ASSESSMENT OF GENETIC DIVERSITY AMONG *Vicia sativa* L. CULTIVARS BY TOTAL PROTEIN PROFILE, CYTOLOGICAL ANALYSIS AND MOLECULAR CHARACTERIZATION

Vicia sativa L. ÇEŞİTLERİ ARASINDAKİ GENETİK ÇEŞİTLİLİĞİN TOTAL PROTEİN PROFİLİ, SİTOLOJİK ANALİZ VE MOLEKÜLER KARAKTERİZASYON İLE DEĞERLENDİRİLMESİ

Büşra YAZICILAR[1], Assist. Prof. Dr. İsmail BEZİRGANOĞLU[2]

[1,2]Department of Molecular Biology and Genetics, Erzurum Technical University, 25050 Erzurum, Turkey.

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ÖZET

Fiğ (*Vicia* subsp.) çok veya tek yıllık, besin değeri yüksek, bir baklagil yem bitkisidir. Baklagil yem bitkileri içerisinde uzun ömürlü olması çevre şartlarına daha dayanıklı olması nedeniyle en çok tercih edilen bir bitki türüdür. Bu çalışmada 2 tane Yaygın fiğ (*Ankara Moru* ve *Ayaz)* ile 3 tane Macar fiğ (*Ankara Pembesi, Tarım Beyazı* ve *Kansur*) çeşitleri kullanılarak çekirdek DNA, kromozom sayımı ve total protein miktarına bakılarak genetik çeşitliliğinin belirlenmesi amaçlanmıştır.

5 farklı fiğ (*Vicia* subsp.) populasyon tohumları MS ortamına (Murashige Skoog basal medium 1962) ekilerek yaprak örnekleriyle flow sitometri analiziyle çekirdek DNA ve total protein miktarına bakılmıştır. 5 farklı fiğ (*Vicia* subsp.) populasyonlarının kök uçlarından örnek alınarak kromozom sayımı yapılmıştır. SDS PAGE analizi 140 kDa ile 25 kDa arasında bant vardır. Total protein miktarı en çok *Kansur, Tarım Beyazı, Ankara Moru* ve *Ayaz'*dır. Total Protein miktarı en az ise *Ankara Pembesi*'dir. Çekirdek DNA miktarı 3.53 pg ile 11.11 pg arasında değişmiştir. Flow sitometri sonuçlarına göre *Kansur* ve *Tarım Beyazı* ile *Ankara Pembesi* ve *Ankara Moru* çekirdek DNA oranları birbirine yakındır. *Kansur* kromozom sayısı *2n=2x=14*, *Ankara Pembesi, Tarım Beyazı, Ankara Moru* ve *Ayaz* kromozom sayısı *2n=2x=12*'dir. Total protein miktarı ile kromozom sayımı sonuçları birbirini doğrulamıştır. Proteince zengin ve genom boyutu en büyük olan *Kansur* hattımızdır.

Anahtar Kelimeler: Fiğ (*Vicia* subsp.), Flow Sitometri, Kromozom Sayımı, SDS PAGE

ABSTRACT

Faba bean (*Vicia faba* L.) is an important food and feed legume because of the nutritional value of its seed protein and starch content, high biomass, and resistant environmetal conditions. It is commonly grown as a crop for human consumption. In this study, seeds of 5 cultivars of *Vicia faba* were evaluated for the nuclear DNA, chromosome count, and total protein amount.

5 different vicia faba seeds were cultured on MS basal medium at a temperature of 23°C ± 2°C under a 16 h light photoperiod with the light intensity of 97 µmol m^{-2} s^{-1} for 2 weeks. The amount of nuclear DNA was measured by flow cytometry analysis with leaf samples. Chromosome count was made by taking samples from root tips of 5 different faba bean (*Vicia faba*) cultivars. SDS PAGE analysis was produced bands between 140 kDa and 25 kDa. SDS PAGE also analysis displayed the accumulation of proteins in leaves. In terms of SDS PAGE analysis, *Kansur* exhibited the most abundant protein, followed by *Tarım Beyazı* and *Ankara Moru*. Total protein amount is the lowest in *Ankara Pembesi*. The amount of nuclear DNA content ranged from 3.53 pg to 11.11 pg. According to flow cytometry results, there was no significant variation between nuclear DNA contents of *Kansur* and *Tarım Beyazı*, *Ankara Pembesi* and *Ankara Moru*. The four *Vicia faba* plants showed the same chromosome numbers as *2n= 2x= 12*. However, *2n= 2x= 14* number was detected in the root tips of the *Kansur*. *Kansur* displayed that nucleat DNA content and protein accumulation was higher the other cultivars.

