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DNA and Protein Content Variations of Turkish Pea (*Pisum sativum* L.) Genotypes

Büşra YAZICILAR[1*], Merve ŞİMŞEK[2], İsmail BEZİRGANOĞLU[3], Doğan İLHAN[4]

[1,2,3] Erzurum Technical University, Department of Molecular Biology and Genetics, Erzurum, TURKEY
[4]Kafkas University, Department of Molecular Biology and Genetics, Kars, TURKEY

[1]https://orcid.org/0000-0003-2465-7579
[2]https://orcid.org/0000-0002-4088-183X
[3]https://orcid.org/ 0000-0003-4079-5998
[4]https://orcid.org/ 0000-0003-2805-1638
* Corresponding author: busra.yazicilar21@erzurum.edu.tr

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ABSTRACT

Pea (*Pisum sativum* L.) is an annual plant with a high nutritional value and dietary fibers. The aim of the present study is to determine of genome size variation of some pea cultivars and ecotypes by DNA, protein, and nucleus. Tested samples demonstrated a small different value of nuclear content via flow cytometry analysis. These tested plants have verified the existence of expected ploidy levels as diploid plants. Nuclear DNA content ranged from 4.25 to 4.53. Comparison of globular protein accumulation in between pea cultivars and populations, cultivars revealed that globular protein accumulation was higher than populations samples; on the other hand, albumin protein accumulation showed a similar level for all the tested samples. Globular protein amount was between 35 kDa and 100 kDa.

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INTRODUCTION

Pea (*Pisum sativum* L.) is one of the earliest grain legumes domesticated in world with a long history, and they are widely consumed as part of animal and human nutrition (Zohary and Hopf 2000; Faostat 2007). Pea is cultivated for its rich source of proteins, carbohydrates, vitamins, minerals and dietary fibers (Bastianelli et al., 1998). The nutritional and functional quality of pea seed depend primarily on their protein content and composition. Seeds of pea including digestible starch (50%), soluble sugars (5%), fibbers minerals and vitamin were in rich in protein (23-25%). Pea is self–crossed, annual and diploid (*2n =2x= 14*) with a genome size of 4.4 Gbp grown all over

world (Bennett and Leitch 1997; Kaur et al., 2012; Zhu et al., 2005). It has more than 650 genera and 18,000 species based on morphological structures and agronomical traits as determined by traditional classification. The genetic diversity of pea has been determined using morphologic traits and pedigree data (Sharma 2002; Singh et al., 2003), protein markers (Julier et al., 2003), and molecular markers consisting of RAPDs (Ahmad et al., 2012; Yadav 2004), and simple-sequence repeat markers (SSRs) (Burstin et al., 2001; Hanci 2019). Flow cytometry is one option to quickly determine the amount of DNA content. It has been provided as a rapid tool for assessing of ploidy for genome sizes studies requiring large sample numbers (Dirihan et al., 2013). Protein profiles content have provided convenient evidence for determining genetic diversity at the molecular level in plants. The main pea storage protein known as globulin, convicilin, vicilin (7S) and legumin (11S) (Barac et al., 2010). Several reports have been tested on the changes of protein bands of pea (Barac et al., 2010; Guleria et al., 2009). Most of genetic variations studies in pea are mainly dependent on morphological traits in germplasm collections using selection techniques, but these techniques have still some limitations for being closely related populations and cultivars for germplasm. Moreover, during the selection process, many natural germplasm resources and distant relatives may have lost which has ultimately resulted in a decrease in genetic variations (Hagenblad et al., 2012; Leino et al., 2013). Genetic variation is defined the number of different alleles of quantitative traits in the germplasm of any populations, therefore genetic variations in species are greatly useful and significant for the sustainable agriculture and food security. Conventional breeding and molecular marker technique information have been used widely for improvement of cultivars for various crops. Thus, information on genetic variation is a vital properties in new cultivars improvement. The aim of this study is to evaluate the DNA and protein content variations by using flow cytometry and SDS PAGE analyses, of seven pea cultivars, *Özkaynak* (11), *Ürünlü* (16), *Kirazlı* (14), *Töre* (4), *Ulubatlı* (10), *Taşkent* (3), *Maro*

Tarım (17) and eleven populations.

