



## Determination of morphological variability of local pea genotypes

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### ABSTRACT

This study was conducted to determine morphological variability of 40 pea genotypes obtained from Plant Gene Bank of Aegean Agricultural Research Institute and collected from five districts of Black Sea Region and divided according to seed colour and shape. Genotypes were sowed in the field area of Agricultural Faculty of Ondokuz Mayıs University in the autumn rearing period. 45 different traits were observed taking into account the list for identification suggested by UPOV and EU-CPVO. Principal Component Analysis (PCA) was done in order to determine morphological variability. 13 principal component axis were obtained by the analysis. These components represented 85.61% of total variation among genotypes. Eigen value of the first 13 principal components ranged between 1.12-7.60 and 41.97% of the variation was explained. Properties of seed coat colour and leaf colour on the varieties with anthocyanin at the second main component axis and characteristics of dry leaf weight, dry stem weight, dry leaf / dry stem ratio at the third main component have larger values than  $\pm 0.3$ . As the eigen value of the genotypes was greater than 1, dendrogram was created by using Cluster analysis. Genotypes could be clustered in 8 groups based on Cluster analysis. Group A was found to be having the most genotypes with 14 numbers in these 8 groups. In this study it was determined that plant height varied between 57.5-173.2 cm, branch number per plant 1.4-7.8, pod number per plant 10.6-43.0, pod length 4.9-9.9 cm, seed number per pod 4.0-7.6, seed yield per plant 5.3-30.0 g, 100 seed weight 10.3- 36.4 g and crude protein rate 16.3-23.6 %. The range of variation in observed traits suggested the usability of the genotypes in the variety development and breeding studies.

**Keywords:** local pea, morphological variability, cluster, principal component analysis

### Introduction

Pea is a plant which is accepted as cool climatic plant among legumes. Although pea has the opportunity of being cultivated in lots of area including coastal segments and interior regions of our country surrounded on three sides by the sea pea's sowing area is quite a little. In fact, our country is the gene center of pea (Akçin 1988). In accordance with FAOSTAT 2012, the sowing area of dry pea is 1219 ha, with average yield 2173 kg/ha and total production 2650 t in Turkey. Whereas, in our country, there is no registered variety intended for the use of pea as dry seed purpose, until today. Out of

11 registered or production permitted variety oriented for fresh consumption has got involved in the market, only one is registered in our country.

Collection, characterization and conservation of the plant gene sources come at the beginning of the highest priority of research and development studies in terms of the agricultural sustainability. Our country's the wealth of herbal bio-diversity is the one most important advantage for us to catch the developments in this sector in the world. Thus, natural genetic materials in a region, are also important sources especially for the studies of resistance breeding.

To determine of genetic variability among plant materials (genotypes) selected for the variety breeding studies is essential. Recent statistical tools are required to identify variation among genotypes which otherwise is difficult based on morphological variation as it is impacted by genotype x environment interactions. Principal Component, Discriminant and Factor analysis called as multivariate analysis methods give a chance to analyze more than one property together. Cluster analysis has been accepted as one of the multivariate methods to analyze a large number of variables collectively (Rencher 1995). Using cluster analyses, Sözen et al. (2013) found wide variation for qualitative and quantitative traits in local bean population collected from West Black Sea Region. This wide variation built a rich genetic base enables selection of genotypes for developing varieties of sugar grain type which are preferred especially by the majority of our consumers.

We believe that Black Sea Region has an important potential to generalize the agriculture considering the ecological request of pea. To be able to carry out this, suitable sorts should be developed for the region. This study, aimed at identification of pea's gene sources picked from our region and provided by national plant gene bank and to determine morphological variability for finding out its agricultural properties, will throw light on possibilities of breeding and development of a variety in future.

