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Genetic Diversity of Pea (*Pisum arvense* L.) Genotypes According to the Tissue Culture Traits

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ABSTRACT: Sufficient knowledge of genetic variation and germplasm classification is necessary to select suitable parents for breeding purposes. Effective and repeatable tissue culture method is a prerequisite for genetic engineering of pea genotypes (*Pisum arvense* L.). In this study, genetic diversity of forty-two pea genotypes (*Pisum arvense* L.) was evaluated based on callus induction capacity, embryogenic callus production and plant regeneration ability. Significant variation was observed among genotypes based on tissue culture parameters. The results of ANOVA indicated significant (P<0.01) differences among genotypes for traits callus induction, embryogenic callus, responded embryogenic callus, number of somatic embryos, responded somatic embryogenesis, regeneration efficiency and number of regenerated plantlet. Cluster analysis based on the tissue culture traits classified the genotypes into four groups. The highest genetic distance was observed between Subatan and Ovaçevirme-3 genotypes. The relationships among parameters related to tissue culture were investigated by principle component analysis (PCA). The PCA1 and PCA2 axes accounted 80.43% of total variation, mainly distinguish the indices in different groups.

Key words: Cluster analysis, Genetic diversity, PCA, Pea

Bezelye (*Pisum arvense* L.) Genotiplerinin Doku Kültürü Özelliklerine Göre Genetik Çeşitliliğinin Belirlenmesi

ÖZ: Islah amaçlarına uygun ebevenyleri seçmek için gen kaynakları ve mevcut genetik çeşitlilik hakkında yeterli bilgiye sahip olmak gereklidir. Etkin ve tekrarlanabilir doku kültürü sistemi bezelye genetik mühendisliği için ön koşullardan biridir. Bu çalışmada, 42 bezelye (*Pisum arvense* L.) genotipinin genetik çeşitliliği kallus oluşum kapasitesi, embriyogenik kallus oluşumu ve bitki rejenerasyon yeteneğine göre değerlendirilmiştir. İstatistik analiz sonuçlarına göre; genotipler arasında, kallus oluşumu, embriyogenik kallus, cevap veren embriyojenik kallus, somatik embriyogenesis sayısı, cevap veren somatik embriyogenez, rejenerasyon etkinliği ve rejenere bitkicik sayısı özellikleri bakımından önemli (P<0.01) farklılıklar gözlenmiştir. Doku kültürü özellikleri dikkate alınarak yapılan kümeleme analizine göre genotipler 4 gruba ayrılmıştır. En yüksek genetik uzaklık Subatan ve Ovaçevirme-3 genotipleri arasında görülmüştür. Test edilen doku kültürü parametreleri arasındaki ilişkiler temel bileşenler analizi tespit edilmiş ve iki bileşenin (PCA1 ve PCA2) toplam varyasyonun %80.43'lük kısmını açıkladığı gözlenmiştir. Kallus oluşum kabiliyetinde gözlenen yüksek varyasyon, ıslah programlarında ebeveynlerin seçiminde etkin olarak kullanılabileceği sonucuna ulaşılmıştır.

Anahtar Kelimeler: Kümeleme analizi, Genetik çeşitlilik, PCA, Bezelye

INTRODUCTION

Legumes are important crops worldwide, and they have major impacts on agriculture, environment, animal and human nutrition (Graham and Vance, 2003). Plant cell and tissue culture is one of the most significant potential tools to improve plants by application of in vitro selection and recovering useful genetic variants. Plant cell culture has provided an alternative to obtain greater genetic variability relatively quickly without sophisticated technology (Larkin and Scowcroft, 1981; Shu and Lagoda, 2007). Genetic diversity of plants defines their potential for improved efficiency and hence their use for breeding, which eventually may result in enhanced crop production. Plant uniformity, which can be resulted by the use of modern plant breeding techniques, can produce plants, which are more efficient by means of different goals including enhanced resistance under stress, however more research must be performed to indicate the most optimized methods that can be used for the production of efficient plants (Khodadadi et al., 2011).

One of the important approaches to plant breeding is hybridization and subsequent selection. Parents' choice is the first step in plant breeding program through hybridization. In order to benefit transgressive segregation, genetic distance between parents is necessary (Joshi et al., 2004). The higher genetic distance between parents, the higher heterosis in progeny can be observed (Joshi and Dhawan, 1966; Anand and Murrty, 1968). It has been proposed that the differences for studied traits across regions were significantly (P=0.01) different and resulted in nine classes discriminated by geographical regions (Benadeki, 1992).