Keywords: Faba bean (*Vicia* subsp.), Flow cytometry, Chromosome count, SDS PAGE

1. INTRODUCTION

Vicia faba L., is an annual, self fertile forage legume and commonly grown in the cool season regions. It has 166 species, is spread mostly in North America, Asia and Europe extending to the temperate regions of South America and tropical Africa (Maxted, 1993). *Vicia faba* is cultivated for its nutritious seeds and pods which are high in protein. Its important function in crop cycle, powerful nitrogen fixation, soil bioremediation abilities has loong been known (Ye, 2003). The traditional classification of *Vicia faba* is based on seed characters and weights and sizes of seeds and pods. Clasically, various seed sizes cultivars are cultivated in autumn sowing region for animal consumption, while cultivars of large seed size generated in spring sowing region are mainly used for vegetable, food and feed (Ye, 2003). It is doubtful if traditional classification is only based on agronomical and morphologic characters since these characters are either influenced by degree of plant growth and environment factors or they display only restricted variation (Terzopoulos, 2008). *Vicia faba* is a diploid plant and has fewer chromosome number ($2n = 2x = 12$).

However, the nuclear DNA content of *Vicia faba* is remarkably large 13.000 Mb, (Terzopoulos, 2008; Johnston, 1999). Various cytological analysis and protein markers have been successfully used in the characterize genetic diversity, taxonomy classification and population genetic structure in crop plants. Seed storage proteins are one of the most important markers in genotype specific protein markers. Moreover, proves provided by the separation protein components in polyacrylamide gels are mostly informative at the species and intra-specific levels (Signor, 2005). Seed proteins informations have been used to determine genetic variation at intra specific degrees also for varieties identifications [6]. Flow cytometry is a useful method of plant cultivars that identifies ploidy level and analyzes the nuclear DNA content. It has been characterized as a rapid tool for assessing the ploidy level of *Vicia faba* cultivars. Compared to other methods: robust technique, quickly detection of mixed samples, non destructive sampling and endopolyploidy, hence a large samples can be measured in a short time and the presence of subpopulations might be observed (Sammour, 2007).

Chromosome number analysis has been evident to be an important tool for classification and releasing chromosome origin events. The basic principle of cytological analysis is to determine classification and assessment of basic chromosome number of closely related species or other related ones. Although agronomic traits methods have provided significant successes in taxonomic studies, these methods have still some restrictions because of closely related populations and species for germplasm. Molecular marker and cytogenetics techniques can considerably provide more information about chromosome number, genome size, and base pair. The aim of this study is to evaluate the genetic diversity and ploidy levels by using SDS PAGE, chromosome number, and flow cytometry analysis of eight *Vicia faba* varieties. Moreover, it is also thought to be an important material for the improvement of new cultivars through breeding studies in the future.

2. MATERIALS AND METHODS

Plant material

Five *V. sativa* L. cultivars (*Ankara Moru*, *Ankara Pembesi, Ayaz, Tarım Beyazı* and *Kansur*) ecotype was used as the plant material for this study. All cultivars used were obtained from the Department of Molecular Biology and Genetics at Kafkas University.

Chromosome counting

In the mitotic chromosome analysis, five *V. sativa* L. cultivars seeds were planted in each petri dishes. After 3 days, 1.5 cm roots were cut with a scalpel. It was stored in 0.05% colchicine in glass tubes at room temperature for approximately 3 hours. It was then left to wash for 3 hours. Then it was left in fixative (3:1, ethanol:45% acetic acid) for 3 hours at room temperature. Then, it was kept in a 85 ^{0}C bath for 10 minutes in 1 N NaOH in hydrolysis. Hemotoxylin was kept at room temperature for 10

hours. It is then passed through dsu 3 times. Zeiss Axiophot microscope was used and analyzed samples 3 replicates (Agayev, 1998).

Flow cytometry

Nuclear DNA content of *Vicia* subsp. cultivars and ecotypes were carry-out by flow cytometer using fresh plant materials. Genome size analysis was determined by using 3 replicates. Commercial kits (CyStain PI absolute P) of Partec were used in nuclear DNA content analysis. A slightly modified version of the Partec protocol was carried out in the analyses. (2 pg/2C) was obtained as a reference standard. Shortly, the protocol consisted of simultaneously chopping leaf tissues (20 mg each) of *Vicia* subsp. and *Lycopersicon Esculentum* as a control in 0.5 mL nuclei extraction buffer, transferring homogenized tissues into centrifuge tubes through filter, brief centrifugation (20 s), dissolving the pellet in extraction buffer (0.5 mL), adding staining buffer (1 mL) and incubation (30 min) at room temperature. The samples were then analyzed using a Partec CyFlow Space flow cytometer (Munster, Germany) equipped with green laser excitation at 488 nm. The absolute DNA contents of *Vicia* subsp. cultivars and ecotypes were calculated based on the ratios of the G1 peak means of sample and reference standard by using the following formula:

