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Genetic Diversity of the Beet Distributed in Armenia Using Protein Markers

Tatevik Aloyan, Manvel Badalyan and Andreas Melikyan

Scientific Center of Agrobiotechnology, ANAU Armenian National Agrarian University, Teryan 74, Yerevan 0009, Armenia

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Corresponding Author:
Tatevik Aloyan
Scientific Center of Agrobiotechnology, ANAU Armenian National Agrarian University, Teryan 74, Yerevan 0009, Armenia
Email: tatevaloyan22@gmail.com

Abstract: The genetic resources of plants and animals are the wealth of every country. They are a valuable starting material in the breeding processes of cultivated plants and farm animals. In order to improve the existing varieties of crops and obtain new, valuable varieties, it is very important to document, preserve and enrich their gene pool, while preserving local varieties and forms of crops, it is possible to carry out breeding works. From this point of view, the genetic characterization of beet population varieties cultivated in Armenia and their wild relatives using protein markers is up-to-date and has important scientific and practical significance. It is known that the degree of genetic diversity of a population can be estimated if the protein formulas and their frequency of occurrence in the population are known. The purpose of this research was to evaluate the genetic diversity of beet spread in Armenia, formed during the evolutionary development processes, to passport the wild species and population varieties of beet by using protein markers and offering them as starting breeding material. As a result, the preferred raw material forms were proposed in the breeding works in the direction of increasing yield, sugar level, and resistance to powdery mildew and *Cercospora* leaf spot diseases. Further cluster analysis will make it possible to select parental pairs. Future beet breeding efforts in the target direction must make use of the genetic and genomic resources available for efficient improvement.

Keywords: Beet, Population, Protein Formula, Electrophoresis Spectrum, Polypeptide

Introduction

The genetic resources of plants and animals are the wealth of every country. Being the result of natural evolution and human activity, they are a strategic material for food production and at the same time play a major role in the process of maintaining environmental balance. Genetic resources are a valuable starting material for plant and animal breeding processes, contributing to economic growth, national autonomy, and food security in each country (Eastwood *et al*., 2022).

The flora of Armenia is extremely rich and diverse; it is a source of huge reserves of various useful plants. In order to improve the existing varieties of crops and obtain new, valuable varieties, it is very important to document, preserve and enrich their gene pool, while preserving local varieties and forms of crops, it is possible to carry out breeding works. Wild relatives of plants, which have enormous genetic potential, are also considered valuable genetic resources (Melikyan, 2001; Avetisyan *et al*., 2022).

It is known that the wild relatives of world-renowned crops spread in Armenia are also considered to be the source of the creation of a number of varieties of these crops. However, due to various reasons, the flora of Armenia is gradually decreasing, so its preservation is of great strategic importance. Wild relatives of many cultivated plants are still found in the territory of Armenia, making it one of the globally important centers of origin of agrobiodiversity (Dostatny *et al*., 2021; Hübner and Kantar, 2021; Zhukovski, 1971).

Beet is a popular vegetable in eastern and central Europe but is much less important in Western Europe and the USA, where it is known as garden beet. Beetroot is cross-compatible with all the other forms of *Beta vulgaris* L. including the non-cultivated wild species.

Out of eleven wild species in the genus *Beta*, three are distributed in the territory of the Republic of

Armenia (Melikyan, 2001; Takhtajian, 1956). These species include *B. macrorhiza* Stev., *B. lomatogona* Fisch C. A. Meyer, and *B. corolliflora* Zosimovic ex Buttler. They have been found in the floristic regions of Shirak, Aragats, Aparan, Lori, Tavush, Sevan, and Darelegis, with specific distribution areas for each species (Gabrielian and Zohary, 2004; Aleksidze *et al*., 2006; Melikyan, 2012; Avetisyan *et al*., 2022). Wild species and forms of beet in selection terms have a number of valuable properties. They are especially distinguished by cold tolerance, which enables them to sow in autumn, with large seeds, sprouts, and roots, the lack of property time soon blossoming, drought-resistance, salt-resistance, resistance from diseases, etc. Especially should be noted the single-seeded fruit which is typical for the species *Beta lomatogona*. The roots of some species are characterized by stability towards nematodes (Melikyan, 2001).