Estimation of genetic distance is one of appropriate tools for parental selection in plant hybridization programs. Appropriate selection of the parents is essential for crossing to enhance the genetic recombination for potential yield increase (Islam, 2004). Principal components analysis can be considered as a multivariate powerful technique for data reduction that removes interrelationships among components and effective in finding structures of data sets, genotypes grouping and estimation of genetic diversity of breeding materials (Zeinalzadeh-Tabrizi et al., 2011). Determination of genetic diversity is a first step for plant breeding and hence production of more valuable plant varieties under different conditions. The main objective of this study is to screen the pea genotypes based on plant tissue culture traits and capture the potential genetic diversity among pea genotypes by using cluster analysis and cluster analysis-PCA-based methods. The results of present study will be used in selection of appropriate parents for breeding program based at Ataturk University.

MATERIAL AND METHOD

Plant material

Forty-two peas (*Pisum arvense* L.) genotypes collected from different locations of the Eastern Anatolia Region were used as the plant material in the experiments. The ecotypes were named with the location names in where they were collected. Seeds were surface-sterilized in 70% (v/v) ethanol for 5 min, rinsed twice with sterile distilled water, incubated further in commercial bleach (5% sodium hypochlorite) with a two drops of Tween for 35 min, and rinsed twice in sterile distilled water. Sterilized seeds were placed on MS medium without hormone for germination.

Callus initiation

The root explant from 5-days old *in vitro* grown seedlings were cultured on Murashige and Skoog (MS) medium supplemented with 20 mg/L sucrose, 2 g/L phytagel, 1.95 g/L MES and 0.5 mg/l picloram for callus induction for 4 weeks (Bencheikh and Gallais, 1996). Media was adjusted to pH 5.8 with 1 N NaOH. Media solutions containing basal salts and solidifying agent were autoclaved at 121 °C for 15 min for sterilization. Vitamins and plant growth regulators were filter-sterilized. Explants were cultured at 25 °C. The callus induction was determined after four weeks.

Embryogenic callus formation and plant regeneration

For the formation and maturation of embryogenic calli, root explants were cultured to an MS medium to MS medium containing 0.05 mg/l NAA and 0.017 mg/l each of BA, kinetin and TDZ (Lazzeri et al., 1987), 2 mg/l phytagel, 20 g/l sucrose at 25±1˚C 16: 8 days: night photoperiod for 45 days. The embryogenic callus by the number of explants percentage, responded embryogenic callus by the number of explants percentage, number of somatic embryogenesis, responded somatic embryogenesis, regeneration efficiency and number of regenerated plantlet were determined after four weeks.

Rooting

Plantlets transferred to rooted medium containing MS medium with 0.2 mg/l NAA, 2 mg/l phytagel, 20 g/l sucrose at 25±1˚C 16: 8 days: night photoperiod for 45 days.

Statistical analysis

This study was carried out in complete randomized experimental design with 4 replicates. Analysis of variance (ANOVA) was performed using the general linear model (GLM) procedure in SPSS version 20 (SPSS. Chicago, USA). Each petri dish was considered as an experimental unit and 15 root explant were cultured in each petri dish. Means treatments was compared using Fisher's Duncan test. Cluster analysis based on ward's method using squared Euclidian distance (Kumar et al., 2009) and identification the cutting point using discriminate analysis were performed using the statistical software SPSS version 20 (SPSS. Chicago, USA) program. PCA was also performed using SPSS version 20 software.

Results

Plant tissue culture

Analysis of variance showed that there were significant differences among 42 genotype based on callus induction, embryogenic callus by the number of explants percentage, responded embryogenic callus by the number of explants percentage, number of somatic embryogenesis, responded somatic embryogenesis, regeneration efficiency and number of regenerated plantlet ($P<0.05$) (Table 1). Genotype in tissue culture was critical factor which determining the efficiency and the characteristics of callus. Callus appeared two weeks after culture initiation and in following weeks' different callus types were formed. Based on mean values of CI for among genotypes, the highest mean CI% was observed the Çamlıçatak-2. Değirmencik-1, Görele-1, İncili-2 and Oburcak genotypes (100.00%) whereas the lowest mean CI% was observed in the Selamverdi genotype (43.75%) (Table 1).