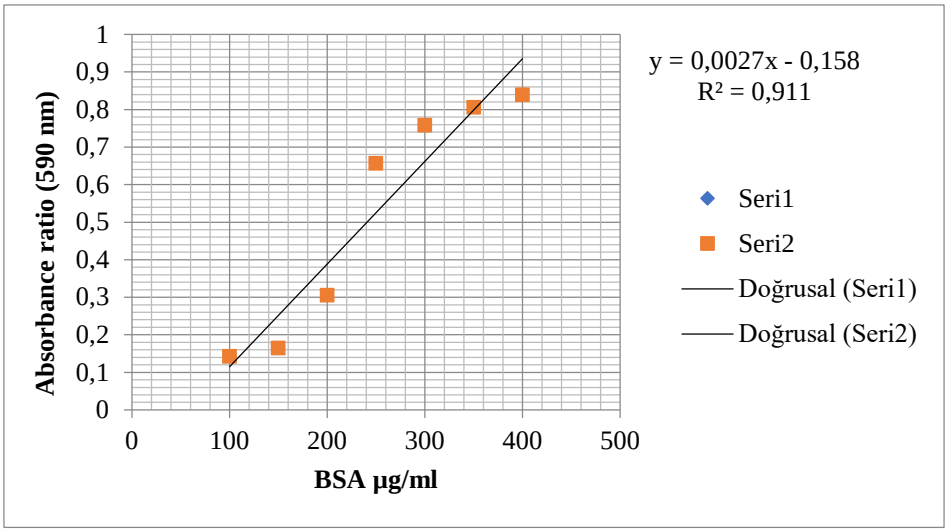
Sample 2C DNA content= sample G1 peak mean $standard\ G1\ mean$ x2C DNA content (pg)

Nuclear DNA content values were converted from pg to bp by using formulas. The C-values of the species were compared using t-test.

Total proteins analysis

For *Vicia* subsp. total protein isolation, 0.03 grams of leaf sample was weighed. It was taken into 2 mL eppendorf tube and homogenized by adding 200 µL sample buffer sample. It was kept in a water bath at 100 ^{0}C for 3 minutes. It was then centrifuged at 10.000 rpm for 5 minutes. The supernatant portion was transferred to another 2 mL eppendorf tube. Later, a standard graphic was created using the Bradford method (Table 1). 20 µg of the protein were separated in 10% sodium dodecyl sulfate (Laemmli, 1970).

Table 1. Standard plot of Bradford protein



3. RESULTS

Chromosome number and ploidy level

Out of the 5 cultivars tested, the four cultivars were found to be entirely diploid with basic chromosome number *2n=2x=12* (*Ankara Moru*, *Ankara Pembesi*, *Ayaz* and *Tarım Beyazı*). Although *Kansur* ecotype was detected to be diploid, basic chromosome numbers were detected as *2n=2x=14* (Figure 1).

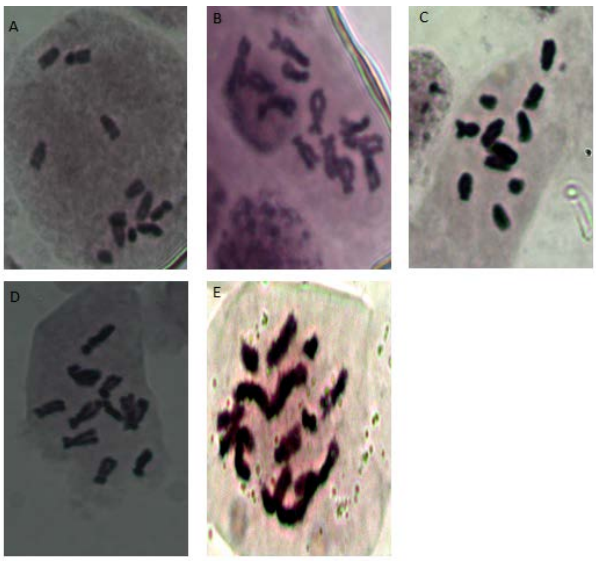


Figure 1. Chromosome numbers analysis of *Vicia* L. the population using microscopy. Representative of the ploidy levels of population **A.** *Ankara Moru 2n=2x=12* **B.** *Ankara Pembesi 2n=2x=12* **C.** *Ayaz 2n=2x=12* **D.** *Tarım Beyazı 2n=2x=12* **E.** *Kansur 2n=2x=14*.

