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Full Length Research Paper

The phenotypic diversity and fruit characterization of winter squash (*Cucurbita maxima*) populations from the Black Sea Region of Turkey

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Winter squash are one of the most important *Cucurbit* crops in Turkey. Winter squash populations show great diversity in morphological characteristics, particularly fruit length, fruit diameter, fruit shape, fruit brightness, skin thickness , flesh thickness and colour in the Black Sea region of Turkey. In this research, 115 populations of winter squash, *Cucurbita maxima* Duch, were collected from different provinces of the Black Sea region in 2006 and 2007 and phenotypic diversity in their fruit characters was assessed. The collection showed appreciable phenotypic variation in fruit shape, fruit colour, fruit brightness, fruit dimension and fruit weight. Cluster and principal component analysis (PCA) were performed to determine relationships among populations and to obtain information on the usefulness of those fruit characters for the definition of groups. Cluster analysis based on 14 quantitative and 7 qualitative variables identified 10 different groups. The first five principal component axes accounted for 65.0% of the total multivariate variation among the populations. The greater part of variance was accounted for by fruit weight, fruit diameter, fruit length, length of seed cavity and flesh thickness. This evaluation of fruit trait variability can assist geneticists and breeders to identify populations with desirable characteristics for inclusion in variety breeding programs.

Key words: Winter squash, genetic resources, fruit characterization, multivariate analysis, Turkey.

INTRODUCTION

Cucurbita spp. is collectively ranked among the 10 leading vegetable crops worldwide. China and India are the world leading producers. Other major producers are U.S.A, Egypt, Mexico, Ukraine, Cuba, Italy, Iran and Turkey (FAOSTAT, 2007). Squashes (*Cucurbita* spp.) are members of the economically important *Cucurbitaceae*. There are three economically important *Cucurbita* species, namely *Cucurbita pepo* L., *Cucurbita maxima* Duch. and *Cucurbita moschata* Duch., which have different climatic adaptations and are widely distributed in agricultural regions worldwide (Robinson and Decker-Walters, 1997; Paris and Brown, 2005; Wu et al., 2007). The *Cucurbita* genus is of American origin. *C. maxima* is the most diverse *Cucurbita* species after *C. pepo* and is found throughout tropical and temperate regions. Phenotypic diversity within populations of *Cucurbita* is high and includes variation in shape, size and colour of fruits; number and size of seeds; quality, colour and thickness of fruit flesh and precocity in fruit production, among other traits (Whitaker and Robinson, 1986; Hernandez et al., 2005). The centre of diversity, *C. maxima*, lies in South American temperate zones, where landraces exhibit an array of interesting traits. Many landraces of this species are also found in North America, Australia and different countries of Africa (Zambia and Nigeria); Asia (China, India, and Iran) and Europe (Spain and Turkey) (Ferriol and Pico, 2008).

Turkey is one of the important diversity centers for cultivated cucurbits because of their adaptation to diverse ecological conditions as a result of natural selection and also farmers' selections (Sari et al., 2008). Only one improved cultivar of winter squash is currently grown commercially in Turkey. Farmers have maintained local

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Figure 1. Picture of the diversity fruit size, shape and color for *Cucurbita maxima* populations which were collected from Black Sea Region in Turkey.

population of winter squash and exchanged seeds with surrounding areas, mainly at local markets. It is a traditional vegetable often grown in small gardens. However, for decades, the old winter squash landraces have progressively been replaced by new cultivars, including Arican 97, which ensure higher yields and incomes and meet the requirements of processors and consumers (Sari et al., 2008; Balkaya et al., 2009). However, the traditional landraces are the important genetic resources for plant breeders because of their considerable genotypic variations. These variations are maintained by deliberate selection for specific traits by farmers. At the research level, the diversity of genetic resources in collections may increase the efficiency of efforts to improve a species (Geleta et al., 2005). Determination of the degree of variation of quantitative and qualitative traits present in genetic resources is important for vegetable breeding programs (Escribano et al., 1998). Plant breeders can use genetic similarity information to complement phenoltypic information in the development of breeding populations (Greene et al., 2004; Balkaya et al., 2009).

With respect to variation in characters among populations, cluster analysis has been used to identify morphological variability in different crop species (Decker and Wilson, 1986; Escribano et al., 1991; Cartea et al., 2002; Balkaya et al., 2005; Balkaya and Ergun, 2008). In analysing genetic variation among populations and determining the most important variables contributing to this variation, it appeared that principal component analysis is most useful (Ozdamar, 2004). In analyzing genetic diversity, studies indicated that cultivated species are built up from different genetic groups. A dendrogram of 21 *Cucurbita* species constructed from 93 phenotypic characters aggregated cultivated species into five different groups (Whitaker and Bemis, 1975). Genetic diversity in pumpkins has been reported (Babu et al., 1996; Kale et al., 2002; Kumar et al., 2006) and fruit size, shape and colour vary greatly among cultivated cucurbits (Robinson and Decker-Walters, 1997).

Morphological characterization is the first step in the description and classification of genetic resources (Smith and Smith, 1989). There has been no comprehensive program for the collection and characterization of the winter squash genetic resources of the Black Sea region of Turkey and there are no reported studies (Yanmaz, 2002). In the current paper, the authors characterized winter squash populations collected from different parts of the Black Sea region of Turkey. This represents the first collection and characterization of Turkish *C. maxima* germplasm. The second aim of the present study was to assess the genotypic variation among the 115 populations of winter squash determined.

MATERIALS AND METHODS

Winter squash seeds from four provinces (Samsun, Amasya, Sinop and Bolu) of the Black Sea region of Turkey were collected before and during harvest time between September 2004 and February 2006. A total of 115 populations of fruit and/or seeds were collected (Figure 1). The numbers of the populations based on geographical

Table 1. Fruit traits used winter squash (*C. maxima* Duch) population characterization.