Table 1: Callus induction (CI), mean embryogenic callus by the number of explants percentage (ECNEP), mean responded embryogenic callus by the number of explants percentage (RECNEO), mean number of somatic embryo (NSE), mean responded somatic embryo by the number of explants percentage (RSE) and mean regeneration efficiency (RE) and mean Regenerated plant number (NRP) of peas (*Pisum arvense* L.).

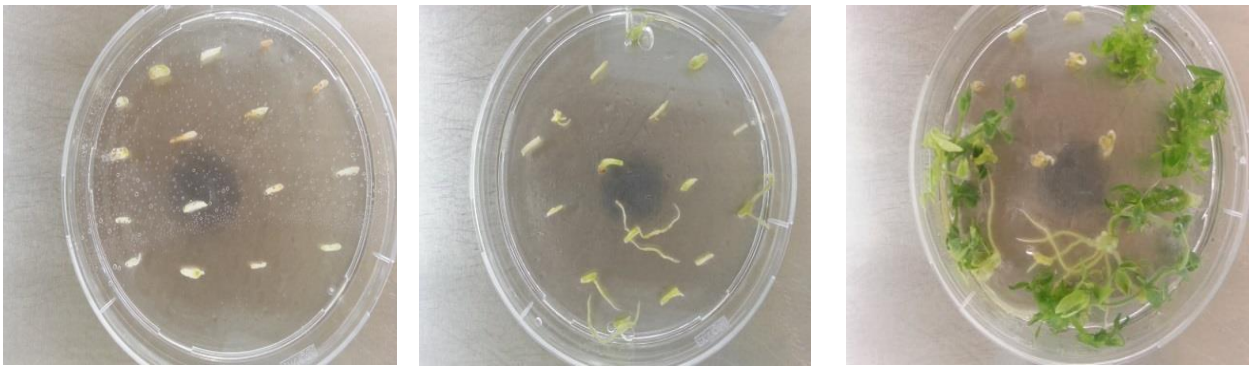
Genotype	CI %	ECNEP %	RECNEP %	NSE (number)	RSE (number)	RE (number)	NRP (number)
Ardahan Merkez-1	90.62 a-e	67.18 e-j	60.93 e-k	40.00 c-f	36.50 b-d	0.00 b	0.00 b
Ardahan Merkez-2	96.87 a	82.81 b-e	70.31 c-g	55.75 b	39.5 b-c	0.00 b	0.25 b
Ardahan Merkez-3	85.93 a-g	73.43 c-g	50.00 e-m	16.75 k-p	10.75 i-m	0.05 b	0.25 b
Aşağıcambaz	71.87 f-m	59.37 f-l	43.75 g-o	18.25 k-p	16.00 g-l	0.13 b	0.00 b
Aşağıkırzı	81.25 a-h	70.31 e-i	65.62 d-h	36.00 c-g	32.75 b-f	0.00 b	0.00 b
Avcılar	90.62 a-e	75.00 c-f	60.93 e-k	28.00 g-l	24.75 d-h	0.00 b	0.00 b
Balçeşme	73.43 e-k	60.93 f-k	46.87 f-n	16.00 l-p	12.50 h-m	0.00 b	0.00 b
Camlıçatak-1	60.93 i-m	40.62 m-p	14.06 o-s	15.25 k-p	9.50 i-m	0.00 b	0.00 b
Camlıçatak-2	100.00 a	56.25 g-n	62.5 e-j	23.75 g-o	22.50 e-i	0.00 b	0.00 b
Cayağzı	56.25 k-m	1.562 q	0.00 s	8.50 p-q	0.00 m	0.00 b	0.25 b
Ciğdemtepe	96.87 a	79.68 b-e	60.93 e-k	16.75 k-p	11.50 h-m	0.02 b	0.00 b
Cumhuriyet	59.37 j-m	51.56 j-n	43.75 g-o	14.75 m-p	9.00 i-m	0.00 b	0.00 b
Değirmencik-1	100.00 a	73.43 c-g	100.00 a	44.25 c	44.25 b	0.00 b	2.25 a
Doğruyol	46.87 m	42.18 l-p	35.93 j-q	22.75 i-o	8.00 j-m	0.56 a	0.00 b
Döşeli-1	51.56 l-m	29.68 p	18.75 o-s	12.75 o-q	3.00 l-m	0.00 b	0.00 b
Giresun Merkez	65.62 h-i	42.18 l-p	34.37 k-q	26.25 g-m	20.75 f-j	0.00 b	0.00 b
Görele-1	100.00 a	100.00 a	96.87 a-b	32.25 d-i	31.00 c-f	0.00 b	0.00 b
Incili-1	45.31 m	32.81 0-p	20.31 n-s	24.75 j-o	3.00 l-m	0.00 b	0.00 b
