

Turkish Journal of Agriculture and Forestry

http://journals.tubitak.gov.tr/agriculture/

Diversity pattern among agromorphological traits of the Swiss chard (Beta vulgaris L. subsp. vulgaris) genetic resources of Turkey

M. Kadri BOZOKALFA*, Dursun EŞİYOK, Tansel KAYGISIZ AŞÇIOĞUL

Department of Horticulture, Faculty of Agriculture, Ege University, Bornova, İzmir, Turkey

Received: 21.12.2015 • Accepted/Published Online: 19.05.2016 • Final Version: 02.11.2016	
--	--

Abstract: Swiss chard (Beta vulgaris L. subsp. vulgaris) is a nutritionally rich leafy vegetable of moderate economic value. Despite being the first species among the genus Beta to be cultivated, knowledge regarding its biodiversity and agronomic and morphological properties is limited. The objective of this study was to evaluate the agromorphological characteristics of the Turkish Swiss chard genetic resources, and to determine their genetic relationships and diversity. To achieve this, a total of 52 Swiss chard accessions from a wide range of environmental and geographical origins in Turkey and two cultivars (one local and one foreign) were investigated. Fourteen qualitative and 13 quantitative agromorphological traits of these accessions were analyzed over the two consecutive growing seasons. Principal component analyses (PCA) explained 77.26% of the total variations for the agromorphological traits, while the hierarchical agglomerative clustering methods separated the accessions into four clusters and leaf weight, petiole width, petiole thickness, lamina length, and lamina width were the primary characteristics to distinguish the Swiss chard accessions. An extremely high degree of agromorphological diversity was observed in the Turkish Swiss chard genetic resources, and promising germplasm was identified to improve the cultivars for yield and leaf traits.

Key words: Agromorphological characterization, genetic diversity, multivariate analyses, Swiss chard, variability

1. Introduction

The genus Beta comprises four sections: Beta, Corollinae, Nanae, and Procumbentes (Shen et al., 1998), which include both wild and cultivated forms (Letschert et al., 1994). The cultivated forms comprise Beta vulgaris, including sugar beet, fodder beet, and chard (leaf beets) (Shen et al., 1998). While the native distribution of the genus Beta is primarily in Europe, the category Nanae is distributed in Greece, and Procumbentes is in the Canary Islands. Beta and Corollinae enjoy wider geographical distribution extending into Turkey (Frese et al., 2001).

Frese et al. (2001) recorded that the domestication of beets began in the Euphrates and Tigris basins of Turkey and in Greece, from where the cultivated beets were introduced to northern Europe (Boughey, 1981); however, Cheng et al. (2011) indicated different European and Mediterranean regions as the first center of domestication. Oyen (2004) supported the view that wild beet had been cultivated for its leaves in the 9th century in Mesopotamia. According to Sun (1994), cultivated beet has existed in China since the 5th century and was grown in Europe since ancient times. At present, beet is cultivated globally, in northern India, South America (Tindall, 1983), the

Mediterranean countries, and the USA (Bozokalfa et al., 2011).

The leaves and stems form the edible parts of the Beta plant and contain high amounts of health-promoting compounds such as phenolic acid and flavonoids (Pyo et al., 2004). Among the leafy vegetables, Swiss chard (Beta vulgaris L. subsp. vulgaris) is considered a good source of vitamins A, B, and C, and minerals including calcium, iron, and phosphorus (Huxley et al., 1992; Tindall, 1993; Maynard and Hochmuth, 1997; Bozokalfa et al., 2011).

Plant genetic resources are characterized by both morphological and molecular markers because the assessment of the genetic variation among and within the germplasm is fundamental for plant breeding and germplasm conservation (Pagnotta et al., 2011). Shen et al. (1998) reported genetic relationships within 11 taxa of the genus Beta with random amplified polymorphic DNA (RAPD) analysis. Some accessions collected from Turkey were members of different species/subspecies of the section Corrolinae, and the results noted that lomatogona, trigyna, and macrorhiza subsp. Turkish accessions were not well separated and further trigyna and macrorhiza accessions were clustered together. Genetic variation of

^{*} Correspondence: mehmet.kadri.bozokalfa@ege.edu.tr

the *Beta* species was underlined in several articles and leaf beet germplasm exhibited a relatively broad range of cytoplasmic genotypes while mitochondrial haplotype diversity was low in garden beet germplasm (Cheng et al., 2011). Desplanque et al. (1999) evaluated about 300 individuals composed of cultivated beets, table beet, weed beet, and wild coastal beet collected from several regions of France and a high degree of polymorphism was reported within all groups except for cultivated beets and indicated wild *B. vulgaris* species observed significant diversity. Furthermore, the population was grouped based on habitats and geographical characteristics.

Among the wild and cultivated *Beta* species, Swiss chard is a relatively valuable crop because the genetic structure contains genes valuable for crop improvement including disease and pest resistance (Goldman and Navazio, 2003). Moreover, Swiss chard served as a bridge species to introduce the desired traits in sugar beet by yielding viable hybrids from crosses with both *B. procumbens* and *B. webbiana* (Gaskill, 1954; Goldman and Navazio, 2003).

Swiss chard has considerable variability; however, after long-term cultivation, there are no concrete reports on the distribution of the genetic diversity or its relationship with agromorphological diversity. Turkey is the center of the origin and natural distribution area of the Swiss chard; however, there is a lack of information regarding the genetic diversity and relationships among the collections. The main objective of the present study was to preliminarily estimate the extent of genetic diversity of the Turkish Swiss chard genetic resources using agromorphological markers to assess the genetic relationships among the populations and to evaluate the morphological and agronomic characteristics of the collections.

