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Characterization of the biochemical composition and antioxidant activity of *Spinacia oleracea* L. and *Spinacia turkestanica* Iljin.: a comparative study

Diana V. Sokolova *, Alla E. Solovieva

Federal Research Center the N.I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR)
42-44 Bolshaya Morskaya St.,
St. Petersburg, 190000, Russia

***Corresponding Author:** dianasokol@bk.ru

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Abstract

Spinach is an economically important vegetable crop widely cultivated and consumed worldwide. This early ripening leafy vegetable is rich in bioactive components, fiber, micro and macro elements, vitamins, and has high antioxidant activity. Results of numerous studies on the effects of spinach on human health confirm its beneficial effect. The species *S. oleracea* L. is cultivated commercially. The ancestor of cultivated spinach is *S. turkestanica* Iljin, which has a breeding potential for different economically valuable traits. Its biochemical composition has been studied extremely little. The present article offers a comparative evaluation of the biochemical profile and antioxidant activity of cultivated and wild spinach species. The material for the study was a representative sample of 48 collection accessions of spinach from the N.I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR). The accessions were grown in 2019 and 2020 in the open ground of the Pushkin and Pavlovsk Laboratories of VIR. The antioxidant activity was studied spectrophotometrically by the DPPH assay of free radical scavenging at a wavelength of 515 nm. A significant similarity of the two species in most biochemical parameters was revealed, which confirms their phylogenetic relationship. Significant differences were found in the content of phenolic elements, which determine the elevated values of antioxidant and antiradical activity of *S. turkestanica*. The article presents correlation matrices of species biochemical composition, describes general trends, negative relationships and conjugated factors. The identified promising accessions of both cultivated and wild spinach are recommended for breeding for increased content of phenolic compounds, ascorbic acid and antioxidant activity. The result of the study helps to reveal the potential of the crop as a valuable source of bioactive components and high antioxidant activity.

Keywords: spinach; *Spinacia oleracea* L.; *Spinacia turkestanica* Iljin.; antioxidant activity; phenolic compounds

Характеристика биохимического состава и антиоксидантной активности *Spinacia oleracea* L. и *Spinacia turkestanica* Iljin.: сравнительное исследование

Д.В. Соколова *, А.Е. Соловьева

Федеральный исследовательский центр Всероссийский институт генетических ресурсов растений им. Н. И. Вавилова (ВИР)
190000, Россия, г.Санкт-Петербург,
ул. Б. Морская,д .42,44

***Автор для переписки:** dianasokol@bk.ru

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Аннотация

Шпинат - экономически значимая овощная культура, широко возделываемая и потребляемая во всем мире. Этот скороспелый листовой овощ богат биологически активными компонентами, клетчаткой, микро- и макроэлементами, витаминами, обладает высокой антиоксидантной активностью. Многочисленные результаты изучения влияния шпината на здоровье человека подтверждают его благотворное действие. В производстве культивируют вид *S. oleracea* L. Прародителем культурного вида шпината является вид *S. turkestanica* Iljin., обладающий потенциалом для селекции по различным хозяйственно-ценным признакам. Его биохимический состав изучен крайне мало. В настоящей работе дана сравнительная оценка биохимического профиля и антиоксидантной активности культурного и дикого видов шпината. Материалом для исследования послужила репрезентативная выборка из 48 образцов коллекции шпината ВИР. Образцы выращивались в 2019 и 2020 году в условиях открытого грунта Пушкинских и Павловских лабораторий Всероссийского института генетических ресурсов растений имени Н.И. Вавилова. Антиоксидантную активность изучали методом DPPH на спектрофотометре путем поглощения свободных радикалов раствора, измеряемое при длине волны 515 нм. Выявлено значительное сходство двух видов по большинству биохимических показателей, что подтверждает их филогенетическое родство. Существенные различия обнаружены в содержании фенольных элементов, определяющих повышенные значения антиоксидантной и антирадикальной активности *S. turkestanica* Iljin. В статье приведены корреляционные матрицы видового биохимического состава, описаны общие тенденции, отрицательные взаимосвязи и сопряженные факторы. Выделившиеся перспективные образцы как культурного, так и дикого видов шпината рекомендованы для селекции на увеличение содержания фенольных соединений, аскорбиновой кислоты и антиоксидантной активности. Результат исследования помогает раскрыть потенциал культуры, как ценного источника биологически-активных компонентов и высокой антиоксидантной активности.