Incili-2	100.00 a	84.37 a-e	68.75 c-h	43.00 c-d	41.00 bc	0.00 b	0.25 b
Incili-3	82.81 a-h	73.43 c-g	56.25 e-l	19.25 j-p	11.00 i-m	0.03 b	0.00 b
Kartalpınar	76.56 c-j	42.18 l-p	35.93 j-q	17.00 k-p	7.00 k-m	0.00 b	0.00 b
Kenarbel	70.31 g-k	60.93 f-k	40.62 i-p	16.00 k-p	8.25 j-m	0.00 b	0.00 b
Koyunpınarı	93.75 a-c	90.62 a-c	71.87 b-e	33.00 c-i	26.50 d-g	0.00 b	0.00 b
Oburcak	100.00 a	71.87 d-h	73.43 b-e	31.00 e-j	21.00 e-j	0.00 b	0.00 b
Ovaçevirme-1	82.81 a-h	79.68 b-e	75.00 b-e	35.50 c-h	34.00 b-e	0.00 b	0.00 b
Ovaçevirme-2	85.93 a-g	81.25 b-e	53.12 e-l	18.75 j-p	17.50 g-k	0.00 b	0.00 b
Ovaçevirme-3	93.75 a-c	89.06 a-d	93.75 a-c	78.00 a	77.25 a	0.00 b	0.00 b
Ovaçevirme-4	71.87 f-k	59.37 f-l	60.93 e-k	18.50 k-p	13.0 h-m	0.00 b	0.00 b
Ovaçevirme-5	68.75 g-i	59.37 f-l	25.00 m-s	17.25 k-p	6.50 k-m	0.00 b	0.00 b
Paslı	98.43 a	96.87 a-b	90.62 a-d	42.25 c-e	39.75 b-c	0.00 b	0.00 b
Sayvan	59.37 j-m	53.12 i-n	34.37 k-q	26.25 g-m	24.75m	0.00 b	0.00 b
Selamverdi	43.75 m	29.68 p	9.37 q-s	2.25 q	0.50 m	0.00 b	0.25 b
Senkaya Merkez	92.18 a-d	54.68 h-n	7.81 r-s	13.25 n-q	4.25 k-m	0.08 b	0.00 b
Serhat	78.12 b-i	73.43 c-g	65.62 d-h	14.75 m-p	11.25 i-m	0.00 b	0.00 b
Seyitören	95.31 a-b	75.00 c-f	21.87 n-s	9.25 p-q	1.5 m	0.00 b	0.00 b
Subatan	76.56 c-j	57.81 f-m	50.00 e-m	17.00 k-p	10.25 i-m	0.00 b	0.00 b
Sulakyurt	75.00 d-j	53.12 i-n	46.87 f-n	17.25k-p	11.75 h-m	0.00 b	0.00 b
Tahtakıran	89.06 a-f	67.18 e-j	56.25 e-l	28.75 f-k	21.50 f-j	0.00 b	0.00 b
Tepeköy	71.87 f-k	25.00 p	17.18 o-s	15.00 m-p	4.75 k-m	0.00 b	0.50 b
Tepeler	78.12 b-i	39.06 n-p	29.68 l-r	18.75 j-p	11.75 h-m	0.08 b	0.00 b
Yamçılı	93.75 a-c	79.68 b-e	65.62 d-h	23.50 h-o	16.75 g-k	0.00 b	0.25 b
Yolgeçmez	68.75 g-i	48.43 k-o	42.18 h-o	26.00 g-o	9.25 i-m	0.04 b	0.00 b
F value (Genotype)	9.22**[2]	15.27**	9.82**	0.95**	14.27**	15.18*	0.94*

