

## DIVERSITY OF SAINFOIN (*Onobrychis Mill.*) COLLECTION SAMPLES ACCORDING TO THE SPECTRA OF STORAGE PROTEINS

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Bulatova K., R. Massonichich-Shotunova, G. Meirman, S. Mazkirat (2015):  
*Diversity of sainfoin (Onobrychis Mill.) collection samples according to the spectra of storage proteins.* - Genetika, Vol 47, No. 2, 375 -382.

Sainfoin (*Onobrychis Mill.*) is a valuable forage plant of the legume family (*Fabaceae*). In Kazakhstan the sainfoin introduced as field fodder in the 50s of the XX century and because of its biological features widely spread as a promising crop for forage resources increasing. The electrophoresis of seed storage proteins of *Onobrychis viciifolia*, *Onobrychis arenaria* and *Onobrychis transcaucasica* species was carried out. Considerable diversity of the number, mobility and intensity of components was determined.

*Key words:* diversity, electrophoresis, sainfoin, seed proteins

### INTRODUCTION

Sainfoin (*Onobrychis Mill.*) is a valuable forage crop with more than 130 species distributed in many parts of the world, including West Asia, Europe, the western part of the United States and Canada (KARAMIAN and RANJBAR, 2008; AVCI and DEMIR KAYA, 2013).

In the republics of former Soviet Union sainfoin is represented by 62 species, three of them are widely used in the culture: *Onobrychis viciifolia* Scop. – Common sainfoin (sativa, european) introduced into culture about 500 years ago; *Onobrychis arenaria* Kit. D.C. first introduced into culture in the Ukraine in the beginning early of the XX century; and *Onobrychis*

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*transcaucasica (antasiatica) Khin.* the most ancient sainfoin species with more than a thousand year history of cultivation in Transcaucasia (MASSONICHICH-SHOTUNOVA, 2014).

In comparison with other species of grass the sainfoin is less demanding to the soil and in presence of moisture in the soil produces high yields even in the poorest soils. This plant has a high resistance to cold, drought, and responds favorably to moisture, high adaptive flexibility and high nutritive value.

Green mass of the sainfoin is nutritionally good raw material for haulage and silage. Thus, on average 100 kg sainfoin green mass contain 21.7 feed units and 3.1 kg of digestible protein, 100 kg of hay fodder contains 54 units and 10.1 kg of digestible protein and 2.5 g of carotene. In contrast from alfalfa feeding by fresh green form of sainfoin does not cause animal timpani. Protein content of sainfoin hay yields only alfalfa hay and exceeds clover hay. Sainfoin hay contains a great amount of calcium and mineral salts which are necessary for the normal development of productive animals (FITZEV, 2003).

Sainfoin crops improve soil structure and enrich the soil with humus and nitrogen, prevent erosion and soil degradation, normalize water regime of agro-ecosystems, expand agroecocenosis biodiversity, increase stability of agro-ecosystems to drought and desertification, strengthen agro-landscapes, improve ecological conditions and the environment.

In Kazakhstan the sainfoin introduced into field fodder in the 50s of the XX century and because of its biological features it is widely spread as a promising crop for forage resources increasing (BULATOVA *et al.*, 2014).

In connection with above mentioned the study aimed to explore the gene pool of the sainfoin at the genus, species and population levels.

Protein markers, in particular components of the storage proteins, are widely used in evaluation of the genetic diversity of crops and identification of intra-cultivar heterogeneity and polymorphism. However there are no data of intra-population characterization of cultivars of the species *Onobrychis viciifolia* and other species of the genus *Onobrychis Mill.*, and information of inter-population diversity is insignificant.

Profile of reserve proteins of sainfoin was analyzed for identification of interspecific variation and taxonomic revisions (EMRE *et al.*, 2007). The authors have not found clear differences among eight analyzed species. In the other set of samples of the genus *Onobrychis Mill.* comprising 10 species stable and variable protein bands of the electrophoretic spectrum were determined with high level of similarity within the analyzed species (ARSLAN and ERTUGRUL, 2010).

The aim of our researches was to study the composition of seed storage proteins of collection samples of *Onobrychis arenaria* and *Onobrychis transcaucasica* and to estimate their genetic diversity.

#### MATERIALS AND METHODS

The objects of our investigations were collection consisted of 9 samples of *Onobrychis arenaria* and 8 samples of *Onobrychis transcaucasica*. The list and origin of these samples are presented in Table 1.

