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Full Length Research Paper

Study on genetic variation in Iranian castor bean (*Ricinus communis* L.) accessions using multivariate statistical techniques

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Genetic diversity on 12 accessions of castor bean *(Ricinus communis* L.) collected from different origins of Iran was assessed under filed conditions. Accessions were evaluated in a randomized complete block design with three blocks. The data on 5 individuals in each block were recorded for 32 agro-morphological traits. The descriptive statistics for each one of 32 studied traits were calculated. Clustering of accessions into similarity groups was performed using Ward's hierarchical algorithm based on squared Euclidean distances. Discriminant function analysis used to confirm the accuracy of grouping that produced by cluster analysis. In order to identify the patterns of morphological variation, principal component analysis (PCA) was conducted. Studied accessions showed high coefficient of variation for hollow seed number on primary raceme, secondary and tertiary branch fresh weight, secondary and tertiary branch dry weight, lamina leaf length and leaf length traits. The accessions based on studied traits were classified in 3 groups. Our results showed that, the most of studied accessions (75%) have been clustered together in group 2 indicating relatively low genetic variability in castor bean germplasm. Principal component analysis (PCA) revealed that the first six principal components accounted for 93% of the total variation. Among the studied traits, seed number on primary raceme as a yield component in castor bean showed positive correlation with the first component (PC1). Hollow seed number on primary raceme showed positive correlation with the second component (PC2). Oil percent presented negative correlation with PC2. According to breeding goal, breeders can chose accessions by considering appropriate PCs values.

Key words: Agro-morphological traits, descriptive statistics, cluster analysis, discriminant function analysis, genetic diversity, principal component analysis.

INTRODUCTION

Castor bean (*Ricinus communis* L.), a monotypic species in the spurge family (Euphorbiaceae) with 2n = 20 chromosomes, is an important non-edible oilseed crop (Hayes, 1985). The seeds of castor bean contain more than 45% oil and its oil is rich (80 to 90%) in an unusual hydroxyl fatty acid, ricinoleic acid (Jeong and Park, 2009). Castor bean oil is widely used for its lubricating properties and medicinal purposes. In industry, castor bean oil is used for the manufacturing of soaps, lubricants, hydraulic and brake fluids, paints, dyes, coatings, inks, cold resistant plastics, waxes and polishes, nylon, pharmaceuticals and perfumes (Franz and Jaax, 1997; Duke, 1998). Castor bean is considered to be native to tropical Africa (Vavilov, 1951; Zeven and Zhukovsky, 1975), but it is cultivated in many tropical and subtropical regions of the world (Govaerts et al., 2000). Once established, castor bean can quickly spread and is considered an invasive weed in many countries (Weber, 2003). It occurs in dense stands and is frequently found along roadsides and disturbance areas. Castor bean is both self- and cross-pollinated by wind, but controlled

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Table 1. List of the 12 studied castor bean accessions collected from various locations of Iran.

Number	Code in gene bank	Location	Latitude	Longitude	Altitude (m)
P1	80-23	Tafresh (Markazi State)	34º 24´	49º 43´	1735
P2	80-31	Ashtian (Markazi State)	34º 30´	50º 04´	2450
P3	80-25	Arak (Markazi State)	34º 20´	49º 49´	1753
P4	80-12-1	Sahreza (Isfahan State)	32º 11´	51º 37´	1750
P5	80-29	Toyserkan (Hamedan State)	36º 30´	48º 16´	1910
P6	80-18	Taft (Yazd State)	31º 32´	54º 15´	2000
P7	80-16-1	Fasa (Fars State)	28º 58´	51º 41´	1382
P8	80-17	Ashtian (Markazi State)	32º 24´	50º 14´	1775
P9	80-7	Mehriz (Yazd State)	30º 05	54º 17´	1550
P10	80-11-1	Sahreza (Isfahan State)	32º 14´	51º 32´	1750
P11	80-4	Jiroft (Kerman State)	28º 40´	57º 44´	685
P12	80-22	Tafresh (Markazi State)	34º 27´	49º 38´	1727