### Materials and methods

In this study, material comprised 40 pea genotypes procured from National Plant Gene Bank included in Aegean Agricultural Research Institution and collected from local sources from 5 districts in Black Sea Region. The material then was distinguished with regard to seed colour and type. Among these, 24 belong to Black Sea Region, 10 Marmara Region and 1 Mediterranean Region (Karayel and Bozoğlu 2008). These materials were sown in 5m long rows at 50x15cm density at Ondokuz Mayıs University, Agricultural Faculty research and application field in 2004-2005 period. CAN fertilizer was applied with 4 kg/da N in February. In the trial, harvest time was determined considering that plants's stems and leaves have dried and seeds have ripened. 45 different traits were determined on each genotypes (Table 1). 13 were quantitative and 32 qualitative from among these traits. These traits were identifies from morphological characterization list determined by UPOV (The International Union for the Protection of New Varieties of Plants) and EU-CPVO (Community Plant Variety Office) (URL 2003). In order to characterize pea genotypes, obtained data was subjected to Principal Components Analysis first

to determine morphological variability and then to clustering analysis to compose dendrogram and to see classification. JMP 5.0.1 software was used for Cluster and ABA analysis.

### Results and discussion

First Principal Components Analysis was carried out using obtained data to characterize and determine morphological variation among pea genotypes. Variances for scatter around principal components were calculated separately for every component. These are called eigen value. In the conclusion of ABA obtained PC axis and eigen values belonging them, variance and cumulative variance ratios with factor coefficient indicating weighted factor values at principal components occurring on the basis of trait as given at Table 2. 13 principal component axes which were independent from each other were obtained related to traits observed in the conclusion of principal component analysis. 13 principal component axes accounted for 85.61% of total variation in respect of local pea genotypes. The initial 3 of principal component's eigen value ranged between 1.12-7.60, the third principal component axis explained 41.97% of the variation (Table 2). If the weight values at the principal components of traits observed at the Principal Component Analysis are over  $\pm 0.3$ , they are accepted to have a significant weight (Brown 1991). When weight degrees at second principal component axis were observed, pink or purple spots on testa at varieties with anthocyanin and the colour of testa at the varieties with anthocyanin were determined to attain greater than  $\pm 0.3$  value. Besides, when weight degrees were observed at the third principal component axis, leaf area, the weight of dry leaf and the weight of dry stem; at the fourth principal component axis, the maximum wideness of vexillum; at the fifth principal component axis, dry leaf/dry stem ratio, curvature degree of pod, pod colour and density of green colour at pod; at the sixth principal component axis, the length of stipule, the wideness of stipule and the density of green colour at pod properties have gotten greater value than  $\pm 0.3$  (Table 3). Because of this, traits which have been mentioned above are represented for the initial six principal component axis.

Eigen values of Local pea genotypes picked from our region and procured from National Plant Gene Bank were bigger than 1(1.12) which shows that principle component weight degrees dealt are reliable and can be carried out on Cluster Analysis (Mohammadi and Prasanna 2003). In the conclusion of Cluster Analysis, genotypes have been accumulated into 8 groups at dendrogram. The A group has had the most genotypes with 14 genotypes. The E and G groups have followed

it with 6 genotypes. Dendrogram obtained in the conclusion of the Cluster Analysis is given at Figure 1 and the distribution of groups and sub-groups composed in the conclusion of the dendrogram are given at Table 4.

By determining genotypes included in main and sub-groups indicated in the conclusion of the Cluster Analysis, severities of proximity between genotypes were found. It was determined that the relationship severities of Bz23 and Bz38 genotypes were higher than other genotypes, Bz29 and Bz35 genotypes followed them, Bz1 and Bz10 genotypes were the farthest in terms of relationship severities.

For plant height 40 genotypes ranged between 57.5-173.2 cm. Gülümser et al. (2008) have qualified that the ones shorter than 75cm are short, the ones between 75-125 cm are medium and the ones higher than 125 cm are long for pea. The plant height average of 3 genotypes represented for the group H that they have the shortest plant height average (63 cm) among 8 groups composed through Cluster Analysis and these genotypes comprise short group. Group G has 6 genotypes and follows group H with 68.4 plant height average. On the contrary, it has been determined that group F has 1 genotype and the longest plant height average with 173.2 cm.

