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AGRICULTURE AND ENVIRONMENTAL BIOTECHNOLOGY

EVALUATION OF GENETIC SIMILARITY AND AGRONOMIC TRAITS OF CASTOR BEAN POPULATIONS NATURALLY GROWN IN THE EASTERN MEDITERRANEAN REGION OF TURKEY

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ABSTRACT

Genetic characterization of wild castor bean genotypes collected from the eastern Mediterranean region of Turkey was evaluated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of seed storage proteins. Five distinct groups were identified from the cluster analysis of the castor bean genotypes studied at 0.80 coefficient level. Cluster analysis showed that some of the genotypes from the same location were grouped separately. Genetic similarity coefficients ranged from 0.36 to 0.95 among genotypes. The highest similarity (95%) was obtained between Iskenderun-1 and Iskenderun-2; Adana-4 and Iskenderun-2; Iskenderun-4 and Mersin-1. In addition to genetic diversity, plant characteristics such as stem color, seed color, days to maturity, plant height, main spike length, 100-seed weight and seed yield per plant were recorded. The results of both genetic similarity and agronomic analysis of castor bean genotypes would help in planning breeding programs for improving high yielding cultivars.

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Keywords: castor bean, cluster analysis, gel electrophoresis, genetic similarity, *Ricinus communis*, SDS-PAGE, seed storage proteins

Introduction

Castor bean (*Ricinus communis* L., family *Euphorbiaceae*) is an annual or perennial sometimes tree-like non-edible oil seed crop grown in low rainfall regions of the semi-arid tropics and sub-tropics (14, 18). Castor bean seeds contain 50-55% oil composed of the unusual hydroxylated fatty acid ricinoleic acid, which is used in industrial lubricants, paints, coatings, plastics, nylon fibers, artificial leather and bullet-proof glass (4). Castor is also a potential raw material for biodiesel production. It is native to the Ethiopian-East African region but has naturalized in moist tropical and subtropical regions throughout the world (28). The plants are also grown as ornamentals due to their prolific growth, distinctive vibrant leaves and striking spike color.

Weiss (28) stated that castor bean was introduced into Turkey by the ancient Romans to cultivate it for its oil, which they used as illuminant and for other purposes. After World War II, castor bean genotypes that were introduced for ornamental purposes escaped from landscapes and became wild. Castor bean grows wild in the roadside, field edges, bushy areas and riverbanks in all parts of the Aegean, Mediterranean and Southeastern Anatolia regions of Turkey. The cross pollination habit of the plant created the genetically mixed populations throughout the eastern Mediterranean region. Although Turkey is not the origin of castor bean, wild castor bean populations show great variation. There has been no attempt to breed the wild castor bean population in Turkey.

Molecular and biochemical markers are useful tools for estimation of genetic variability since they are not influenced by environmental factors (3). In addition to the high oil level, castor bean seeds also contain 15-20% protein. The predominant seed storage proteins of castor bean are 2S albumins, 7S lectins (ricin and its homologue *R. communis* agglutinin) and 11S globulins (7, 13, 19, 31). Among the numerous biochemical markers available for assessing the genetic variability and relatedness, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of seed storage proteins represents a valid alternative and has become an extensively used biochemical technique due to its simplicity and effectiveness for estimating genetic diversity (17). Seed storage proteins are highly polymorphic, largely independent of environmental factors and have been widely used for identification of genetic similarity and diversity (8, 9, 21). Therefore, the seed storage protein profiling of germplasms is used for several purposes such as legal plant cultivar protection, registration, certification, cultivar purity testing, crop origin and evolutionary studies (5, 10, 26, 30).

The information on the characteristics of castor bean genotypes is essential for its utilization in breeding programs aiming to improve the performance of castor bean as a crop. The purpose of the present study was to evaluate the genetic diversity using seed storage proteins electrophoresis (SDS-PAGE) and to evaluate the agronomic characteristics of the wild castor bean populations in the Eastern Mediterranean region of Turkey. To the best of our knowledge, this is the first detailed study to determine the genetic diversity and plant characteristics of wild castor bean populations in Turkey.