crossing studies suggest that out crossing is a frequent mode of reproduction (Meinders and Jones, 1950; Brigham, 1967). Although, the genus Ricinus is considered monotypic, castor bean varies greatly in its growth habit, color of foliage and stems, seed size and oil content (Weiss, 2000; Li et al., 2008). Due to increased demand for castor bean in many countries, improvement of varieties is drawing great attention from breeders (Sujatha et al., 2008). Success in breeding for yield superiority is limited by a low genetic variability for productivity traits and sources of resistance to diseases and pests (Weiss, 2000; Hegde et al., 2003). It is necessary to characterize the genetic diversity present across *R. communis* germplasm from different geographic regions to develop a genotyping scheme that links castor bean evidence to a particular source, geographic region, or batch (Hinckley, 2006).

Morphological characterization is the first step in the description and classification of germplasm collections (Smith et al., 1991). The multivariate analyses are useful for characterization, evaluation and classification of plant genetic resources when a number of accessions are to be assessed for several characters of agronomic and physiological importance (Peeters and Martinelli, 1989). Different types of analysis such as cluster analysis and principal component analysis (PCA) can be used to obtain idea about how identify groups of accessions that have desirable traits for breeding, and enlightening the patterns of variation in germplasm collection, to identify relationships among accessions and possible gaps (Camussi et al., 1985; Cowen and Frey, 1987; Peeters and Martinelli, 1989). Principal component analysis (PCA) is a method of data reduction that original variables decreased to a limited number of uncorrelated new variables. This has been widely used in the studies of variability in germplasm collections of many species (Julier et al., 1995; Veasey et al., 2001; Naghavi and Jahansouz, 2005; Bhargava et al., 2007; Nooryazdan et al., 2010). The aims of the present study were to assess the genetic diversity present at the morphological and agronomical traits in 12 Iranian castor bean accessions. Information presented herein, will help the breeder to develop high yielding castor bean hybrids.

MATERIALS AND METHODS

Plant materials and agro-morphological traits

The experimental material comprised 12 accessions of *R. commonis* kindly provided by The Seed and Plant Improvement Institute (SPII), Karaj, Iran (Table 1). The experiment was carried out at Urmia Agricultural Research Center in 2009. The latitude and longitude of region is 37º 44´ N and 45º 10´ E. Climate of the region is cold and semidry and the average rainfall and the area temperature according to 16 years statistics are 184 mm and 12°C, respectively. Soil type of the experimental site was clay loam with pH 7. Accessions were evaluated in a randomized complete block design with three blocks. Experimental units in each block comprised of 3 lines of 6.5 m long. Row to row and plant to plant spacing was 0.60 and 0.50 m, respectively. The data on 5 individuals in each block were recorded for 32 morphological and agronomical traits. The agro-morphological characters evaluated in this study are listed in Table 2. Number of leaf was recorded as the number of fully developed leaves per plant at the end of the growing season. Leaf dry weight was measured as the dry weight of all fully developed leaves per plant at the end of the growing season. Lamina leaf length (from the leaf top to the leaf base), lamina leaf width (from the widest part of the leaf lamina) and petiole leaf length for each plant were measured in centimeter from the mean of 6 sampled leaves per plant at the end of the growing season.

Statistical analyses

In the present work, the descriptive statistics such as mean, standard deviation and coefficient of variation for each one of 32 studied traits were calculated. Clustering of accessions into similarity groups was performed using Ward's hierarchical algorithm based on squared Euclidean distances. Prior to squared Euclidean distance calculation, the data were standardized by variable to have a mean of zero and a variance of one. In order to identify the

Table 2. Basic statistics for 32 agro-morphological traits record in 12 castor bean accessions.