MATERIALS and METHODS

Plant material

Seven pea cultivars, *Özkaynak* (11), *Ürünlü* (16), *Kirazlı* (14), *Töre* (4), *Ulubatlı* (10), *Taşkent* (3), *Maro Tarım* (17), and eleven populations were used as the plant material for this study (Table 1). All cultivars and populations used were obtained from Department of Molecular Biology and Genetics at Kafkas University. Pea seeds were sown in pots containing soil. They were maintained at 25/27^{0}C (day/night) growth-chamber with a 16 h day length for 8 days to initiate seedling. After 2 weeks, the leaves of plants were collected from cultivars and populations for further analysis.

Table 1. The Number, Id, Types and Origin of pea cultivars and populations

Number	Id	Type	Origin
1	Population 1	Population	Turkey
2	Population 2	Population	Turkey
3	*Taşkent*	Cultivar	Turkey
4	*Töre*	Cultivar	Turkey
5	Population 5	Population	Turkey
6	Population 6	Population	Turkey
7	Population 7	Population	Turkey
8	Population 8	Population	Turkey
9	Population 9	Population	Turkey
10	*Ulubatlı*	Cultivar	Turkey
11	*Özkaynak*	Cultivar	Turkey
12	Population 12	Population	Turkey
13	Population 13	Population	Turkey
14	*Kirazlı*	Cultivar	Turkey
15	Population 15	Population	Turkey
16	*Ürünlü*	Cultivar	Turkey
17	*Maro Tarım*	Cultivar	Turkey
18	Population 18	Population	Turkey

Flow cytometry

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. *Vicia sativa* L. (2 pg/2C) was used as a reference standard. Shortly, the protocol consisted of simultaneously chopping leaf tissues (20 mg each) of *Pisum sativum* and *Vicia sativa* 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 pea cultivars and populations were calculated based on the ratios of the G1 peak means of sample and reference standard by using the following formula:

$$Sample\ 2C\ DNA\ content = \frac{Sample\ G1\ peak\ mean}{Satandard\ G1\ peak\ mean\ x\ satandard\ 2C\ DNA\ content\ (pg)}$$

Globular and Albumin Protein Analysis

The pea seeds were weighed at 0,035 grams. It was homogenized by adding 1 mL of hexane. It was centrifuged at 6000 rpm at 25 ^{0}C. Supernatant was thrown and 1 mL of Water Extraction (10 mM $MgCl_2$, 10 mM $CaCl_2$ pH: 8.0) was added to the pellet part. It was centrifuged at 3000 rpm for 15 minutes at 4 ^{0}C. This stage was done twice. The supernatant part was albumin. The pellet part was the globular protein. Salt Solution Extraction (100 mM Tris-Cl pH: 7.5, 10% NaCl, 10 mM EDTA, 10 mM EGTA) was added to the pellet part. It was centrifuged at 30000 rpm for 5 minutes at 4 ^{0}C. The supernatant part formed the globular protein (Nadal et al., 2011). Globular and albumin proteins were analyzed by loading an equal concentration of 15% SDS PAGE gel (Laemmli 1970).

RESULTS and DISCUSSION

All cultivars and populations were found to be entirely diploid with basic chromosome number *2n=2x=16* except a cultivar Özkaynak (11) (Fig.1). Additionally, a pea cultivar calling 11 (Özkaynak) was poliploid with *2n=4x=3*2. Nuclear content value for cultivars was 4.28 pg for *Özkaynak* (11), 4.30 pg for *Ulubatlı* (10), 4.40 pg for *Töre* (4), 4.31 pg for *Ürünlü* (16) and 4.42 pg for *Kirazlı* (14) respectively. Whereas, the genome size of the tested populations ranged from 4.25 pg in 7 to 4.44 pg in 15, which is a diploid *2n=2x=16* among populations (Table 2, Fig. 1).