Ключевые слова: шпинат; *Spinacia oleracea* L.; *Spinacia turkestanica* Iijin.; антиоксидантная активность; фенольные соединения

Introduction

The conditions of modern life put forward new requirements for human nutrition. In the market, there is a growing demand for natural products with high nutritional value and revitalizing properties. Cultivated spinach (*Spinacia oleracea* L.) is an economically important vegetable crop widely cultivated and consumed worldwide. According to FAOSTAT, global production of spinach is growing every year, having reached 35 million tons in 2021 (FAOSTAT) [1]. Spinach is consumed fresh, used in the canning industry for the production of juices, purees, in baby and diet foods, as well as for the production of a green dye [2,3]. This leafy vegetable is rich in bioactive components and fiber [4]. It is valued for its high content of ascorbic acid, carotenoids, vitamins B1, B2, B3, B6, B9, H, K, E, P, and PP [5]. Also, it contains iron, sodium, potassium, calcium, magnesium, phosphorus, sugars, and protein. Due to the high content of various organic acids, the nutritional value of spinach does not change during canning and drying [6,7]. Spinach contains various active compounds such as flavonoids and other polyphenolic active ingredients that act synergistically as anti-inflammatory, antioxidant and anti-cancer agents. Epidemiological and preclinical data from studies on the health effects of spinach confirm its beneficial effects [8,9,10,11,12,13].

According to the APG II Classification System (2003) [14], *Spinacia* L. genus belongs to the *Chenopodioideae* subfamily of the Amaranthaceae family. In an earlier classification, spinach belonged to the Chenopodiaceae family. The genus is represented by three species: two 2003wild ones, *S. turkestanica* Iljin and *S. tetrandra* Stev., and cultivated *S. oleracea* L. The species *S. tetrandra* was first described by Christian von Steven, a Russian botanist of Swedish origin, while studying the flora of the Caucasus (1809) [15], and was long considered the only wild spinach species. The species *S. turkestanica* was isolated by M.M. Ilyin in 1934 as an independent species of wild spinach growing in Central Asia [16]. The distribution area of *S. tetrandra* is located mainly in Transcaucasia, while that of *S. turkestanica* is found in East and Central Asia, Uzbekistan, Turkmenistan, Afghanistan, and Iran [17,18,19].

The exact origin and the earliest date of *S. oleracea* cultivation are still unknown. It is believed that spinach was introduced into culture about two thousand years ago in Iran (former Persia), from where it spread to China, Europe, North Africa and America [20]. These assumptions were confirmed by recent transcriptome sequencing of 120 spinach accessions. It was shown that the most likely progenitor of cultivated spinach is *S. turkestanica* [21,22].

Wild *S. turkestanica* is of considerable interest for spinach breeding for such economically valuable traits as cold and drought tolerance, resistance to the most common diseases, abiotic stresses, as well as soil salinity and acidity. Its biochemical composition has been studied little. In light of the growing interest to the nutritional value and composition of spinach, the present study is relevant.

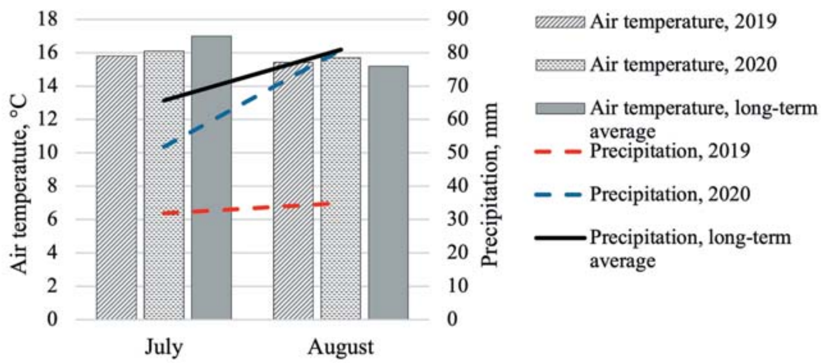
The present work was aimed at revealing features of the biochemical profile and antioxidant activity in the cultivated spinach species (*S. oleracea*) and its wild predecessor (*S. turkestanica*).