Code	Traits	Mean	Minimum	Maximum	CV
X1	Main stem length (cm)	46.65	32.80	82.80	24.87
X2	Secondary branch number	2.53	2.00	5.20	24.63
X3	Tertiary branch number	2.42	0.00	4.25	39.34
X4	Secondary branch length (cm)	28.54	10.73	53.83	33.92
X5	Tertiary branch length (cm)	15.11	0.00	34.90	45.67
X6	Primary raceme length (cm)	27.82	16.40	51.80	27.46
X7	Secondary raceme length (cm)	18.33	7.00	30.25	27.61
X8	Tertiary raceme length (cm)	8.83	0.00	19.25	52.36
X9	Female flower length (cm)	18.90	11.00	37.00	33.33
X10	Male flower length (cm)	8.77	2.33	18.80	44.51
X11	Leaf number	20.96	8.60	40.00	34.78
X12	Leaf dry weight (g)	43.68	11.00	119.20	55.22
X13	Main stem fresh weight (g)	84.89	36.00	232.00	49.46
X14	Main stem dry weight (g)	26.68	10.87	73.96	48.80
X15	Main stem diameter (cm)	1.60	1.20	2.24	16.37
X16	Secondary and tertiary branch fresh weight (g)	63.96	7.60	271.00	75.50
X17	Secondary and tertiary branch dry weight (g)	21.46	2.42	81.32	69.11
X18	Secondary and tertiary racemes weight (g)	59.31	18.27	144.83	62.37
X19	Total primary raceme weight (g)	59.19	11.11	128.94	41.61
X20	Primary raceme weight without Capsule and seed	3.29	1.13	11.50	60.74
X21	Capsule weight on primary raceme (g)	22.14	6.22	52.13	40.42
X22	Total seed weight on primary raceme (g)	31.21	6.82	62.43	39.73
X23	10 seed weight on primary raceme (g)	2.69	1.93	4.37	19.58
X24	Seed number on primary raceme	115.17	26.60	185.60	32.74
X25	Hollow seed number on primary raceme	4.43	0.20	19.80	97.23
X26	Leaf area per plant (cm^2)	1175.00	700.00	1944.60	22.94
X27	Lamina leaf dry weight (g)	10.01	5.97	30.88	43.85
X28	Lamina leaf length (cm)	21.15	17.11	27.50	13.34
X29	Lamina leaf width (cm)	22.38	17.87	28.90	12.92
X30	Petiole leaf length (cm)	15.35	9.41	25.00	20.00
X31	Leaf length (lamina leaf length + petiole leaf length) (cm)	29.02	21.83	42.50	15.26
X32	Oil content (%)	2.07	0.46	4.22	40.54

CV: Coefficient of variation.

patterns of morphological variation, principal component analysis (PCA) was conducted. Those PCs with eigenvalues >1.0 were selected, as proposed by Jeffers (1967). Data were processed using statistic program Minitab 14 (Minitab version 14, Minitab Inc., State College, PA, USA).

Discriminant function analysis used to confirm the accuracy of grouping that produced by cluster analysis. Canonical discriminant analysis was performed with the PROC CANDISC command of SAS software (SAS Institute Inc, NC, USA). Canonical variables are linear combinations of the original quantitative measurements that there is not any correlation among them. The mean values of the canonical variables are referred to as group centroids. The difference between centroid values of two groups is the Mahalanobis distance (D^2) (Mahalanobis, 1936) and is calculated as:

$$D^2 = \left(\overline{X}_1 - \overline{X}_2\right)' S^{-1}\left(\overline{X}_1 - \overline{X}_2\right)$$

Where S^{-1} is the inverse of the pooled sample variance-covariance matrix, and $\overline{X}_1$ and $\overline{X}_2$ are the respective vectors of measurements on groups 1 and 2.