Cultivated beets, including leaf beets, garden beets, fodder beets, and sugar beets, which belong to the species *Beta vulgaris*, are economically important edible crops that have originated from a halophytic wild ancestor (Yolcu *et al*., 2021). The red juice from table beet is an important source of natural pigments (e.g., betalains), which play a role in free-radical scavenging and have economic value due to their use in the health, pharmaceutical, and food industries (Babarykin *et al*., 2019; Sawicki *et al*., 2016). Sugar beet stands as the second largest source of refined table sugar after sugar cane, which accounts for approximately 30-40% of global sugar production (Hussein *et al*., 2019; Zhang *et al*., 2016). Fodder beet has a higher yield potential than any other arable fodder crop. It can provide huge palatable yields for livestock or energy for anaerobic digesters (Al-Jbawi, 2020).

The widespread use of genetically uniform crop varieties has caused agricultural crops to lose some of the genetic diversity present in their wild ancestors. CWR offers important sources of useful agronomic traits, including tolerance for cold, salt, and drought conditions, and resistance to diseases (Zhang *et al*., 2017).

In order to increase work efficiency, and reduce costs and time in the selection works with plants, a new method of selective breeding has started in recent years: "Molecular" or "Marker-Assisted" Selection (MAS). In this case, the work is carried out in a very specific directionat the level of genes forming this or that characteristic (Nigmatullina *et al*., 2018; Sukhareva and Kuluev, 2018). From this point of view, the genetic characterization of beet population varieties cultivated in Armenia and their wild relatives using protein markers is up-to-date and has important scientific and practical significance.

The application and protection of biodiversity have become easier and more proficient with the utilization of biochemical (protein) and molecular (DNA) markers. Biochemical markers can reveal the polymorphism of sequences of specific proteins as well as indirectly identify polymorphism of the DNA sequences from which they are translated (Ismail *et al*., 2020). These markers are based on the change in the sequence of amino acids in a protein molecule (White *et al*., 2007; Yuasa and Umetsu, 2005).

The genetic information included in the genome is practically carried out only through proteins, which is why their supremacy as genetic and phylogenetic markers is obvious. Protein traits, as a rule, are inherited by the principle of co-dominance, and genotype analysis is directly possible with the protein phenotype (Konarev *et al*., 2000; Bojórquez-Velázquez *et al*., 2021; Nosenko *et al*., 2021).

The application of genomic tools could help uncover new traits in CWRs. Such diversity can be disclosed using high-throughput methodologies to identify new genomic information for breeding applications. Such innovative tools will provide crucial genetic elements to breeding programs (Monteiro *et al*., 2018).
The purpose of this research was:

- To evaluate the genetic diversity of beet spread in Armenia, formed during the evolutionary development processes
- To passport the wild species and population varieties of beet by using protein markers
- To offer them as starting breeding material

Materials and Methods

Sample Collection

Field works were conducted in natural habitats of wild beets: Hrazdan in Kotayk marz (Aparan floristic region) for *Beta corolliflora*, areas around Vardenyats Mountain Pass in Gegharkunik marz (Sevan floristic region) for *Beta macrorhiza*, as well as neighborhoods of Akunq village in Aragatsotn marz (Shirak floristic region) for *Beta lomatogona* (Fig. 1).

All cultivated beet varieties have a biennial development cycle. In the first year, they produce a leaf rosette and a fleshy root, in the second year they form a generative stem and seeds. Cultivated beet (table, sugar, fodder) seeds with extremely different root crops were taken from different regions of the RA and sown in conditions of the Voskehat teaching-experimental farm functioning under the Armenian National Agrarian University (ANAU) in order to evaluate the plant's morphological and biological characteristics (Aloyan, 2022).