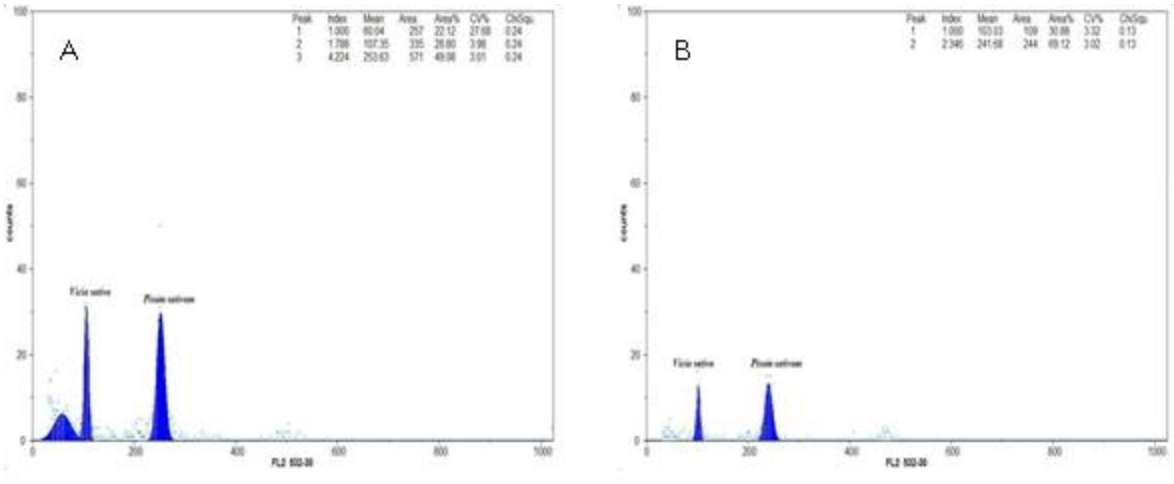

Figure 1. Flow cytometric analysis of PI stained nuclei *Pisum sativum* L. using *Vicia sativa* L. as internal standart A: *Maro Tarım* (17), B: *Özkaynak* (11)

Table 2. Nuclear DNA content of pea cultivars and populations

***Pisum sativum* L.**	**Sample peak**	**Standart peak**	**Standart DNA content(pg)**	**Test DNA content(pg)**	**CV1 (*Vicia sativa* L.)**	**CV2 (*Pisum sativum* L.)**
1	277.24	113.79	3.65	4.44	2.48	3.20
2	269.36	115.15	3.65	4.26	4.56	4.91
Taşkent	272.22	113.75	3.65	4.37	2.62	3.23
Töre	272.57	112.89	3.65	4.40	3.57	3.27
5	271.30	114.01	3.65	4.34	4.71	3.60
6	260.71	110.97	3.65	4.4	3.11	3.75
7	251.86	108.04	3.65	4.25	3.12	3.03
8	317.48	127.91	3.65	4.53	4.04	4.16
9	255.70	107.48	3.65	4.35	4.10	3.65
Ulubatlı	262.01	111.04	3.65	4.30	3.28	3.28
Özkaynak	254.92	107.43	3.65	4.32	3.11	3.68
12	243.88	104.21	3.65	4.27	2.83	3.83
13	224	97.65	3.65	4.41	4.11	3.82
Kirazlı	294.10	121.53	3.65	4.42	3.10	2.56
15	273.74	113.83	3.65	4.38	3.72	3.37
Ürünlü	260.50	110.36	3.65	4.31	3.99	3.62
Maro Tarım	264.77	110.75	3.65	4.36	3.76	4.03
18	250.55	102.81	3.65	4.44	2.60	3.71

The analysis demonstrated that the globular and albumin protein are easily recordable in the tested pea samples. Bands of the expected sizes were detected between 35 kDa and 100 kDa in the seeds of the pea plants (Fig. 2a). Comparison of globular protein accumulation in between pea cultivars and populations, cultivars, revealed that globular protein accumulation was higher than populations samples; on the other hand, albumin protein accumulation showed a similar level for all tested samples. According to the result of globular band profiles, *Kirazlı* (14) exhibited the most abundant protein, followed by *Özkaynak* (11), and *Ürünlü* (16). However, weak accumulation of globular proteins were detected in the *Maro Tarım* (17) cultivars (Fig. 2b.).