RESULTS AND DISCUSSION

Descriptive statistics

The mean, maximum, minimum and coefficient of variation for 32 agro-morphological variables are presented in Table 2. Studied accessions showed high coefficient of variation for hollow seed primary raceme number, secondary and tertiary branch fresh weight, secondary and tertiary branch dry weight, lamina leaf length and leaf length traits as well as moderate variation for oil percent whereas they showed low coefficient of variation for some other traits. Descriptive statistics

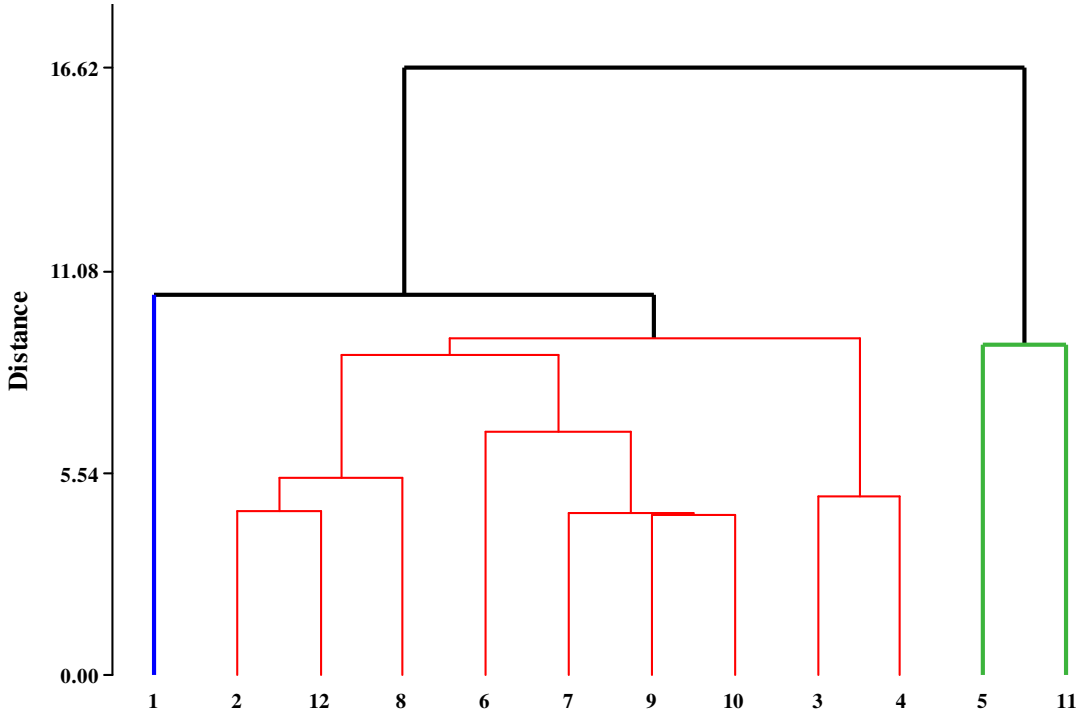

Figure 1. Ward's dendrogram of 12 castor bean accessions using squared Euclidean distances.

analysis was also used for studying genetic variability in some other crops, such as garlic (*Allium sativum* L.) (Panthee et al., 2006); groundnut (*Vigna subterranea* (L.) Verdc) (Ntundu et al., 2006) and melon (*Cucumis melo* L.) (Lotti et al., 2008). Genetic variability is the raw material of crop breeding industry on which selection acts to evolve superior genotypes. The higher amount of variation present for a character in the breeding materials, greater is the scope for its improvement through selection.

Cluster analysis

The 12 castor bean accessions based on 32 morphological traits were classified in 3 groups using Ward's hierarchical algorithm as shown in Figure 1. Cluster 1 contained only one accession originated from Markazi state. Cluster 2 was comprised nine accessions originated from Markazi, Yazd, Fars and Isfahan states. The third cluster included 2 accessions from Hameden and Kerman states. Accuracy of produced groups by cluster analysis was done using discriminant function analysis (Figure 2). It has been observed that total success rate of the discriminant function was %100 showing the success of discriminant function in cluster grouping or distancing among various groups. This result is coincident with findings of some researches such as Balochi et al. (2001) and Jaynes et al. (2003) in barley and corn, respectively. Moreda et al. (2003) grouped 85 tea samples into Asian and African groups with cluster analysis using Ward's minimum variance method and squared Euclidean distance. Then, discriminant function analysis manifest that 94.4% of grouping was correct.