Table 1: Conditions necessary for electrophoresis of 11S-globulin protein

Protein	Gel, %	Gel length, cm	Sample titer	Buffer		Power voltage, V	Phoresis duration, hours
				Gel	Electrode		
11S-globulin	10	12	1:1	0.05 M tris HCl, pH = 8,8	0.025 M tris -glycine, pH = 8,3	230	1,5

Fig. 1: *Beta lomatogona, Beta corolliflora, and Beta macrorhiza* species in their natural habitats

Experimental Studies

Molecular studies were performed in the biological research laboratory of the agrobiotechnology scientific center branch of ANAU. Within each species/variety, marking and formation of experimental groups were carried out. 30 randomly selected plants from each population were certified according to the electrophoresis spectrum of storage protein 11S-globulin.

Seeds of beet were powdered. Then, the powder was mixed with 10-fold of 0.2 M NaCl buffer by dipping it at 45°C for 1 h, followed by centrifugation at 8000× g for 30 min to remove the precipitation, and the pH of the supernatant was adjusted to 6.4 with 2 M HCl. After overnight storage at 4°C, the precipitate was collected by centrifugation (6000× g, 20 min, 4°C) and frozen at 24 °C for 24 h. After that, approximately 10 g of beet 11S-globulin and 90 g of deionized water (10°C) were mixed in uniformity using a glass rod, and the solution was homogenized twice. The supernatant was discarded. The precipitate was dissolved in a minimum volume of a buffer solution containing 0.4 g of Tris, 3 ml of 1 M HCl,1 g of Sodium Dodecyl Sulfate (SDS), 5 g of sucrose, 18 g of urea, 2.5 mL of mercaptoethanol and 0.25 g of bromophenol blue in 100 mL of water, centrifuged and used for electrophoresis.

Electrophoresis was performed on 10% polyacrylamide gel using the Davis method (Davis, 1964), with the Multigel-long phoresis apparatus of the German company Biometra. After the end of phoresis, the gel was fixed for 60 min in ethanol, acetic acid, and distilled water (40:10:60), after which it was stained with Kummas G-250 dye for 30-60 min and then washed 3 times with washing buffer (10% acetic acid solution) (Table 1).

Data Analysis

The results of the phoresis were compared with the reference spectrum of 11S-globulin. The frequency of meeting electrophoretic spectra was calculated by the following formula: $P_i = \frac{n}{N}$ where P_i is the frequency of spectra, n is the number of plants with a given spectrum, *and* N is the total number of plants.

Results

The 11S-globulin electrophoresis spectrum of the studied beet species and cultivars was compared with the total or reference spectrum of 11S-globulin, as a result

of which the protein formulas of the specified cultivars and their frequency of occurrence were deciphered (Table 2). The intensity of each polypeptide in the protein formula was evaluated by points: 3 is very intense, and 1 is weakly intense.

In the case of *B. corolliflora* (*Bc*) species, two different electrophoretic spectra of 11S-globulin were recorded, conventionally designated *Bc1* and *Bc2*. In the case of the *Bc1* spectrum, the total number of polypeptides is 5, with the following protein formula: 1, 5, 7, 10, 16 and the frequency of meeting the specified spectrum in the selected group is equal to 0.18 or 18%. In the case of spectrum *Bc2*, the total number of polypeptides is again 5, with the following protein formula: 1, 5, 6, 11, 16. The frequency of meeting the specified spectrum is equal to 0.82. The total number of polypeptides per species was 10, of which 30% were rated as low intensity, 30% as intense, and 40% as very intense.

In the case of *B. macrorhiza* (*Bm*) and *B. lomatogona* (*Bl*) species, one spectrum was observed in each, of which the total number of polypeptides is 6. Moreover, polypeptides were generally evaluated as intense and very intense. Their frequency of meeting is 1.00 (100%).

Three different electrophoresis spectra were observed in the Aparan population (*TAp*) of table beet: *TAp1*, *TAp2*, and *TAp3*. In the case of the *TAp1* spectrum, the number of polypeptides is 10. The frequency of the spectrum meeting is 0.12. In the case of the *TAp2* spectrum, the total number of polypeptides is 11, with a meeting frequency of 0.17. The polypeptide number of the *TAp3* spectrum is again 10. The frequency of the spectrum meeting is 0,71. The total number of polypeptides in the population was 31, of which 13% were rated as low intensity, 48% as intense, and 39% as very intense.