2. Materials and methods

2.1. Plant material and experimental site

The material for this study consisted of 52 Swiss chard (*Beta vulgaris* L. subsp. *vulgaris*) accessions representing all the Swiss chard accessions collected from Turkey by the National Gene Bank of the Aegean Agricultural Research Institute (AARI), İzmir, Turkey, where the plant materials have been stored, along with two reference cultivars (Table 1). All accessions were grown and analyzed over two consecutive vegetation periods (2007 and 2008) in the experiment field at Ege University, Agriculture Faculty, Department of Horticulture, İzmir, Turkey, located at 38°28'N, 27°15'E, and at an altitude of 25 m above sea level. The climatic conditions of the experimental site are shown in Figure 1.

2.2. Experimental set-up

The seeds were sown in plots in the first week of October to raise 20 plants per 4.5 m^2 in both years. The field layout followed a completely randomized block design with three replications for each accession. Traditional cultivation practices were implemented, and fertilizers were not used during the cultivation. Weeds were manually controlled, and furrow irrigation performed on a weekly basis until the beginning of the rainy season. Plant samples were harvested when the leaves reached full maturity or were ready to eat.

2.3. Agromorphological characterization and data collection

Agromorphological traits were evaluated on 15 plant samples using the International Board for Plant Genetic Resources descriptors (IBPGR 1991) for Beta, with minor adaptations for some agronomic traits. The quantitative agromorphologic data measured were leaf weight (g): total weight of the harvested leaves divided by the number of leaves; plant height (cm): height of the main shoot from the ground level to the top during the flowering period; plant canopy (cm): average plant width measured during the flowering period; petiole length (cm): measured across the petiole; petiole width (mm): the petiole width across the widest portion of the petiole; petiole thickness (mm): the thickest point of the petiole; lamina length (cm): measured across the leaf; lamina width (cm): lamina width across the widest portion of the leaf; and leaf lamina color measured using a CR-300 colorimeter (Minolta, Osaka, Japan), and the data evaluated according to the Commission Internationale de l'Eclairage (CIE) system. The standard color determination by CIE is L*a*b, where L* is the light factor (lightness) and hue (°H): the values were calculated using the formula $^{\circ}H = \tan - 1(b/a)$; chroma (C^*): the values were calculated using the formula $C^* = \sqrt{(a^2 + b^2)}$. Other parameters analyzed included seed weight (g) calculated and expressed as 100 seeds and leaf dry matter (%) determined by oven-drying the leaf laminas and petioles at 65 °C until the weight loss between the measurements was <0.05 g and subsequently calculating the dry matter based on the difference between the fresh and dry weights.

The qualitative agromorphological observations of leaf pigmentation, petiole color, leaf curliness, leaf hairiness, cuticle thickness, annuality, plant growth habit in the flowering stage, tepal shape, keel formation, tepal attitude, bract distribution on the seed stalk, stem color, stem pigmentation, and the number of seeds per seed coat were scored and classified according to the descriptor (IBPGR, 1991).

2.4. Statistical analysis

Quantitative and qualitative scales of the agromorphological traits were used to prepare a raw data set of both years. Principal component analysis (PCA) was performed for combined quantitative and qualitative agromorphological data, and the total variation was calculated as the sum of the extracted eigenvalues. Estimates for the Euclidean

BOZOKALFA et al. / Turk J Agric For

Accession number	Collection locale	Country of origin	Altitude (m)
TR 30741	Gaziantep	Turkey	
TR 35012	Çanakkale	Turkey	
TR 35065	Bursa	Turkey	300
TR 35137	Tokat	Turkey	630
TR 35164	Kayseri	Turkey	1050
TR 35180	Sivas	Turkey	
TR 35278	Şanlıurfa	Turkey	
TR 35821	Mardin	Turkey	550
TR 35289	Mardin	Turkey	550
TR 35316	Hakkari	Turkey	1550
TR 35331	Kahramanmaraş	Turkey	
TR 35354	Muğla	Turkey	600
TR 35355	Muğla	Turkey	120
TR 35393	Hatay	Turkey	155
TR 40459	Siirt	Turkey	1320
TR 43621	Sakarya	Turkey	30
TR 46354	Kayseri	Turkey	1260
TR 51154	Hatay	Turkey	140
TR 51160	Adana	Turkey	15
TR 51169	Mersin	Turkey	10
TR 51170	Mersin	Turkey	20
TR 51194	Muğla	Turkey	
TR 51199	İzmir	Turkey	
TR 52424	Erzurum	Turkey	1400
TR 52488	Artvin	Turkey	410
TR 55632	Giresun	Turkey	1100
TR 55633	Giresun	Turkey	1200
TR 55664	Giresun	Turkey	150
TR 55689	Trabzon	Turkey	350
TR 55756	Rize	Turkey	50
TR 55767	Rize	Turkey	250
TR 55773	Rize	Turkey	350
TR 55778	Rize	Turkey	350
TR 55787	Rize	Turkey	50
TR 55800	Rize	Turkey	10
TR 55821	Rize	Turkey	400
TR 55832	Artvin	Turkey	20
TR 55848		Turkey	4
TR 55866	Artvin	Turkey	75
	Artvin	1	
TR 55879	Artvin	Turkey	200
TR 55889	Artvin	Turkey	20
TR 55931	Rize	Turkey	10
TR 55936	Rize	Turkey	4
TR 55983	Trabzon	Turkey	500
TR 55993	Trabzon	Turkey	750
TR 55999	Trabzon	Turkey	100
TR 56010	Trabzon	Turkey	700
TR 56017	Giresun	Turkey	10
TR 56046	Ordu	Turkey	20
TR 71077	Kayseri	Turkey	1463
TR 73437	Turkey	Turkey	
TR 73438	Turkey	Turkey	
Local cultivar	Pinaper seed	Turkey	
Foreign cultivar	Freya	Germany	

Table 1. List of accessions, collection locale and origin of Swiss chard used in present study.