Materials and Methods

Plant material

A total of 17 castor bean genotypes, 16 from Adana, Mersin and Hatay provinces and one (GP-3 PI 631156) from the Plant Genetic Resources Conservation Unit, Griffin, GA, USA, were evaluated for genetic similarity and agronomic traits. The plant characteristics of the selected genotypes are shown in **Table 1**.

TABLE 1

Plant characteristics of selected castor bean genotypes grown in the eastern Mediterranean region of Turkey

Genotype	Stem color	Relative time of maturation	Seed color
Iskenderun-1	Green	Midseason	Brown
Iskenderun-2	Mix	Late	Tan
Iskenderun-3	Mix	Midseason	Tan
Iskenderun-4	Red	Midseason	Tan
Iskenderun-5	Mix	Midseason	Tan
Dortyol-1	Green	Late	Brown
Dortyol-2	Mix	Late	Brown
Reyhanli	Red	Midseason	Tan
Adana-1	Red	Late	Brown
Adana-2	Red	Late	Brown
Adana-3	Mix	Midseason	Tan
Adana-4	Red	Late	Brown
Adana-5	Red	Midseason	Tan
Mersin-1	Red	Midseason	Brown
Mersin-2	Mix	Midseason	Tan
Mersin-3	Red	Late	Tan
GP-3 (PI 631156)	Green	Midseason	Brown

Morphological and agronomic studies

The field experiment was carried out in the research farm of Mustafa Kemal University located in the eastern Mediterranean region of Turkey (36° 39′ N, 36° 40′ E; 83 m elevation) in 2008-2009. Castor bean seeds were planted in four 6 m rows with an inter-row spacing of 0.65 m and inner-plant spacing of 0.25 m. The experimental design was a randomized complete block with three replications. The crop was fertilized with 75 kg·ha^{-1} of N and 75 kg·ha^{-1} of P_2O_5. Proper irrigation and cultural practices were followed throughout the cropping season. The soil of the experimental plots was a clay silt loam with pH of 7.4, with 1.1% organic matter, 0.11% total nitrogen content, and water holding capacity of 0.36 cm^3. The long-term monthly mean temperatures from January to December were 8.3, 9.5, 13.2, 17.1, 21.2, 24.8, 27.2, 27.6, 25.6, 20.9, 14.1 and 9.4 °C, respectively. The long-term monthly mean precipitations from January to December were 172.6, 156.7, 141.4, 101.6, 90.5, 21.8, 23.1, 8.0, 39.9, 74.1, 114.3 and 172.2 mm, respectively. Investigated plant parameters were stem color, relative time of maturation, plant height (cm), raceme length (cm), seed color, 100-seed weight (g), seed yield (g/plant) and oil content (%).

Protein extraction

For the extraction of seed storage proteins, the seat coats of whole seeds were removed and ground with mortar and pestle. To remove fats, 0.01 g castor bean seed flour was mixed with 500 µl n-Hexane and then centrifuged at 15,000 rpm for 10 minutes at room temperature. After centrifugation, the supernatant was discarded and the pellet was resuspended in 400 µl of protein extraction buffer (0.5 M Tris-HCl, pH 6.8, 2.5% SDS, 5 M urea, 10% glycerol, 2% 2-mercaptoethanol) followed by vortexing for 5 min. The samples were kept at 40 °C overnight and then were centrifuged at 13,000 rpm for 10 min at room temperature. Bromophenol Blue was added to the sample buffer as a tracking dye. The extracted crude proteins were recovered as clear supernatant and stored at -20 °C until electrophoresis.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

The separating gel was prepared by mixing 10.0 ml 1.5 M tris HCl pH 8.8, 5.6 ml distilled water, 20 ml of 30% acrylamide and 0.8% N, N'-methylenebisacrylamide, 4.0 ml of 10% ammonium persulphate (APS) and 160 µl TEMED. The stacking gel was prepared by mixing 1 ml of 0.6 M tris HCl pH 6.8, 7.2 ml distilled water, 1.66 ml of 30% acrylamide, 100 µl of 10% SDS, 80 µl of 5% APS and 9 µl TEMED. The glass plates, silicon tube and comb were cleaned with ethanol. The resolving gel was poured up to 15 cm and layered with water. After removing the water, the staking gel was poured and a comb was inserted into the staking gel. The glass plate was fixed in the electrophoresis apparatus and the upper tank was filled with SDS running buffer. Twenty microliters of protein sample was loaded in each start. Molecular weight marker SM0661 (Fermentas) was used for rough estimation of the protein band sizes. Electrophoresis was performed at constant voltage (300 volts, 30 mA) till the tracking dye reached the bottom of the gel. After completing electrophoresis, the gel was stained with 0.5% coomassie brilliant blue (CBB) R-250 in acetic acid-methanol-water (3:22:25 volume ratio) for 6 h with continuous shaking. After staining, the gel was washed with water and put into decolorizing solution (7% acetic acid, 5% methanol) until the background disappeared. The gel was photographed by gel documentation system. To determine the banding pattern of each genotype two electrophoretograms were scored.