[1] Mean values marked with the same letter are not significantly different at ($p \leq 0.05$).

[2] *significant at the p $p \leq 0.05$. **significant at the $p \leq 0.01$.

Both embryogenic and nonembryogenic calli were observed in the embryogenic callus initiation medium. The embryogenic callus was characterized by compact presence of globular somatic embryos and a light yellow color (Figure 1).

In addition, great variation was determined in mean embryogenic callus by the number of explants percentage among genotypes and the highest mean embryogenic callus by the number of explants percentage was observed the Görele-1 genotype (100%) whereas the lowest mean embryogenic callus by the number of explants percentage % was observed in the Cayağzı genotype (1.56%) (Table 1). In respect to result of mean responded embryogenic callus by the number of explants percentage among genotypes, the highest mean responded embryogenic callus by the number of explants percentage was observed in the Değirmencik-1 genotype (100%) whereas the lowest mean responded embryogenic callus by the number of explants percentage % was observed in the Çamlıçatak-1 genotype (14.06%) (Table 1).



A **B** **C**

Figure 1. Root explants on culture (A), Morphogenesis (B), and Plant regeneration from root explants

According to mean number of somatic embryo among pea genotypes, the highest mean number of somatic embryo was demonstrated the Ardahan Merkez-2 genotype (55.75) whereas the lowest mean number of somatic embryo was observed in the Selamverdi genotype (2.25) (Table 1). In addition, significant variation was observed among genotypes in terms of responded somatic embryogenesis. The highest mean responded somatic embryo by the number of explants percentage was determined in Incili-2 genotype (41) whereas the lowest mean responded somatic embryo by the number of explants percentage was observed in the Cayağzı genotype (1.56) (Table 1). Regeneration was observed in genotypes Ardahan Merkez-3, Aşağıcambaz, Ciğdemtepe, Doğruyol, Incili-3, Senkaya Merkez, Tepeler and Yolgeçmez. In terms of mean regeneration efficiency, the highest mean regeneration efficiency was demonstrated the Doğruyol genotype (0.56) whereas the lowest mean regeneration efficiency was observed in the Ciğdemtepe genotype (0.02) (Table 1). In terms of number of regenerated plantlet, the highest mean number of regenerated plantlet was demonstrated the Doğruyol genotype (2.25) (Table 1An important factor in successful callus induction and plant regeneration in plant tissue culture was dependent to genotypes responding (Ahloowalia, 1982; Carman et al., 1987; Maheshwari et al., 1995) and our results agreed with this finding. Maddock et al., (1983) found that embryo formation and shoot regeneration varied from 12% to 96% in 25 cultivars. He et al., (1990) reported that production of plants from embryogenic and nonembryogenic calli were genotype dependent.

Principal component analysis

The purpose of the principal components analysis is to obtain a small number of linear combinations, which account for most of the variability in used data. In this study, two components have been identified, the result showed that two components had Eigen values greater than or equal to 1. They accounted for 80.43 % of the variability in the original data (Table 2).

Table 2. Principal Components Analysis of pea (*Pisum arvense* L.) genotypes.

Component Number	Eigen value	Percent of Variance	Cumulative Percentage
1	3.67	52.42	52.42
2	***1.96***	***28.01***	***80.43***
3	0.79	11.36	91.80
4	0.30	4.32	96.13
5	0.17	2.46	98.59
6	0.08	1.21	99.80
7	0.01	0.19	100.00

Maruthi Sankar et al., (1999) have assessed the variability of eight plant traits for growth of sunflower and reduced the dimensionality to two principal components, which extracted about 80% of variance in the original data.

The portion of each two components was approximately 52.42 and 28.01 percent of total variance, respectively. Greatness of these variances influences good separation of genotypes. If there would be correlations among traits or similarities among genotypes, these components can provide suitable grouping and separate same genotypes in distinct groups (Zeynalzadeh-Tabrizi et al, 2011).

Table 3. Component Weights of Principal Components Analysis of pea (Pisum arvense L.) genotypes

Weights	**Component 1**	**Component 2**
CI %	**0.778**	-0.156
ECNEP %	**0.859**	-0.089
RECNEP %	**0.894**	-0.058
NSE (number)	-0.055	**0.993**
RSE (number)	**0.834**	**0.064**
RE (number)	**0.882**	**0.014**
NRP (number)	-0.044	**0.992**

Table 4 shows each component weight. It is considerable that mean responded embryogenic callus by the number of explants percentage (%) has a highest weight in first component and from the aspect of this trait; genotypes can be grouped using this component. Mean regeneration efficiency, mean embryogenic callus by the number of explants percentage (%)**,** mean responded somatic embryo by the number of explants percentage (number) and callus induction (%) were other traits having higher weights, also and can be explained by the first component. Second component was more associated with mean number of somatic embryo, mean responded somatic embryo by the number of explants percentage and mean regeneration efficiency and mean Regenerated plant number. Kroonenberg (1995) concluded that the angle of vectors shows correlations of vectors and therefore, among traits.

