100), population 38529 of *V. sativa* var.angustifollia, *V. sativa* var.sativa (24631, 29802, 32900), 28061, 34212 and 14561 of *V. villosa*, with high amount of vegetative traits were collected in one group. Cluster G_4 contained seven populations: five belong to species *V. sativa* (11763, 11764, 11774, 38526, 38527), population 34295 of *V. sativa* var.cordata and *V. monantha* (32845). These were classified with the highest plant height, stems number and vegetative traits compared with other clusters. Cluster G_5 was the largest one with 17 populations, nine from *V. sativa* (5321, 33456, 38517, 38523, 38528, 38531, 38532, 38533, 38536), five from *V. sativa* var.angustifollia (38524, 38525, 38534, 38535, 38537), *V. michauxii* (2944), *V. michauxii* var.stenophylla (37129) and *V. narbonensis* (34878).These were classified as highest vegetative and pod traits populations.

The principal component analysis (PCA) of the 12 quantitative traits is summarized in Table 7. The first five PCs had eigenvalues >1 and they explained more than 80 % of the total variation for the vegetative and phenology traits. Day to sprout, days to first flowering, days to total flowering and days to maturity were loaded highly in PC1 and they accounted for 25.7 % of the total variation. In PC2, Biomass yield and dry weight accounted for 21 % of the total variation. In PC3, plant height and internode length accounted for 14.3 % of the total variation. PC4 contributed 11.2 % of the total traits variation in these populations with plant length and stems number loading highly. PC5 accounted for 9.8 % of total variation with length, width and pod index. Generally, for the 12 vegetative and phenology traits studied, PC1 and PC2 constituted more than 46 % of the total traits variation with most phenology traits and yield-related traits. This indicated that these traits can be used to classify the populations under study.

Table 2: Analysis variance of eight morphological traits of 58 vetch (*Vicia* spp.) populations

Source of Variations	Degrees of freedom (d.f)	Plant height	Internode length	Stem number	Pod length	Pod width	Pod index	Biomass yield	Dry weight
Taxon	8	3770.70**	48.92**	25.93**	5.23**	0.47**	9.19**	3809.63**	223.70**
Population	48	905.59**	12.11**	15.62**	1.89**	0.06 ns	7.76**	1967.42**	202.36**
Error	150	346.70	2.28	2.58	0.39	0.05	1.30	218.67	18.08
CV %		35.88	29.18	32.38	22.77	39.08	22.53	44.56	48.83

*, ** significant at 0.05 and 0.01 levels, respectively; [ns] not significant.

Table 3: Means comparison of 8 traits in different species of *Visia* spp.

Species	Plant height (cm)	Internode length (cm)	Stems number	Pod length	Pod width (cm)	Pod index	Biomass yield (g)	Dry weight (g)
V. michauxii (*Vmi*)	63.38 [cd]	6.56 [bc]	2.81 [b]	2.54 [bc]	1.14 [a]	4.36 [b]	9.59 [cd]	5.46 [bc]
V. michauxii var. stenophylla	100.0 [b]	15.0 [a]	5.0 [b]	2.5 [bc]	1.1 [a]	2.27 [c]	20.0 [bd]	5.0 [bc]
V. monantha (*Vmo*)	150.0 [a]	8.0 [b]	9.0 [a]	4.0 [a]	0.6 [b]	6.67 [a]	0.06 [d]	0.01 [c]
V.narbonensis (Vn)	24.50 e	4.75 [cd]	3.0 [b]	3.3 [ab]	0.65 [b]	5.1 [ab]	5.13 [cd]	1.15 [c]
V. sativa (*Vs*)	48.41 [d]e	4.8 [cd]	5.08 [b]	2.88 [bc]	0.56 [b]	5.33 [ab]	35.06 [b]	9.16 [ab]
V. sativa var. angustifollia	45.07 [d]e	6.9 [bc]	4.6 [b]	3.27 [ab]	0.60 [b]	5.48 [ab]	19.03 [bd]	5.18 [bc]
V. sativa var. cordata (*Vsc*)	85.13 [bc]	8.69 [b]	8.75 [a]	1.06 [d]	0.29 [b]	3.75 [bc]	27.92 [bc]	6.92 [bc]
V. sativa var. sativa (*Vss*)	54.67 [c]e	3.29 [d]	4.58 [b]	2.0 [cd]	0.5 [b]	4.0 [bc]	41.08 [ab]	9.32 [ab]
V. villosa (*Vv*)	63.56 [cd]	3.36 [d]	4.5 [b]	2.17 [c]	0.51 [b]	4.26 [b]	60.12 [a]	15.63 [a]

Table 4: Means comparison of 12 traits of 58 populations of different species of *Vicia* spp.