Data analysis

Genetic similarity of each genotype was estimated based on the presence (1) or absence (0) of protein bands. A distance matrix was calculated using the Jaccard index (24) and dendrogram was built using the unweighted pair group method with arithmetic mean (UPGMA) algorithm of the statistical software NTSYSPC, version 2 (20). Agronomic traits were subjected to analysis of variance using the GLM procedure of SAS. Means were separated using Fischer's Protected *LSD* test ($P < 0.05$).

Results and Discussion

Evaluation of genetic similarity based on SDS-PAGE of seed storage proteins

In the current study, seed storage protein variations of 16 castor bean genotypes from the eastern Mediterranean region of Turkey and one from the Plant Genetic Resources Conservation Unit, Griffin, GA, USA, were analyzed. The analyzed castor bean genotypes showed similar protein profiles based on SDS-PAGE of seed storage proteins (**Fig. 1**). A total of 22 protein bands with molecular weight ranging from 10 to 150 kD were recorded. There was high degree of homogeneity in the 5 major protein bands. The uniformity in the major bands among various genotypes indicated that the genes coding these proteins are highly conserved (12). However, there were variations in the minor bands. The similarity coefficient based on seed storage protein distances among castor bean genotypes ranged from 0.364 to 0.955 (**Table 2**). Maximum similarity (95.5%) was recorded between Iskenderun-1 and Iskenderun-2, between Adana-4 and Iskenderun-2, and between Iskenderun-4 and Mersin-1, showing a reduced level of polymorphism in the genetic make-up. Genotypes Iskenderun-3 and Iskenderun-5 showed maximum divergence (63.6%) from each other, demonstrating increased level of polymorphism in the genetic make-up.

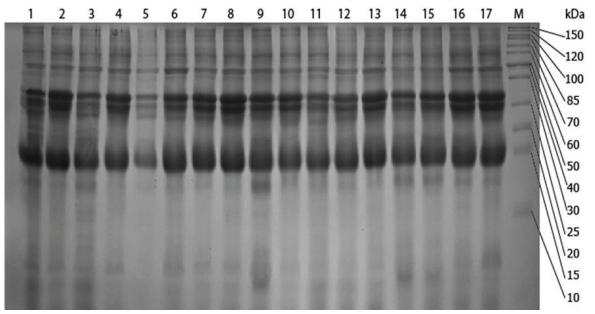

Fig. 1. Gel electrophoretograms of 17 castor bean genotypes grown in Eastern Mediterranean regions of Turkey. Lanes – **1:** Iskenderun 1; **2:** Iskenderun 2; **3:** Iskenderun 3; **4:** Adana 2; **5:** Adana 4; **6:** Adana 3; **7:** Adana 5; **8:** Adana 1; **9:** Mersin 2; **10:** Mersin 3; **11:** Mersin 1; **12:** Iskenderun 4; **13:** Iskenderun 5; **14:** Dortyol 1; **15:** Dortyol 2; **16:** Reyhanli; **17:** GP-3.

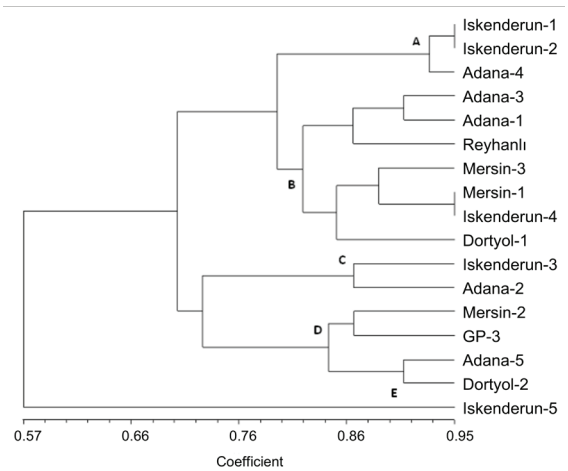

Fig. 2. UPGMA dendrogram of 17 castor bean genotypes based on SDS-PAGE of seed storage proteins.