S/N	Traits
1	Fruit length (cm)
2	Fruit diameter (cm)
3	Peduncle length (cm) (1, short; 2, medium; 3, long)
4	Peduncle diameter (cm)
5	Number of fruit grooves (1, absent; 2, present)
6	Fruit shape (1, globular; 2, oval; 3, transverse elliptical; 4, ovoid 5, top-shaped; 6, cylindrical)
7	Number of colours of skin (1, one colour; 2, two colour intensities two colour hues; 3, more than two colour)
8	Main colour of skin (1, dark green; 2, light green; 3, green; 4, light white; 5, white; 6, cream)
9	Secondary colour of skin (1, light speckled white; 2, speckled white; 3, light speckled pink; 4, speckled pink; 5, pink; 6, speckled green; 7,absent)
10	Fruit brightness (1, light; 2, medium; 3, heavy)
11	Skin thickness of fruit (mm)
12	Flesh colour (1, orange; 2, dark orange; 3, light orange; 4, light yellow; 5, yellow)
13	Flesh thickness (mm) (1, thin; 2, medium; 3, thick)
14	Fruit firmness (kg) (1, loose; 2, medium; 3, firm)
15	Total soluble solid content (TTSC ^{o}Brix) (1; high; 2, medium; 3, low)
16	Length of seed cavity (cm)
17	Fruit number per plant (1; few; 2, medium; 3, many)
18	Fiber weight per fruit (g)
19	Average fruit weight (kg) (1, light (1-5kg); 2, medium (5-10 kg); 3, heavy (>10 kg)
20	Fiber index (%) (mean fiber weight per mean fruit weight)
21	Sensory testing score (1, medium 3.0-5.0; 2, good 5.0-7.0; 3, very good 7.0-10.0)

distribution were 47 from Samsun, 21 from Bolu, 29 from Sinop and 18 from Amasya. These genetic materials were collected from well-established, traditional, open-pollinated populations grown by local farmers.

The field component of this study was carried out in the Bafra district of Samsun province in 2006 and 2007. The experimental site was located at 41° 35’ N, 35° 54’ E. This site is situated in the north of Turkey and has a humid climate with annual relative humidity of 72.0% and average annual rainfall of 780.2 mm (TSMS, 2008). The soil of the experimental area was sandy loam with a pH of 6.5.

The seed of all populations was sown into plug trays containing peat, organic manure and sand mixed in the ratio of 4:2:1 on 20 April, 2006 and 10 April, 2007. Twenty seedlings from each population were field planted at the 4 - 5 leaf stage at a spacing of 2.8 x 2.0 m in the middle of May of each year. Soil tests were done before and after planting. Standard fertilization and weed control practices were applied. The plants were harvested manually at full maturity. The harvest period began at the end of September and lasted until the middle of October of each year because the populations had different maturation periods.

Characterization

All observations and analyses were carried out on 18 plants from each population. All characters were measured in the field and at the normal harvest time. The selected characters were included in the description form developed for *Cucurbita maxima* Duch. by UPOV (The International Union for the Protection of New Varieties of Plants) with the reference number TG/155-4 (Table 1) (UPOV, 2007). To standardize the interpretation of fruit characters, the same researcher did all the classifications in this study. The fruit characters studied at maturity included length, diameter, shape, brightness, colour, skin and flesh thickness. Fruit and flesh colour were measured as per the CIELAB (L*a*b) colour code using a Minolta model CR-300 colourimeter. Three readings were taken for both fruit and flesh colour. Lightness (L*) was determined within the L* range, from 0=black and 100=white. The number of fruits per plant and total fruit weight per plant were determined for each population. Mean fruit weight was calculated by dividing the total weight of fruits by the fruit number. In addition, total soluble solid content (TSSC, ^{o}Brix) were measured and sensory testing was done. TSSC (^{o}Brix) content for each population was determined with a hand–held refractometer. Samples for the sensory testing panel were prepared by cutting five squash per population into stalk to calyx segments that were 25 mm across at the widest point. Samples from each population were cooked together by steaming over boiling water for 25 min or until tender when tested, with the seed cavity side facing the boiling water. Samples were kept uncovered as they cooled before assessment, to reflect normal usage conditions (Corrigan et al., 2001). Ten panelists rated samples on a 1-10 numerical scale, according to technical definitions describing brittleness, cohesiveness, hardness, adhesiveness, mouth-feel, moistness and fibrousness. Statistical analysis was performed using the statistical package SPSS (13.0 for Windows).

Data analysis

In principal component analysis (PCA), the data were used to generate Eigen values, the percentage of the variation accumulated by PCA and the load coefficient values which relate the values (eigen values are proportional to the amount of total variation among the populations that is associated with the axis). Those principal components (PC) with Eigen values >1.0 were selected and those characters with load coefficient values >0.6 were considered highly relevant for that PC (Jeffers, 1967). To develop a better insight into

Table 2. Frequency distribution of discrete description in 115 winter squash progenies.

Descriptor	Score code	Descriptor state	Frequency (%)
Fruit shape	1	Globular	49.6
	2	Oval	28.2
	3	Transverse elliptical	10.3
	4	Ovoid	6.0
	5	Top-shaped	5.1
	6	Cylindrical	0.9
Main colour of skin	1	Dark green	60.7
	2	Light green	20.5
	3	Green	14.5
	4	Light white	1.7
	5	White	1.7
	6	Cream	0.9
Average fruit weight (kg)	1	Light (1 - 5)	7.7
	2	Medium (5 - 10)	81.2
	3	Heavy (>10)	11.1
Flesh colour	1	Orange	47.9
	2	Dark orange	35.0
	3	Light orange	15.4
	4	Light yellow	0.9
	5	Yellow	0.9
Skin thickness (mm)	1	Thick (> 6)	51.3
	2	Medium (4.3 - 6.0)	32.5
	3	Thin (2.9 - 4.3)	16.2
Fruit firmness (kg)	1	Firm (2.6 - 2.9)	59.8
	2	Medium (2.9 - 3.2)	32.5
	3	Loose (3.2 - 3.5)	7.7
Flesh thickness (mm)	1	Thin (21.2 - 32.5)	64.1
	2	Medium (32.5 - 43.8)	33.3
	3	Thick (43.8 - 55.1)	2.6
Total soluble solid content (°Brix)	1	High (11.0 - 15.0)	23.9
	2	Medium (7.0 - 11.0)	47.0
	3	Low (3.0 - 7.0)	29.1
Sensory testing score	1	Medium (3.0 - 5.0)	16.2
	2	Good (5.0 - 7.0)	46.2
	3	Very good (7.0 - 10.0)	37.6
Fruit number per plant	1	Few (1.2 - 1.7)	44.4
	2	Medium (1.8 - 2.3)	42.8
	3	Many (2.4 - 2.9)	12.8

the diversity of their winter squash populations, the authors also used cluster analysis. Hierarchical cluster analyses were performed using Ward's criteria, minimizing the total sum of squared distances of objects to cluster centers. Ward's criteria were preferred because they tend to produce desirable compact clusters (Zewdie and Zeven, 1997).