Population	Plant Height (cm)	Internode Length (cm)	Stems Number	Pod Length (cm)	Pod Width (cm)	Pod Index	Biomass Yield (g)	Dry Weight (g)	Day to Sprout	Days to first Flowering	Days to total Flowering	Days to Maturity
Vmi2944	78.56 c-e	7.94 b-e	2.67 g-i	2.87 c-j	0.82 ab	3.72 k-p	10.15 j-n	2.66 i-n	28 a	95 a	115 c	158 c
*Vmis*37129	100 b	15 a	5 f-i	2.5 f-k	1.1 a	2.27 op	20.00 h-n	5.00 g-n	28 a	95 a	115 c	158 c
*Vmo*32845	150 a	8 b-e	9 b-d	4 a-c	0.6 bc	6.67 a-h	0.06 n	0.01 n	28 a	95 a	115 c	158 c
*Vn*34878	24.50 i-k	4.75 f-p	3.00 g-i	3.30 b-g	0.65 bc	5.10 f-n	5.13 l-n	1.16 k-n	28 a	95 a	120 b	162 b
*Vs*5321	43.88 e-k	4.00 i-p	2.50 hi	2.14 g-l	1.06 a	2.00 p	2.07 mn	0.55 l-n	28 a	95 a	115 c	158 c
*Vs*6646	44.33 e-k	2.83 n-p	4.67 f-i	2.83 c-j	0.50 bc	5.67 c-k	61.83 b-d	14.61c-f	28 a	95 a	115 c	158 c
*Vs*6654	28.33 h-k	2.00 p	4.00 f-i	2.00 h-l	0.50 bc	4.00 j-p	42.67 e-i	10.83 d-i	28 a	95 a	115 c	158 c
*Vs*6681	63.00 c-h	3.33 l-p	5.00 f-i	3.97 a-d	0.50 bc	7.93 ab	74.83 bc	18.43 cd	28 a	95 a	115 c	158 c
*Vs*11760	56.67 c-k	4.50 g-p	4.67 f-i	2.00 h-l	0.50 bc	4.00 j-p	53.00 c-g	15.27 c-f	28 a	95 a	115 c	158 c
*Vs*11761	66.67 b-g	6.17 c-l	5.33 f-h	2.50 f-k	0.50 bc	5.00 f-n	83.00 ab	26.00 b	28 a	95 a	115 c	162 b
*Vs*11762	37.67 f-k	3.67 j-p	6.00 e-g	2.50 f-k	0.50 bc	5.00 f-n	19.00 h-n	5.06 g-n	28 a	95 a	115 c	158 c
*Vs*11763	80.00 b-e	6.33 c-i	11 bc	3.33 b-g	0.50 bc	6.67 a-h	45.00 e-h	9.33 e-k	28 a	95 a	120 b	162 b
*Vs*11764	61.67 c-i	5.17 e-o	11.67 b	2.67 e-k	0.50 bc	5.33 e-m	51.17 c-g	9.03 e-l	28 a	95 a	115 c	158 c
*Vs*11771	52.33 d-k	4.83 f-p	4.33 f-i	2.00 f-k	0.50 bc	4.00 j-p	56.67 b-f	11.43 d-h	28 a	95 a	115 c	158 c
*Vs*11772	65.00 b-h	3.17 m-p	4.67 f-i	2.00 f-k	0.50 bc	4.00 j-p	41.50 e-i	8.93 e-l	28 a	95 a	120 b	162 b
*Vs*11774	68.33 b-f	4.33 h-p	15.00 a	2.67 e-k	0.50 bc	5.33 e-m	53.00 c-g	15.27 c-f	28 a	95 a	115 c	158 c
*Vs*24062	40.00 f-k	1.83 p	4.67 f-i	2.00 f-k	0.50 bc	4.00 j-p	83.00 ab	26.00 b	28 a	95 a	120 b	162 b
*Vs*24069	41.00 f-k	2.50 n-p	5.33 f-h	3.83 a-e	0.50 bc	7.67 a-d	37.17 e-k	10.43 d-j	28 a	95 a	115 c	158 c
*Vs*24074	49.67 d-k	4.17 h-p	6.00 e-g	4.00 a-c	0.47 bc	8.67 a	44.00 d-h	10.43 d-j	28 a	95 a	115 c	158 c
*Vs*24076	41.33 f-k	3.50 k-p	4.33 f-i	2.50 f-k	0.47 bc	5.50 d-l	41.50 e-i	8.93 e-l	28 a	95 a	120 b	162 b
*Vs*24084	55.00 c-k	5.33 d-o	4.67 f-i	2.67 e-k	0.50 bc	5.33 e-m	44.00 d-h	10.43 d-j	28 a	95 a	115 c	158 c
*Vs*24097	51.33 d-k	3.00 n-p	6.00 e-g	2.00 f-k	0.50 bc	4.00 j-p	45.00 e-h	8.77 e-m	28 a	95 a	115 c	158 c
*Vs*32972	44.67 e-k	2.83 n-p	3.00 g-i	4.00 a-c	0.57 bc	7.11 a-f	48.00 c-h	15.10 c-f	28 a	95 a	120 b	162 b
*Vs*33456	43.43 e-k	6.07 c-m	4.86 f-i	4.04 a-c	0.64 bc	6.39 b-i	21.29 h-n	5.30 g-n	28 a	95 a	115 c	158 c
*Vs*38517	34.67 f-k	6.17 c-l	3.33 f-i	2.50 f-k	0.53 bc	4.72 g-n	11.27 j-n	3.05 h-n	28 a	95 a	115 c	158 c
*Vs*38523	43.33 e-k	7.33 c-g	3.67 f-i	3.17 b-h	0.60 bc	5.28 f-m	25.87 g-n	7.17 f-n	28 a	95 a	115 c	158 c
*Vs*38526	86.17 b-d	6.50 c-j	6.33 f-i	2.73 e-k	0.40 bc	6.18 b-j	18.92 h-n	5.50 g-n	28 a	95 a	115 c	158 c
*Vs*38527	90.00 bc	9.83 b	3.00 g-i	4.53 a	0.60 bc	7.56 a-e	20.41 h-n	3.91 h-n	28 a	95 a	115 c	162 b
*Vs*38528	53.40 c-k	8.00 b-e	6.40 d-f	3.63 a-f	0.63 bc	5.75 b-k	34.49 e-l	8.91 e-l	28 a	95 a	115 c	158 c