Two different electrophoresis spectra were observed in the Aramus population (TAr): *TAr1* and *TAr2*. In the case of the *TAr1* spectrum, the total number of polypeptides is 12, the meeting frequency is 0.76. The number of polypeptides in the *TAr2* spectrum is 11 with a meeting frequency of 0.24. The total number of polypeptides in the population was 23, of which 13% were rated as low intensity, 48% as intense, and 39% as very intense.

In the case of the Martuni population (*TMa*), 3 different electrophoresis spectra were observed: *TMa1*, *TMa2* and *TMa3*. The total number of polypeptides in the *TMa1* spectrum is 11. The spectrum meeting frequency is 0.15 or 15%. In the case of the *TMa2* spectrum, the total number of polypeptides is 10. The meeting frequency of this spectrum is 0,74. The number of polypeptides in the *TMa3* spectrum is the least with the following protein formula: 9: 2, 5, 8, 10, 11, 13, 15, 16, 18. The meeting frequency is 0.21. The number of polypeptides in the population was 30, of which 17% were rated as low intensity, 47% as intense, and 36% as very intense.

In the case of the Echmiadzin population (*TEj*), 3 different electrophoresis spectra were also observed: *TEj1*, *TEj2*, and *TEj3*, all of which had the same number of polypeptides 10. For the *TEj1* spectrum, the frequency of the spectrum meeting is 19%. For the *Tej2* spectrum, the meeting frequency is 0.18. The *TEj3* spectrum with 1, 5, 7, 8, 10, 11, 13, 15, 16, and 18 protein formulas had the highest frequency of meeting (0.63 or 63%). The number of polypeptides in the population is 30, of which 17% were scored as low intensity, 43% as intense and 40% as very intense.

Artik population (*TAt*) has the highest number of spectra among table beet populations, it forms 4 different electrophoretic spectra, *TAt1*, *TAt2*, *TAt3*, and *TAt4*, with the presence of 10-11 polypeptides. The frequency of meeting of the *TAt1* spectrum is 0.31, the *TAt2* spectrum is 0.09 or 9%, the *TAt3* is 0.47 or 47%, which is the highest in the population, and the *TAt*4 spectrum is 0,13. The total number of polypeptides in the population was 42, of which 19% were rated as low intensity, 36% as intense, and 45% as very intense.

In the case of the Abovyan population (*TAb*), 3 different electrophoresis spectra were observed: *TAb1*, *TAb2*, and *TAb3*. For the *TAb1* spectrum, the total number of polypeptides is 10. The frequency of spectrum meeting is the highest in the population, it is 0.63. The number of polypeptides in the *TAb2* spectrum is 12 with a meeting frequency of 0.16. The number of polypeptides in the *TAb3* spectrum is 10 with a meeting frequency of 0.21. The total number of polypeptides in the population was 32, of which 15% were rated as low intensity, 38% as intense, and 47% as very intense.

In the case of the Vardenis population (*TVd*), 3 different electrophoresis spectra were also observed: *TVd1*, *TVd2*, and *TVd3*. In the *TVd1* spectrum, the number of polypeptides is 11. The frequency of the spectrum meeting is 0.17. The total number of polypeptides in the *TVd2* spectrum is 8. This is the spectrum separated by the least number of polypeptides among table beet populations, with a frequency of 72%. For the *TVd3* spectrum, the number of polypeptides is 11 with a frequency of meeting 0.11. The total number of polypeptides in the population is 30, of which 13% were scored as low intensity, 40% as intense, and 47% as very intense.