RESULTS

The current study evaluated the variability of winter squash populations on the basis of 115 open-pollinated populations collected from the Black Sea region of Turkey. Substantial genetic diversity was recorded and frequency distribution of the resulting data on a percentage basis is given in Table 2. Morphological variation was most apparent in fruit shape varied from globular to cylindrical. Most populations in this study had globular (49.6%), oval (28.2%) or transverse-elliptical fruit (10.3 %). Fruit colour was also quite variable (Table 2). Skin colour of the collected genotypes was mainly dark-green (60.7%) and light green (20.5%) tones. In this study, flesh colour was generally orange. Most populations in the

Table 3. Variability of some fruit traits of the Black sea region winter squash populations.

Trait	Mean ± SD*		
	Minimum values	Maximum values	Average values of populations
Fruit length (cm)	26.0	49.8	38.5±0.5
Fruit diameter (cm)	35.1	56.5	45.1±0.4
Peduncle length (cm)	5.2	15.9	9.7±0.2
Peduncle diameter (cm)	20.5	56.3	34.0±0.5
Skin thickness of fruit (mm)	2.9	9.0	5.7±0.1
Flesh thickness (mm)	21.2	55.1	34.9±0.5
Length of seed cavity (cm)	13.9	25.0	19.4±0.2
Fruit firmness (kg)	2.6	3.5	2.8±0.02
Total soluble solid content (TSSC ^{o}Brix)	3.5	15.0	9.0±0.2
Sensory testing score	2.8	8.6	6.1±0.1
Average fruit weight (kg)	3.2	11.8	7.4±0.2
Fiber weight per fruit (g)	45.0	444.0	187.8±7.7

*Table shows minimum, maximum and mean values and standard deviation (SD) for 115 local populations.

current study were of orange tones (Table 2). For the 115 populations; the maximum, minimum, mean values and standard deviations in some fruit characteristics are shown in Table 3. The results showed a large variation among populations. Fruit length and fruit diameter varied widely, from 26.0 to 49.8 cm, and from 35.1 to 56.5 cm, respectively. In terms of skin thickness and flesh thickness, there were big differences among the populations. They showed a range of 2.9 - 9.0 mm for skin thickness and 21.2 - 55.1 mm for flesh thickness (Table 3). TSSC ranged from 3.5 ^{o}Brix to 15.0%. Analysis of sensory test results showed variation of scores from 2.8 to 8.6 across the populations. The average value of fruit weight across populations was 7.4 kg and average fruit weight of individual populations varied from 3.2 to 11.8 kg (Table 3).

The original set of twenty one variables (Table 1) was reduced by PCA to fourteen, which indicated about 65% of the total genetic variation. The degrees of association of characters with this axis were also obtained and are given as their factor scores or Eigen-vectors (Table 4). This information was used to construct three dimensional ordinations of winter squash genotypes (Figure 2). PCA results indicated that the first three PCs explained 46.9% of the total variation. The principal component 1 (PC1) had the largest Eigen-value (3.446) and accounted for the greatest amount of variance in the original data, while PC2 accounted for the greatest amount of variation in the residual variation, which was unaccounted for by the first principal axis. PC3 accounted for the greatest amount of variation in the residual variation unaccounted for by PC2. The same process unfolded for principal axes 4 and 5 (Table 3). Characters with higher coefficients on the PC axes should be considered more important, thus eigen vectors above 0.60 (Jeffers, 1967; Balkaya and Ergun, 2008) are shown in bold in Table 4. Fruit weight, fruit diameter, fruit length, length of seed cavity and flesh thickness had higher coefficients on the first PC axis than on the other axes, the second axis had the highest coefficient for total soluble solids and sensory testing scores, the third axis had the highest coefficients for fruit shape and skin thickness and the fourth PC axis had the highest coefficient for fruit number per plant. Finally, PC5 was mainly related to skin colour.

Characters with high coefficients in the first and second PCs should be considered more important since these axes explain nearly half of the total variation. Results stated earlier indicated that populations could be distinguished by fruit morphological and weight traits, both of which had the highest coefficients on the first PC axis.

To better understand the overall diversity of the winter squash populations, the data were analyzed by cluster analysis which revealed the distribution of genetic diversity displayed in Table 5. Cluster analysis was performed to group populations according to their variability. Ten clusters were obtained. The means and standard deviations for the traits for each cluster are presented in Table 6.

General characteristics of the investigated winter squash populations are as follows:

Group A: The first cluster included sixteen populations collected from Samsun and Amasya provinces, except for G-68 (Sinop province) (Table 5). This group was divided into four subgroups that were formed by three, six, three and four populations. Populations had an average fruit length of 33.5 cm and fruit diameter of 43.0 cm. The number of grooves in the fruit was higher than for other groups, except for Group I (Table 6). Fruit colour was

Table 4. Principal component analysis (PCA) of characters associated with 115 winter squash (*C. maxima* Duch) populations. Proportions of variations are associated with first five PC axes, which correspond to Eigen values greater than 1.

Principal component analysis	**PC axis**				
	1	**2**	**3**	**4**	**5**
Eigen values	3.446	1.744	1.377	1.333	1.202
Explained proportion of variation (%)	24.6	12.5	9.8	9.5	8.6
Cumulative proportion of variation (%)	24.6	37.1	46.9	56.4	65.0
Traits	**Eigen vectors**				
Fruit weight (kg)	**0.94**	-0.03	0.06	-0.10	0.01
Fruit diameter (cm)	**0.82**	0.04	0.21	-0.05	0.20
Fruit length (cm)	**0.70**	-0.21	-0.22	-0.36	-0.18
Length of seed cavity (cm)	**0.68**	-0.09	0.21	0.17	0.24
Flesh thickness (mm)	**0.61**	0.37	-0.37	-0.05	-0.28
Fiber weight fruit (g)	0.58	-0.34	0.15	0.10	-0.10
Total soluble solids (%)	-0.28	**0.75**	0.08	0.01	0.05
Sensory testing score	-0.01	**0.64**	0.06	-0.34	0.11
Fruit firmness (kg)	0.03	0.58	-0.04	0.28	-0.34
Fruit shape	0.01	-0.20	**-0.78**	0.18	0.06
Skin thickness (mm)	0.32	-0.12	**0.62**	0.16	-0.15
Fruit number per plant	0.03	0.02	0.04	**0.86**	0.05
Number of fruit grooves	0.20	0.29	0.22	-0.42	-0.02
Main colour of skin	0.10	0.01	-0.18	0.08	**0.91**