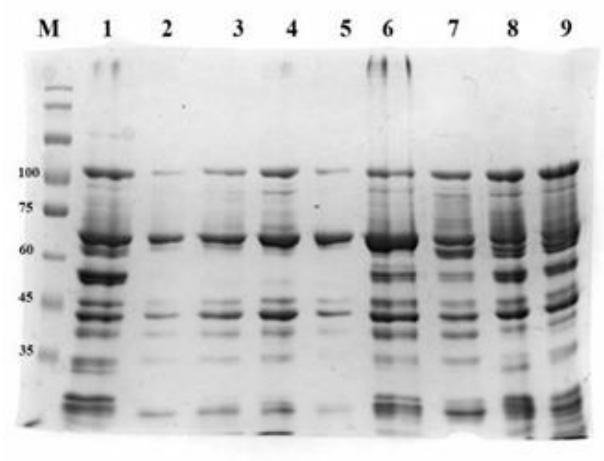

Figure 2a. SDS PAGE profiles of globular proteins from pea cultivars and populations. M: Marker (kDa), 1: 15, 2: *Bayburt* 3: *Taşkent* 4: *Töre* 5: 14, 6: 8, 7: 43, 8: 11, 9: 45.

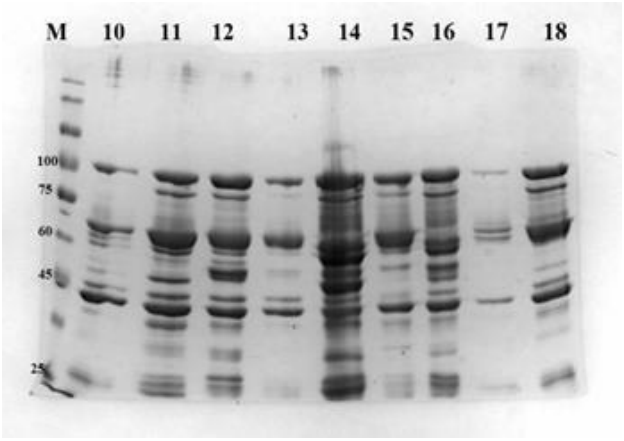

Figure 2b. SDS PAGE profiles of globular proteins from pea cultivars and populations. M: Marker (kDa), 10: *Ulubatlı*, 11: *Özkaynak* 12: *Yusufeli* 13: 17 14: *Kirazlı* 15: 10 16: *Ürünlü* 17: *Maro Tarım* 18: *Kars*

Genetic variation has become one of the fundamental tools of plant genetic research. Alternative putative lines are necessary for flaw adjustment of commercial cultivars and improvement of novel cultivars. A substantial amount of reports in plant genetic variations has been focused to understand how natural selection operates in the quantitative traits. It is widely employed in the phenotypic character, conservation and improvement of new cultivars (Bhandari et al., 2017; Rao and Hodgking 2002). Most of these reports have included analyses of marker gene methods that are likely to be

the aims of variation in natural populations. To the best of our knowledge, there is no report on genetic variation, working collectively on flow detection methods and protein profiles in combination with a comprehensive analysis of pea samples. *P. sativum* has scientific and economic significance, making it potentially an excellent plant to be used for genetic diversity (Ahmad et al., 2012). Morphological definitions are easy, fast, inexpensive, and they do not need special technology. In addition, most of these definitions are necessary for human laborers and they are time-consuming. Cytological markers in plants have been routinely used by chromosome numbers of stained root tips using microscopy; however, these have limited implementations in genetic variation analysis on account of their low resolution and limited number (Bhandari et al., 2017). The nuclear DNA content and protein profiles of 11 populations and 7 cultivars of pea have been detected by flow cytometry and SDS PAGE. These two properties found to be different both between and within species. Pea is strongly various in terms of phenotypes, and pea breeding could profit from extensive crosses, including introgressions from wild relatives (Ladizinsky and Abbo 2015). Flow cytometry analysis exhibited different genome sizes among different cultivars and populations of pea in the experiment. Flow cytometry is used when chromosome number information data can be interpreted by microscopy (Pellicer et al., 2017). Changes in genome sizes inducued a wide range of morphological alters (Chung et al., 2014; Dolezel et al., 2007). Leaves of plants of larger genome sizes were significantly thicker, rounder and taller than smaller plants as well as with a better photosynthetic capacity (Cai et al., 2015). The pea genome is large, likely resulting from a long-time self-pollination and diversification of transposable elements (Ellis and Poyser 2002). In the present investigation, flow cytometry analysis on cultivars and populations of pea were tested and the present study demonstrated slightly to be different in terms of nuclear content values. Our observations confirm that the level of nuclear content is sufficiently different to clarify diverse lines in pea. The nuclear