Table 2: Protein formulas of studied beet wild species and population varieties

Wild species or population varieties	The type of spectrum	Meeting frequency, n	Number of fractions	Protein formulas																	
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Wild beet																					
B. corolliflora	*Bc1*	0.18	5	2				3		2			1						3		
	Bc2	0.82	5	2				3	1					1					3		
B. macrorhiza	*Bm*	1.00	6		2			3			3					3			2		1
B. lomatogona	*Bl*	1.00	6	2					3		2		3			2		3			
Table beet																					
Aparan	*TAp1*	0.12	10	2				2		3	1		3		2		3	2	2		3
	TAp2	0.17	11		3			2	1	2	1		3		2		2	2	3		3
	TAp3	0.71	10	3				3		2			2		1	2	3	3		2	2
Aramus	*TAr1*	0.76	12	3	2			3	2	1	2		2	3			2	3	3		2
	TAr2	0.24	11	2	3			2	2	3	1		3		2		2		3	1	
Martuni	*TMa1*	0.15	11	1	2				3	2	1		2	3	2	3		2		3	
	TMa2	0.74	10	2	2			1		3			2		2		3	1	2		3
	TMa3	0.21	9		1			3			2		2	3		2		2	3		3
Ejmiatsin	*TEj1*	0.19	10	2	1			1		2	3			2		3		2		2	1
	TEj2	0.18	10		2			2			2		3		1		3	3	3	2	1
	TEj3	0.63	10	3				3		2	3		2	2		3		3	2		3
Artik	*TAt1*	0.31	10		1				1		3		3	2		1	2		2	3	3
	TAt2	0.09	11	2	3			3		2	1			3	2		3	3		2	1
	TAt3	0.47	11	3	2			2	1		3		3		2		2		3	2	3
	TAt4	0.13	10		1			3	1		3		2		3		3	2		2	3
Abovyan	*TAb1*	0.63	10		2			2	3		3		3		2	1		3	3		2
	TAb2	0.16	12	3	3			3	1	2	1		2		3	2	3		3	3	
	TAb3	0.21	10	2	1				2				3		2	1	3	3		2	2
Vardenis	*TVd1*	0.17	11	3	3			2			3		1		3	2	3	1	3		2
	TVd2	0.72	8	2				3			2				2		3	2	2		3
	TVd3	0.11	11	1	1				3		2		3		2	3		2	3	2	3
Sugar beet																					
Hrazdan	*SHr1*	0.43	8		2			2			3		1		3	1		2		3	
	SHr2	0.57	8	2				2			3		1		3	2		2		3	
Artik	*SAt1*	0.51	8		2			2			2		2		2	1		3			3
	SAt2	0.49	9		2			3			3		1		3	2		2		2	2
Fodder beet																					
Sevan	*FSv1*	0.37	9	2	3						2		3		2	3		3	2	3	
	FSv2	0.36	8	2	3						3		1		3		2		3	3	
	FSv3	0.27	9	2	3						2		3		2		3	3		3	2
Shirak	*FSh1*	0.23	9		3			2					3		3	2	3		3	2	1
	FSh2	0.17	8		2			3					2			3		3	2	2	2
	FSh3	0.39	8		2			2							1		3	2	2	3	3
	FSh4	0.21	8	3				3					2		2		3	3	3		2

* 1-slightly intense, 2-intense, 3-very intense

Both sugar beet populations exhibit 2 spectra each. In the case of the Hrazdan population (*SHr*), *SHr1* and *SHr2* spectra were formed, each with the presence of 8 polypeptides. For the *SHr1* spectrum, the frequency of the spectrum meeting is 0.43. In the case of the *SHr2* spectrum, the protein formula is as follows: 1, 5, 8, 10, 12, 13, 15, 17 with a frequency of 0.57. The total number of polypeptides in the population was 16, of which 19% were scored as low intensity, 44% as intense, and 37% as very intense.

The *SAt1* and *SAt2* spectra were generated for the Artik population (*SAt*). In the case of the *SAt1* spectrum, the number of polypeptides is 8. The spectrum meeting frequency is 0.51. The total number of *SAt2* spectrum polypeptides is 9. The frequency of this spectrum meeting is 0.49. The total number of polypeptides in the population was 17, of which 12% were rated as low intensity, 59% as intense, and 29% as very intense.