The pod number per plant is one of the most important traits affecting the yield for legumes positively (Tiwari et al. 2001; Gülümser et al. 1994; Karayel and Bozoğlu 2009), It ranged between 10.6-43.0 number/plant for observed pea genotypes in this study. It was observed that the pod number on pea ranged between 6-14 number in the similar ecological studies (Gülümser et al. 1994). Group C included 4 genotypes took place on the top with 28.7 pod number average, the maximum pod number, among all groups. However, group H took place at the bottom as having the fewest pod number with 13.8 number/plant of average of 3 genotypes' for pod number per plant.

Seed number per pod is among the important traits affecting yield. Karayel and Bozoğlu (2009), determined that there is very important and positive relationship ( $r=0.363^{**}$ ) between seed yield and seed number per pod of pea. In our study, seed number per pod ranged between 4.0 and 7.6 number/pod. Toğay et al. (2006) found that seed number per pod of pea ranged between 3.69-5.23 number under Van conditions, Alan and Geren (2012) reported that it ranged between 4.9 and 7.2 under İzmir conditions. Two groups which have the highest average of seed number per pod (7.0), were obtained upon conclusion of the Cluster Analysis. These groups were group C that had 4 genotypes and Group G that had 6 genotypes.

There is a wide variation in pea from small grained types to big grained types. In variety and cultural

application studies for pea, thousand seed weight ranged between 139.5 and 147.0 g as reported by Toğay et al. (2006), between 153.3 and 189.7 g by Öz and Karasu (2010) and between 150.7 and 335.1 g by Alan and Geren (2012), hundred seed weight changed between 15.06 and 31.09 g reported by Gülümser et al. (1994), between 10.8 and 17.3 g by Demirci and Ünver (2005) and between 14.01 and 17.84 g by Kaya (2000). In our study hundred seed weight of genotypes ranged between 10.3 and 36.4 g. Group H had 3 genotypes and the most 100 seed weight average in the conclusion of the Cluster Analysis. Genotypes in this group have been seen as short type. On the contrary, group C included 4 genotypes having the lowest 100 seed weight average (12.4 g). From these genotypes, Bz4 is Aydın's material and Bz7, Bz15 and Bz16 are Muğla's materials.

One of the most important properties discriminating legumes family from other cultivated plants is the high protein ratio in their dry seeds. In studies, the protein ratio of pea ranged between 20.3 and 37.9% as reported by Perez et al. (1993), between 17.56 and 25.24% by Kaya (2000) and between 17 and 23.5% by Timuroğlu et al. (2004). Raw protein ratios of 40 genotypes was used in our study ranged between 16.3 and 23.6%. It was determined that group H included 3 genotypes having the highest average of raw protein ratio. This group has the highest average of 100 seed weight and pod length at the same time and genotypes in this group are short.

Leaves are the most important assimilation organs of a plant. Area of leaves was measured to their areas as well as their numeric values and to determine their relationship with yield as it is beneficial and especially on the grounds that to be an important criterion for pea types that can be a crib. Bhatt and Chanda (2003) reported that area of leaves' should be determined for plant growth analysis and envapotranspiration studies, and also they reported leaves' area is required because of eclipsing of the light, the activity of radiation usage and find the index of leaf area which is an important value for the plant growth. Garnier et al. (2001) have reported the property of leaf area can be used to compare species. Leaf area of genotypes in our study ranged between 1577.4-16984.6 cm<sup>2</sup>/plant. Group B had 3 genotypes having the highest average of leaf area.