Nuclear DNA content

The genome size of the tested samples ranged from 10.98 pg in *Tarım Beyazı* to 11.11 pg in Kansur, which is a tetraploid. The genome size of the tested samples ranged from 3.53 pg in *Ankara Moru* to 8.83 pg in *Ankara Pembesi* and, *Ayaz* 6.87 pg which is a diploid. All samples have too small genome. Significant differences among the samples were observed. There was intraspecific diversity in genome size among tested. Overall, tested samples demonstrated the significant different value of genome size (Table 2, Figure 2).

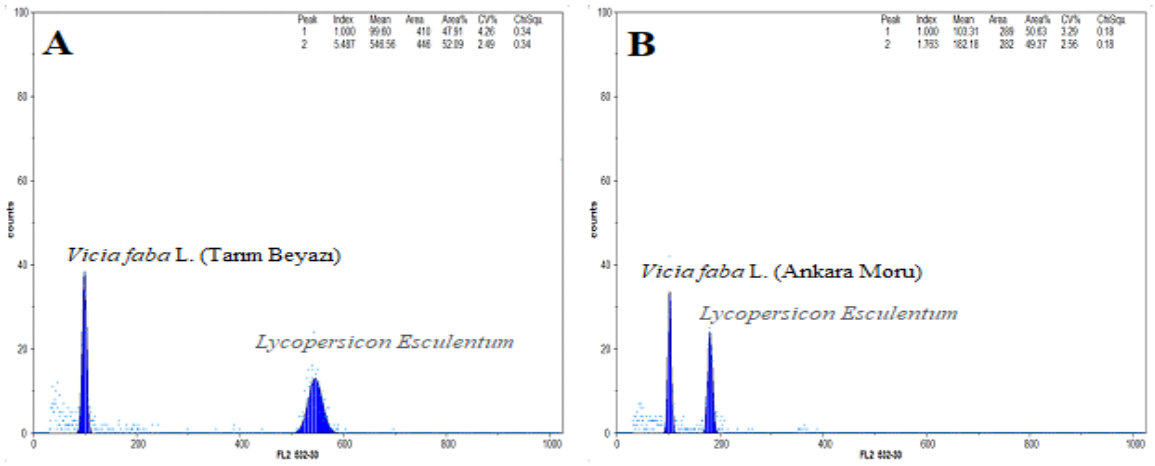


Figure 2. Flow cytometric analysis of PI stained nuclei *Visia faba* L. using *Lycopersicon Esculentum* as control. **A.** *Tarım Beyazı*, **B.** *Ankara Moru*.

Table 2. Nuclear DNA content of *V. sativa* L. cultivars and ecotypes.

Vicia faba L.	Sample peak	Standart peak	Standart DNA content(pg)	Örnek DNA content(pg)	CV1 (*Alfalfa*)	CV2 (*Lycopersicon Esculentum*)
Ankara Pembesi	451.75	102.32	10.65	8.83	3.1	2.71
Ankara Moru	182.18	103.31	10.65	3.53	3.29	2.56
Kansur	656.97	118.29	10.65	11.11	3.11	2.68
Tarım Beyazı	546.56	99.6	10.65	10.98	4.26	2.49
Ayaz	351.75	102.30	10.65	6.87	3.1	2.71

Total proteins analysis

Total protein isolated from leaves of 5 cultivar was subjected to 12% SDS PAGE analysis. A amounts of total protein molecular weight were determined in all tested samples from 140 kDa by 25 kDa. According to the band intensity results, the highest protein levels were observed for *Kansur*, *Tarım Beyazı* and *Anakar Moru* cultivars. The lowest protein levels were observed for *Ayaz* and *Anakara Pembesi* cultivars (Figure 3).

