Comparative analysis of species of the genus *Rosa* L. on the territory of the Eastern European Plain

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Abstract. The methods of gel electrophoresis with some modification have been developed on the types of rose hips. Identification of a species, as well as determining the degree of its intraspecific polymorphism, includes analysis of individual seeds taken from a random sample of different populations. For identification and analysis of the purity of the composition of the seeds of cultivars, an analysis of at least 100–150 seeds of each cultivar is recommended. Spectra were recorded using a special digital (large-scale) scale, which allows fixing the position (“addresses”) of individual polypeptide components at positions from 1 to 122. Components of low intensity were taken for 1 point, and strong ones (bright) for 2 points. The high sensitivity of the polyacrylamide gel electrophoresis (PAGE) method in the presence of sodium dodecyl sulfate (DS-Na, SDS) and 2-mercaptoethanol to genetic differences at the level of small taxa is clearly manifested in rosehip species. The research results allow us to clarify the taxonomy of the genus *Rosa*.

1. Introduction

Among the shrub plants of the Orenburg region, dogrose occupies a special place due to the content of biologically active substances in the fruits. The fruits of many types of rose hips have a high content of vitamins C (ascorbic acid), B1, B2, K, E and carotenoids [1]. Rosehip is widely known as a valuable forest reclamation and ornamental shrub, widely used as a stock for cultivated rose varieties.

The Ural region, the territorial basis of which is the Orenburg region of the Russian Federation and the adjacent territory of the Republic of Kazakhstan, has 5 species of dogrose, of which the most common in this territory are the *Rosa canina* L. and the *R. majalis* Herrm. Other species – *R. glabriifolia* C.A. Mey. ex Rupr., *R. acicularis* Lindl. and *R. pimpinellifolia* L. – have limited distribution.

In the Orenburg region, the total area of natural thickets with a high share of *Rosa canina* L. and *R. majalis* Herrm. hips is about 13.7 thousand ha, the net area of dog rose thickets is about 136 ha, the total fruit stock is estimated at 42 tons annually.

2. Statement of the problem

The study of dogrose scientifically is of great theoretical and practical importance, their systematic position remains problematic to date. Most botanists working with wild rosehip species note their ability for mutual hybridization, due to the common areas of growth.

Therefore, despite the huge number of works devoted to dogrose, and the huge number of described species and intraspecific taxa, the systematics still remain a lot of obscurity [2]. The words...
of the founder of botanical taxonomy Karl Linnaeus "species rosarum difficillime distinguuntur, difficilius determinantur", that is, "species of roses are difficult to distinguish and can hardly be determined", and now they have not lost their relevance [3]. And according to I.A. Shantser, the description of many new species and intraspecific taxa based on purely morphological characters, without a detailed study of their variability, we greatly confused the system of wild rose and it led to almost complete chaos in their nomenclature [4]. In addition, the selection of species by taxonomists is often based on controversial taxonomic characters that vary greatly within the range of one species.

Being components of plant formations of various origins, rose hips are promising for analyzing the origin and development of the flora of the region under study. In connection with the wide spread of rosehips in the Orenburg region, there was an urgent need for identification, registration of the existing gene pool, the establishment of its authenticity and originality.

The solution to the above problem is possible with reliable biological tests to evaluate the source material.