content value of the 5 pea cultivars ranged between 4.30 pg and 4.42 pg (Table 2). (Baranyi and Greilhuber 1996) reported the chromosome numbers and nuclear DNA contents of 15 *P. sativum* cultivars, landraces and wild accessions using chromosome counts and flow cytometry. In their study, this could be a strong evidence to prove absence of significant nuclear content variation in the *P. sativum.* Their outcome confirms that nuclear content variation is originally present in the genus and requires further analysis. In our cases, all the tested samples were found to be tetraploid by flow cytometry analysis. The reports are different from those of (Sakiroglu et al., 2011) who detected that the 2C DNA genome size of *M. sativa subsp*. *varia* ranged from 2.85 pg to 4.9 pg. The large variation value could be a result of different sub-species within one species. In contrast, values of genome size were tightly clustered to 4.25 pg to 4.53 pg in our study. The high level of genome size protection is notable and may be correlated with a low amount of chromocentric constitutive heterochromatin, which is known as a variable component in plant genomes. Genome size variations can be influenced on conservations occur in different ways in various plant species. These are very common in outcrossing than self-pollinations. Nuclear content varies significantly among land plants and the highest ploidy level was found in 11. We suggest that total protein bands be considered to explain the taxonomy specific to the *Legume* group and other species. SDS PAGE analysis of pea cultivars and populations revealed weak, medium and high level of banding profiles, and flow cytometry analysis detected nuclear content from these cultivars and populations. The degree of protein-band content of the tested samples is correlated to the value of nuclear content, and gel results of globular proteins in pea samples suggest such correlation. For example, *Kirazlı* (14) had a significantly larger nuclear content with 4.53 pg followed by 4.44 tested samples *Kirazlı* (14), 11, and 8 with multiple band intense of the globular proteins was found to have high value to nuclear content, when compared with the tested samples (13, *Maro Tarım* (17) and *Kars* (18)) with few band intense of globular

proteins. It is likely that the degree of nuclear DNA content is associated with the level of protein band content, which is related to the band number of the pea samples. In our case, the band number of *Kirazlı* (14) and 8 in the protein profiles was observed with an extra band. However, it seems that this statement does not quite concur with our albumin protein band observations (data not shown). The 7 line in our experiment has a genome of about 4.25 pg. It showed that an adversely relation exists between nuclear content and albumin protein on the contrary globular protein. Highly variable banding pattern were observed in these tested samples. As observed through total protein analysis, the amount of protein in the diploid plant is correlated to the ploidy level. This confirms that nuclear DNA content is the total amount of DNA contained within one copy of a single entire genome. These findings are in consistent with those published by (Bourgeois et al., 2009) in the study on phenotypic plasticity and seed protein composition of *P. sativum*. Their results on seed storage protein composition demonstrate that large genetic variation results both from gene diversity and post-translational processing.

CONCLUSION AND SUGGESTIONS

The stability in ploidy levels and the band patterns of the globular proteins can potentially contribute to the knowledge for genetic variations in pea samples. This study provides key findings for the classification, conservation and innovation of pea (*Pisum sativum* L.) germplasm resources.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

Contribution Rate Statement Summary of Researchers

İB contributed to the project idea, design and execution of the study. BY, MŞ, İB, Dİ conducted the laboratory analyses. İB and Dİ supervised the experiment and wrote the manuscript.

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