Based on the genetic similarity coefficients, the total seed storage proteins fell into five clusters at the 0.80 coefficient level (**Fig. 2**). Cluster A consists of 3 genotypes – Iskenderun-1, Iskenderun-2 and Adana-4. Seven genotypes (Adana-3, Adana-1, Reyhanli, Mersin-3, Mersin-1, Iskenderun-4 and Dortyol-1) constitute the major cluster B. Cluster C consists of 2 genotypes – Iskenderun-3 and Adana 2. Cluster D consists of 4 genotypes – Mersin-2, GP-3, Adana-5 and Dortyol-2. Cluster E consists of 1 genotype – Iskenderun-5. According to these analyses, the following genotypes had over 90% similarity (Iskenderun-1 and Iskenderun-2; Adana-4 and Iskenderun-1; Adana-4 and Iskenderun-2; Adana-3 and Adana-4; Iskenderun-4 and Mersin-3; Dörtyol-1 and Mersin-1).

These results clearly indicated that the clustering patterns of castor bean genotypes collected from the eastern Mediterranean region might be attributed to cross pollination and introduction of genotypes for ornamental purposes. As a whole, the dendrogram showed high genetic similarity. Foster et al. (6) also reported high genetic similarity and low genetic diversity and structure for castor bean populations from a wide range of sources. High levels of genetic similarity in castor bean are consistent with comparatively high genetic similarity in some of the cultivated plants (11, 27, 29). In a study with soybean, Sihag et al. (23) found no definite relationship between genetic diversity and geographic diversity and they concluded that genetic diversity and geographic distribution were independent of each other.

Agronomic traits of castor bean populations

The analyzed genotypes differ in stem color, relative time of maturation and seed color. Stem color of the genotypes was red, green, and a mix between green and red (**Table 1**). Seed color of genotypes was brown and tan. When the relative maturation time was considered, the genotypes were either midseason or late season. The analysis of variance showed that there were significant differences among the 17 castorbean genotypes for raceme lenght, plant height, 100-seed weight, seed yield and curude oil content (**Table 3**). Plant height values varied between 47.0 and 202.3 cm. The highest and the lowest plant height values were obtained from Adana-1 and Dortyol-1, respectively. Genotype Adana-4 had the maximum seed yield (168.2 g/plant), followed by Iskenderun-3 and Iskenderun-2 (153.3 and 141.2 g/plant, respectively), while Dortyol-1 and Dortyol-2 had the lowest seed yields (31.3 and 35.7 g/plant, respectively). The oil content varied from 41.2% to as much as 54.0%. Dortyol-1 had the highest oil content, while Adana-3 had the lowest.

The investigated genotypes differed in stem color, relative time of maturation, raceme lenght, plant height, 100-seed weight, seed yield and crude oil content. The genotypes collected from the eastern Mediterranean are uncultivated wild perennial plants, but castor bean is grown as an annual crop for seed production (16). Except for plant height, the main raceme lenght, 100-seed weight, seed yield and oil content were higher than those previously reported for castor bean (22). Castor bean

TABLE 2

Similarity coefficients of 17 castor bean genotypes based on SDS-PAGE of seed storage proteins