Population	Plant Height (cm)	Internode Length (cm)	Stems Number	Pod Length (cm)	Pod Width (cm)	Pod Index	Biomass Yield (g)	Dry Weight (g)	Day to Sprout	Days to first Flowering	Days to total Flowering	Days to Maturity
*Vs*38531	46.00 e-k	6.40 c-k	5.20 f-i	2.84 c-j	0.60 bc	4.68 h-n	13.00 i-n	3.95 h-n	28 a	95 a	115 c	158 c
*Vs*38532	29.80 g-k	7.60 b-f	4.80 f-i	2.00 f-k	0.64 bc	3.27 l-p	8.35 k-n	2.00 i-n	28 a	95 a	115 c	162 b
*Vs*38533	33.30 f-k	4.74 f-p	3.40 f-i	3.66 a-f	0.62 bc	5.93 b-k	10.54 j-n	2.29 i-n	28 a	95 a	115 c	158 c
*Vs*38536	36.33 f-k	7.00 c-h	3.67 f-i	2.23 g-k	0.50 bc	4.47 h-o	3.04 mn	0.83 k-n	28 a	95 a	115 c	158 c
*Vs*40310	33.33 f-k	3.17 m-p	3.67 f-i	3.33 b-g	0.50 bc	6.67 a-h	55.00 c-g	15.77 c-e	28 a	95 a	115 c	158 c
*Vs*40315	38.33 f-k	2.50 n-p	3.67 f-i	3.17 b-h	0.50 bc	6.33 b-i	51.33 c-g	14.63 c-f	28 a	95 a	115 c	158 c
*Vs*40326	44.67 e-k	2.33 op	4.33 f-i	3.50 a-f	0.50 bc	7.00 a-f	103.67 a	36.33 a	28 a	95 a	120 b	162 b
*Vs*40334	31.33 f-k	1.83 p	4.67 f-i	3.33 b-g	0.43 bc	7.83 a-c	41.50 e-i	8.93 e-l	28 a	95 a	115 c	158 c
*Vs*43100	22.00 j-k	1.83 p	4.33 f-i	1.50 kl	0.50 bc	3.00 n-p	9.57 j-n	0.33 mn	28 a	95 a	120 b	162 b
*Vsa*38524	29.60 g-k	7.70 b-f	4.40 f-i	3.16 b-h	0.78 ab	4.07 j-p	7.18 l-n	1.95 j-n	28 a	95 a	115 c	158 c
*Vsa*38525	50.44 d-k	8.28 b-d	5.56 f-h	4.22 ab	0.61 bc	6.96 a-g	19.92 h-n	5.62 g-n	28 a	95 a	115 c	162 b
*Vsa*38530	65.33 b-h	6.67 c-i	2.00 i	3.17 b-h	0.53 bc	5.69 c-k	29.33 e-n	10.98 d-h	28 a	95 a	115 c	162 b
*Vsa*38534	19.00 k	5.50 d-n	5.00 f-i	3.25 b-h	0.50 bc	6.50 b-i	26.75 f-n	5.80 g-n	28 a	95 a	115 c	162 b
*Vsa*38535	36.50 f-k	6.50 c-j	2.50 hi	2.75 d-k	0.65 bc	4.29 i-o	4.55 l-n	1.25 k-n	28 a	95 a	115 c	158 c
*Vsa*38537	43.67 e-k	8.00 b-e	5.67 f-h	2.93 c-i	0.60 bc	4.89 f-n	4.70 l-n	1.05 k-n	28 a	95 a	115 c	162 b
*Vsa*4740	49.67 d-k	3.50 k-p	5.00 f-i	2.00 h-l	0.50 bc	4.00 j-p	45.00 f-h	9.33 e-k	21 b	90 b	107 d	150 d
*Vsa*7243	54.33 c-k	5.17 e-o	4.33 f-i	2.67 e-k	0.50 bc	5.33 e-m	18.67 h-n	5.67 g-n	21 b	90 b	107 d	150 d
*Vsa*38529	30 f-k	4 i-p	3 g-i	0.6 m	0.3 c	2.00 p	3.10 mn	0.60 l-n	28 a	95 a	115 c	158 c
*Vsc*34295	85.13 b-d	8.69 bc	8.75 c-e	1.06 l	0.29 c	3.75 k-p	27.92 e-n	6.92 f-n	28 a	95 a	115 c	158 c
*Vss*1862	44.67 e-k	4.33 h-p	4.33 f-i	2.00	0.50 bc	4.00 j-p	31.67 e-m	9.00 e-l	21 b	90 b	107 d	150 d
*Vss*24631	51.00 d-k	2.33 op	5.00 f-i	1.83 i-l	0.50 bc	3.67 k-p	56.67 b-f	11.43 d-h	21 b	90 b	125 a	167 a
*Vss*29802	67.33 b-g	3.50 k-p	4.33 f-i	2.50 h-l	0.50 bc	5.00 f-n	41.50 e-i	8.93 e-l	28 a	95 a	107 d	150 d
*Vss*32900	55.67 c-k	3.00 n-p	4.67 f-i	1.67 j-l	0.50 bc	3.33 l-p	34.50 e-l	7.93 e-n	28 a	95 a	107 d	150 d
*Vv*315	55.00 c-k	2.83 n-p	4.33 f-i	2.00 h-l	0.50 bc	4.00 j-p	53.83 c-g	13.08 c-g	21 b	90 b	107 d	150 d
*Vv*322	100.33 b	4.00 i-p	4.33 f-i	2.50 f-k	0.50 bc	5.00 f-n	108.33 a	38.60 a	28 a	95 a	115 c	158 c
*Vv*6268	47.67 e-k	3.17 m-p	4.67 f-i	1.67 j-l	0.53 bc	3.17 m-p	83.50 ab	19.73 bc	21 b	90 b	107 d	150 d
*Vv*14561	58.33 c-j	4.50 g-p	4.67 f-i	2.67 e-k	0.50 bc	5.33 f-n	57.71 b-e	10.83 d-i	28 a	95 a	115 c	158 c
*Vv*28061	51.67 d-k	2.00 p	4.33 f-i	2.00 h-l	0.50 bc	4.00 j-p	18.00 h-n	3.73 h-n	28 a	95 a	115 c	158 c
*Vv*34212	68.33 b-f	3.67 j-p	4.67 f-i	2.17 g-l	0.53 bc	4.06 j-p	39.33 e-i	7.80 e-n	28 a	95 a	115 c	158 c