Quantitative as well as qualitative traits were considered for the Cluster Analysis carried out to group 40 local pea genotypes. 17 from 40 genotypes have been with anthocyanin and could be included in part in A, B, C groups in Cluster Analysis. In 4 genotypes testa did not have pink or purple spots in variety with anthocyanin, one of the qualitative properties and they were grouped in group C. It was seen that scala value (1=reddish brown, 2=brown, 3=brownish green)

belonging to colour of testa variation with anthocyanin was also encountered in materials of our study

Pea is grown to meet mainly house and local market need at small areas in our region, but it is grown almost everywhere from east to west of our region. Pea is a product which bring the industry to areas being cultivated widely depending upon its agriculture, because of that especially its frozen fresh ones and fresh and dry seed are raw material of canned food processing industry. Because of this reason, on the purpose of also bringing agricultural industry to our region, pea's cultivation should be promoted in large areas and at the commercial level. Suitable varieties should be developed for the region for realization of it. So, the properties of pea genotypes were found and their variability were determined in this study.

Dendrogram composed in the conclusion of clustering analysis showed rather wide variation in terms of 45 qualitative and quantitative traits investigated. The wideness of this variation put forward that we have a material which forms a rich genetic base for selection studies onwards. Selection for pea genotypes will be continued regarding pink or purple spots on testa among the varieties with anthocyanin, for testa colour with anthocyanin, leaf area, dry leaf weight, dry stem weight, the maximum wideness of vexillum on flower, dry leaf/dry stem rate, curvature degree of pod, pod colour, density of green colour of pod, stipule length and stipule width properties that these have got higher value than  $\pm 3$  at the initial six principal component.

Table 1. Qualitative and quantitative traits considered for Cluster and ABA analysis

Trait number	Morphologic properties	Trait number	Morphologic properties
1	Plant height (cm)	24	Wideness of stipule (3-5-7)
2	Branch number per plant	25	Density of spot on stipule (1-3-5-7-9)
3	Pod number per plant	26	Time of flowering (1-3-5-7-9)
4	Pod length (cm)	27	Max number of flowers per node (1-2-3-4-5-6-7)
5	Seed number per pod	28	Density of alea colour in the red-pink flowers varieties (3-5-7)
6	Leaf number per plant	29	Density of vexillum colour in the red-pink flowers varieties (3-5-7)
7	Leaf area per plant (cm <sup>2</sup> )	30	Colour of vexillum in the anthocyanin varieties (1-2-3)
8	Dry leaf weight per plant (g)	31	Max wideness of vexillum (3-5-7)
9	Dry stem weight per plant (g)	32	Shape of base of vexillum (1-3-5-7-9)
10	Dry leaf weight/Dry stem weight	33	Density of waving of vexillum (1-3-5-7-9)
11	Seed yield per plant (g)	34	Wideness of sepal (3-5-7)
12	100 seed weight (g)	35	Shape of upper of sepal (1-2-3)
13	Crude protein rate (%)	36	Size of pod (1-3-5-7-9)
14	Seed shape (1-2-3-4-5-6)*	37	Max wideness of pod (1-3-5-7-9)
15	Pink or purple spots on testa of varieties with anthocyanin (1-2-3)	38	Curvature degree of pod (1-3-5-7-9)
16	Colour of testa of varieties with anthocyanin (1-2-3)	39	Pod colour (1-2-3-4)
17	Foliage colour (1-2-3)	40	Density of green colour of pod (3-5-7)
18	Intensity of foliage colour (3-5-7)	41	Number of ovules in pod (3-5-7)
19	Leaflet size (1-3-5-7-9)	52	Density of green colour of immature seed (3-5-7)
20	Leaflet length (3-5-7)	43	Seed maturation time (1-3-5-7-9)
21	Leaflet wideness (3-5-7)	44	Degree of wrinkling of cotyledon (3-5-7)
22	Distance of broadest from the bottom of leaflet (3-5-7)	45	Seed weight (1-3-5-7-9)
23	Length of stipule (3-5-7)		

\* Scale values of UPOV and EU-CPVO

Table 2. Factor coefficients of examined traits in the conclusion of principal components analysis