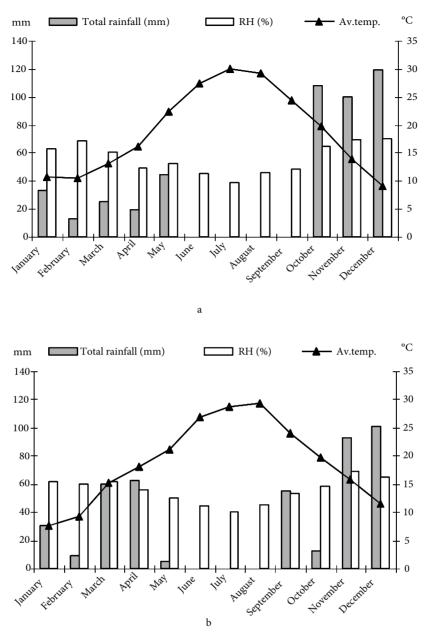


Figure 1. Climatic condition during (a) 2007 and (b) 2008.

dissimilarity coefficients for the agromorphological data were used to assess the relationships between the genotypes, and hierarchical agglomerative clustering was subsequently performed on the principal component (PC) axes obtained using the unweighted pair-group method with arithmetic average (UPGMA) (Sneath and Sokal, 1973; Rabbani et al., 1998). Principal coordinate analyses were performed based on a distance matrix and a three-dimensional (3D) scatter plot was then prepared with the first three principal coordinates to visualize the relationship explaining the quantitative and qualitative agromorphological traits. Descriptive value, frequency of traits, and frequency distribution for total accessions were determined. The mean, maximum, and minimum values and standard deviations of 14 agromorphological quantitative traits were calculated. All data analyses were performed using the software Statistica.

3. Results

The accessions characterized displayed great diversity for agromorphological traits. Both qualitative and quantitative characters evaluated in the experiment showed a large degree of variation.

3.1. Agromorphological characters

Descriptive statistics including mean, maximum, minimum, and standard deviation values for the quantitative agromorphological characters of Swiss chard accessions are shown in Table 2. The range of variation for the quantitative characters examined revealed high variability for all agromorphological characters. Large variations, indicating a high level of diversity, were determined in the plant traits including leaf weight (8.25–51.87 g), plant height (67.0–157.67 cm), plant canopy (40.64–140.59 cm), petiole width (0.64–2.22 cm), petiole length (5.71–17.63 cm), and chroma (118.65–136.63).

Some of the 14 qualitative agromorphological traits were highly variable in their descriptive value, frequency, and frequency distribution and Table 3 shows remarkable differences among the accessions. Leaf color varied from yellow to light green with red veins. Most of the accessions demonstrated extremely sparse leaf hairiness, and only 4 accessions (TR 55778, TR 55866, TR 55773, TR 55787) produced medium cuticle thickness. A total of 44 accessions revealed erect plant growth habits. All the accessions showed concave tepal shapes with ecarinate keel formation and semierect/connivent tepal attitude. Seed bract was distributed at the top of the plant and stem color was yellow to light green in the populations examined. The number of seeds per seed coat depended on the accessions; 8 accessions exhibited monogerm or bigerm, 30 accessions ranged from 2 to 4 seeds, and 16 showed more than 5 seeds per seed coat.

3.2. Principal component analysis (PCA)

Quantitative and qualitative agromorphological characters, variation proportion, component loading, and eigenvalues are listed in Table 2. PCA explained over 77% of total variation for 27 quantitative and qualitative

Table 2. Minimum (Min), mean (Mn), maximum (Max), and standard deviation (SD) values for quantitative agromorphological characters and eigenvalues proportion of variability of agromorphological traits contributed to the first eight PCs of Swiss chard accessions.