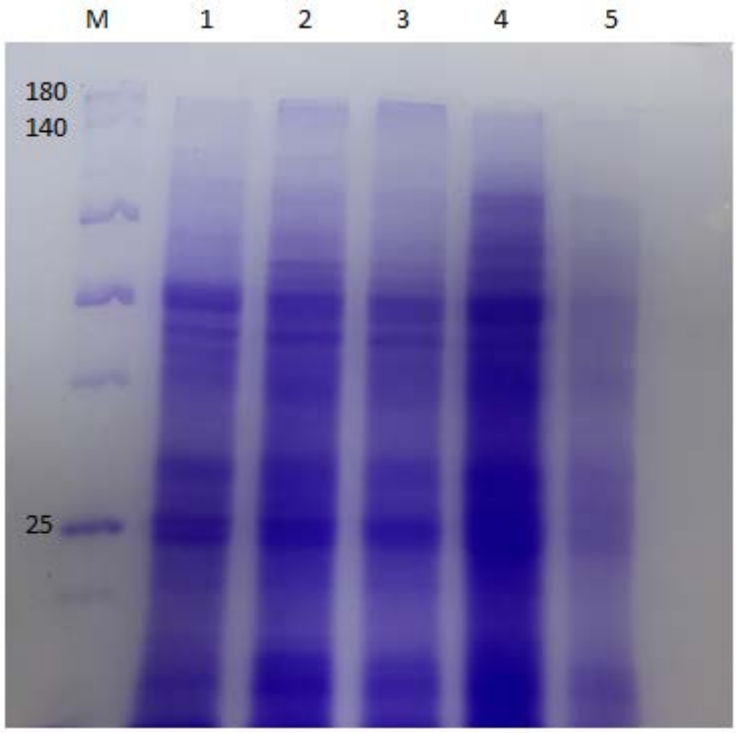


Figure 3. SDS PAGE profiles of total proteins from *Vicia* L. cultivars and ecotypes. M: Marker (kDa), 1: *Ankara Moru* 2: *Tarım Beyazı*, 3: *Ayaz*, 4: *Kansur*, 5: *Ankara Pembesi*.

4. DISCUSSION

The genetic diversity and relationship of a small number samples from different *Vicia faba* ecotype regions cultivated in Turkey were analyzed in this study. The high variability of *Vicia faba* L. species and the events of inter- and intra-specific diversity result in the expansion of its genetic variations. In cytological numbers experiment, the analyzed samples exhibited various levels of chromosome numbers between cultivars. Chromosome counting analysis were mainly found to be diploid among samples. A sample that have shown deviated chromosome numbers were reported for *Kansur* cultivar. These results are similar with one of the reports on faba bean population (Cooper, 2017). It is proved that in Turkey there is extensive gene flow among variants at the the same chromosome level in wild representatives of the *Vicia faba* species. The alterations of chromosome numbers can be explained a result of aneuploidy, which plays an essential role in genetic variations. Our findings recommended that such relations among varieties indicate that the deviation numbers enclose *2n=2x=14* chromosomes instead of *2n=2x=*12 chromosomes. The estimated results confirm that the genome sizes of the tested five cultivars is remarkably different from the smallest nuclear content of 3.53 pg in the cultivars *Ankara moru* obtained in the variety and the biggest nuclear DNA content of 11.11 pg C DNA in the cultivar *Kansur* which results in an average of 10.65 pg C DNA at 2C DNA per nucleus (Table 2 and Figure 2). Highly various values were observed among tested cultivars, it is not consistent that obtained results from the chromosome numbers analysis thereby verifying the different ploidy level of the tested varieties. Cytogenetic analysis displayed that the *Vicia faba* chromosome number was *2n=2x=12* expect *Kansur* (Figure 1). It was displayed that an positive correlation exists between the chromosome numbers and nuclear DNA content. Nuclear DNA content estimation displayed that *Vicia faba* species have very large genomes and this trait enables the classification of all cultivars. These results are very far determination of nuclear DNA content variation among *Vicia sativa* cultivars by flow cytometry (Kubaláková,2003). This could be due to differences in endopolyploidy, which may influence the chromatin density. In the present study high genetic diversity was detected for the amount of total protein of the seed storage whereas the variation in the number of bands in seeds of the *Vicia faba* cultivars under study is relatively narrow. These genetic diversity may be displayed in the polymorphism exhibited in the minor bands and the major bands intensities, which gave each cultivar its specific elctrophoregram for protein bands. The elctrophoregram analysis of the tested cultivars can be used as a passport data for their genetic identity. Similar results were obtained using elctrophoregram methods the protein patterns of 10 faba bean population from Indian. Their results show that the elctrophoregram is a useful selection method for determining intra-species degree within *faba bean*. Total protein analysis, the amount of protein in the diploid plant is correlated to the chromosome numbers. Moreover, *Ankara moru, Tarım beyazı* and *Ayaz* cultivars were also nearly the similar amounts in terms of their protein bands, Although *Ankara Pembesi* was considered as diploid with respect to ploidy levels in which the *Kansur* cultivars exhibited the highest level of protein. This is confirms that an positive correlation exists between the chromosome numbers and genome sizes. The cytological and electrophoregram used in this study seem to be beneficial in the genetic studies of *Vicia faba*. This work provides important findings for the classification, conservation and innovation of *Vicia faba* populations.

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