Principal Component Axis													
	PCA1	PCA2	PCA3	PCA4	PCA5	PCA6	PCA7	PCA8	PCA9	PCA10	PCA11	PCA12	PCA13
<b>Eigen Value</b>	7.60	6.55	4.74	3.52	3.35	2.25	2.18	1.71	1.57	1.42	1.31	1.21	1.12
<b>Variance (%)</b>	16.88	14.55	10.53	7.83	7.44	4.99	4.84	3.80	3.50	3.15	2.91	2.68	2.48
<b>Cumulative Variance (%)</b>	16.88	31.44	41.97	49.80	57.24	62.24	67.09	70.89	74.38	77.54	80.44	83.13	85.61

Table 3. Principal component values of examined traits

Trait number	PCA1	PCA2	PCA3	PCA4	PCA5	PCA6	PCA7	PCA8	PCA9	PCA10
1	-0.21257	0.20241	0.04503	-0.08225	-0.23182	-0.00208	-0.05576	0.18932	-0.15801	0.09969
2	-0.17626	0.03965	0.25770	0.15776	0.00741	0.06438	0.11961	0.01532	0.21024	-0.08940
3	-0.18073	-0.05006	0.20591	0.10485	0.06051	0.12778	-0.15885	-0.08657	0.00706	-0.03851
4	0.26603	0.08709	0.10966	-0.16181	0.15340	0.07309	0.02504	0.10151	0.05321	0.02272
5	-0.08903	-0.03473	0.16101	-0.00228	0.28678	-0.04426	0.06007	0.13832	0.31431	-0.32656
6	-0.22257	0.09603	0.26629	0.14531	-0.02877	0.04550	0.13937	-0.03344	0.13358	0.03680
7	-0.12504	0.19841	0.33528	0.05204	-0.05182	-0.06646	0.11819	-0.02870	-0.05135	0.00982
8	-0.13203	0.17259	0.33442	0.10684	-0.00433	-0.05547	0.08302	-0.05096	-0.07147	0.06110
9	-0.14810	0.17394	0.30948	0.03626	-0.12171	-0.02114	0.08732	-0.01869	-0.04902	0.12941
10	0.05039	-0.07428	0.14330	0.23720	0.32783	-0.18028	0.10640	-0.02133	0.07139	-0.13630
11	0.05572	-0.03038	0.24300	-0.00323	0.03684	0.16083	-0.34681	-0.08419	-0.06333	-0.31588
12	0.26653	0.18323	0.03177	-0.14314	-0.02863	0.12395	-0.05522	0.02179	-0.03184	-0.00516
13	0.06548	-0.12885	0.07878	-0.12219	-0.08831	0.07537	0.38325	0.30676	-0.07626	0.03808
14	0.04998	0.17942	-0.01366	-0.16056	0.29693	0.11797	0.11855	-0.09560	0.17389	-0.08398
15	-0.08276	0.31442	-0.16925	0.00766	0.00918	-0.06736	-0.06873	-0.01405	0.09020	-0.05747
16	-0.04923	0.31507	-0.10831	0.02929	0.03592	0.01593	-0.03240	-0.01265	0.06197	-0.06680
17	0.07089	-0.25042	0.09361	-0.05978	0.06498	0.01625	-0.09210	0.06937	0.29960	0.14486
18	-0.11398	-0.08911	-0.04167	0.11400	0.01781	0.01858	0.06780	0.05865	-0.01951	0.45635
19	0.10962	0.11854	0.20299	-0.14490	0.01184	-0.11548	-0.12006	-0.19227	-0.25266	0.09870