The average inter-cluster distance ranged from 10.27 to 7.054. The largest inter-cluster distance (10.27) was observed between cluster 1 and 3 and the lowest one (7.05) was observed between cluster 2 and 3 (Table 3). Crossing between individuals from clusters with maximum inter-cluster distance may result in high heterosis. Even though the genetic mechanisms that explain heterosis are not fully understood, it is well documented that crosses between unrelated, and consequently genetically distant parents, show greater hybrid vigor than crosses between closely related parents (Stuber, 1994).

It was obvious that genetic relationships among studied landraces did not have force tendency to associate with their geographic origins. Murthy and Arunachalam (1966) showed that genetic drift and selection in different environments can cause greater diversity among genotypes than geographic distance cause. Therefore, selection of parental material for hybridization in breeding programs simply based on geographic diversity may not be rewarding. One possible reason for the genetic similarity among germplasm from different regions is that the materials might have originally been introduced from the same region.

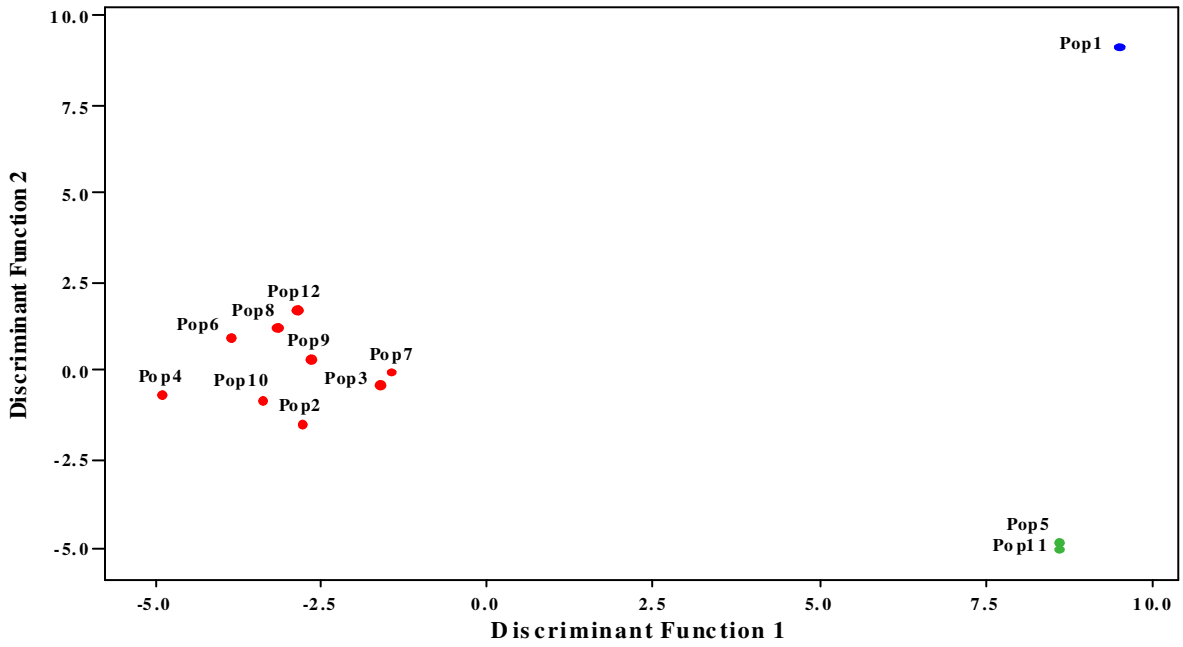

Figure 2. Scatter plot of the discriminant function analysis showing position of the 12 castor bean accessions.

Table 3. Inter cluster divergence (D^2) among 3 clusters involving 12 accessions of castor bean.