Materials and methods

The object of the study were two spinach species, *S. oleracea* and *S. turkestanica* (Fig. 1). The material for the experiment were 48 accessions from the spinach collection of the N.I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR). The plants were grown in the open field of the "Pushkin and Pavlovsk Laboratories of VIR" Research and Production Base (59°7111275′N, 30°43032647′E) in 2019, 2020. Seeds were sown manually on July 15 in a row with a 10 cm distance between plants and 70 cm between rows. Biomass was sampled for analyzing in the rosette phase on day 40 from the sprouts emergence. Soils in Pushkin are predominantly sod-podzols and sandy loams. The accessions were grown against a natural background without the use of fertilizers and pesticides.

The weather conditions of the second half of the summer of 2019 and 2020 were generally favorable for growing spinach and were characterized by moderate air temperatures at the level of long-term mean values (Fig.2). The growing season of 2020 was characterized by a large amount of precipitation: in June by 20 mm, in August - by 45 mm. Irrigation was carried out if necessary. The weather conditions of both years of testing made it possible to obtain plants identical in habit and weight of one plant with the closest possible biochemical parameters.

Fig.1. Experimental spinach species: S. oleracea L. (left) and S. turkestanica Iljin. (on right).
Рис.1. Виды шпината в опыте: S. oleracea L. (слева) и S. turkestanica Iljin. (справа).



Source: PGR Automated Information Systems Department. Hydrometeorological station of VIR.

Fig. 2. Climatic characteristics of the growing seasons 2019, 2020 (Pushkin)
Рис. 2. Климатическая характеристика вегетационных периодов 2019, 2020 годов (г. Пушкин)

Biochemical analysis was carried out in the Department of Biochemistry and Molecular Biology of VIR. Accessions were processed and analyzed as described in Ermakov et al. [23]. Dry weight (in %) was measured gravimetrically. A 50 g portion of fresh substance was dried in a thermostat at 80 °C for 12 hours, and then at 105 °C for 1 hour to a constant weight. The content of ascorbic acid was determined by direct extraction from plants (10 g) with 1% HCl solution (according to I.K. Murri) followed by titration with 2,6-dichloroindophinol (Thielman's reagent) and expressed in mg/100 g. The total sugars content was determined by the Bertrand method. A 25 g sample was taken for the analysis. Oligosaccharides were preliminarily hydrolyzed with 10% HCl solution. The amount of cuprous oxide precipitate strictly corresponded to the amount of sugar in the solution. The settled precipitate of cuprous oxide was dissolved with iron sulfate (oxide) in the presence of sulfuric acid. In this case, copper oxide is oxidized completely, and ferrous oxide, in turn, is quantitatively oxidized with a titrated solution of potassium permanganate. The data are presented in percent. To measure the total acidity, a 25 g sample of fresh substance was homogenized in 250 ml of hot distilled water, then filtered, and 10 ml was titrated with 0.1 N alkali in the presence of an indicator. The results are expressed as a percentage, recalculated as oxalic acid. The protein content was measured by the Kjeldahl method [24]: a sample of dried and ground material was mineralized by heating with concentrated sulfuric acid at 420 °C for one and a half hours. Nitrogen was determined using a Kjeltec 2200 semi-automatic analyzer (FOSS, Sweden) followed by titration with 0.1 N sulfuric acid solution The total protein content was calculated from nitrogen with a factor of 6.25 (for vegetable crops). Pigments were isolated with 100% acetone, and their absorption was measured on an Ultrospec II spectrophotometer (England) at different wavelengths (nm): 662 and 645 for chlorophylls a and b, 440 for carotenoids, and 454 for *β*-carotene. The total amount of soluble phenolic compounds was determined by Folin-Ciocalteu spectrophotomety (phenolic compounds were extracted with 80% alcohol and kept for 12^h in the dark at room temperature; absorption measured at 765 nm) modified by Singleton and Rossi [25]. The result was expressed as mg of gallic acid equivalent (GAE) per 100 g. For assessing antioxidant activity, free radical colorimetry was used as a method based on the reaction of the ethanol-dissolved DPPH (2,2-diphenyl-1-picrylhydrazyl ($C_{18}H_{12}N_5O_6$, M = 394.33)) with an antioxidant sample [26]. The result was expressed as ascorbic acid equivalent (AAE). All data are given in terms of crude matter.