					PC axis							
					1	2	3	4	5	6	7	8
Eigenvalues					4.76	2.70	2.03	1.66	1.55	1.26	1.23	1.04
Explained proportion of variation (%)					22.66	12.84	9.65	7.91	7.40	6.00	5.86	4.95
Cumulative proportion of variation (%)					22.66	35.50	45.15	53.05	60.45	66.45	72.31	77.26
Characters					Eigenval	ıes						
Petiole color					-0.278	0.510	0.311	0.480	0.065	-0.076	0.301	0.062
Plant growth habit at flowering stage					-0.061	0.462	0.148	0.424	0.309	0.177	-0.101	-0.378
Cuticle thickness					-0.002	0.172	-0.168	-0.642	-0.135	0.029	-0.325	-0.329
Leaf pigmentation					-0.015	0.491	0.230	0.320	-0.520	-0.045	-0.075	0.255
Stem pigmentation					-0.039	0.275	-0.041	0.122	0.485	-0.460	-0.472	0.186
Leaf hairiness					0.033	0.317	-0.362	0.176	-0.449	-0.161	-0.234	0.292
Leaf curliness					-0.182	-0.215	0.030	-0.270	0.009	-0.478	0.476	0.336
Number of seed per seed coat					-0.389	0.261	-0.032	0.023	0.037	0.473	0.418	-0.065
	Min	Mn	Max	SD								
Leaf weight (cm)	8.25	22.91	51.87	7.58	0.881	-0.165	-0.011	0.044	0.053	0.132	-0.059	0.086
Petiole width (cm)	0.64	1.18	2.22	2.60	0.812	-0.242	0.032	0.040	-0.029	0.021	-0.081	0.058
Petiole thickness (cm)	0.46	0.82	1.36	0.17	0.801	-0.217	-0.045	0.128	-0.069	0.028	0.122	0.220
Lamina length (cm)	12.54	19.01	26.09	2.66	0.764	-0.303	0.121	0.165	0.197	-0.020	0.154	-0.112
Petiole length (cm)	5.71	12.29	17.63	3.24	0.673	0.082	-0.253	-0.022	0.082	0.407	-0.014	0.189
Lamina width (cm)	9.78	13.98	22.10	2.25	0.729	0.219	0.308	0.154	-0.199	0.059	-0.049	-0.006
Chroma	118.65	123.41	136.63	2.35	0.203	0.344	-0.456	-0.112	0.420	-0.149	0.339	0.163
Hue°	15.63	22.73	37.87	3.13	-0.472	-0.630	0.382	0.035	-0.099	0.012	-0.094	0.090
Lightness	36.42	41.20	49.94	2.39	-0.487	-0.605	0.167	0.178	-0.058	0.229	-0.102	0.213
Plant height (cm)	67.00	120.65	157.67	22.16	0.209	0.418	0.617	-0.333	0.220	-0.015	-0.165	0.248
Plant canopy (cm)	40.64	99.28	140.59	22.83	0.224	0.278	0.604	-0.578	-0.017	0.085	0.147	0.051
Seed weight (g)	1.01	1.83	3.47	5.48	-0.292	-0.268	0.110	0.134	0.526	0.246	-0.222	0.304
Leaf dry matter (%)	9.02	10.89	18.53	1.48	0.373	-0.314	0.260	0.237	0.003	-0.484	0.095	-0.450

BOZOKALFA et al. / Turk J Agric For

Table 3. Qualitative agromorphological t	raits, descriptive value, frequen	cy of traits, and frequency	y distribution for total accessions.

					Cluster				
No.	Trait	Description	Class	Frequency (%)	Cluster 1 2 3 4				
	Leaf pigmentation	Absent	0	51.8	1.8		25.9	24.1	
1		Red vein	2	48.1	25.9	20.4		1.8	
2	Petiole color	Yellow/light Green	2	24.1	3.7	5.6	13.0	1.8	
		Green	3	66.7	18.5	14.8	9.3	24.1	
		Pink	4	7.4	3.7		3.7		
		Red vein	5						
	Leaf curliness	None	1	13.0	1.8		7.4	3.7	
		Smooth	3	25.9	9.3	9.3	1.8	5.6	
3		Medium	5	46.3	14.8	9.3	1.0	11.1	
		Curled	7	14.81	1.8	1.8	5.6	5.6	
	Leaf hairiness	Glabrous	1	72.2	22.2	1.8	16.7	14.8	
			3	14.8	1.8	1.8	7.4	3.7	
4		Very sparse	5	14.8	3.7		1.8	7.4	
		Hairy Norm haire	7						
	Cratial a thirdrease	Very hairy	5					25.9	
5	Cuticle thickness	Medium	7	92.6	25.9	18.5	22.2		
	A	Thick Annual		7.4	1.8	1.8	3.7		
6	Annuality	Biennial	1	100.0	27.8	20.4	26.0	25.9	
	Direct over the back it to the second second		2						
	Plant growth habit at flowering stage	Erect	1 2	81.5	20.4 7.4	20.4	18.5 5.6	22.2	
7		Erect and procumbent		14.8				+	
		Procumbent	3	1.8			1.8		
	Turliburg	Erect/rostrate	4	1.8				1.8	
8	Tepal shape	Straight	1 2						
	K al Gammatian	Concave curved	1	100.0	27.8	20.4	25.9	25.9	
9	Keel formation	Ecarinate	1 2	100.0	27.8	20.4	25.9	25.9	
	77 1 w 1	Carinate					ł		
10	Tepal attitude	Erect	1						
		Semierect connivent	2	100.0	27.8	20.4	25.9	25.9	
	Distribution of bracts on the seed stalk	Bract absent	0						
11		Some present on the lower part	1						
		Inflorescence half way up	5 9						
	Chan eilen	Bract to the top		100.0	27.8	20.4	25.9	25.9	
10	Stem color	White	1						
12		Yellow/light green	2	100.0	27.8	20.4	25.9	25.9	
13	Channa di successi di suc	Green	3						
	Stem pigmentation	Absent	0	59.3	13.0	14.8	16.7	14.8	
		Striped	1	36.0	11.1	5.6	7.4	11.1	
		Entire red	2	3.7	1.8		1.8		
		All	3	1.8	1.8				
	Number of seed per seed coat	Monogerm	1						
14		Monogerm and bigerm	3	14.8	3.7	3.7	5.6	1.8	
		Multigerm (2–4)	5	77.8	24.1	13.0	16.7	24.1	
		Multigerm (>5)	7	7.4		3.7	3.7		

agromorphological characters. The first PC (22.66% of the total variation) was chiefly related to leaf size parameters, of which leaf weight, petiole width, petiole thickness, lamina length, petiole length, and lamina width are the most discriminate characters among the plant traits examined. The second PC explained 12.84% of the total variation and petiole color, plant grown habit at flowering stage, leaf pigmentation, chroma, hue, and lightness showed the highest eigenvalues. The third PC (9.65% of the total variation) mainly comprised plant height, plant canopy, and chroma. The fourth PC (7.91% of the total variation) emphasized the cuticle thickness, plant canopy, and petiole color. The highest eigenvalues in the fifth PC, which explained 7.40% of the total variation, mainly related 100 seed weight, leaf pigmentation, stem pigmentation, and leaf hairiness. The sixth PC (6.0% of the total variation) mainly comprised leaf curliness, stem pigmentation, and leaf dry matter. The remaining two axes, PC 7 and PC 8, were related to stem pigmentation and leaf dry matter, and explained 5.86% and 4.95% of the total variation, respectively.