Three different electrophoresis spectra were observed in the fodder beet Sevan population (*FSv*): *FSv1*, *FSv2*, and *FSv3*. The total number of *FSv1* spectrum polypeptides is 9. The frequency of the spectrum meeting is 0.37. For the *FSv2* spectrum, the total number of polypeptides is 8 with a 0.36 meeting frequency. For the *FSv3* spectrum, the total number of polypeptides is 9 with a 0.27 frequency of meeting. The number of polypeptides in the population was 26, of which 4% were rated as low intensity, 38% as intense, and 58% as very intense.

In the Shirak population (*FSh*), 4 different spectra were observed: *FSh1*, *FSh2*, *FSh3*, and *FSh4*. For the *FSh1* spectrum, the total number of polypeptides is 9 with a 0.23 frequency of meeting. For the other three spectra, the total number of polypeptides is 8 in each. In the *FSh2* spectrum, the frequency of meetings is 17%. The frequency of *FSh3* spectrum meeting is 39%. For the FSh4spectrum, the protein formula is 1, 5, 10, 12, 14, 15, 16, and 18 with a frequency of 21%. The total number of polypeptides in the population was 33, of which 6% were rated as low intensity, 45% as intense, and 49% as very intense.

Most economically valuable traits are controlled by many genes and their variability depends on the allelic state of a small number of genes. According to the literature data, large amounts of a polypeptide with a molecular weight of 39 kDa (P-39) were found in beetroots during the period of active growth in size by researchers (Battey *et al*., 1994; Parpinello *et al*., 2004).

Other authors have found that enzymes of carbohydrate metabolism are associated with the growth potential of roots in beet plants (Jammer *et al*., 2020). In this regard, it becomes relevant to study the relationship between protein formulas and economically useful traits.

Table 3: The level of expression of some bio-economic characteristics of plants with different protein spectrums of beet studied species/varieties

Wild species or a population varieties	The type of spectrum	The average weight of the root, kg M±m	The sugar content, %	The presence of powdery mildew	The presence of *Cercospora* leaf spot
Wild beet					
B. corolliflora	*Bc1*	10.2±1.59	-	-	-
	Bc2	9.8±1.12	-	-	-
B. macrorhiza	*Bm*	8.5±0.96	-	-	-
B. lomatogona	*Bl*	3.2±0.55	-	-	-
Table beet					
Aparan	*TAp1*	0.41±0.09	13,5	-	-
	TAp2	0.29±0.11	9,8	+	+
	TAp3	0.32±0.07	9,7	-	-
Aramus	*TAr1*	0.22±0.07	6,9	+	-
	TAr2	0.25±0.04	10,3	-	-
Martuni	*TMa1*	0.38±0.15	11,4	+	-
	TMa2	0.36±0.19	8,1	-	-
	TMa3	0.31±0.08	9,6	-	-
Ejmiatsin	*TEj1*	0.19±0.04	7,6	-	-
	TEj2	0.26±0.07	10,4	-	+
	TEj3	0.21±0.07	7,2	-	+
Artik	*TAt1*	0.29±0.08	7,2	-	-
	TAt2	0.36±0.11	12,8	+	+
	TAt3	0.32±0.17	10,6	+	-
	TAt4	0.35±0.13	7,4	-	-
Abovyan	*TAb1*	0.26±0.06	8,0	-	-
	TAb2	0.28±0.02	11,6	-	+
	TAb3	0.20±0.02	7,4	-	-
Vardenis	*TVd1*	0.27±0.08	8.6	-	-
	TVd2	0.28±0.05	7,9	-	-
	TVd3	0.35±0.10	9,3	-	-
Sugar beet					
Hrazdan	*SHr1*	0.94±0.17	16.9	+	+
	SHr2	0.76±0.12	15,9	+	-
Artik	*SAt1*	0.98±0.18	17,2	-	+
	SAt2	0.86±0.16	16,6	-	-
Fodder beet					
Sevan	*FSv1*	1.25±0.96	5,2	-	-
	FSv2	0.85±0.99	7,8	-	-
	FSv3	1.50±0.74	6,5	+	+
Shirak	*FSh1*	1.10±0.48	5,9	-	-
	FSh2	1.62±0.69	8,0	+	-
	FSh3	1.30±0.47	7,3	-	-
	FSh4	1.10±0.94	7,6	-	-

«+» - is present, «-» - not present

The level of expression of several beneficial economic traits and natural resistance to diseases in plants with different 11S-globulin spectra of beet species and cultivars was also studied (Table 3).