The data were statistically processed in the Statistica 10.0 program and in the R environment. The descriptive statistics (mean values, standard error of the mean, and coefficient of variation) were calculated for all parameters. Pearson correlation coefficient values of $r<0.5$ were considered as low, those in the range of $0.51>r\geq 0.7$ as medium, in the range of $0.71>r\geq 0.9$ as high, and those of $r\geq 0.9$ as very strong.

Results and discussion

The VIR spinach collection is represented by three known species and features a wide variety of genotypes both in terms of origin and year of inclusion in the collection, and in terms of morphological characteristics. All the studied accessions are of European or Asian origin. The largest number of accessions belongs to the species *S. oleracea*; 39 accessions are from European countries, Russia and Japan (Fig.3). *S. turkestanica* was represented by 9 accessions from Armenia, Kazakhstan, Uzbekistan, Tajikistan and China, which is consistent with historical data on the center of crop origin and ways of its spreading [19].

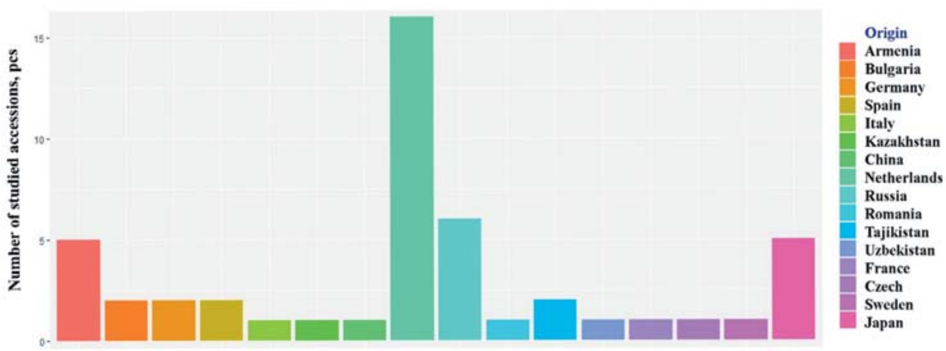


Fig. 3. Origin and number of the studied spinach accessions
Рис. 3. Происхождение и количество образцов шпината в опыте

Dry matter content. The content of dry matter is one of important indicators for judging the quality of vegetable raw matter. In the present study, the dry matter content did not differ significantly ($p < 0.05$) between accessions of the two species and averaged 10.5% for *S. oleracea* and 10.31% for *S. turkestanica* (Table 1). A weak variability of this trait is characteristic (*Cv*=11-14%). The maximum content of 14.6% was noted in 'Sp. Riccio DAmerica' (k-942, Italy), an accession of *S. oleracea*. Dry matter content above the average was shown by 55.5% of the accessions of *S. turkestanica*.

Ascorbic acid content. A marked variation in the levels of ascorbic acid (vitamin C) among the tested genotypes was observed in the range from 24.8 to 62.0 mg/100 g. On the average, it was 40.9 mg/100 g. The maximum content of 62 mg/100 g was found in an accession of *S. turkestanica* from Tajikistan (k-942). No significant differences in the content of ascorbic acid were found between the two species. Similar data were obtained for *S. oleracea* by other authors when studying various genotypes grown in open ground conditions [27,28,29]. However, in protected ground conditions the differences between genotypes were much stronger. For instance, a study of a set of 34 *S. oleracea* genotypes in China has revealed a significant difference in the content of ascorbic acid [30]. This is explained by lighting conditions that affect the concentration of ascorbic acid in fruits and vegetables: a decreased light intensity usually leads to a decrease in its concentration and genotypic differences [31,32]. It may be assumed that the genotypes of *S. turkestanica*, which formed under conditions of increased photosynthetic activity in the center of origin of the crop, are able to synthesize ascorbic acid more actively. Several components, including ascorbic acid and phenolic compounds, have been reported to inhibit nitrite toxicity in spinach [33]. No doubt that spinach, which accumulates more ascorbic acid, will be more beneficial for human health.