3. Materials and methods

*R. canina* and *R. majalis* from different ecological populations throughout the Orenburg region. *R. acicularis*, seeds of this species were taken from the populations of the Sol-Iletsk district at the edges of forests, *R. glabrifolia* seeds were collected in the Belyaevsk district in floodplain meadows; seeds of *R. pimpinellofolia* – on the rocky slopes of the Sakmara region. In addition, for electrophoretic research, rosehip seeds from various geographical habitats were used. Seeds were provided to us from the seed collection of the All-Russian Institute of Plant Production named after Vavilov (St. Petersburg) and the main Botanical Garden of the Russian Academy of Sciences (Moscow). For comparison, there are raspberries (*Rubus idaeus* L.) and strawberries (*Fragaria Vesca* Dum.). The collection was carried out in such a way that the environmental conditions of the collection sites were sharply different from each other. To obtain more accurate results, seeds were collected from 50–100 individuals for each species of wild-growing populations from the territory of the Urals. For electrophoresis, a vertical electrophoresis device with gel plates 120 × 130 mm in size and 1 mm thick was used. A 12.5 % polyacrylamide gel is used as a working gel for electrophoretic separation of seed globulins into polypeptides. The polymerization was carried out at room temperature for 20–30 minutes. After removing excess SDS, the plates are immersed in cuvettes with a Coomassie brilliant blue R-250 dye. The duration of staining in fresh dye is 5 minutes; as the dye is used, the duration of staining is increased. For more uniform staining, it is advisable to install the cuvette on an electric rocking chair. At the end of staining, the dye is removed and filled with 7 % acetic acid.

Moreover, on a millimeter scale, the positions of the components 22, 37, 65, and 108 correspond to molecular weights of 65, 45, 25, and 17.5 kDa, respectively [5]. This method is convenient in that it allows you to compactly display all the polypeptide components. The resulting circuit can be scanned and printed on a computer. The second (tabular) method is the simplest, but at the same time it is necessary to place the most informative components in the table for compactness, omitting part of the stable components of 7S-globulins. Components of weak intensity are taken for 1 point, and strong intensity (bright) for 2 points.

In the gel pockets zone, the components of high molecular weight albumin proteins are concentrated on the electrophoregram, which many plants do not take into account when marking. They cannot be separated on the electrophoregram even with the use of 9% PAGE.

4. The results of the study

The protein water-salt extract of rosehip seeds contains reserve proteins – albumin, 12S– and 7S-globulins. Practice shows that the polypeptide spectra of flowering plant globulins are usually distributed on an electrophoregram into 3 zones: the lower low molecular weight zone of the main polypeptides, the middle zone of acidic polypeptides (11S or 12S globulins), and the upper zone of 2S or 7S globulins.

On the electrophoregrams of rosehip species, 3 main zones of polypeptides are also distinguished: the main 12S (up to 23 kDa), acidic 12S (up to 45 kDa) and 7S-globulin polypeptides.
It is known that the main polypeptides of legumin and legumin-like proteins are species-specific, i.e. represent a stable species radical (taxonomic trait), and therefore they must be carefully recorded.

Often species-specific polypeptides of vicilin and vicilin-like 7S-globulins. Acid polypeptides are polymorphic, which is associated with allelic variation in the genes encoding these proteins. The polymorphism of such polypeptides allows identification (certification) of individual genotypes (individuals, varieties) or their groups in populations. As for the specific polypeptides of the supraspecific rank (section, subgenus, genus, subfamily, etc.), they are less common in flowering plants and are usually represented by individual components throughout the electrophoregram zone [6].

The high sensitivity of the SDS page electrophoresis method to genetic differences at the level of small taxa is clearly manifested in rosehip species. It is known that many species easily interbreed with each other [7], and in this regard are, in fact, only nomenclature (formal) species. Apparently, hybridization is the main reason that species, spaced in sections according to morphological characters [8], by protein markers show a slightly different degree of kinship. Thus, the species R. fedtschenkoi from the Cinnamomeae section is very close to typical species of the Caninae section (R. canina, R. glabriifolia, R. corymbifera, R. gorinensis, R. pomifera, R. glauca). At the same time, the species R. agrestis (Caninae) clearly gravitates toward some species of the section Cinnamomeae (R. acicularis, R. beggeriana) and the section Pimpinellifolia (R. pinninellifolia). Species R. subpomifera, isolated by V.G. Khzhanovsky and placed in the Caninae section is very specific in that in addition to the set of polypeptides 90, 92, 95 in the radical zone (Table 1), the electrophoregram also has bright additional components 100, 105, and 110. The species R. spinosissima is just as specific, and, as noted, the Ural R. pinninellifolia sharply differs from it. Of the studied typical species of the Cinnamomea section, only the closest species R. majalis and R. kakanica remain as a result.