Genotype	Iskenderun-1	Iskenderun-2	Iskenderun-3	Adana-2	Adana-4	Adana-3	Adana-5	Adana-1	Mersin-2	Mersin-3	Mersin-1	Iskenderun-4	Iskenderun-5	Dortyol-1	Dortyol-2	Reyhanli
Iskenderun-2	0.955															
Iskenderun-3	0.773	0.818														
Adana-2	0.727	0.682	0.864													
Adana-4	0.909	0.955	0.773	0.727												
Adana-3	0.818	0.864	0.773	0.727	0.909											
Adana-5	0.773	0.818	0.818	0.864	0.864	0.864										
Adana-1	0.727	0.773	0.682	0.727	0.818	0.909	0.864									
Mersin-2	0.773	0.818	0.727	0.682	0.773	0.682	0.818	0.682								
Mersin-3	0.818	0.864	0.682	0.545	0.818	0.818	0.682	0.818	0.773							
Mersin-1	0.773	0.818	0.636	0.591	0.864	0.864	0.727	0.864	0.636	0.864						
Iskenderun-4	0.727	0.773	0.591	0.545	0.818	0.818	0.682	0.818	0.682	0.909	0.955					
Iskenderun-5	0.500	0.545	0.364	0.409	0.591	0.591	0.545	0.682	0.455	0.591	0.727	0.682				
Dortyol-1	0.682	0.727	0.636	0.591	0.773	0.773	0.636	0.773	0.545	0.773	0.909	0.864	0.727			
Dortyol-2	0.682	0.727	0.727	0.773	0.773	0.773	0.909	0.773	0.909	0.682	0.636	0.682	0.455	0.545		
Reyhanli	0.727	0.773	0.591	0.636	0.818	0.818	0.773	0.909	0.773	0.818	0.864	0.818	0.682	0.773	0.773	
GP-3	0.636	0.682	0.591	0.636	0.727	0.636	0.773	0.727	0.864	0.727	0.682	0.727	0.500	0.591	0.864	0.818

TABLE 3

Combined data over two years (2008 - 2009) for plant height, main raceme length, 100-seed weight, seed yield and oil content of castor bean genotypes

Genotype	Plant height (cm)	Main raceme length (cm)	100-Seed weight (g)	Seed yield (g/plant)	Oil content (%)
Iskenderun-1	128.7	37.3	73.0	128.6	51.4
Iskenderun-2	171.7	37.3	74.2	141.2	51.6
Iskenderun-3	124.6	32.0	73.8	153.3	48.2
Iskenderun-4	172.5	39.0	61.9	104.3	49.9
Iskenderun-5	152.0	36.2	75.2	98.9	52.4
Dortyol-1	47.0	20.0	17.8	31.3	54.0
Dortyol-2	48.7	22.5	19.0	35.7	53.7
Reyhanli	123.3	34.0	69.6	117.8	53.3
Adana-1	202.3	40.7	59.9	104.6	46.3
Adana-2	124.0	17.0	76.0	110.2	51.3
Adana-3	166.0	35.7	53.3	56.9	41.2
Adana-4	151.3	30.7	72.7	168.2	42.9
Adana-5	152.3	39.7	70.7	118.9	49.1
Mersin-1	133.0	11.3	69.3	77.1	43.2
Mersin-2	101.0	40.0	68.0	101.7	43.0
Mersin-3	188.8	30.7	90.2	133.7	52.1
GP-3	74.0	17.3	39.9	79.2	53.2
LSD 5%	42.62	12.94	5.16	17.9	1.66

cultivars with short stature are prefered over taller cultivars due to ease of harvest. Except for Dortyol-1, Dortyol-2 and GP3, the rest of the genotypes had plant heights higher than 100 cm. Similar plant height results of castor bean collected from the Mediterrenean and South Eastern Anatolia regions were reported by Babagiray (1). Although the investigated genotypes were wild and have not been geneticaly improved, their seed yiled values were greater than the avarage seed yield values (850 and 1250 kg/ha) of most of the genotypes available in castor bean producing countries (**2, 15, 25)**. Our findings for seed yield were within the range of 1440 - 3150 kg/ha as earlier reported by Babagiray (1). However, Among the tested plant parameters, plant height is one of the main obstacles for cultivation of wild genotypes growing in the eastern Mediterrenean region and therefore should be improved.

Conclusions

Seventeen wild castor bean genotypes naturally growing in the eastern Mediterranean region of Turkey were successfully differentiated using SDS-PAGE of seed storage proteins. The dendrogram built on the basis of presence or absence of protein bands clustered all genotypes in five phylogenetic groups. The similarity coefficients varied between 0.364 and 0.955. The analyzed wild castor bean genotypes showed great variability for most of the investigated plant characteristics and agronomic traits, which makes them a promising source of genetic material for future breeding programs**.**

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