Content of chlorophylls and total acidity. The content of organic acids and chlorophylls in the studied accessions of the two species was similar and had no significant differences ($p < 0.05$).

Protein content. In terms of protein content, the group of *S. oleracea* accessions was superior to *S. turkestanica*. The maximum value of 29.11% was recorded for the 'Ratnik' variety (k-916, Russia). A general regularity was observed in the negative correlation between the content of protein and sugars (Fig. 4, 5). At the same time, this relationship was more significant (r=– 0.72 (p<0.05)) in accessions of *S. turkestanica* vs. r = – 0.57 (p<0.001) in *S. oleracea*.

Phenolic Content. Phenolic compounds are among the most common secondary plant metabolites [34]. Phenolic compounds found in spinach have a strong antioxidant effect due to the ability of their hydroxyl groups to scavenge free radicals. Extensive conjugation in the structure of flavonoids and numerous hydroxyl groups enhance their antioxidant properties [35]. Previous studies of spinach have shown that kaempferol (54%) predominates among

Table 1. Comparative characteristics of biochemical indicators of S. oleracea and S. turkestanica
Таблица 1. Сравнительная характеристика биохимических показателей образцов видов S. oleracea L. и S. turkestanica Iljin.

Biochemical indicators	S. oleracea	S. turkestanica	LSD05 [d]
	M±SE [a] (Cv[b], %), Median (min÷ max)[c]		
Dry matter, %	10.5±0.2 (14.0%)	10.31±0.4 (11.0%)	1.18
Ascorbic acid, mg/100 g	41.0±1.3 (20.2%)	40.5±3.7 (27.2%)	7.32
Total sugars, %	0.46 (0.28÷1.86)[c]	0.83 (0.45÷2.38) c	-
Titrated acidity, %	0.17±0.01 (15.5%)	0.17±0.01 (14.6%)	0.02
Chlorophyll A, mg/100 g	82.4±2.92 (22.1%)	80.8±6.1 (22.5%)	15.2
Chlorophyll B, mg/100 g	32.7±1.1 (21.8%)	31.9±2.6 (24.7%)	6.05
Chlorophylls, mg/100 g	115.1±4.1 (22.0%)	112.8±8.6 (23%)	21.13
Carotenoids, mg/100 g	32.0±1.0 (20.0%)	32.0±1.9 (17.8%)	5.21
β-carotene, mg/100 g	5.44±0.2 (22.0%)	5.35±0.4 (21.7%)	0.99
Proteing, % of dry matter	25.4±0.3 (6.8%)	23.2±1.1 (14.3%)	1.74
Phenolic compounds, mg GAE/100 g	238.7 (82.9÷750.9)[c]	429.7 (112.6÷656.5)[c]	-
AOA [e], μg AAE/100 g	166.3±10.0 (37.5%)	177.0±25.2 (42.7%)	54.0
DPPH[f], %	56.8±2.9 (31.6%)	60.0±7.8 (39.1%)	15.8

[a] M±SE – mean±standard error; [b] Cv – coefficient of variation; [c] standard distribution difference;
[d] Least Significant Difference (LSD); [e] Antioxidant activity; [f] Antiradical activity