It is interesting that, according to morphological characteristics, the modern species R. majalis, understood by taxonomists quite widely, is related to the species R. glabriifolia and R. gorinensis, but only the last 2 species of rose hips are close. Thus, along with combining the known types of rosehips into close groups, the electrophoresis method in PAGE allows these species to be clearly distinguished.

The last 3 species are, in essence, morphological twin taxa, so the electrophoresis method also allows them to be detected efficiently. Despite the close proximity of the grouped species, each of them, as well as individual genotypes within the species, differ well in the zones of acidic 12S-globulins and 7S-globulins.

The data on protein markers allow us to interpret them in terms of taxonomy as follows. In the genus Rosa L., there are only semi-species, which are aggregates of close taxa of a lower (subspecific) level. These are R. canina L., R. pimpinellifolia L., R. spinosissima L., R. majalis Herrm. and possibly R. subpomifera Christan. It should be noted that all studied rosehip taxa have components 90, 80, and 50, partly 92, which are also characteristic of other genera of the subfamily Pink Rosoidae – Rubus idaeus L. and Fragaria vesca L. There is every reason to consider these components as molecular markers of this subfamily [Peace, Norelli, 2009].

Considering that among the studied semi-species, component 92 is primarily inherent in R. canina, we can conclude that it is a taxon that originated simultaneously with the genera Rubus L., Fragaria L. from a common ancestor. In other words, R. canina can be considered as an archaic (ancient) evolutionary branch of the genus Rosa.

The second important task was to give a comparative analysis of Rosa L. species by protein marking. The studied objects relate mainly to three well-known sections of the genus Rosa: species R. canina L., R. glabriifolia C.A. Mey. Ex. Rupr., R. pomifera Herrm., R. corymbifera Borkb., R. glauca Pourr., R. gorinensis Bess., R. agrestis Savi belong to the section Caninae Crep., Species R. majalis Herrm., R. acicularis Lindl., R. kokanica (Regel Juz., R. beol schenoana Regel., R. beggeriana Schrenk – to the section Cinnamomeae Crep., Species R. spinosissima L. – to the section Pimpinellifolia.
| Component Positions on the Scale | 12S-globulins, polypeptides | 7S-globulins |
|----------------------------------|-----------------------------|-------------|
| basic, up to 23 kDa              | acidic, up to 45 kDa        |             |
| 29                               | 28                          |             |
| R. Canina L., Asia Minor and Southern Europe, North Caucasus, Urals | 2 1 1 2 1 1 1 2 1 |  |
| 1 1                               |                             |             |
| R. glabriolida C.A. Mey. ex Rupr., Eastern Europe, Urals | 2 2 1 2 1 1 2 1 |  |
| 1 1                               |                             |             |
| R. Corymbifera Borkh., Eastern Europe | 2 2 1 2 1 2 1 2 1 |  |
| 1 1                               |                             |             |
| R. Gorinkensis Bess., Eastern Europe, Volga Region | 1 1 2 1 1 2 1 2 1 |  |
| 1 1                               |                             |             |
| R. Pomifera Herrm., North Caucasus | 1 2 2 1 1 2 2 1 1 |  |
| 1 2                               |                             |             |
| R. glauca Pourr., Western Europe, Volga region | 1 2 2 1 1 2 1 1 1 |  |
| 1 1                               |                             |             |
| R. Fedtschenkoana Regel, Central Asia (Northern Tien Shan) | 1 2 1 2 1 2 |  |
| 1 2                               |                             |             |
| R. Agrestis Savi, Western Europe | 1 2 2 1 1 2 1 2 1 |  |
| 1 1                               |                             |             |
| R. Aicuarius Lindl., Urals, South and East Siberia, Far East and North America (Texas, USA) | 1 2 1 1 1 1 1 1 1 |  |
| 1 1                               |                             |             |
| R. Beggeriana Schrenk, South Kazakhstan (Tarbagatai) | 1 2 1 1 1 2 |  |
| 1 1                               |                             |             |
| R. Pimpinellifolia L., Urals (Orenburg Region of the Russian Federation) | 1 2 1 1 1 1 2 1 |  |
| 1 1                               |                             |             |
| R. Subpomifera Chrzan., Eastern Europe (Vologda Oblast of the Russian Federation) | 1 2 1 1 1 1 2 1 |  |
| 1 1                               |                             |             |
| R. Majalis Herrm., Eastern Europe, Volga and Urals, South Siberia and North America (Texas, USA) | 1 2 1 1 1 1 1 1 1 |  |
| 1 2                               |                             |             |
| R. kakanica (Regel) Juz., Central Asia (Northern Tien Shan) | 1 2 1 1 1 1 1 2 |  |
| 1 1                               |                             |             |
| R. spinossissima L., Southern Europe, Southern Siberia and the Eastern Tien Shan (China) | 1 2 1 1 1 1 1 1 1 |  |