At preparation of protein samples the sainfoin seeds were separated from the pericarp, dried and milled in the mortar, each seed was grinded and placed into a separate Eppendorf tube. Extraction of proteins was carried out by means of 0.0618 M Tris-HCl buffer solution containing: Na-SDS 3%, 10% glycerol, 4% mercaptoethanol and bromophenol blue dye.

Extraction was being carried out for two hours on a shaker at room temperature; the extract was alkylated, heated for two minutes in a boiling water bath and was loaded by 14  $\mu$ l into the pockets of 10% polyacrylamide gel. Preparation of gels and electrophoresis was carried by means of Laemmli method (1970), in modification of Bulatova (1985). As molecular weight marker was used Thermo scientific set (Lithuania) (170 kDa, 130 kDa, 100 kDa, 70 kDa, 55 kDa, 40 kDa, 35 kDa, 25 kDa, 15 kDa, 10 kDa). Processing of the results was carried out using cluster analysis by means of Ward method.

Table 1. Collection samples of the sainfoin investigated by spectrum of seed reserve proteins

No	Catalogue number	Name, its origin, year of yield
1	42305	<i>Onobrychis arenaria</i> , Kyrgyzstan, 2010
2	38747	<i>Onobrychis arenaria</i> , Kazakhstan, 2002
3	40817	<i>Onobrychis arenaria</i> , improved, Kazakhstan, 2002
4	17012	<i>Onobrychis arenaria</i> , wild, Kazakhstan, 2002
5	29651	<i>Onobrychis arenaria</i> , Krasnoyarskiy, 2009
6	28312	<i>Onobrychis arenaria</i> , Bashkortostan, 2008
7	109(29192)	<i>Onobrychis arenaria</i> , hybrid, the Ukraine, 2008
8	40824	<i>Onobrychis arenaria</i> , Donetskiiy-21, the Ukraine, 2008
9	42304	<i>Onobrychis arenaria</i> , Sazonovskiiy, the Ukraine, 2008
10	40930	<i>Onobrychis transcaucasica</i> , Gruziya-1, 2005
11	40929	<i>Onobrychis transcaucasica</i> , Natekhterskiy, Georgia, 2005
12	38626	<i>Onobrychis transcaucasica</i> , local, Nagorno-Karabakh, 2005
13	41619	<i>Onobrychis transcaucasica</i> , local, Armenia, 2005
14	37208	<i>Onobrychis transcaucasica</i> , local, Azerbaijan, 2005
15	30618	<i>Onobrychis transcaucasica</i> , Baltasist Hungary, 2010
16	30093	<i>Onobrychis transcaucasica</i> , Azerbaijan, 2005
17	37212	<i>Onobrychis transcaucasica</i> , Krymskiy-89, the Ukraine, 2008

## RESULTS AND DISCUSSION

Spectrum analysis of storage proteins of 8 samples of *Onobrychis transcaucasica* and 9 samples of *Onobrychis arenaria* gathered from geographically remote regions (Figure 1) showed its extraordinary diversity both by mobility in gel and by intensity of manifestation of the components.

In general, in the protein spectra 25-37 polypeptides with molecular weight of 10-115 kDa were determined. The least variable zone was the one with the polypeptides having molecular weight 10-20 kDa, by the zone of slowly moving components (75-115 kDa) the majority of seeds demonstrated affinity, but, nevertheless, individual seeds demonstrated differences in the number and mobility of the components in the gel. The most heterogeneous and specific for each seed was a zone with the components having molecular weight 25-70 kDa.

Significant diversity of sainfoin is known by morphological, anatomical characteristics, and molecular markers (ZARRABIAN *et al.*, 2013; HEJRANKESH, 2014).

However, the specific features of spectrum for individual sainfoin species have not been identified. By means of the cluster analysis method the component composition of proteins the analyzed genotypes identified by the presence-absence of certain bands and their intensity in the

spectrum, were divided into 3 main clusters including samples of *Onobrychis transcaucasica* and *Onobrychis arenaria*, respectively (Figure 2).