As the results show, all wild species were resistant to powdery mildew and *Cercospora* leaf spot diseases and no signs of infection were recorded. As for table beet populations, it should be noted that the Vardenis population stands out for its resistance to both diseases. The plants of ejmiatsin and Abovyan populations were not infected with Powdery Mildew disease and the plants of Aramus and Martuni populations were not infected with *Cercospora* leaf spot. In other populations, variation was observed according to the protein spectrum. The most infectious plants of the Aparan population had the *TAp2* spectrum with the corresponding protein formula. Plants with the *TAr1* spectrum of the Aramus population were susceptible to powdery mildew disease. In the case of the Martuni population, only plants with the *TMa1*spectrum were infected with the powdery mildew and so on.

As for sugar beet, all the plants of the Artik population showed resistance to the Powdery Mildew disease, and all the plants of the Hrazdan population were infected with

that disease. Plants with *SHr2* and *SAt2* spectra were not infected with *Cercospora* leaf spot disease.

In the case of fodder beet, the plants with the *FSv*3 spectrum of the Sevan population were susceptible to both diseases and in the case of the Shirak population, only the plants with the *FSh2* spectrum were infected with the Powdery Mildew and the population was generally stable against *Cercospora* leaf spot disease.

From the point of view of the root weight, the plants with the *TAp1* spectrum of the Aparan population of table beet stand out, which also have the highest level of sugar content. In the case of fodder beet, for which the weight of the beetroot is one of the most important indicators, it was the largest in the plants of the *FSv3* spectrum of the Sevan population and the *FSh2* spectrum of the Shirak population.

Plants with the *SHr1* spectrum of the Hrazdan population and the *SAt1* spectrum of the Artik population were distinguished by sugar level indicators, which are characteristic of sugar beet populations.

All these data can serve as a marker for the selection of parental forms for breeding works.

Discussion

It is known that the degree of genetic diversity of a population can be estimated if the protein formulas and their frequency of occurrence in the population are known (Ayala, 1984).

Methods for marking breeding materials using protein markers aimed at identifying biotypes, predicting genetic compatibility, and controlling hybridization in beets, however, required active development. For beets, it is promising to use genes that determine the synthesis of individual enzymes as biochemical markers. The advantage of using isoenzymes as phenotypic markers compared to genetically determined morphological traits is that enzymes are direct products of gene activity and therefore are less affected by the external environment. The storage proteins of seeds, 11S-globulins, are the most widely used as markers in sugar beet.

The diversity of 11S-globulin as a biological feature of the protein is demonstrated by electrophoresis, where the amount of polypeptides and electrophoretic mobility determines the features and origin of genotypes (Konarev, 1983, Konarev *et al*., 2000).

It is possible that the coincidence of individual spectra in beet breeding materials is due to cross-pollination.

The absence of polymorphism in some lines in terms of the number of protein components indicates their high homozygosity and allows the use of electrophoretic spectra in studies of beet inbreeding (Lesnevich, 1997).

Since proteins really reflect the genotype of a plant, classification using them is more reliable in the process of analyzing the original breeding material (Lesnevich, 1997).

Our results are consistent with the data of (Konarev *et al*., 2000), who also notes that the structural composition of proteins can be recommended to exclude heterozygous plants at the first stages of selection and preserve the ratios of morphologically indistinguishable genotypes characteristic of the variety in populations.