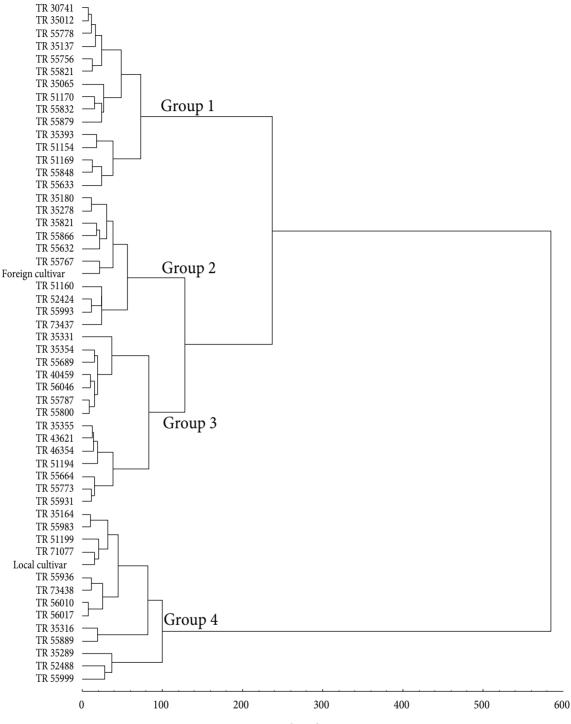
3.3. Cluster analysis

The multivariate cluster analysis performed for all the agromorphological plant traits categorized the Swiss chard accessions examined into four independent clusters (Figure 2). The first cluster comprised 15 accessions and were characterized by the highest plant height (146.5 cm), plant canopy (114.7 cm), lamina width (14.8 cm), and chroma (124.2), and the lowest leaf dry matter (10.8%). The second cluster included 11 accessions and were characterized by lower leaf weight (19.5 g), lamina length (18.9 cm), petiole thickness (0.77 cm), petiole width (1.13 cm), and chroma (122.6). The third cluster was made up of 14 accessions and represented the highest leaf weight (25.7 g) and petiole length (13.2 cm) and showed the lower values in lightness (39.7), hue (21.5), and 100 seed weight (1.5 g). The fourth cluster consisted of 14 accessions characterized by higher lamina length (20.6 cm), petiole thickness (0.98 cm), petiole width (1.34 cm), lightness (43.4), hue (25), 100 seed weight (2.1 g), and leaf dry matter (12.4%), while registering lower values for plant height (92.3 cm), plant canopy (75.9 cm), petiole length (7.7 cm), and lamina width (12.3 cm).

In order to visualize the relationships of the quantitative morphological traits examined, principal coordinate analyses (PCoA) were applied and the first three PCoA are given as 3D screen plots (Figure 3).

4. Discussion

Among the *Beta* sections, Swiss chard is grown as a minor crop compared with the other species within this genus. However, the significance of this plant has increased because of its genetic structure (i.e. resistance to drought conditions, tolerance of moderate salinity), and Swiss chard is gaining in importance as a horticultural crop for a Beta species breeding program in the future. Leaf beet and garden beet have desirable resistance against the biotic and abiotic stress agents that can be considered for sugar beet crop enhancement programs (Santamaria, 1999; Baranski et al., 2001; Pyo et al., 2004). Swiss chard also contains several health-promoting compounds and is extremely rich in mineral composition (Bozokalfa et al., 2011). The plant is used for medicinal purposes (Bolkent et al., 2000; Ninfali et al., 2007), and also contains betalains (Kugler et al., 2004), polysaccharides (Potter and Long, 1965), ascorbic acid (Schudel et al., 1979), and flavonoids (Dijioux et al., 1995), and colored petioles utilized in food coloring due to their large quantities of betalains. Breeding programs can considerably increase the betalain content of the Swiss chard (Kugler et al., 2004). However, Cheng et al. (2011) emphasized that leaf beet has never been bred intensively, thereby resulting in the assumption that plenty of variation is still possible in the leaf beet cultivars/landrace and detailed morphological and agronomic properties and genetic diversity are yet to be comprehensively investigated. This paper is the first attempt to identify the Turkish Swiss chard genetic resources with respect to the agromorphological quantitative and qualitative characters and to analyze the diversity of the collections examined. Quantitative characters are more useful in the assessment of varietal descriptors and variety identification, while the quantitative characters are mainly related to variety improvement programs (Panthee et al., 2006). A large variability in the agromorphological traits was observed among the accessions between those of the different geographical locations. Leaf weight, petiole width, petiole thickness, lamina length, petiole length and lamina width are the most discriminating characters among the plant traits examined. Plant height (67.0-157.67 cm) and petiole width (0.64-2.22 cm) are consistent with those reported by Pokluda and Koben (2002), who stated that statistically significant differences in plant weight, plant height (42.5-57.9 cm), stalk width (1.36-3.55 cm), leaf number, and yield were found influenced according to the variety. The diversity exhibited among the accession based on morphologic traits was confirmed by Baranski et al. (2001), who emphasized the significant differences observed among garden beets in terms of leaf weight, leaf pigmentation, yield, morphology, and the chemical composition of the roots. Furthermore, they stated that morphological traits enabled the accessions to be distinguished into different classes. Distinctive morphological characteristics were reported with respect to flower, leaf, and root properties of the Beta section (Ford Lloyd and Williams, 1975).