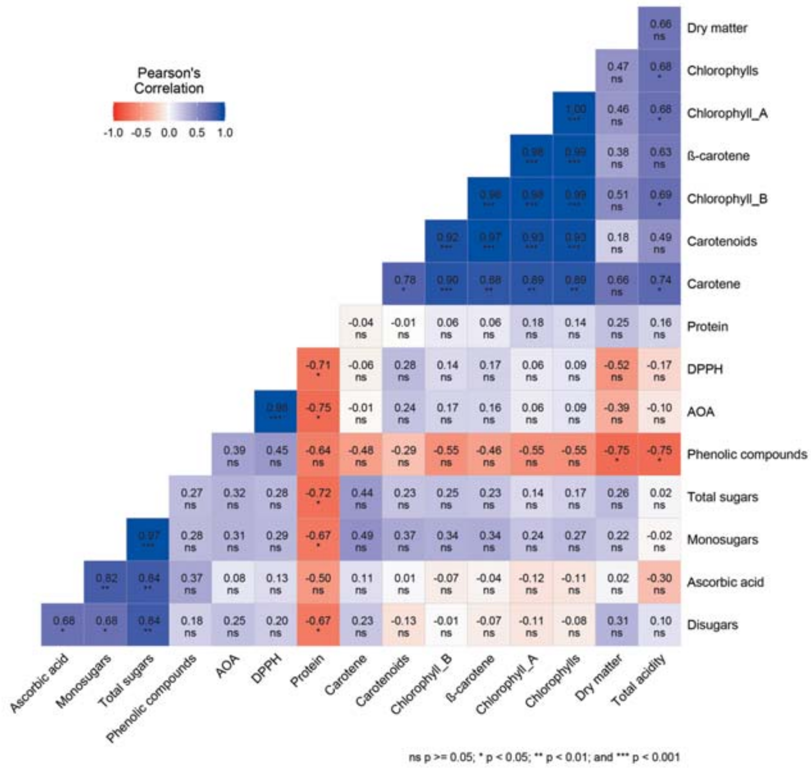


Fig. 4. Correlation matrix of biochemical parameters in genotypes of S. turkestanica
Рис. 4. Корреляционная матрица биохимических показателей генотипов вида S. turkestanica lijin

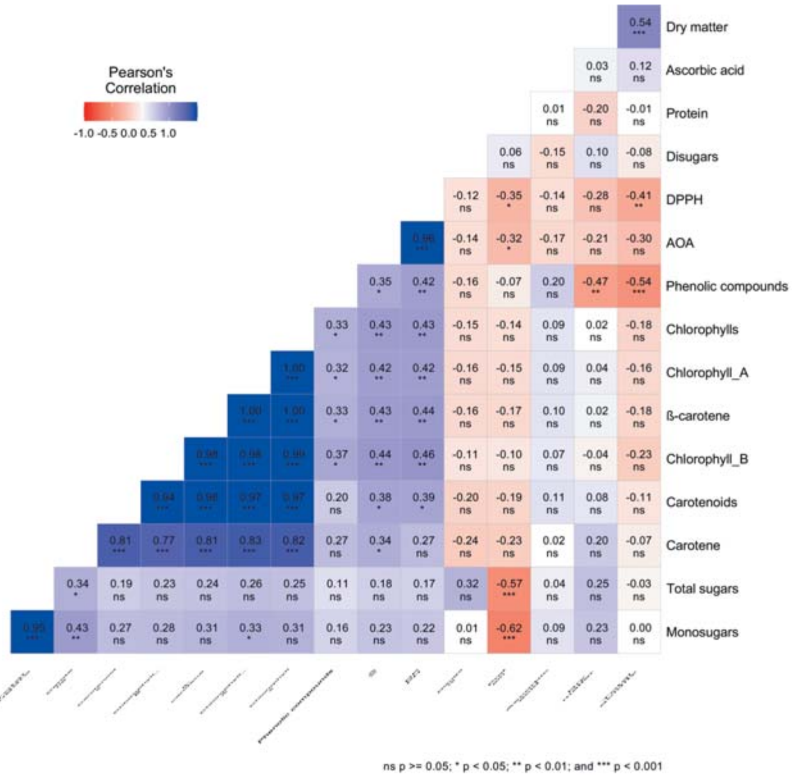


Fig. 5. Correlation matrix of biochemical parameters in genotypes of S. oleracea
Рис. 5. Корреляционная матрица биохимических показателей генотипов вида S. oleracea L.