Cluster	1	2	3
1	0		
2	7.504	0	
3	10.27	8.125	0

Our results showed that, the most of studied accessions (75%) have been clustered together in group 2 indicating relatively low genetic variability in castor bean germplasm. Worldwide genetic diversity in 200 individuals comprising 41 castor bean accessions was assessed using amplified fragment length polymorphism and simple sequence repeat markers (Allan et al., 2008). They found that, despite surveying five continents and 35 countries, genetic diversity in castor bean germplasm is relatively low compared to estimates of genetic diversity in other plant species. Furthermore, at least one of their studied accessions was monomorphic at all AFLP loci and homozygous at all SSR loci.

The low levels of genetic variability could be the result of selective cultivation, domestication and long-term propagation of one or a few castor bean cultivars, which are nonetheless morphologically divergent (Allan et al., 2008). In addition, given that castor bean is used for both industrial and medicinal purposes, it is possible that the low-genetic diversity is the result of historical anthropogenic influence (Allan et al., 2008). Thus, the variation currently observed may be indicative of an overall reduction in genetic diversity mediating by human for particular cultivars followed by widespread planting and intercontinental trade of castor bean seed that might diffuse or obliterate local genetic differentiation. This theme of reduced genetic variation following domestication has been observed in other cultivated plant species such as soybeans and wheat, and is largely believed to be the result of intense selection for favorable traits. Concerning to castor bean, one can postulate that human-mediated selection for special characters, such as high-oil performance, may have helped shape the genetic variability of this species (Allan et al., 2008), as similar selective practices have dramatically altered the genetic makeup and diversity of other cultivated crops such as soybean (Hyten et al., 2006) and rice (Zhu et al., 2007). Another possible explanation for the low genetic variability in castor bean gene pool is that genetic diversity in genera of Euphorbiaceae may be comparatively lower than other dicots. Studies of natural populations of more distantly related Euphorbiaceae genera report higher levels of genetic diversity compared to castor bean (Park, 2004; Figueiredo-Goulart et al.,

Table 4. Eigenvalues, proportion of total variability and eigenvector of the first six principal components (PCs) with respect to 12 Iranian castor bean accessions used in present study.

Variable	PC1	PC2	PC3	PC4	PC5	PC6
x1	-0.22	0.06	-0.22	-0.07	-0.14	0.14
x2	-0.14	-0.22	-0.22	-0.11	0.18	-0.12
x3	*0.17*	-0.13	0.20	-0.06	*0.26*	0.23
x4	-0.14	-0.12	0.04	-0.19	-0.24	*0.49*
x5	*0.15*	0.19	*0.26*	-0.13	0.03	0.14
x6	-0.18	0.19	-0.21	-0.01	-0.10	0.20
x7	-0.10	0.06	*0.32*	-0.14	-0.34	0.20
x8	*0.19*	0.19	0.19	-0.21	0.00	-0.04
x9	-0.08	*0.27*	-0.19	0.14	0.13	*0.37*
x10	-0.24	-0.04	-0.08	-0.15	-0.22	-0.10
x11	-0.09	-0.16	-0.03	-0.02	*0.54*	-0.01
x12	-0.21	0.02	0.03	-0.25	0.32	0.01
x13	-0.26	0.12	-0.04	-0.06	0.07	0.03
x14	-0.25	0.12	-0.08	-0.08	0.00	0.03
x15	-0.16	*0.27*	0.11	-0.04	0.20	-0.08
x16	-0.25	0.01	0.22	0.07	0.01	0.01
x17	-0.25	0.03	0.20	0.02	-0.02	-0.04
x18	-0.22	0.02	*0.24*	0.11	-0.04	-0.23
x19	*0.15*	0.25	*0.22*	0.15	0.02	-0.04
x20	-0.11	0.24	-0.15	-0.19	-0.14	-0.27
x21	*0.16*	*0.27*	0.15	0.12	-0.05	-0.11
x22	-0.01	*0.36*	-0.17	0.13	0.03	0.12
x23	-0.22	0.17	-0.05	-0.09	-0.14	-0.26
x24	*0.15*	0.24	-0.17	0.13	0.09	*0.33*
x25	0.01	*0.28*	-0.14	*0.32*	0.08	-0.15
x26	-0.17	0.11	*0.28*	0.04	0.24	-0.02
x27	-0.26	0.04	-0.05	0.03	0.21	0.09
x28	-0.08	0.08	0.20	-0.30	0.18	0.11
x29	-0.19	0.02	0.17	*0.35*	-0.04	-0.04
x30	-0.20	-0.12	0.14	*0.29*	-0.04	0.18
x31	-0.18	-0.15	0.22	*0.28*	-0.09	0.08
x32	-0.06	-0.24	-0.13	*0.36*	0.02	0.04
Eigenvalue	12.60	6.16	4.24	2.87	2.29	1.56
Proportion	0.39	0.19	0.13	0.09	0.07	0.05
Cumulative	0.39	0.59	0.72	0.81	0.88	0.93