Thus, the study of the parental components of hybrids made it possible to reveal the genetic structure of the lines, which is characterized by the ratio of different biotypes. The variability of the studied materials according to the electrophoretic spectra of 11S-globulin seed storage protein was revealed, which can be used as one of the criteria for choosing a further direction of selection and complement the existing methods for evaluating breeding material used in breeding.

During the studies, two diseases were recorded and observed: Powdery mildew (*Erysiphe Beta* (Vanha) Weltzein) (Weltzien, 1963) and *Cercospora* leaf spot (*Cercospora beticola* Sacc.) (Weiland and Koch, 2004), against which the evaluation was performed.

Plants of beet in the first and second year are affected. Disease appears on all above-ground parts of the plant (on leaves, and stalks of beet) as a white bloom. In the beginning, the bloom is gentle and web-like; then it quickly expands, becoming white, dense, and powdering. The affected parts of plants get a powdered kind (Park *et al*., 2012).

Leaf spot disease caused by *Cercospora beticola* Sacc. is the most damaging foliar disease threatening beet production worldwide. The wide spread of disease incurs a reduction of yield and economic losses. It reduces the quality of beet and results in a loss exceeding 30% of the yield (Tan *et al*., 2023). The symptom development of leaf spots on beet crops is a gradual progress. In the first stage, necrotic spots emerge on the primary leaves. Then the primary foliage of the plant starts to wilt and fall off. This can lead to a vegetative regrowth to maintain photosynthetic capacity (Skaracis *et al*., 2010).

Cluster analysis can be used as a method for assessing the automatic classification of the studied genetics by the sum of features, or assessing the information content of the features themselves, as a result of which the so-called “redundant” or superfluous features can be excluded (Cormack, 1971). The results of the further cluster analysis can be used for the optimal selection of parental pairs and breeding work.

Conclusion

Plants with the *TAp1* spectrum of Aparan, *TMa1* of Martuni, *TAt2* of Artik, and *TVd3* of Vardenis populations of table beet can be used as parental forms in selection works aimed at increasing the yield. In the case

of sugar beet, preference should be given to plants with the *SHr1* spectrum of Hrazdan and *SAt1* spectrum of Artik populations. For fodder beet, the plants with the *FSv3* spectrum of the Sevan and the *FSh2* spectrum of the Shirak populations should be selected.

Plants with the spectrum *SHr1* of the Hrazdan population and *SAt1* of the Artik population can be used as parental forms in selection works aimed at increasing sugar content.

All 3 wild species of beet can be used as parental forms in the selection works aimed at resistance to the Powdery Mildew (*Erysiphe betae* (Vanha) Weltzein) disease. In the case of table beet, plants with all spectra of Ejmiatsin, Abovyan, and Vardenis populations, as well as plants with *TAp1* and *TAp3* spectra of Aparan population, *TAr2* of Aramus, *TMa2* and *TMa3* of Martuni, *TAt1* and *TAt4* spectra of Artik populations. Sugar beet can be used Artik population, for fodder beet plants with *FSv1* and *FSv2* spectra of the Sevan population and *FSh1*, *FSh3*, and *FSh4* spectra of the Shirak population.

All 3 wild species of beet can be used as parental forms in the selection works aimed at resistance to the *Cercospora* leaf spot (*Cercospora beticola* Sacc.) disease. Also can be used for plants with all spectrums of Aramus, Martuni, and Vardenis populations of table beet, as well as plants with *TAp1* and *TAp3* spectra of the Aparan population, *TEj1* of Ejmiatsin, *TAt1*, *TAt3* and *TAt4* of Artik, *TAb1* and *TAb3* of Abovyan populations. For sugar beet, choose plants with the *SHr2* spectrum of the Hrazdan population and with the *SAt2* spectrum of the Artik population, for fodder beet plants with all spectra of the Shirak population and plants with *FSv1* and *FSv2* spectra of the Artik population.

Further cluster analysis will make it possible to select parental pairs and start breeding work in the target direction. Future beet breeding efforts must make use of the genetic and genomic resources available for efficient improvement.

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Author's Contributions

All authors equally contributed in this study.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues are involved. There is no conflict of interest.

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