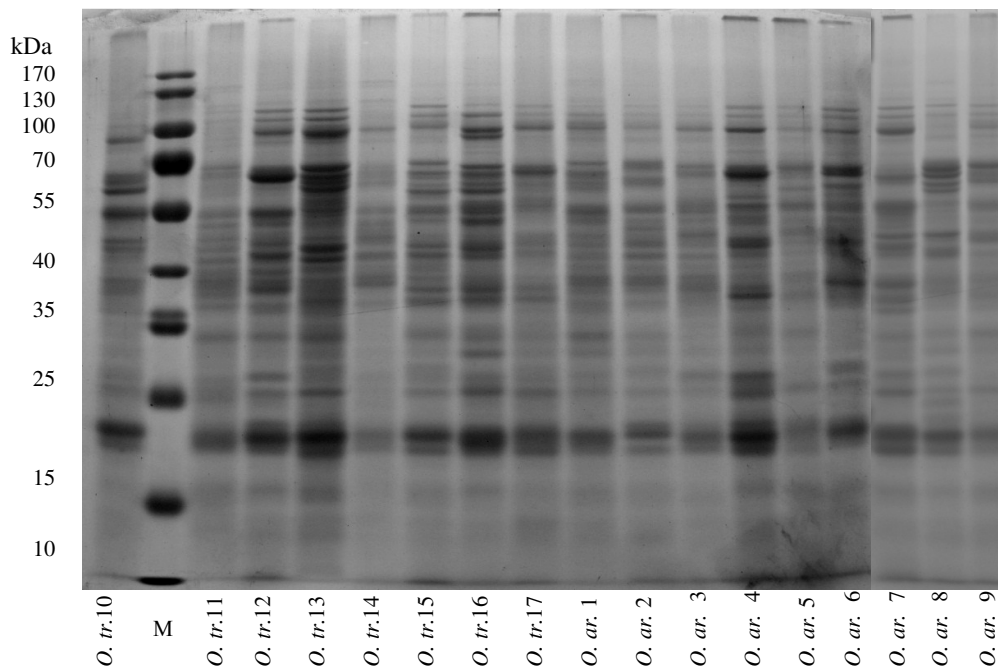


Figure 1. Spectrum of single seeds storage proteins of sainfoin *Onobrychis transcaucasica* and *Onobrychis arenaria*.

Since for analysis of intraspecific polymorphism of collection samples of *Onobrychis transcaucasica* and *Onobrychis arenaria* the individual seeds were used the separate collection samples (*Onobrychis transcaucasica*, cv. Natekhterskiy, Georgia and *Onobrychis arenaria*, cv. Donetskiiy-21, the Ukraine) were evaluated by the composition of proteins using larger amount of seeds (Figure 3). In the electrophoregram for comparison the molecular weight marker (M) and the spectrum of soybean globulin (s) are shown.

High level of diversity identified within collection samples, is in agreement with results of the genetic variability evaluation of different populations of *Onobrychis viciifolia* by means of RAPD marker analysis where the greatest diversity was concentrated within the population, rather than between populations (NOSRATI *et al.*, 2012; RASOULI *et al.*, 2013). The authors connected the high level of inter- and intra-population polymorphism of the sainfoin with cross-pollination of the species, although not always variability of traits within species and populations is related to the pollination nature of plants. Thus, alfalfa (*Medicago sativa*) is also a cross-pollinating crop, at the same time its intervarieties and intravariety polymorphism according to storage proteins is insignificant (KROCHKO *et al.*, 2000; HABIBBI *et al.*, 2012). Another supposed reason of high genetic variability of populations is stressful conditions under which the sainfoin

is often grown. Thus there are studies showing that populations grown in adverse natural conditions characterized by high genetic variability (BROCK *et al.*, 2007; HAGENBLAD *et al.*, 2012).

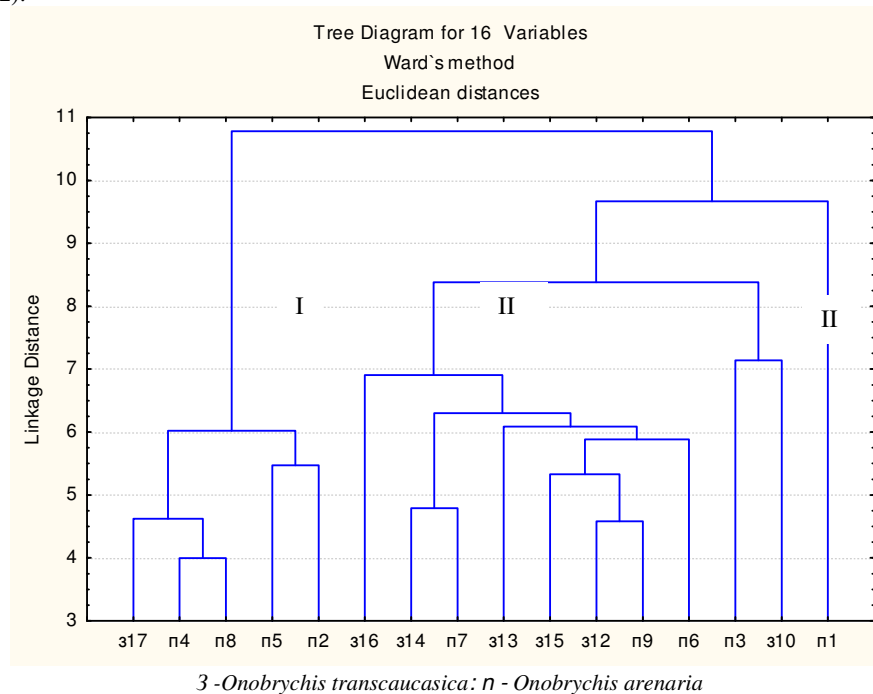


Figure 2. Dendrogram of the sainfoin collection samples distribution by similarity-difference of component composition of seed storage proteins.