Different letters indicate significant differences among different populations for the same species. P <0.05.
V. michauxii (*Vmi*), *V. michauxii* var. stenophylla (*Vmis*), *V. monantha* (*Vmo*), *V.narbonensis (Vn)*, *V. sativa* (*Vs*), *V. sativa* var. angustifollia (*Vsa*), *V. sativa* var. cordata (*Vsc*), *V. sativa* var. sativa (*Vss*), *V. villosa* (*Vv*).

Table 5: Simple correlation matrix for the 12 traits of *Vicia* spp. populations

Traits	Plant height	internode length	Stems number	Pod length	Pod width	Pod index	Biomass yield	Dry weight	Day to sprout	Days to first flowering	Days to total
Internode length	0.43**										
Stems number	0.38**	0.11^{ns}									
Pod length	0.13^{ns}	0.24*	0.03^{ns}								
Pod width	0.10^{ns}	0.51^{ns}	-0.22^{ns}	0.23*							
Pod index	0.09^{ns}	-0.06^{ns}	0.16^{ns}	0.86^{ns}	-0.26*						
Biomass yield	0.11^{ns}	-0.46^{ns}	0.14^{ns}	-0.03	-	0.20					
Dry weight	0.13^{ns}	-0.38**	0.07^{ns}	0.03^{ns}	-0.28*	0.22	0.95^{ns}				
Day to sprout	0.04^{ns}	0.19^{ns}	0.06^{ns}	0.28*	0.11^{ns}	0.23*	-0.16^{ns}	-0.09			
Days to first flowering	0.04^{ns}	0.19^{ns}	0.06^{ns}	0.28*	0.11^{ns}	0.23*	-0.16^{ns}	-0.09	1^{ns}		
Days to total flowering	-0.08^{ns}	-0.03^{ns}	0.05^{ns}	0.17^{ns}	0.03^{ns}	0.15	0.04^{ns}	0.07^{ns}	0.46^{ns}	0.46^{ns}	
Days to maturity	-0.07^{ns}	0.13^{ns}	0.02^{ns}	0.26*	0.06^{ns}	0.21	-0.03^{ns}	0.02^{ns}	0.50^{ns}	0.50^{ns}	0.92^{ns}

*, ** significant at 0.05 and 0.01 levels, respectively; ^{ns} not significant.

Table 6: Means comparison of 12 traits of five vetch groups produced in Figure 2

Groups	Plant height (cm)	Internode length (cm)	Stems number	Pod length (cm)	Pod width (cm)	Pod index	Biomass yield (g)	Dry weight (g)	Day to sprout	Days to first flowering	Days to total flowering	Days to maturity
G_1	50.27^{b}	3.80^{c}	4.53^{c}	2.07^{c}	0.51^{b}	4.10 d	46.53^{b}	11.36^{b}	21.00^{c}	90.00^{c}	107.00^{c}	150.00^{c}
G_2	50.97^{b}	3.40^{c}	4.36^{c}	3.24^{a}	0.50^{b}	6.51^{a}	63.15^{a}	18.94^{a}	28.00^{a}	95.00^{a}	116.15^{a}	159.54^{a}
G_3	49.50^{b}	3.43^{c}	4.60^{b}	2.04^{c}	0.49^{c}	4.14 d	37.73^{c}	8.20^{c}	27.56^{b}	94.69^{b}	115.56^{b}	158.31^{b}
G_4	88.76^{a}	6.98^{b}	9.25^{a}	3.00^{b}	0.48^{c}	5.93^{b}	30.92^{c}	7.14^{c}	28.00^{a}	95.00^{a}	115.71^{b}	159.14^{a}
G_5	43.91^{c}	7.12^{a}	4.21^{c}	3.01^{b}	0.68^{a}	4.72^{c}	13.43 d	3.44 d	28.00^{a}	95.00^{a}	115.29^{b}	159.18^{a}

^{abc} Different letters indicate significant differences among different populations for the same species. P<0.05.

Table 7: Eigenvalues, the proportion of variance, and morphological traits that contributed to the first five principal components (PC)

Variable	PC1	PC2	PC3	PC4	PC5
Plant height	0.058	-0.029	0.466	0.441	-0.267
Internode length	0.208	0.302	0.428	0.055	-0.242
Stems number	0.050	-0.107	0.176	0.668	0.075
Pod length	0.310	-0.154	0.389	-0.244	0.378
Pod width	0.141	0.303	0.243	-0.301	-0.357
Pod index	0.230	-0.332	0.265	-0.084	0.533
Biomass yield	-0.144	-0.53	-0.004	0.062	-0.236
Dry weight	-0.101	-0.541	0.043	-0.042	-0.321
Day to sprout	0.464	-0.017	-0.102	0.045	-0.075
Days to first flowering	0.464	-0.017	-0.102	0.045	-0.075
Days to total flowering	0.380	-0.129	-0.338	0.057	-0.164
Days to maturity	0.419	-0.100	-0.267	0.014	-0.151
Eigenvalue	3.340	2.723	1.856	1.452	1.279
Proportion	0.257	0.210	0.143	0.112	0.098
Cumulative	0.257	0.467	0.610	0.721	0.820

Figure 1: Two principal components showing the relationship among 12 traits of 58 populations of *Vicia* spp.