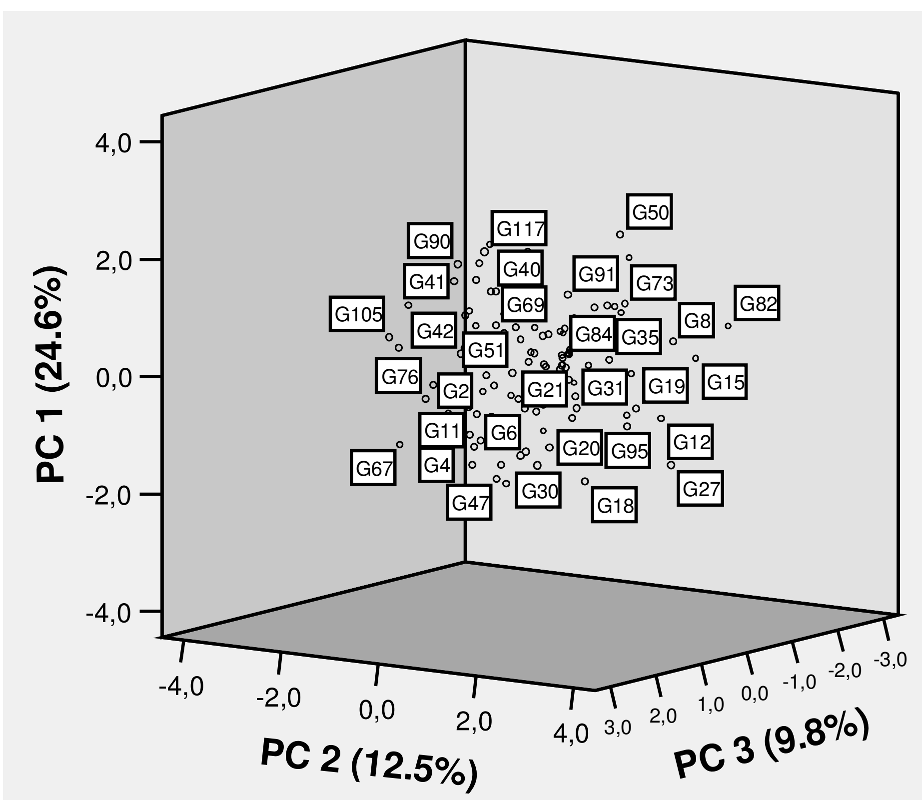

Figure 2. Scatter plot constructed at the basis the first three principal component axes, which contain 46.9 % of the total variation.

mainly green tones. The populations in this group had the highest sensory testing scores, except for Group G. In this group, fruit shape was globular, oval or transverse elliptical. Average fruit weight was 6.1 kg (Table 6). Compared to other groups, fruit weights were in the medium range. Group A fruits are used for consumption as dessert.

Group B: This group was clustered into three subgroups consisting of seven populations. Fruits were dark green and globular or oval shaped. The fruits of this group had a long peduncle with a large diameter. In addition, fruit yield was high (Table 6). Fiber weight averaged 254.2 g per fruit and was higher than in all other groups, except for Group I. The fiber index greatly influences consumer appeal. For this group, the fiber index value was 3.7, which was the highest of all groups (Table 6). It should be noted that consumers prefer a low fiber percentage for dessert dishes. After sensory test analysis, winter squash from this group were not selected for dessert consumption because their fiber weight and fiber index ratio were very high.

Group C: This was the smallest group. It was composed of three populations in one subgroup with an average fruit length of 30.0 cm. It was the lowest among all the groups (Table 6). The fruits were oval shaped and dark green in colour and their flesh was dark orange. This type of fruit is preferred by consumers. Fruit firmness was greater than for other groups. The length of seed cavity averaged 16.7 cm which was the lowest of all groups (Table 6). The mean TSSC content was 10.5 ^{o}Brix, the second highest of all groups. In addition, fiber weight and fiber index values of this group were the lowest in all groups. These populations were very suitable for fresh consumption.

Group D: There were nine populations which were clustered into four subgroups. The plants had a short peduncle with a small diameter. The peduncle length and diameter values of this group were the lowest of all groups (Table 6). Fruit diameter averaged 41.0 cm and fruits were the narrowest of all groups. Fruit shapes were globular or oval. The colour of fruits varied from green to dark green. Flesh thickness averaged 27.1 mm. This was the lowest of all groups (Table 6). The fruit yield per plant was low, mean fruit weight was 5.8 kg and fruit size was medium. Group D fruits were not selected for fresh consumption in this study.

Group E: Four populations constituted this group. There were three populations in the first subgroup and one in the second. Fruits of this group were ovoid, which was very different from the other groups. Skin thickness averaged 4.9 mm. Fruit skin thickness was the lowest of all groups (Table 6), as was the TTSC of 6.6 ^{o}Brix. The lowest sensory test scores (4.6) were also seen in this group (Table 6). As a result of sensory test analysis, fruits from this group were not selected for dessert consumption.

Group F: Group F consisted of seven populations in three subgroups. On average, these genotypes had fruits with a mean length of 38.0 cm, diameter of 44.2 cm, weight of 7.1 kg and TSSC of 7.3 ^{o}Brix. Fruits were globular or transverse elliptical in shape. Fruit colour ranged from light green to dark green tones. Fiber weight averaged a very high 216.8 g. In addition, the populations in this group had the highest fiber index scores, except for Group B. These populations were not selected for fresh consumption because of low fruit quality characteristics.

Group G: There were 16 populations clustered in three subgroups. Fruit shape was globular, oval, or round shaped. The main skin colour was green tones and flesh colour was dark orange. Mean flesh thickness was 38.3 mm. This value was higher than for all other groups. The TSSC of 12.5 ^{o}Brix was the highest recorded in all groups. These populations also had the highest sensory testing scores (7.4) (Table 6). For on-going variety breeding, these genotypes were promising.

Group H: This largest group was composed of 20 populations; the majority originating from the Black Sea region (Samsun, Sinop and Bolu provinces). This group was clustered into three sub-groups which had the largest fruit dimensions, except for Group I. In addition, peduncle length averaged 36.3 mm (Table 6), which ranked it second for all groups. Fruit shapes varied a great deal in this group. Main skin colour was dark green and flesh colour was dark orange tones. Flesh thickness was the second highest among the groups. Average fruit weight was 8.1 kg and the fruits were classified as medium size.