It should be noted that the genetic diversity revealed by morphological and physiological, anatomical, biochemical, and other plant indicators, as well as at the level of isozyme and DNA markers, which involved vegetative organs, characterizes the state of the genome of plants realizing at the time of screening the genetic potential transmitted through the seed phase. However, the inter- and intrapopulation diversity measured by the spectrum of seed reserve proteins provides information about the degree of polymorphism and heterozygosity of future cultivar populations.

To solve the qualitatively new problems of sainfoin breeding a huge genetic diversity of species and populations revealed by means of protein and molecular markers should be taken into account. This genetic diversity may be associated with economically valuable traits of plants, comprehensive and deep study of biological and economically valuable features not only of samples but also of each individual plant using traditional and modern methods of marker assisted breeding.

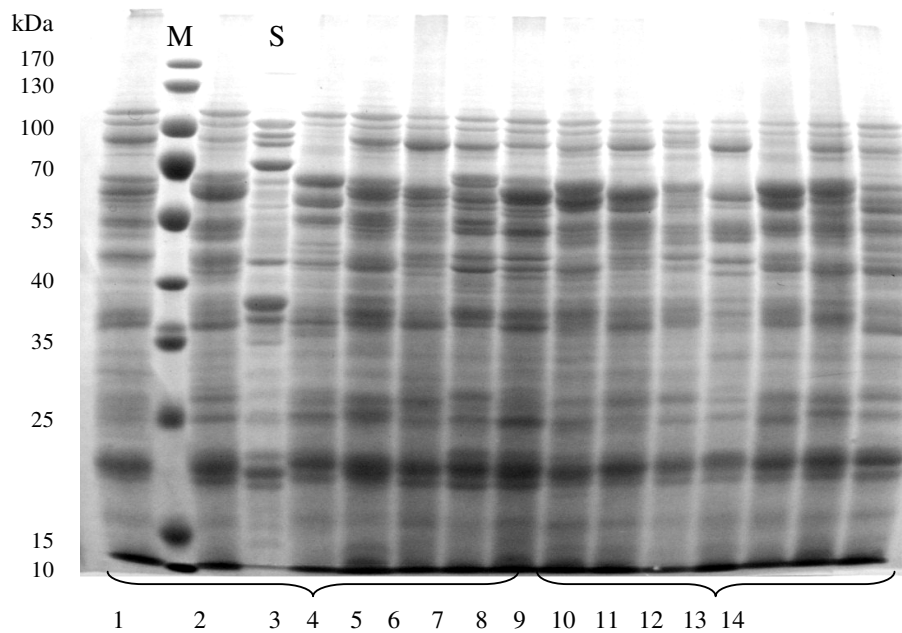


Figure 3. Spectrum of storage proteins of individual seeds of *Onobrychis transcaucasica* (cv. Natekhterskiy, Georgia: 1-7) and *Onobrychis arenaria* (cv. Donetskiiy-21, the Ukraine: 8-14)

Received September 02<sup>th</sup>, 2014

Accepted December 25<sup>th</sup>, 2014

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**DIVERGENTNOST UZORAKA ESPARZETE (*Onobrychis Mill.*) KOLEKCIJE  
PREMA SPEKTRU REZERVNIH PROTEINA**

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Izvod

Esparzeta (*Onobrychis Mill.*) je važna biljka za stočnu hranu u porodici leguminoza (*Fabaceae*). U Kazahstan je uvedena u gajenje u poljskim uslovima sredinom 20. Veka i zbog bioloških osobina široko gajena u kao perspektivan izvor za proizvodnje stočne hrane. Analiza rezervnih proteina *Onobrychis viciifolia*, *Onobrychis arenaria* i *Onobrychis transcaucasica* vrsta je vršena gelelektroforezom. Utvrđena je začajna divergentnost broja, mobilnosti i intenziteta pojedinih komponenata.

Primljeno 02. IX. 2014.

Odobreno 25. XII. 2014.