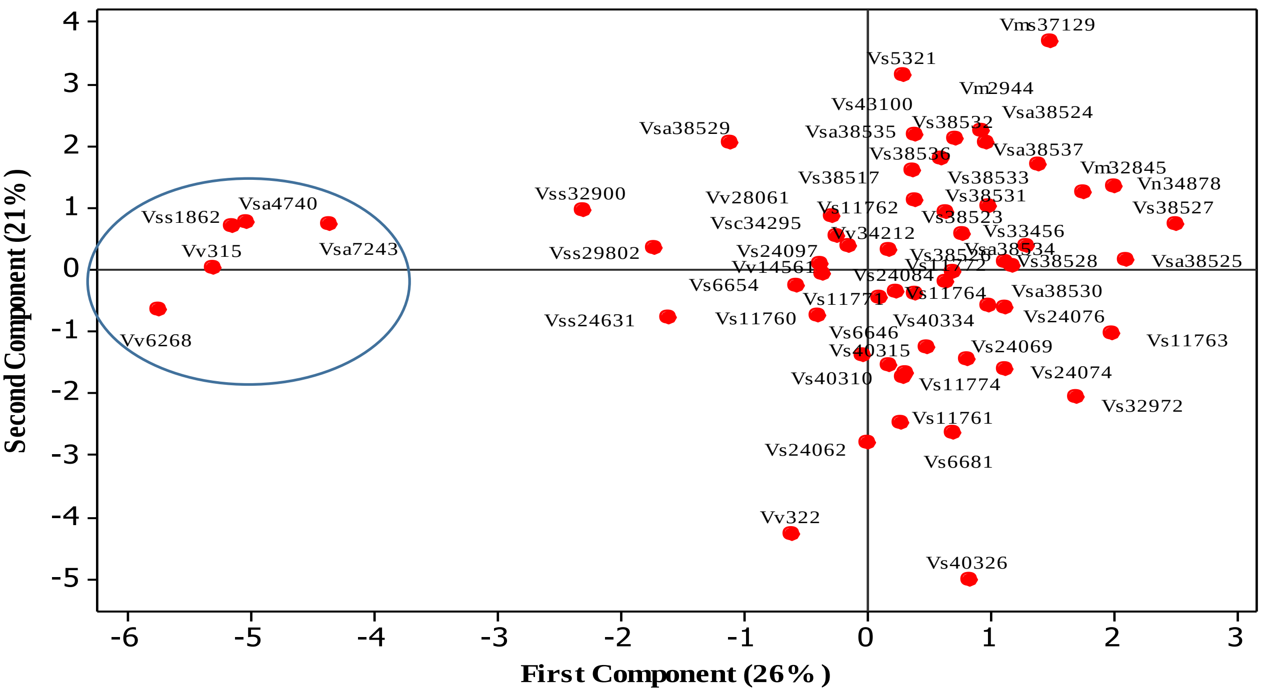