Linkage distance

Figure 2. Unweighted pair-group method with arithmetic mean (UPGMA) dendrogram based on the dissimilarity matrix constructed from 27 agromorphological traits for 54 Swiss chard accessions.

Although some accessions were collected from considerably close geographical locations, the multivariate analyses of the 54 Swiss chard accessions revealed a high variability for quantitative agronomic plant traits, particularly in the foliage properties. PCA distinguished 21 agromorphological traits into 8 components and explained 77.26% of the total variations. The cluster and PC analyses results revealed that the Swiss chard

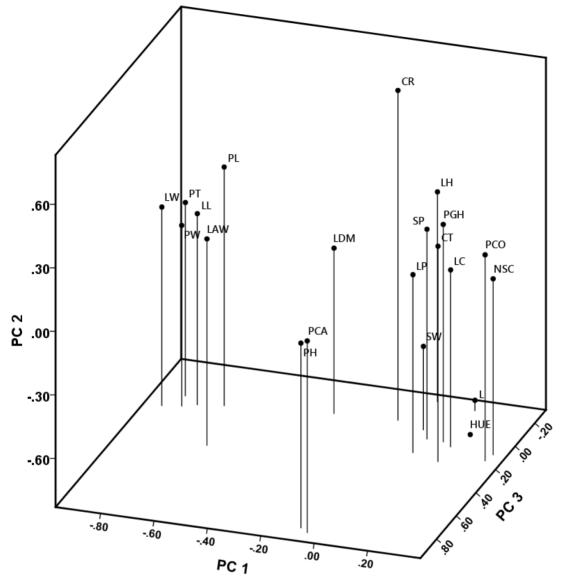


Figure 3. Principal coordinate analysis of discriminating agromorphological traits among Swiss chard accessions. PC1, PC2, and PC3 the first second and third principal components explaining 22.6%, 12.84%, and 9.65% of the total variation respectively. (LW: leaf weight, PW: petiole width, PT: petiole thickness, LL: lamina length, PL: petiole length, LAW: lamina width, CR: chroma, HUE: hue°, L: lightness, PH: plant height, PCA: plant canopy, SW: 100 seed weight, LDM: leaf dry matter, PCO: petiole color, PGH: plant growth habit at flowering stage, CT: Cuticle thickness, LP: leaf pigmentation, SP: stem pigmentation, LH: leaf hairiness, LC: leaf curliness, NSC: number of seed per seed coat).

populations examined had different agromorphological plant properties and, therefore, the accessions were grouped under four clusters. Even if several accessions were collected from the same vicinity, the clusters did not correlate well with the geographical origin. Similar results in terms of correlating the genetic variations with the leaf traits among the leaf beet germplasm were reported by Frese (1991), who evaluated the germplasm from several countries and clustered the accessions under five groups based on leaf color, leaf curliness, leaf length, plant height, and petiole length. The genetic variability among the Swiss chard genotypes was reported not only for the agromorphological traits, but also emphasized for the mineral concentration and nutritive value; and the variability observed for the nutrient composition among the cultivars, and nonhybrid and wild forms of Swiss chard and the varietal differences were reported for vitamin C concentration and the phenolic compounds in the white and red stem chard cultivars (Rozycki et al., 1997; Gil et al., 1998; Pyo et al., 2004). In fact, our earlier studies indicated that the present Swiss chard accessions revealed a high degree of variation for the primary and secondary nutrient compositions, and using the hierarchical agglomerative clustering method they were grouped by accession into five major clusters for the concentration of the 12 nutritive elements and it is this great variability that can be responsible for the genetically diverse populations (Bozokalfa et al., 2011). Furthermore, to evaluate the genetic structure of the present populations and documented comprehensive explanation on the variability of accessions, analysis of variance was calculated for the agronomic traits. The results revealed significant variations among all the accessions studied, in all the plant traits, at P > 0.01 (Eşiyok et al., 2011). Phenotypic coefficients of variation were higher for all the plant traits than the corresponding genotypic coefficients of variability; in addition, the lamina length, lamina width, petiole thickness, petiole width, lightness, chroma, and leaf dry matter showed high heritability with high genetic advancement in a percent mean, which provides valuable data for evaluating the agronomic traits and accessions for phenotypic selection (Eşiyok et al., 2011). Identification diversity of the Swiss chard gene pools may contribute to valuable scientific knowledge in terms of new breeding programs for the improvement of targeted agronomic or health benefit compounds and leaf beet can be considered a potentially valuable source of the desirable characters absent in the sugar beet breeding programs (Baranski et al., 2001).

The results contributed to a better understanding of the genetic relationships existent among the Turkish Swiss chard genetic resources. Besides the research findings, there are increased expectations of high diversity among the species and/or subgroups of the Beta section in Turkey that are located in the center of the origin and domestication area of the genus Beta (Tan et al., 1999; Frese et al., 2001; Biancardi et al., 2012) and that are rich in wild and cultivated forms. Swiss chard has been grown as a home garden plant along with the other Beta species and while limited cultivars are available in the markets, cultivations by farmers are mostly based on landraces by exchange of genetic material, the local varieties being empirically selected by growers who failed to apply any isolation protocols for seed production over time and those well adapted to the environmental conditions. The large diversity may be explained by gene flow and introgression observed in the cultivated beets, i.e. sugar beet, garden beet, and leaf beet, which are cross-compatible with the wild taxon, i.e. sea beet (Bartsch et al., 1999). In addition, Barocka (1985) concluded that B. vulgaris is generally selfincompatible, wind-pollinated, and highly out-crossed, resulting in increased genetic diversity.