Group I: This group had 15genotype clustered into five subgroups and had the highest values for fruit length and fruit diameter (Table 6). This group also had large fruit diameter and the highest fruit volume. The plants of this group had a long peduncle with a large diameter. Fruit was light green in colour and globular or oval shaped.

Average skin thickness was 7.1 mm. This value was higher than for other groups. These populations also had the highest length of seed cavity (22.0 cm). Average fruit weight was 10.3 kg and the group was characterized by the heaviest fruits (Table 6). Because the fruit dimensions of this group were larger than for other groups, the average weight of fiber was the highest (260.1 g) of all groups (Table 6). On the other hand, it had lower fiber index than all groups.

Group J: There were a total of 18 populations in this group which were clustered into four sub-groups. Fruit shape was globular or oval and skin colour ranged from green to dark green tones (Table 6). Skin thickness

Table 5. Cluster memberships of 115 winter squash populations.

Group H		Group J		Group A		Group G		Group I		Group D		Group B		Group F		Group E		Group C	
Code	**Origin**	**Cod e**	**Origin**	**Co de**	**Origin**	**Co de**	**Origin**	**Code**	**Origin**	**Code**	**Origin**	**Code**	**Origin**	**Code**	**Origin**	**Code**	**Origin**	**Code**	**Origin**
G5	Samsun	G11	Samsun	G1	Samsun	G8	Samsun	G37	Samsun	G10	Samsun	G2	Samsun	G54	Amasya	G55	Amasya	G3	Samsun
G92	Sinop	G42	Samsun	G47	Samsun	G16	Samsun	G40	Samsun	G65	Amasya	G101	Bolu	G110	Bolu	G70	Sinop	G113	Bolu
G36	Samsun	G74	Sinop	G76	Sinop	G82	Sinop	G78	Sinop	G111	Bolu	G48	Amasya	G84	Sinop	G112	Bolu	G27	Samsun
G99	Bolu	G13	Samsun	G4	Samsun	G15	Samsun	G80	Sinop	G67	Amasya	G72	Sinop	G97	Bolu	G63	Amasya		
G100	Bolu	G75	Sinop	G7	Samsun	G26	Samsun	G41	Samsun	G45	Samsun	G115	Bolu	G98	Bolu				
G38	Samsun	G106	Bolu	G6	Samsun	G12	Samsun	G96	Sinop	G108	Bolu	G29	Samsun	G104	Bolu				
G109	Bolu	G21	Samsun	G9	Samsun	G19	Samsun	G53	Amasya	G102	Bolu	G34	Samsun	G107	Bolu				
G66	Amasya	G25	Samsun	G14	Samsun	G18	Samsun	G117	Bolu	G56	Amasya								
G83	Sinop	G28	Samsun	G58	Amasya	G17	Samsun	G60	Amasya										
G59	Amasya	G23	Samsun	G22	Samsun	G95	Sinop	G62	Amasya										
G114	Bolu	G71	Sinop	G32	Samsun	G33	Samsun	G69	Sinop										
G43	Samsun	G24	Samsun	G52	Amasya	G35	Samsun	G90	Sinop										
G44	Samsun	G79	Sinop	G39	Samsun	G20	Samsun	G105	Bolu										
G46	Samsun	G81	Sinop	G64	Amasya	G30	Samsun	G88	Sinop										
G73	Sinop	G77	Sinop	G51	Amasya	G31	Samsun	G103	Bolu										
G86	Sinop	G89	Sinop	G68	Sinop	G49	Amasya												
G85	Sinop	G50	Amasya																
G87	Sinop	G91	Sinop																
G93	Sinop																		
G94	Sinop																		

averaged 6.0 mm. This value was the highest, except for Group I. Flesh colour was dark orange and the TSSC was 9.4 ^{o}Brix. In sensory testing, the fruits were given 7.0 points by the degustators. These genotypes were selected for the winter squash variety breeding study.

DISCUSSION

Turkey is very rich in cucurbit genetic resources due to its diverse geography and ecology (Sari et al., 2008). Ekinci (1976) emphasized Anatolia's great genetic diversity for melons, watermelons and squash. The same author also reported that the origin of melons, watermelons and some squashes grown in the Ukraine and Russia is Anatolia and proposed that the cantaloupe melons of Europe originated from the Van area in eastern Anatolia. Pitrat et al. (1999) reported that Anatolia belongs to a secondary centre of genetic diversity of melons. Şensoy et al. (2007) also found a large degree of genetic variation in melon genotypes in Turkey, which was proposed as a secondary centre of genetic diversity for the species. In almost all regions of Turkey, landraces of *Cucurbitaceae* were highly variable in morphology and taste (Balkaya and Karaağaç, 2005).

Knowledge of the extent of genetic diversity and the identification, differentiation and characterization of genotypes and populations, provided information tool for the detection of duplicates in collections and also better characterization and utilization in breeding (Hornokova et al., 2003). Phenotypic characterization in *Cucurbita* has traditionally been based on seed and fruit characteristics (Balkaya et al., 2005; Ferriol and Pico, 2008). These have proved useful in distinguishing related species (Gwanama et al., 2000). Since

Table 6. Mean trait values used in winter squash (*C. maxima* Duch) group identification.