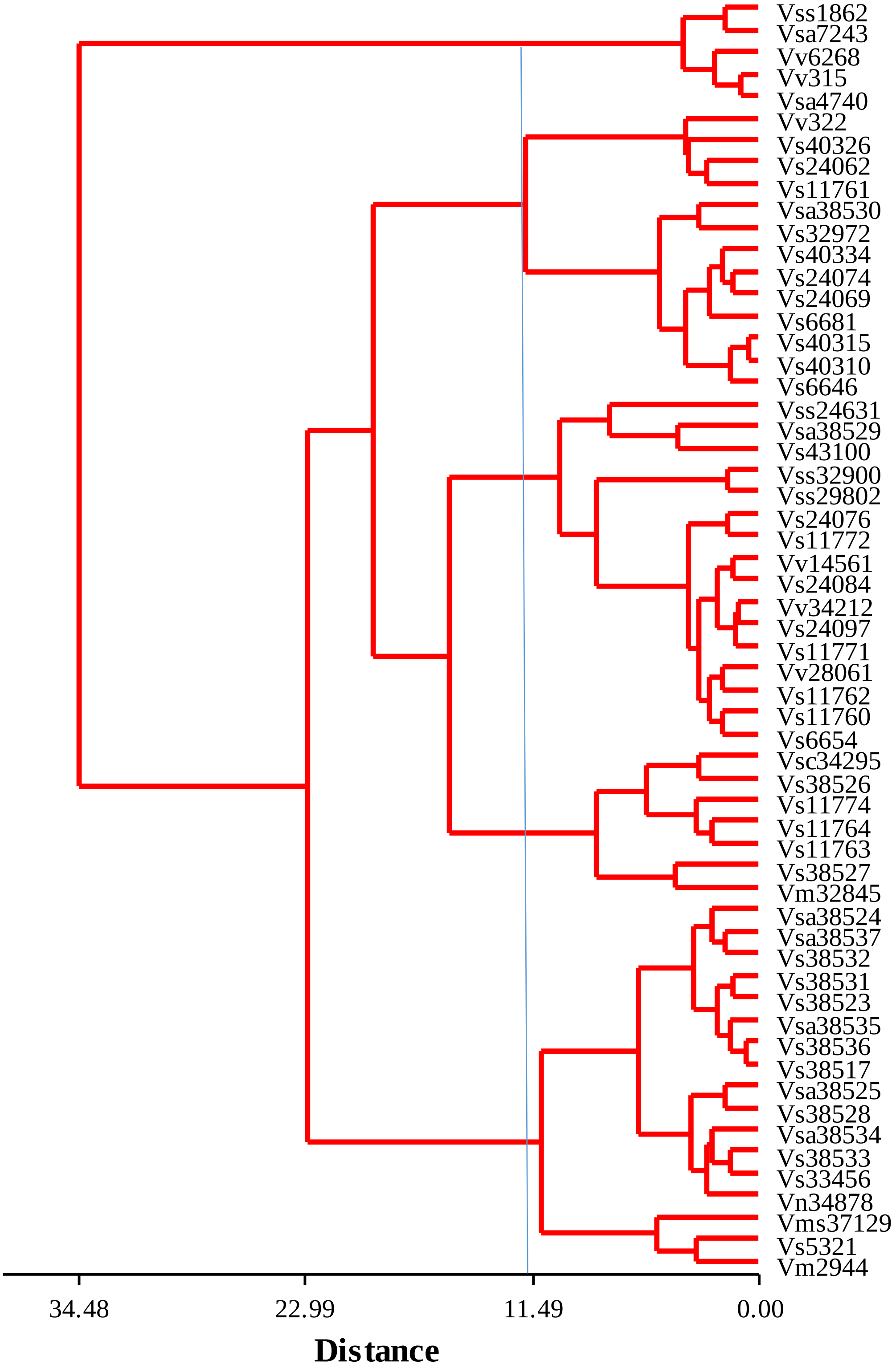