the phenolic compounds, the content of gallic acid is 26% and that of galangin is 18% [36]. The content of phenolic elements in the studied accessions varied over a wide range. It should be noted that genotypes with a high content of phenolic elements were more common in *S. turkestanica*. The group with the above-average values included 23% of all *S. oleracea* and 34% of *S. turkestanica* accessions. Among accessions of the cultivated species, only those of Dutch and Russian origin turned out to be the leaders. The maximum value of 750.0 mg GAE/100 g was found in the accession ‘Gb. 25784’ (k-941, the Netherlands). Among *S. turkestanica* accessions, one from Armenia (vk-935) with 656.5 mg GAE/100 g and another one from Tajikistan (vk-942) with 604.3 mg GAE/100 g were singled out regarding this indicator. A general trend demonstrated by the correlation matrix for the crop is that the higher the content of dry matter and total acidity in plants, the less phenolic elements they contain. The negative correlation of these indicators in genotypes of the wild species was more significant: r=–0.75 (p<0.05) vs. r=–0.47-0.54 (p<0.01) in *S. oleracea* accessions. It was previously noted that the content of phenolic compounds in plants increases under conditions of high photosynthetically active radiation [37], as well as with the advance of the crop to northern latitudes [38,39]. We assume that the origin of *S. turkestanica* genotypes, which formed in climatic regions with active solar radiation and thus got adapted to such conditions, is reflected in the ability to accumulate phenolic elements.

Antioxidant and antiradical activity. Spinach is one of the most valuable green crops with pronounced antioxidant properties [36,40]. Antioxidant activity (AOA) is the ability to inhibit the oxidation process, and antiradical activity (DPPH) reflects the ability of compounds to react with free radicals. There is strong evidence for the role of the vegetable antioxidant components in health maintenance and disease prevention [41,42,43]. Compared with lettuce and kale, the AOA and DPPH values in spinach are higher by 39.5% and 24.2%, respectively, and slightly lower than in broccoli [44]. Literature confirms that along with blueberries, spinach has a high ability to scavenge free radicals [45,46,47]. In our studies, high AOA levels in representatives of *S. turkestanica* were more common (observed in 67% of accessions), while it was true for only 46% of *S. oleracea* accessions. The maximum values were noted in *S. turkestanica* from Kazakhstan (k-775), Tajikistan (k-942) and Armenia (k-960) – 244.04, 243.81 and 256.10 μg AAE/100 g, respectively. These accessions were distinguished by a high content of sugars, a low content of protein, oxalic, citric and pyruvic acids, fatty acids and alcohols. In general, AOA of spinach negatively correlated with protein content and was high in *S. turkestanica* accessions. (r=–0.75, p<0.05).

AOA of spinach is determined mainly by the pigment composition, i.e., chlorophylls and carotenoids, as well as by phenolic compounds. In the present study, the content of carotenoids in two spinach species did not have significant differences and averaged 32.0 mg/100 g. As shown in Figure 6 a close positive correlation between chlorophylls and carotenoids associated with AOA. Since the values of the pigment composition in the wild and cultivated species are close, it can be assumed that the increased AOA and DPPH values in spinach are associated with the accumulation of phenolic compounds.

It is known that the main carotenoid found in spinach leaves is lutein, which averages 39% of the total carotenoids [30]. This pigment is a natural protective filter for the eyes, maintaining visual acuity. The human body is not able to synthesize lutein, so its intake into the body is directly related to nutrition. Spinach is a promising crop, a source of lutein and high AOA. Its promotion and consumption will contribute to the revitalization of the population.

Conclusions

The conducted studies showed that the comparison of biochemical parameters of *S. oleracea* and *S. turkestanica* revealed a significant similarity of the two species in most biochemical parameters, which confirms their phylogenetic relationship. A negative correlation between the content of protein and sugars was noted to be characteristic of both species. Significant differences were found in the content of phenolic elements, which determine the increased values of the antioxidant and antiradical activity of *S. turkestanica*. The maximum content of phenolic elements (750.0 mg GAE/100 g) was recorded for an *S. oleracea* accession 'Gb. 25784' (k-941, the Netherlands). In *S. turkestanica*, the highest values were demonstrated by accessions from Armenia (656.5 mg GAE/100g (vk-935) and Tajikistan (604.3 mg GAE/100 g) (k-942). These genotypes are of interest for breeding for an increased content of phenolic elements and AOA.