2005; Mwase et al., 2006). This comparison suggests that genetic diversity in natural populations of Euphorbiaceae is not limited, and that castor bean may be a unique case. However, studies of germplasm from citrus (Barkley et al., 2006), soybean (Powell et al., 1996), barley (Russell et al., 1997), cassava (Chavarriaga-Aguirre et al., 1999; Colombo et al., 2000) and chickpea (Sethy et al., 2006) show that levels of genetic diversity are considerably higher than those observed for castor bean. In contrast, germplasm collections of watermelon (Levi and Thomas, 2001) and peanut (Kochert et al., 1991; Lanham et al., 1992; He and Prakash, 2001) typically report levels of genetic diversity that are as low, or lower than those found in castor bean. These results suggest that genetic diversity in germplasm collections may in fact differ substantially and that the differences observed in genetic variation may simply be taxon specific.

Principal component analysis (PCA)

Principal component analysis (PCA) on 32 agro-morphological traits of 12 Iranian caster bean accessions was presented in Table 4. The first six principal components had eigen values more than one and

accounted for 93% of the total variation. The first component (PC1), which explained 39.4% of the total variation, was positively correlated with tertiary branch number, tertiary raceme length, total primary raceme weight, capsule weight on primary raceme, seed number on primary raceme whereas it was negatively correlated by primary raceme length, male flower length, secondary and tertiary branch fresh weight, secondary and tertiary branch dry weight, secondary and tertiary raceme weight, leaf and main stem characteristics. PC2 explained 19.3% of the total variation and positively influenced by female flower length, main stem diameter, total primary raceme weight, primary raceme weight without capsule and seed, capsule weight on primary raceme, total seed weight on primary raceme, seed number on primary raceme and hollow seed number on primary raceme whereas it was negatively correlated by secondary branch number and oil content. This means that the genotypes with high values of PC2 have low oil content and vice versa. PC3 accounted for 13.3% of the total variation and positively correlated with the secondary raceme length, tertiary branch length, secondary and tertiary raceme weight and leaf area but negatively correlated by total primary raceme weight and main stem length, secondary branch number and primary raceme length. According to breeding goal, castor bean breeders can select accessions by considering appropriate PCs values. Principal component analysis has been widely used in studying genetic variability in germplasm collections of many species (Julier et al., 1995; Veasey et al., 2001; Naghavi and Jahansouz, 2005; Bhargava et al., 2007; Nooryazdan et al., 2010).

Conclusion

In conclusion, studied accessions showed high coefficient of variation for hollow seed primary raceme number, secondary and tertiary branch fresh weight, secondary and tertiary branch dry weight, lamina leaf length and leaf length traits as well as moderate variation for oil present. The accessions based on studied traits were classified in 3 groups.

Our results showed that, the most of studied accessions (75%) have been clustered together in group 2 indicating relatively low genetic variability in castor bean germplasm. Principal component analysis (PCA) revealed that the first six principal components accounted for 93% of the total variation. Among the studied traits, seed number on primary raceme as a yield component in castor bean showed positive correlation with the first component (PC1). Hollow seed number on primary raceme showed positive correlation with the second component (PC2). Oil percent presented negative correlation with PC2. According to breeding goal, breeders can chose accessions by considering appropriate PCs values.

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