Trait	A	B	C	D	E	F	G	H	I	J
1*	33.5 ± 1.1	37.8 ± 0.5	30.0 ± 3.2	36.3±1.1	41.0 ± 0.4	38.0 ± 1.9	35.6 ± 1.1	39.6 ± 0.8	42.8 ± 0.7	41.2 ± 0.9
2	43.0 ± 0.7	43.7 ± 0.8	41.8 ± 1.3	41.0±1.2	42.4 ± 0.8	44.2 ± 2.1	43.4 ± 0.6	47.0 ± 0.6	51.1 ± 0.7	46.5 ± 0.7
3	9.1 ± 0.4	8.9 ± 0.9	8.2 ± 0.1	7.3±0.9	7.9 ± 1.2	8.8 ± 0.5	10.1 ± 0.4	9.2 ± 0.3	10.7 ± 0.3	12.3 ± 0.4
4	32.6 ± 0.9	34.3 ± 1.1	29.7 ± 1.4	29.2±1.9	30.6 ± 2.4	31.6 ± 1.3	34.7 ± 1.2	36.3 ± 1.4	37.3 ± 1.0	34.6 ± 0.9
5	10.0 ± 0.1	9.8 ± 0.2	3.8 ± 2.8	9.6±0.3	9.5 ± 0.2	1	8.7 ± 0.5	9.3 ± 0.2	10.2 ± 0.3	9.1 ± 0.5
6	1,2	1, 2	1	1,2	4	1,3	1,2,5	1,3,5	1,2	1,2
7	2	1,2	1	1,2	2	2	1,2	2	1,2	1
8	1,3	2	3	1,2	1,2,3	1	1,2,3	1	1,2	1,2
9	1,2,7	7	7	4,5,6,7	2,3	1,2	1,2,7	1,2	2	7
10	2,3	3	2	2,3	1,2	2,3	2	3	3	2
11	5.3 ± 0.2	5.3 ± 0.6	5.5 ± 0.7	5.5 ± 0.4	4.9 ± 0.7	5.5 ± 0.6	5.6 ± 0.4	5.5 ± 0.3	7.1 ± 0.3	6.0 ± 0.3
12	1,2,3	2,3	2	1,2	1	1,2	2,3	1,2	1,2	2,3
13	31.5 ±1.2	34.5 ± 1.1	29.2 ± 1.2	27.1 ± 1.5	35.1 ± 1.2	32.5 ± 1.9	38.3 ± 1.5	37.5 ± 1.1	36.6 ± 1.2	37.2 ± 1.2
14	2.8 ± 0.04	2.9 ± 0.04	3.4 ± 0.3	2.8 ± 01.	2.8 ± 0.04	2.8 ± 0.1	2.9 ± 0.1	2.8 ± 0.1	2.8 ± 0.05	2.8 ± 0.04
15	9.2 ± 0.5	8.0 ± 0.81	10.5 ± 1.1	8.6 ± 0.8	6.6 ± 0.8	7.3 ± 0.4	12.5 ± 0.4	8.2 ± 0.5	7.2 ± 0.4	9.4 ± 0.5
16	19.4 ± 0.3	19.5 ± 0.8	16.7 ± 0.4	18.1 ± 0.4	17.0 ± 1.1	21.3 ± 1.0	17.2 ± 0.3	20.0 ± 0.4	22.0 ± 0.5	19.6 ± 0.4
17	2.0 ± 0.1	2.2 ± 0.2	1.8 ± 0.2	1.3 ± 0.1	1.9 ± 0.2	2.1 ± 0.2	1.7 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	1.4 ± 0.1
18	170.7 ± 11.0	254.2 ± 38.5	80.3 ± 19.1	115.3 ± 20.1	109.7 ± 15.7	216.8 ± 32.4	116.4 ± 13.3	232.3 ± 14.5	269.1 ± 16.6	181.6 ± 15.4
19	6.1 ± 0.3	6.8 ± 0.2	5.0 ± 0.2	5.8 ± 0.4	6.6 ± 0.5	7.1 ± 0.7	6.4 ± 0.2	8.1 ± 0.3	10.3 ± 0.3	8.0 ± 0.4
20	2.9 ± 0.2	3.7 ± 0.5	1.6 ± 0.3	2.1 ± 0.4	1.6 ± 0.2	3.1 ± 0.4	1.8 ± 0.2	3.0 ± 0.3	2.6 ± 0.1	2.3 ± 0.2
21	6.8 ± 0.3	4.2 ± 0.2	6.3 ± 0.4	5.1 ± 0.4	4.6 ± 0.5	5.1 ± 0.4	7.4 ± 0.1	5.8 ± 0.2	5.8 ± 0.2	7.0 ± 0.1

*Fruit traits are given at Table 1.

Cucurbitaceae is one of the most important families in Turkey for its genetic resources, there is a need for collecting, characterizing and evaluating the remnant local populations before they disappear. This task is made more urgent by the considerable information gap concerning collection, classification and evaluation of all genetic resources in Turkey (Balkaya and Yanmaz, 2001). In this study, winter squash populations were collected from the Black Sea Region of Turkey. The collection protocol was designed to ensure that the collected genotypes effectively represented the winter squash populations present in the Black Sea region. The seeds of populations collected and characterized in this study were preserved at -20°C for long term storage in the Turkish seed gene bank (AARI) for use in future breeding programmes. They are also stored at 4°C at the Horticultural Department of Ondokuz Mayis University in Samsun, Turkey.

Up to date, multiple traits have been used for the evaluation of plant diversity. Objective descriptors based on morpho-agronomic characters were considered reliable traits to verify or assess genetic distance or conformity among populations (Hunter, 1993). Researchers have found that genetic diversity within landraces and populations of squash is high, including variation in shape, size and colour of fruits; number and size of seeds; quality, colour and thickness of fruit flesh; tolerance to pests and precocity in fruit production, among other traits (Nerson et al., 2000; Ferriol et al., 2003; Paksoy and Aydin, 2004; Hernandez et al., 2005). Reliable information on character variability within germplasm collections is very useful to breeders in planning crop improvement programs.

As well as morphological characterization, classification using molecular markers is also useful for genetic diversity analysis by extending knowledge of the genetic base, exploiting heterosis, identifying cultivars and selecting parental varieties for breeding (Ferriol et al., 2003). Other researchers have also suggested that the use of morphological traits should be complemented with more accurate techniques, in order to achieve a reliable evaluation and characterization of species diversity (Escribano et al., 1997). In recent years, molecular markers have become recognized as powerful tools in the assessment of genetic diversity within and among plant populations (Gwanama et al., 2000; Ferriol et al., 2004; Okumus and Balkaya, 2007). The current study characterized winter squash populations by using morphological traits and also the promising *C. maxima* genotypes have been molecularly studied. Most of the analysis of genetic variability within *C. maxima* has focused on the establishment of the phylogenetic relationships with the other *Cucurbita* species by using isozyme and allozyme markers (Decker-Walters et al., 1990; Puchalski and Robinson, 1990) and comparison of sequences and RFLP markers with genomic chloroplast and mitochondrial DNA (Ferriol et al., 2003). More recently, other molecular markers such as microsatellites have been used (Katzir et al., 1996; Stift et al., 2004).