Plant genetic resources are the primary sources for plant breeding; therefore, agromorphological and molecular characterizations are the first step in the identification and evaluation of the existing germplasm. In-depth analyses of plant properties increase the effectiveness of genetic conservation and plant breeding programs. Identification of the accessions within the Turkish Swiss chard germplasm with regard to useful agronomic and morphological traits is of particular importance for future research and breeding programs on this species. Geographical differentiation of the distribution area accompanied by the ecological conditions (such as latitude, altitude, temperature, and moisture) has resulted in ecotypical differentiation that has further affected the development of the wild Beta relatives. Resistance to biotic and abiotic stress conditions could partly contribute to the wide diversity (Rao and Hodgkin, 2002). The introduction of new germplasm containing potentially useful characteristics increases the genetic base. Genetic variability in the Swiss chard accessions is relatively high in Turkey, and reliable data on such variability are essential for future studies aimed at the conservation, improvement, and management of Swiss chard. Evaluation of the germplasm based on the agromorphological traits, mineral concentrations (Bozokalfa et al., 2011), and genetic variability combined with the character associations accompanied by heritability (Eşiyok et al., 2011), and the current paper demonstrate the importance of the Turkish Swiss chard genetic collection in the context of biodiversity, which includes the germplasm containing rich and valuable plant material. This study presents valuable information on the national genetic resources, e.g., accessions with high plant growth rate, higher leaf weight, or number of leaves per plant, with the potential for yield enhancement. Some late flowering (data not shown) accessions can be considered to delay the production in late spring or early summer, implying that the germplasm could be used to further improve the cultivars possessing desirable characters.

Acknowledgments

The authors thank the Aegean Agricultural Research Institute Gene Bank, İzmir, Turkey, for providing the seeds samples. Part of this work was supported through funds from the Ege University Scientific Research Fund under grant number 2008-ZRF-002.

References

- Baranski R, Grzebelus D, Frese L (2001). Estimation of genetic diversity in a collection of the Garden Beet Group. Euphytica 122: 19-29.
- Barocka KH (1985). Zucker und Futterrüben. In: Fischbeck G, Plarre W, Schuster W, editors. Lehrbuch der Pflanzenzüchtung Landwirtschaftlicher Kulturformen, Bd, 2, Spezieller Teil. Berlin, Germany: Paul Parey, pp. 245-287.
- Bartsch D, Lehnen M., Clegg J, Pohl-Orf M, Schuphan I, Ellstrand NC (1999). Impact of gene flow from cultivated beet on genetic diversity of wild sea beet populations. Mol Ecol 8: 1733-1741.
- Biancardi E, Panella LW, Lewellen R (2012). Beta Maritima: the Origin of the Beets. Springer Verlag. New York/Heidelberg, pp. 293.
- Bolkent S, Yanardağ R, Tabakoğlu-Oğuz A, Özsoy-Saçan Ö (2000). Effects of chard (*Beta vulgaris* L. var. *cicla*) extract on pancreatic B cells in streptozotocin-diabetic rats: a morphological and biochemical study. J Ethnopharmacol 73: 251-259.
- Boughey CL (1981). Evolutionary and taxonomic studies in wild and cultivated beets. PhD, University of Birmingham, Birmingham, UK.
- Bozokalfa MK, Yağmur B, Kaygısız Aşçıoğul T, Eşiyok D (2011). Diversity in nutritional composition of Swiss chard (*Beta vulgaris* subsp. L. var. *cicla*) accessions revealed by multivariate analysis. Plant Genet Resour-C 9: 557-566.
- Cheng D, Yoshida Y, Kitazi K, Negoro S, Takahashi H, Xu D, Mikami T, Kubo T (2011). Mitochondrial genome diversity in *Beta vulgaris* L. ssp. *vulgaris* (Leaf and Garden Beet Groups) and its implications concerning the dissemination of the crop. Genet Resour Crop Ev 58: 553-560.
- Desplanque B, Boudry P, Broomberg K, Saumitou-Laprade P, Cuguen J, Van Dijk H (1999). Genetic diversity and gene flow between wild, cultivated and weedy forms of *Beta vulgaris* L. (Chenopodiaceae), assessed by RFLP and microsatellite markers. Theor Appl Genet 98: 1194-1201.
- Dijioux MG, Lavaud C, Massiot G, Le Men-Olivier L (1995). Flavonoids from *Beta vulgaris* varieties. Fitoterapia LXVI, 195.
- Eşiyok D, Bozokalfa MK, Kaygısız Aşçıoğul T (2011). Variability heritability and association analysis in plant traits of Swiss chard (*Beta vulgaris* subsp. *cicla*). Genetica 43: 239-252.
- Ford Lloyd BV, Williams JT (1975). A revision of *Beta* section *vulgares* (Chenopodiacea) with new light on the origin of cultivated beets. Bot J Linn Soc 17: 89-102.
- Frese L (1991). Variation patterns in leaf beet (*Beta vulgaris*, Chenopodiacea) germplasm collection. Plant Syst Evol 176: 1-10.
- Frese L, Desprez B, Ziegler D (2001). Potential of genetic resources and breeding strategies for base-broadening Beta. In: Cooper HD, Spillane C, Hodgkin T, editors. Broadening the Genetic Base of Crop Production. Wallingford, UK: CABI Publishing, pp. 295-309.