Note. Due to the indistinct manifestation in a number of species and the compactness of the table on electrophoregrams, stable components of 7S-globulins are not given above position 28 (from 22 to 10).
It should be noted that the systematic position of the fairly well-known species *R. beggeriana* has not yet been determined. V.G. Khrzanovsky includes this species in the Lenconthae M. Pop section. In addition, the author identifies the species *R. subpomifera* Chrishan., which he assigned to the composition of the above section *Caninae*.

A comparative analysis of the obtained data shows that in the studied *Rosa* L. species, proteins are distributed on the electrophoreogram into 2 zones: the lower low molecular weight with a mass of up to 23 kDa and the upper higher molecular weight with a mass of Rosa 40 – 95 kDa.

Analysis of the polypeptide spectra shows that the species of *Rosa* L. from different sections are quite close to each other and this, obviously, reflects the fact of their initial proximity. In particular, all species are characterized by bright components in the region of positions 80, 50, partially 30 and less bright, also characteristic in positions 92, 90 kDa. In the spectra of seeds belonging to the same species, most polypeptides occupy the same positions, i.e. polypeptide spectra of seeds of representatives of the genus *Rosa* L. are species-specific. For example, in the spectra of seeds of *R. grabrifolia*, the main polypeptides in intensity are localized on a “soy” scale at positions 30; 40; 46; fifty; 60; 80; 90; 92, an almost similar characteristic for *R. acicularis* (30; 40; 48; 50; 60; 80; 90), and for *R. spinosisima* 30; 40; 46; 60; 70; 78; 80; 88; 90. Along with the distribution of protein zones characteristic of the species, deviations from the species formula are observed in individual spectra. Deviations relate to the displacement of the polypeptide to a nearby position, its doubling, change in intensity or its absence. Such variability is genetically determined and is a manifestation of seed protein polymorphism [9]. The appearance of protein polymorphism does not overlap the species specificity of the polypeptide spectra. Species features of the protein spectrum are easily distinguished in the analysis of even 3-4 seeds of randomly selected individuals from one or different places of growth.

5. Conclusion

The *R. spinosisima* growing in the Urals is presented according to the modern nomenclature of the subspecies *R. spinosisima* ssp. *Pimpinellifolia* (L.) Soo; moreover, the Ural taxon in polypeptides is very significantly different from the species *R. spinosisima*.