A negative relationship was revealed between dry matter content and total acidity with phenolic elements, which is more significant in *S. turkestanica*. Among the genotypes of the wild species, an accession from Tajikistan (k-942) is a source of high content of ascorbic acid (62 mg/100 g). For breeding for an increased AOA, *S. turkestanica* accessions from Kazakhstan (k-775) and Armenia (k-960) can be recommended.

In general, the biochemical composition of spinach is quite rich and has a beneficial effect on human health. The results of the study help to reveal the value of *S. turkestanica* and recommend its inclusion in breeding programs.

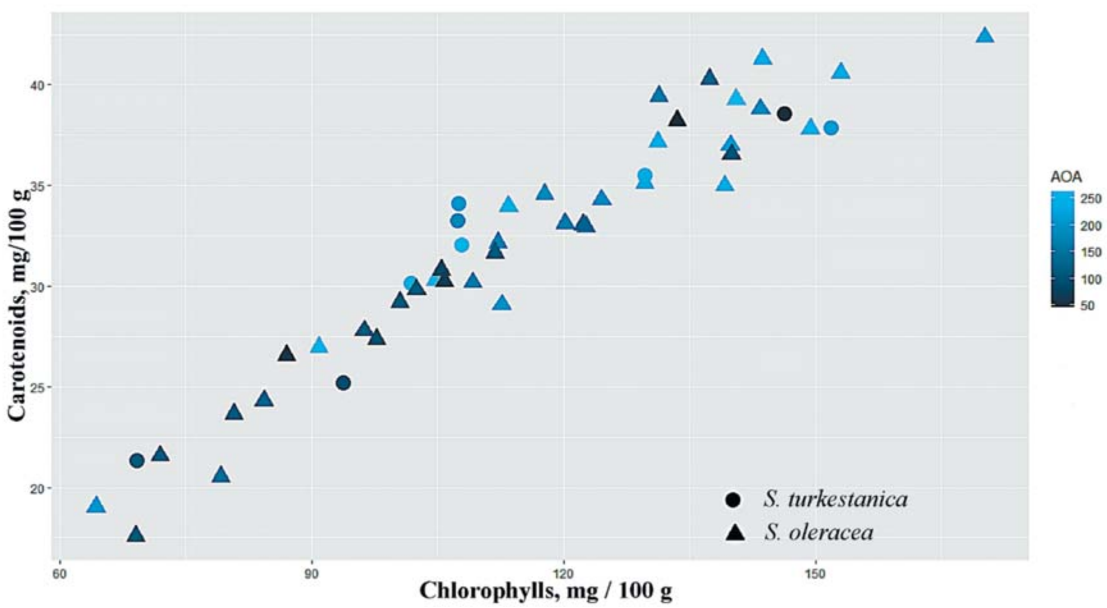


Fig. 6. Scatterplot of spinach chlorophylls and carotenoids
Рис. 6. Диаграмма рассеяния хлорофиллов и каротиноидов шпината. 1 – S. turkestanicalijin., 2 – S. oleracea L.

Об авторах:
Диана Викторовна Соколова – старший научный сотрудник Отдела генетических ресурсов овощных и бахчевых культур, куратор коллекции свеклы, амаранта и шпината, https://orcid.org/0000-0002-9967-7454, автор для переписки, dianasokol@bk.ru
Алла Евгеньевна Соловьева – старший научный сотрудник отдела Биохимии и молекулярной биологии ВИР, https://orcid.org/0000-0002-6201-4294

About the Authors:
Diana V. Sokolova – Senior Researcher, Department of Genetic Resources of Vegetable and Cucurbit Crops, Beet, spinach and amaranth collection curator, https://orcid.org/0000-0002-9967-7454, Correspondence Author, dianasokol@bk.ru
Alla E. Solovieva – Senior Researcher, Department of Biochemistry and Molecular Biology, https://orcid.org/0000-0002-6201-4294

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