Figure 2: Dendrogram of 58 populations of *Vicia* spp. explained by complete linkage clustering of 12 traits



Discussion

In these study, 58 populations of *Vicia* spp. were investigated for genetic diversity based on morphological and phenology traits. Due to, genetic diversity analysis of germplasms using morphological traits is an initial step for crop improvement[19-22]. There was significant (*P*<0.01) genotypic variation among 58 germplasm accessions of *Vicia* spp. for all the measured vegetative and yield traits: plant length, internode length, stems number, pod length, pod width, pod index, biomass yield and dry weight.The estimates of genotypic variation and repeatability for these traits indicated the potential genetic variation available among the germplasm accessions within *Vicia* spp. investigated. Similar results were obtained by the Ebrahimi *et al*[23] on plant and seed morphology traits of white Bean genotypes, Mikic *et al*[12] on forage and seed yields of three lines of common vetch and Berhanu and Abera[24] on forage yield of vetch species investigation.

A comparison between taxon (*V. sativa*: Vs, Vsa, Vsc and Vss, *V. mchauxii*: Vmi and Vmis, *V. monantha*: Vmo, *V. narbonensis*: Vn and *V. villosa*: Vv) showed *V. monantha* (Vmo) with high values of plant height, stems number, pod length and *V. villosa* with high values of biomass yield and dry weight. Berhanu and Abera[24] showed that among the vetch species (*V. sativa*, *V. villosa*, *V. dasycarpa*, and *V. bengalensis*), *V. dasycarpa* and *V. villosa* were the best performing species for forage. Then the vetch species tested in the current study could be used for pasture expansion and forage production, in livestock exclusion areas, in forage strips, as an under-sowing with food crops, or as a backyard forage crop in the pasture of the country.

The populations demonstrated high variation in plant height, internode length, stem number, pod length, biomass yield and dry weight. Populations: Vmo32845, Vv322, Vmis37129 (for plant height), populations: Vmi37129, Vs38527, Vsc34295 (for internode length), populations: Vs11774,Vs11764,Vs11763 (for stems number), populations: Vs38527, Vmo32845, Vsa38525 (for pod length) and populations:Vs11761, Vs24062, Vs40326, Vv322, Vv6268 (for biomass yield and dry weight) showed the highest values of the mentioned traits. However, broadening the genetic base from diverse sources is recommended to include most of the genetic determinants of these traits[25]. This variability can be exploited in fodder breeding programs to select an adapted plant material for the arid and semi-arid areas[26].

Phenology (earliness and lateness) of vetch species has a great effect on seed yield productivity. Late maturity for forage and seed was recorded at 125 and 167 d, respectively. This could be due to high and extended rainfall at the region of populations that encouraged vegetative growth and delayed forage and seed harvesting stages. The results indicated that for vetch populations tested, 107 to 125 and 150 to 167 d were required after the emergence of the seedlings for total flowering and seed maturity, respectively. On average, the difference in harvest forage and seed yield between populations are about 18 and 17 d. This indicates different responses of the tested populations for these important agronomic traits.