It would be very beneficial to complete the description of winter squash genotypes grown in Turkey and to identity all populations with desirable characters for winter squash breeding, using all of the technological tools available. In the current study, multivariate analysis was employed to better understand the diversity of winter squash populations from Turkey, as well as to identify useful characters for use in breeding programs. As a result, the 115 collected winter squash populations from *Cucurbita maxima* were clustered into 10 groups. Across the groups, it was possible to distinguish useful traits for breeding because they possessed a great range of morphological variation. The present work has also identified the relationships among major winter squash groups in the collected genetic material. Phenotypes with similar fruit characteristics were grouped together, irrespective of collection region. No associations of clusters within collection zones were observed. This absence of association may be due to the continuous transport of seed, both deliberate and incidental, by humans. Secondly, the degree of genetic diversity among cultivated crops depends on their reproductive behavior (Geleta et al., 2005). Winter squash plants are monoceious and have high rates of cross pollination. Cross pollination events can change the genetic identity of populations. For this reason, fruit variation is higher in open pollinated populations (Robinson and Decker-Walters, 1997). In the current study, results supported this contention as considerable variability was found among collected populations. It should be noted that the populations used in this study were also maintained as stable inbred lines for variety breeding programs in another study.

To understand the potential of these genetic resources for future breeding efforts, it is important to evaluate the most useful morphological characters. Principal component analysis (PCA) gave supplementary information on the usefulness of the characters of the defined groups. Knowledge of variation found in a cultivated species and its pattern of distribution is important for the development of breeding programs (Gil and Ron, 1992; Balkaya and Ergün, 2007). Within a certain region, variation of plant and fruit types is observed. In this study, winter squash genotypes with similar fruit characteristics clustered together. The greater part of the variation was centered on fruit weight, fruit diameter, fruit length, length of seed cavity and flesh thickness. Groups B, F and I were remarkable for their very high yield, which is very important for fresh fruit production. Additionally, the populations of Groups A, G and J were also remarkable for their fruit quality characteristics (Table 4).

Estimating genetic diversity and determining the relationships between collections are very useful strategies for ensuring efficient germplasm collection and management (Bozokalfa et al., 2009). The genetic diversity of landraces is part of the economic value of global biodiversity and is considered of paramount importance for future world production (Wood and Jenne, 1997; Stoilova et al., 2005) and conservation and maintenance of these valuable genetic resources are important because they are a source of diversity for use in breeding programs (Balkaya et al., 2005). In conclusion, it should be pointed out that the current study revealed considerable variation in multiple fruit characteristics of Turkish winter squash populations. That genetic variation has considerable implications for future collection, storage and breeding efforts with *C. maxima*.

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REFERENCES

Babu VS, Gopalakkrishnan TR, Peter KV (1996).Variability and divergence in pumpkin (*Cucurbita moschata* Poir). J. Trop. Agric. 34: 10-13.

Balkaya A, Yanmaz R (2001). Conservation facilities of plant genetic resources and working systems of seed gene banks. (In Turkish). Ekoloji Çevre Dergisi, 39: 25-30.

Balkaya A, Karaağaç O (2005). Vegetable genetic resources of Turkey. J. Vegetable Sci. 11: 81-102.

Balkaya A, Kurtar ES, Yanmaz R, Ozbakir M (2005). Investigation on collecting, characterization and utilization of winter squash and pumpkin genetic resources in the Black Sea region. (In Turkish). The Scientific and Technical Research (TUBITAK) Project No. 104 O 144. Ankara, Turkey.

Balkaya A, Ergün A (2007). Determination of superior pinto bean (*Phaseolus vulgaris* L. var. Pinto) genotypes by selection under the ecological conditions of Samsun Province in Turkey. Turk. J. Agric. Forest. 31(5): 335-347.

Balkaya A, Ergun A (2008). Diversity and use of pinto bean (*Phaseolus vulgaris*) populations from Samsun, Turkey. N. Z. J. Crop Hortic. Sci. 36: 189-197.

Balkaya A, Yanmaz R, Ozbakır M (2009). Evaluation of variation in seed characters in Turkish winter squash (*Cucurbita maxima* Duch.) populations. N. Z. J. Crop Hortic. Sci. 37(3): 167-178

Bozokalfa MK, Eşiyok D, Turhan K (2009). Patterns of phenotypic variation in a germplasm collection of pepper (*Capsicum annuum* L.) from Turkey. Span. J. Agric. Res. 7(1): 83-95.

Cartea ME, Picoaga A, Soengas P, Ordas A (2002). Morphological characterization of kale populations from north-western Spain. Euphytica. 129: 25-32.

Corrigan VK, Hurst PL, Potter JF (2001). Winter squash (*Cucurbita maxima*) texture: sensory, chemical, and physical measures. N. Z. J. Crop Hortic. Sci. 29: 111-124.

Decker SD, Wilson HD (1986). Numerical analysis of seed morphology in *Cucurbita pepo*. Syst. Bot. 11: 595-607.

Decker-Walters DS, Walters TW, Poluszny U, Kevan PG (1990). Genealogy and gene flow among annual domesticated species of *Cucurbita*. Can. J. Bot. 68: 782-789.

Ekinci AS (1976). Special Vegetable Growing. Ahmet Sait Press. (In Turkish). Istanbul, Turkey.

Escribano MR, Ron AM, Santalla M, Ferreira JJ (1991). Taxonomical relationship among common bean populations from northern Spain. An. Aula Dei. 20: 17-27.

Escribano MR, Santalla M, Ron ADE (1997). Genetic diversity in pod and seed quality traits of common bean populations from northwestern Spain. Euphytica, 93: 71-81.

Escribano MR, Santalla M, Casquero PA, Ron ADE (1998). Patterns of genetic diversity in landraces of common bean *(Phaseolus vulgaris* L.) from Galicia. Plant Breed. 117: 49-56.

FAOSTAT (2007). Agricultural Structure (Production, Price, Value). (available on-line at: http://apps.fao.org/faostat).

Ferriol M, Pico MB, Nuez F (2003). Genetic diversity of some accesisons of *Cucurbita maxima* from Spain using RAPD and SBAP markers. Genet. Resour. Crop Evol. 50: 227-238.

Ferriol M, Pico B, Fernandez de Cordova P, Nuez F (2004). Molecular diversity of a germplasm collection of squash (*Cucurbita moschata*) determined by SRAP and AFLP markers. Crop Sci. 44: 653-664.

Ferriol M, Pico B (2008). Pumpkin and Winter Squash. In Handbook of Plant Breeding. Vol. 1. Vegetables I. (Prohens J, Nuez F, eds) Springer, Heidelberg, pp. 317-349.