Continuing table 3

Trait number	PCA1	PCA2	PCA3	PCA4	PCA5	PCA6	PCA7	PCA8	PCA9	PCA10
20	-0.12936	-0.13208	-0.02927	0.02503	-0.20384	0.09810	-0.00675	-0.14327	-0.02668	-0.28949
21	0.19451	0.11893	0.16377	0.01891	0.10381	-0.24485	-0.07909	0.16457	0.03450	0.13528
22	-0.15575	-0.05212	-0.06916	-0.00778	0.13967	0.10890	0.12685	0.25253	-0.00993	0.13214
23	-0.14015	0.11631	-0.03424	-0.04348	-0.04936	0.33452	0.27778	-0.03021	-0.04182	-0.01178
24	0.12631	0.01772	0.16066	0.04153	-0.00283	-0.40255	-0.22539	0.02956	-0.05645	0.13521
25	-0.02782	0.17821	-0.02492	-0.16642	0.07919	-0.00917	0.31111	-0.14021	-0.18444	0.02272
26	-0.17509	0.02549	0.07288	-0.23447	0.00390	0.09401	-0.28893	0.00424	0.29044	-0.02616
27	0.10057	-0.00945	-0.11320	0.21801	-0.09992	0.11879	0.08331	-0.34176	-0.11954	-0.18038
28	-0.09923	0.26041	-0.23234	0.17475	0.06125	-0.05657	-0.04284	0.02909	0.13110	0.00629
29	-0.09755	0.26288	-0.21117	0.17856	0.05428	-0.05472	-0.05614	0.05122	0.16990	0.01241
30	0.18976	-0.16768	0.24882	-0.00432	-0.05151	0.11453	0.05396	-0.16614	-0.03053	0.10062
31	0.20720	0.05567	0.02092	0.35675	-0.07738	0.12955	-0.04569	0.01743	0.00522	0.01589
32	0.15284	0.05422	0.06029	0.28026	-0.14746	0.14876	-0.01497	0.13664	0.07833	0.00033
33	0.15099	0.06032	-0.09228	0.27975	-0.03925	-0.03298	-0.00094	-0.09130	0.23533	0.27631
34	0.21464	0.10956	0.03329	0.25148	-0.09830	0.19781	-0.00816	0.17206	0.03937	0.03431
35	0.20173	-0.01358	0.10239	0.14913	-0.10435	0.17788	0.00004	0.10968	0.05611	-0.00811
36	0.17061	0.23917	-0.00445	-0.15404	0.09824	0.14362	0.08787	-0.04739	0.04209	0.05994
37	0.14854	0.29593	0.09346	-0.12764	0.05795	0.03376	0.01774	0.02738	-0.03366	-0.08663
38	-0.04356	0.03963	-0.06075	0.10550	0.35044	-0.14829	-0.02002	-0.24005	-0.12962	0.14033
39	-0.07242	-0.00140	-0.00712	0.14956	0.33406	0.23706	-0.16526	0.19883	-0.33295	0.00139
40	-0.04976	-0.02136	-0.02314	0.14058	0.32646	0.30394	-0.15317	0.14733	-0.28228	0.04402
41	-0.06369	-0.06396	0.05466	-0.05634	0.19449	0.25612	-0.06754	-0.39453	0.05129	0.33383
42	0.00883	-0.04078	0.01560	0.04133	0.14125	-0.19579	0.08171	0.29020	-0.25690	-0.16300
43	-0.14872	0.02421	0.03996	-0.22608	-0.04054	0.18505	-0.27029	0.23158	0.12794	0.20938
44	0.21810	-0.09527	0.01385	-0.09672	0.19004	0.03420	0.26655	-0.00840	0.20090	0.04315
45	0.25152	0.19008	0.01732	-0.11472	-0.08658	0.09986	-0.11815	0.01328	-0.02488	-0.03920