According to Getnet *et al*[27], *Vicia narbonensis* and *Vicia sativa* are early maturing and *Vicia villosa* is late maturing species. But in this study two populations of *V. villosa* (315 and 6268) and four populations of *V.sativa* var.angustifollia (4740, 7243), *V. sativa* var.sativa (1862, 29802, 32900) with 107 and 150 d for flowering and seed maturity is recommended for seed production due to earliness, whereas late-maturing species like *V. sativa* var.sativa (24631) should not be advisable to grow for seed purpose.

There is a direct relationship between plant length with internode length and the number of stems, this indicates that tall plants produce long internodes and more number stems. Also, the length and width of the pod have a direct relationship with the number of days of sprout, flowering and seed maturation, which means long and wide pods are produced by late-flowering and seed maturation time. Since, in cereals, the correlation between grain yield and plant height is often negative, but in legumes, this correlation is often positive, because legumes have unlimited growth, therefore, with increasing height, more pods are produced, which has a positive effect on performance, so similar results were obtained in the traits of white Bean genotypes where high grain yields were strongly correlated to days to flowering and plant height[23] and Lens spp.[28].

In PCA, since the first component includes changes that are not explained by the second component and the two components are independent of each other, so the two components were intersected vertically and in the form of a biplot diagram to determine the diversity between different genotypes and determine the far and near genotypes to be used. Phenology traits (day to sprout, days to first flowering, days to total flowering, days to maturity) accounted for the variations recorded in the populations in PC1. On the other hand, yield traits (biomass yield and dry weight) accounted for the variation observed in the populations in PC2. The total cumulative variance in the first two PC was more than 46 %, indicating the high degree of diversity among the traits under study. Furthermore, the traits can be used as phenotypic traits in differentiating the populations. In plot PCA (Figure 1), populations, *V. sativa* var.angustifollia (Vsa7243, Vsa4740), *V. sativa* var.sativa (1862) and *V. villosa* (Vv315, Vv6268), separated from other populations and located on the left of X-axis by containing less of phenology traits (important in the first component). So, these populations recommend for areas with short growth periods. Populations *V. sativa* (40326) and *V. villosa* (322), for containing high value of biomass and dry weight, located on the bottom of Y-axis (negative effect of biomass and dry weight on the second component). As a result, two populations, *V. sativa* (40326) and *V. villosa* (322), produce forage yield more than other populations.

In the present study, the 58 populations of *Vicia* spp. were grouped into five clusters using 12 traits.The populations of cluster G_1 are characterized by the lowest values of days to sprout, flowering, and seed maturity which are the candidate of further evaluations. Also, these populations had a shoter time for these traits. Members of G_1 are similar to the dispersion of these populations in the PCA plot (Figure 1). It is interesting that the population from different climates like Shiraz clustered with populations from Karaj. This pattern of clustering indicates, the diversity of populations within these geographical areas and, the similarity of populations from different geographical areas.

These results agree with the report of Alemayehu and Becker(29) in *Brassica carinata*. Cluster G_2 contained 13 populations belonging to *V. sativa* and *V. villosa* species. These populations had a high value of seed, yield and phenology traits. Member of G_2 due to having a long time for flowering and seed maturity, produce more seed and forage yield. This is the best factor, that can be used for livestock feeding. Cluster G_3 contained mixed 16 populations of *V. sativa* and *V. villosa*. with lowest values of seed and forage yield gather together in a group, that they are not important inbreeding. Cluster G_4 contained seven populations of *V. sativa* and *V. monantha* with high vegetative traits, that recommend livestock feeding and control of erosion. G_5 group with 17 populations of *V. sativa*, *V. michauxii* and *V. narbonensis* were classified as later flowering and seed ripening and containing less amount of yield forage. These populations can be used for areas with a long growth time.

Finally in this study populations were located as five groups based on morphology and phenology traits. Members of each group are similar for mentioned traits and can be recommended for breeding programs. Also, the results indicated no relationship between studied traits and the origin of populations.

Conclusions and implications

The findings showed the high variation of morphology and yield traits in different species and populations of vetch. These differences are very important to select the type of companion crops and methods of integration to improve yields of both crops (food and forage) without significant effect of one on the other. *Vicia sativa* (Kermanshah, Javanrod) and *V. villosa* (Karaj) were superior in terms of fresh and dry forage yields. *V.michauxii* var.stenophylla (Qom), *V. monantha* (Kermanshah), *V. sativa* (Gilan, Astara), and *V. villosa* (Karaj), are recommended by having tall plant and big pods. However, more comprehensive studies and additional experiments are required to complete information for breeding programs.

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