Geleta LF, Labuschagne MT, Viljoen CD (2005). Genetic variability in pepper (*Capsicum annuum* L.) estimated by morphological data and amplified fragment length polymorphism markers. Biodivers. Conserv. 14: 2361-2375.

Gil J, Ron AM (1992). Variation in *Phaseolus vulgaris* in the Northwest of the Iberian Peninsula. Plant Breed. 109: 313-319.

Greene SL, Gritsenko M, Vandewark G (2004). Relating morphological and RAPD marker variation collecting site environment in wild population of red clover. Genet. Resour. Crop Evol. 51(6): 643-653.

Gwanama C, Labuschagne MT, Botha AM (2000). Analysis of genetic variation in *Cucurbita moschata* by random amplified polymorphic DNA (RAPD) markers. Euphytica, 113: 19-24.

Hernandez SM, Merrick CL, Eguilarte L (2005). Maintenance of squash (*Cucurbita* spp.) landrace diversity by farmers activities in Mexico. Genet. Resour. Crop Evol. 52: 697-707.

Hornokova O, Zavodna M, Zakova M, Kraic J, Debre F (2003). Diversity of common bean landraces collected in the western and eastern Carpatien. Czech J. Genet. Plant Breed. 39: 73-83.

Hunter BR (1993). Science based identification of plant genetic material. CSSA, Intellectual Propert Rights: Protection of Plant Materials. Special Publication No.21, pp. 93-99.

Jeffers JNR (1967). Two cases studies in the application of principal component analysis. Appl. Stat. 16: 225-236.

Kale VS, Patil BR, Bindu S, Paithankar DH (2002). Genetic divergence in pumpkin (*Cucurbita moschata*). J. Soil Crops, 12: 213-216.

Katzir N, Danin-Poleg Y, Tzuri G, Karchi Z, Lavi U, Cregan PB (1996). Length polymorphism and homologies of microsatellites in several Cucurbitaceae species. Theor. Appl. Genet. 93: 1282-1290.

Kumar J, Singh DK, Ram HH (2006). Genetic diversity in indigenous germplasm of pumpkin. Indian J. Horticulture, 63: 1, 101-102.

Nerson NH, Paris HS, Paris EP (2000). Fruit shape, size and seed yield in *Cucurbita pepo*. Proc. Cucurbitaceae 2000. Eds. Katzirand N, Paris HS. Acta Hortic. pp. 227-230.

Okumus A, Balkaya A (2007). Estimation of genetic diversty among Turkish kale populations (*B. oleracea var. acephala* L.) using RAPD markers. Russ. J. Genet. 43(4): 409-413.

Ozdamar K (2004). Paket Programlar ile İstatiksel Veri Analizi (Çok Değişkenli Analizler). (In Turkish). 5. Baskı. Kaan Kitapevi. 528s.

Paksoy M, Aydin C (2004). Some physical properties of edible squash (*Cucurbita pepo* L.) seeds. J. Food Eng. 65: 225-231.

Paris HS, Brown RN (2005). The genes of pumpkin and squash, Hort. Sci. 40: 1620-1630.

Pitrat M, Chauvet M, Foury C (1999). Diversity, history and production of cultivated cucurbits. Proc. Ist Int. Symp. On Cucurbits. Eds. Acta Hortic. 492: 21-28.

Puchalski JT, Robinson RW (1990). Electrophoretic analysis of isozymes in *Cucurbita* and *Cucumis* and its implication for phylogenetic studies. In: Bates DM, Robinson RW and Jeffrey C (eds), Biology and Utilization of the Cucurbitaceae. Cornell University Press, pp. 60-76.

Robinson RW, Decker-Walters DS (1997). Cucurbits. New York Cab. International. Crop Prod. Sci. Hortic. p. 226.

Sari N, Tan A, Yanmaz R, Yetişir H, Balkaya A, Solmaz I, Aykas L (2008). General Status of Cucurbit Genetic Resources in Turkey. Cucurbitaceae 2008. Proceedings of the IXth EUCARPIA meeting on genetics and breeding of Cucurbitaceae (Pitrat M.ed.). INRA. Avignon, France, pp. 21-32.

Smith JSC, Smith OS (1989). The description and assessment of distances between inbred lines of maize: the utility of morphological, biochemical and genetic descriptors and a scheme for the testing of distinctiveness between inbred lines. Maydica 34: 151-161.

Stift G, Zraidi A, Lelley T (2004). Development and characterization of microsatellite markers (SSR) in *Cucurbita* species. Cucurbit Genet. Coop. Rep. 27: 61-65.

Stoilova T, Pereira MM, Tavares De Sousa MM, Carnide VI (2005). Diversity in common bean landraces (*Phaseolus vulgaris* L.) from Bulgaria and Portugal. J. Cent. Eur. Agric. 6(4): 443-448.

Şensoy S, Büyükalaca S, Abak K (2007). Evaluation of genetic diversity in Turkish melons (*Cucumis melo* L.) based on phenotypic characters and RAPD markers. Genet. Resour. Crop Evol. 54: 1351-1365.

TSMS (2008). Turkish State Meteorological Service Records of Samsun Province, Turkey.

UPOV (2007). Descriptors for pumpkin (*Cucurbita maxima* Duch.). Guidelines for the conduct of tests for distictness, uniformity and stability. TG/155/4 Rev. (http://www.upov.int/en/publications/tg-rom/tg155/tg_155_4_rev.pdf).

Whitaker TW, Bemis WP (1975). Origin and evolution of the cultivated Cucurbita. Bull. Torrey Bot. Club, 102: 362-365.

Whitaker TW, Robinson RW (1986). Squash breeding. In: BASSETT, M.J. (Ed.). Breeding Vegetable Crops. Westport, Connecticut: Avi, pp. 209-242, 584.

Wood D, Jenne J (1997). The conservation of agrobiodiversity on-farm: Questioning emerging paradigm. Biodivers. Conserv. 6: 109-129.

Wu T, Zhou J, Zhang Y, Cao J (2007). Characterization and inheritance of a bush-type in tropical pumpkin (*Cucurbita moschata* Duchesne). Scientia Horticulturae, pp. 1-4.

Yanmaz R (2002). Bibliography on vegetables in Turkey (1923-1999). (In Turkish). Ankara, Turkey.

Zewdie Y, Zeven AC (1997). Variation in Yugoslavian hot pepper (*Capsicum annuum* L.) accessions. Euphytica, 97: 81-89.