Thus, a different taxon is growing in the Urals. It is quite possible, based on the structure of its polypeptide spectrum that the subspecies should be assigned to the species *R. acicularis*. As a result, our study of *Rosa* L. species by the polypeptide spectra of seed storage proteins allows one, in combination with morphological methods, to clarify the taxonomic structure of the genus. This remains very important today, since, as is known, quite a lot of classifications of the genus *Rosa* have been proposed, but they often contradicted each other. The main reason for the inconsistency was the use for the classification of highly varying (modifying) characters [10,11]. Protein markers, as established by many researchers, are environmentally stable, which makes them promising in taxonomy.

The following conclusion can be drawn from our study. The variation of polypeptide positions in the spectra of seeds of species belonging to the same genus is much higher than their intraspecific variability; nevertheless, a visual analysis of the spectra of the supraspecific level allows us to highlight their specific features. The electrophoretic polypeptide spectra of roship seeds of *R. majalis* and *R. canina* are independent of the ecological conditions of growth. Partial similarity of the spectra of *Rosa* species was revealed; species of *Rubus idaeus*; *Fragaria opinca*, belonging to the subfamily Rosoidae, in the zone of the main polypeptides that reflect the affinity at the subfamily level. The polypeptide spectra of *R. majalis* and *R. canina* are different, which confirms their systematic isolation. The polypeptide spectra of *Rosa* species from different sections for a number of polypeptides are quite close to each other, which reflects the fact of their initial proximity. Judging by the polypeptide spectra, many species of *Rosa* Urals are polymorphic. *A. spinosisima* from the Urals based on the structure of its polypeptide spectrum to the species *R. acicularis*.
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References

[1] Shulaev V, Korban S S, Sosinski B et al 2008 Multiple models for Rosaceae genomics Plant Physiol. 147 985–1003

[2] Peace C and Norelli J 2009 Genomics Approaches to Crop Improvement in the Rosaceae In: Folta K M and Gardiner S E (ed) Genetics and Genomics of Rosaceae. Plant Genetics and Genomics: Crops and Models 6 19–53

[3] Peace C P, Ahmad R, Gradziel T M, Dandekar A M and Crisosto C H 2005 The use of molecular genetics to improve peach and nectarine post-storage quality Acta Horticult. 682 403–10

[4] Potter D, Eriksson T, Evans R C et al 2007 Phylogeny and classification of Rosaceae Plant Syst. Evol. 266 5–43

[5] Schauer N and Fernie A R 2006 Plant metabolomics: towards biological function and mechanism Trends Plant Sci. 11 508–16

[6] Rjabuchina M, Kalyakina R and Friesen N 2019 Phylogeographic analysis of Pinus sylvestris in forest-steppe and steppe zones of the Orenburg Region Turczaninowia 22(2) 110–20 DOI: 10.14258/turczaninowia.22.2.6

[7] Yausheva E, Sizova, Lebedev S et al 2016 Influence of zinc nanoparticles on survival of worms Eisenia fetida and taxonomic diversity of the gut microflora Environ. Sci. Pollut. Res. 23(13) 13245–54 DOI: 10.1007/s11356-016-6474-y.

[8] Rjabuchina M V, Kalyakina R G and Friesen N 2020 Molecular genetic studies of the natural self-renewal of Pinus sylvestris L. populations on the example of the East European Plain and the southern outskirts of the Ural mountain country Turczaninowia 23(1) 116–25 DOI: 10.14258/turczaninowia.23.1.12

[9] Korotkova A, Sizova E, Lebedev S et al 2015. Influence of iron of nanoparticles on induction of oxidative damage in triticum vulgare Ecol., Environ. and Conservat. 21(S) 101–11

[10] Korotkova A, Gavrish I, Lebedev S and Halikov B 2019 Comparative analysis of cell viability of Triticum vulgare after exposure to copper nanoparticles Open Bio. 9 303 DOI: 10.1002/2211-5463.1267

[11] Deryabin D, Galadzhieva A, Kosyan D et al 2019 Plant-derived inhibitors of AHL-mediated quorum sensing in bacteria: Modes of action International Journal of Molecular Sciences 20(22) 5588