Cluster analysis

By incision the dendrogram, the genotypes categorized into four groups. Using discriminant analysis revealed that 95.2% of the members constituted four groups (Figure 2). The first group contained 63.1% of total genotypes, second group including 34.3% of total genotypes, third group including 2.4 % of total genotypes and the fourth group including 0. 005% of total genotypes. The highest genetic distance was observed between Subatan and Ovaçevirme-3 genotypes. According to Rahim et al. (2010) who reported that genotypes with maximum distance resulted in high yield, the cross between these genotypes can be used in breeding programs to achieve maximum heterosis.

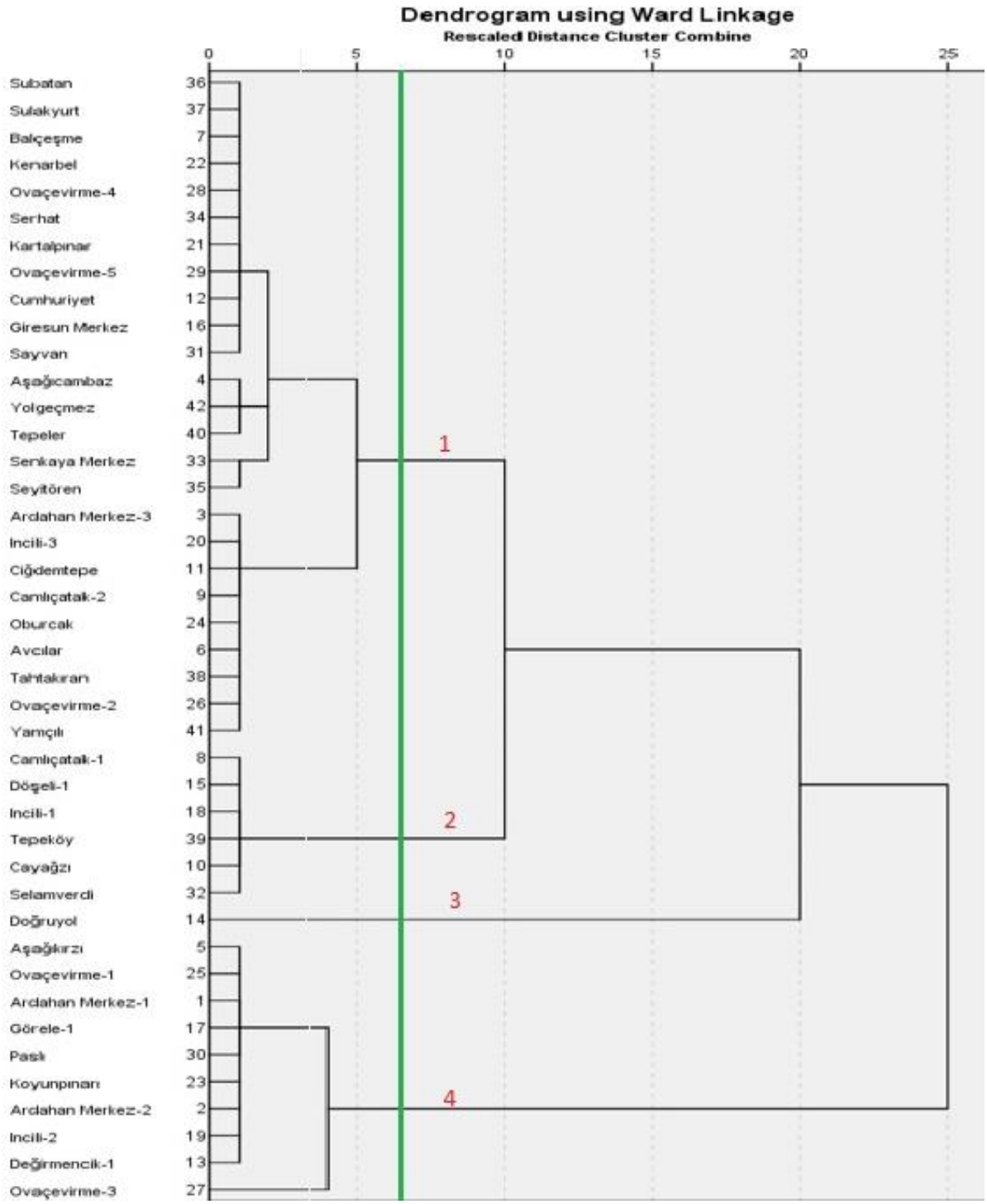


Figure 2. Tree diagram of 42 genotypes for 7 studied variables using hierarchical cluster analysis (ward's method and squared Euclidean distance).

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