Figure 1. Dendrogram obtained from cluster analysis

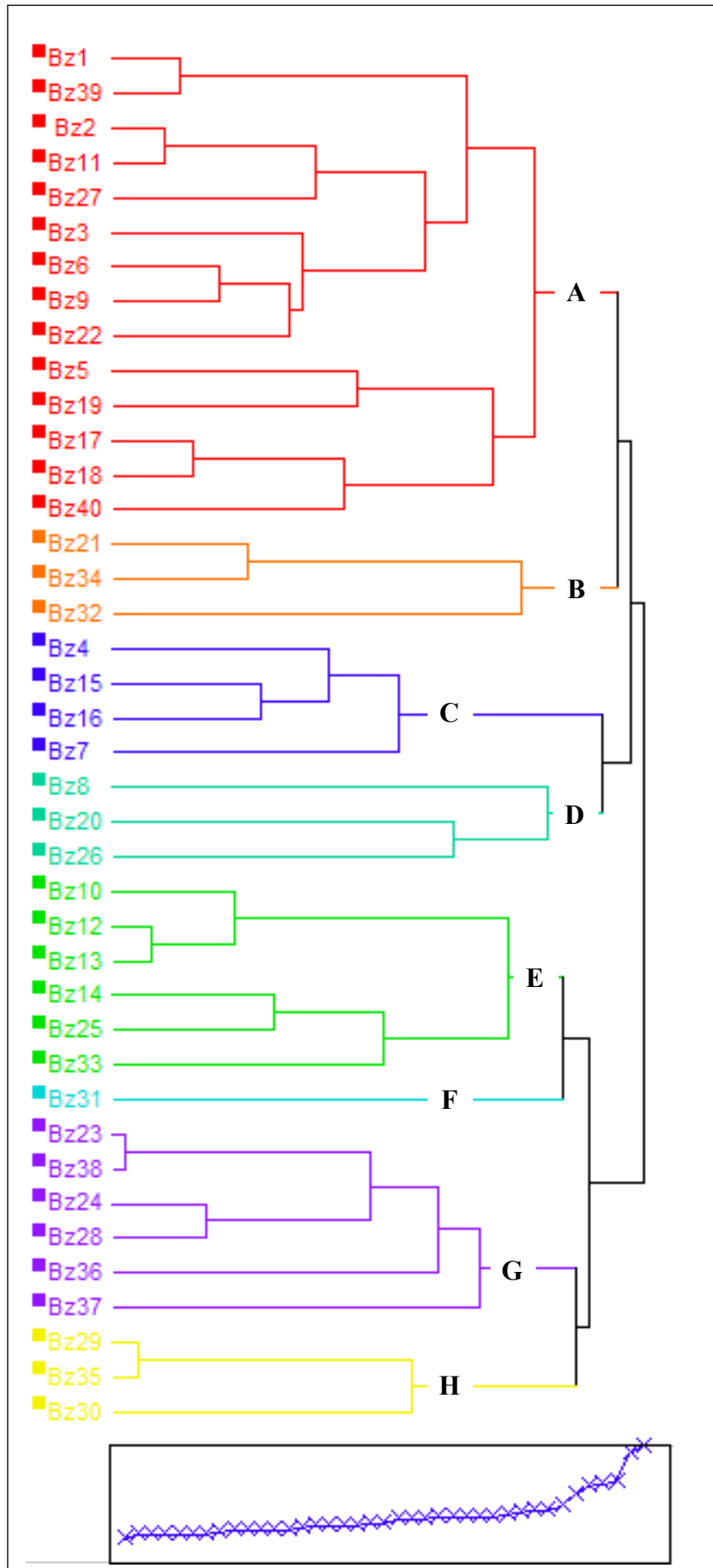


Table 4. Genotypes owned by groups and sub-groups as a result of cluster analysis

Groups	Sub-groups	Genotypes	Genotype number
<b>A</b>	A1	Bz1, Bz39	2
	A2	Bz2, Bz11, Bz27	3
	A3	Bz3, Bz6, Bz9, Bz22	4
	A4	Bz5, Bz19	2
	A5	Bz18, Bz17, Bz40	3
<b>B</b>	B1	Bz21, Bz34	2
	B2	Bz32	1
<b>C</b>	C1	Bz4, Bz15, Bz16	3
	C2	Bz7	1
<b>D</b>	D1	Bz8	1
	D2	Bz20, Bz26	2
<b>E</b>	E1	Bz10, Bz12, Bz13	3
	E2	Bz14, Bz25, Bz33	3
<b>F</b>	F1	Bz31	1
<b>G</b>	G1	Bz23, Bz38	2
	G2	Bz24, Bz28	2
	G3	Bz36	1
	G4	Bz37	1
<b>H</b>	H1	Bz29, Bz35	2
	H2	Bz30	1



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