- Gaskill JO (1954). Viable hybrids from matings of chard with *Beta* procumbens and *B. webbiana*. ASSBT 8: 5.
- Gil MI, Ferreres F, Tomas-Barberan FA (1998). Effect of modified atmosphere packaging on the flavonoids and vitamin C content of minimally processed Swiss chard (*Beta vulgaris* ssp. *cicla* L.). J Agr Food Chem 46: 2007-2012.
- Goldman IL, Navazio JP (2003). History and breeding of table beet in the United States. Plant Breed Rev 22: 357-388.
- Huxley AJ, Griffiths M, Levy M (1992). The New Royal Horticultural Society, Dictionary of Gardening (4). London, UK: The Macmillan Press and The Stockton Press.
- IBPGR (1991). Descriptors for Beta. International Board for Plant Genetic Resources, Rome, Centre for Genetic Resources, the Netherlands.
- Kugler F, Stintzing FL, Carle R (2004). Identification of betalains from petioles of differently colored Swiss chard (*Beta vulgaris* L. ssp. *cicla* [L.] Alef. cv. Bright Lights) by high-performance liquid chromatography electrospray ionization mass spectrometry. J Agric Food Chem 52: 2975-2981.
- Letschert JPW, Lange W, Frese L, Van Den Berg RG (1994) Taxonomy of *Beta* section *Beta*. J Sugar Beet Res 31: 69-85.
- Maynard DN, Hochmuth GJ (1997). Knott's Handbook for Vegetable Growers. 4th ed. New York, NY, USA: John Wiley and Sons.
- Ninfali P, Bacchiocca M, Antonelli A, Biagiotti E, DiGiacchino AM, Piccoli G, Stocchi V, Brandi G (2007). Characterization and biological activity of the main flavonoids from Swiss chard (*Beta vulgaris* subspecies *cycla*). Phytomedicine 14: 216-221.
- Oyen LPA (2004). Vegetables "*Beta vulgaris* L.". In: Grubben GJH, Denton OA, editors. Plant Resources of Tropical Africa 2. Vegetables, PROTA Foundation, Wageningen, Netherlands/ Backhuys Publishers, Lieden, Netherlands CTA, Wageningen, Netherlands, pp. 110-113.
- Pagnotta MA, Annicchiarico P, Farina A, Proietti S (2011). Characterizing the molecular and morphophysiological diversity of Italian red clover. Euphytica 179: 393-404.
- Panthee DR, Rb KC, Regmi HN, Subedi PP, Bhattarai S, Dhakal J (2006). Diversity analysis of garlic (*Allium sativum* L.) germplasm available in Nepal based on morphological characters. Genet Resour Crop Ev 53: 205-212.
- Pokluda R Kuben J (2002). Comparison of selected Swiss chard (*Beta vulgaris* ssp. *cicla* L.) varieties. Hortic Sci 29: 114-118.
- Potter EF, Long MC (1965). Sugar determination in green and leafy plants and dried forages. J Assoc Off Agric Chemist 48: 728-730.
- Pyo Y, Lee TL, Logendra T, Rosen RT (2004) Antioxidant activity and phenolic compounds of Swiss chard (*Beta vulgaris* subspecies *cycla*) extracts. Food Chem 85: 19-26.
- Rabbani MA, Iwabuchi A, Murakami Y, Suzuki T, Takayanagi K (1998). Genetic mustard (*Brassica juncea* L.) germplasm from Pakistan as determined by RAPDs. Euphytica 103: 235-242.

- Rao VR, Hodgkin T (2002). Genetic diversity and conservation and utilization of plant genetic resources. Plant Cell Tiss Org 68: 1-19.
- Rozycki VR, Baigorria CM, Freyre MR, Bernard CM, Zannier MS, Charpentier M (1997). Nutrient in wild vegetable products of the Argentine Chaco. Arch Latinoam Nutr 47: 265-270.
- Santamaria P, Elia A, Serio F, Todaro E (1999). A survey of nitrate and oxalate content in fresh vegetables. J Sci Food Agr 79: 1882-1888.
- Schudel P, Eichenberger M, Augstburger F, Klaey R, Vogtmann H (1979). The effects of compost or nitrogen phosphoruspotassium fertilization on yield, vitamin C and nitrate content of spinach and Swiss chard. Schweiz Landwirtsch Forsch 18: 337-349.
- Shen Y, Ford Lloyd BV, Newbury HJ (1998). Genetic relationships within the genus *Beta* determined using both PCR-based marker and DNA sequencing techniques. Heredity 80: 624-632.

- Sneath PHA, Sokal RR (1973). Numerical taxonomy: the principle and practice of numerical classification. San Francisco, CA, USA: WH Freeman and Co.
- Sun YC (1994). Beta germplasm collection and its application status in China. In: Frese L, Doney DL, editors. International Beta Genetic Resources Network. A Report on the 3rd International Beta Genetic Resources Workshop and World Beta Network Conference. North Dakota State University, Fargo, USA, 4–6 August 1994. International Crop Network Series 11. International Plant Genetic Resources Institute, Rome, pp. 27-29.
- Tan A, Aykas L, İnal A (1999). Status of *Beta* genetic resources in Turkey. In: Maggioni L, Frese L, Germeir C, Lipman E, editors. Report of a Working Group on Beta. 9–10 September 1999.
 Broom's Barn, Higham, Bury St. Edmunds, UK. International Plant Genetic Resources Institute, Rome, Italy.
- Tindall HD (1983). Vegetables in the Tropics. Westport, CT, USA: AVI Publishing Co.