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Evaluation of Morpho-Biological and Phylogenetic Properties of Several Local Populations of Regionalized Beetroot Varieties in Armenia

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ABSTRACT

Identifying the variability of plant genetic resources and selecting valuable genotypes is one of the most important problems in plant growing and plant breeding. The research results show that per their morphological and phylogenetic indices the beetroot populations are combined in 2 varieties: Egyptian flat and Bordeaux 237, those of sugar beet – in 1 variety: Belotserkovskaya single-seeded, and fodder beet – in the variety of Yellow Eckendorf. Each of the studied populations has certain advantages per its economic and ecological characteristics, which can be further used in beet breeding.

Introduction

In the contemporary period of advanced agriculture proper evaluation of the crops genetic resources and their further use in breeding activities along with the development and implementation of new intensive technologies is of utmost significance. Identifying the variability of plant genetic resources and selecting valuable genotypes is one of the most important problems in plant cultivation and breeding (Ahmar, et al., 2020). It has been repeatedly mentioned, that genotype is formed throughout the historical development when being in a relatively stable state, which determines the varietal characteristics.

The beetroot under study belongs to *Chenopodiaceae* family. Four beetroot varieties are known in beet breeding: common beetroot /*Beta vulgaris L. var. esculenta*/, fodder beet /*Beta vulgaris L. var. crassa*/, sugar beet /*Beta vulgaris L. var. saccharifera*/ and leaf beet (mangold) /*Beta vulgaris L. var. vulgaris* and *flavescens*/ (Melikyan, 2005, Goldman and Janick, 2021).

Beetroot is a typical cross-pollinated plant, it can interbreed freely in nature and produce new forms (Goudarzi, et al., 2019). It has been grown in Armenia since very ancient times, while the regionalized varieties have a history of about 100 years. In the absence of a proper seed breeding system, new populations arise naturally, which are considerably different from the original varieties in terms of their morphological indicators. Since beet seed breeding has not been practiced in Armenia in recent decades, we have not chosen specific varieties as the research object, but different populations of varieties common in Armenia, which obviously have external differences. Local populations of beet varieties in Armenia are very diverse. Their common characteristics is the similarity of the old forms, which is manifested through the storage root/root crop with roundish form (Melikyan, 2001).

Populations having been cultivated for years in different ecological environments have been selected as research objects (beetroot – Aparan, Aramus, Martuni, Edjmiatsin, Artik, Abovyan and Vardenis, sugar beet – Hrazdan and Artik, fodder beet – Sevan and Shirak).

The current work aims to morphologically characterize the diverse population of the cultivated beetroot varieties, to select valuable populations which can serve as high-value source materials for breeding activities on the way of producing high-performance varieties.

To achieve the goal the following objectives have been set up:

1. Grouping and identifying populations formed from different beetroot varieties per transition periods of phenophases
2. Determining the variability of the main economically valuable characteristics of these populations in different vegetation years
3. Selecting perspective, valuable populations in reference to breeding activities according to bio-economic properties
4. Indicating the phylogenetic relationships between the studied populations according to core and extracore (plastid) genes
5. Recommending the selected best populations as a selective source material.

Materials and methods

For investigations the populations have been selected per their geographic distribution and root crop appearance. Table beet seeds with extremely different root crops were taken from 7 different regions of the RA and sown in conditions of the Voskehat teaching-experimental farm functioning under Armenian National Agrarian University in order to evaluate the plant's morphological and biological characteristics. From sugar and fodder beets two populations each was selected. The experiments were set up throughout 2019-2020 with 11 options per 3 replications. The size of each estimated experimental bed made 25 m^2. The seeding of the table beet population was implemented with 45 cm interrow and 15 cm intercrop spacing, that of sugar beet population with 45 cm and 25 cm and fodder beet with 50 cm and 30 cm spacing, respectively. The root crops of all population types were planted with the 70x70 cm planting pattern.

Phenological observations were conducted in line with the generally accepted method, every 2-3 days. The collected root crops were planted in the same region as rootstocks in the following year with the same experimental conditions. During the field experiments the plant treatment activities were implemented in accordance with the common agricultural rules.

The leaf area was calculated via scanning and analysis through ImageJ computer software (Schneider, et al., 2012). Yield computation was conducted through weighing the yield of each experimental bed. The mathematical processing of numerical data on yield growth, development and yield calculation was carried out through the method of dispersion analysis developed by Dospekhov.

To identify the populations' phylogenetic relationships, genetic investigations have been carried out in the Holt climate laboratory at the Arctic University of Norway (Tromso). DNA isolation was performed using the E.Z.N.A.® HP Plant DNA kit (Omega Bio-tek, USA). The concentration of isolated DNA was determined with a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). Purity was determined on a 1% agarose gel. For nucleotide sequence determination 2 genes widely used in beet phylogenetic studies (Touzet, et al., 2018) were selected: the chloroplast LF gene with 5′-GGTTCAAGTCCCTCTATCCC′/5′-ATTTGAACTGGTGACACGAG-3′(forward/reverse) primers and core adh gene with 5′-TGTCCTGCCTGTTTTCACTG-3′/5′-TACTGCTCCTAGGCCGAAAA-3′ primers. Raw nucleotide sequence data were read, verified, and aligned using the Clustal Omega multiple sequence alignment program (McWilliam, et al., 2013). Aligned sequences were used to construct phylogenetic trees using the maximum likelihood (ML) algorithm implemented via the Mega X program (Kumar, et al., 2018).

Results and discussions

In general, table beet varieties are classified into 5 groups: Egyptian Flat, Egyptian Round, Bordeaux, Eclipse and Erfrut varieties. The beet varieties with flat root crops are usually early-ripening with average shelf life, reddish-purple coloration of fruit pulp and with somewhat pronounced whitish rings. Roundish and flat-roundish root crops ripen later and have longer storage life. Beet varieties with conical root crops are late-ripening with long shelf life but they contain large amount of cellulose and have fibrous, less juicy fruit pulp (Snapyan, 2001).

For many years a number of beet varieties have been regionalized in the beet breeding farms of Armenia: table beet - Bordeaux 237, Egyptian flat, Egyptian round, sugar beet - Belotserkovskaya single-seeded, Yaltushkovskaya single-seeded, fodder beet – Yellow Eckendorf, Betta Rosa varieties. Anyhow, beet seed breeding hasn't been practiced in our country for already more than 30 years. Considering the fact that beet is a typically cross-pollinated plant, it becomes obvious that decades of cultivation without observing to the rules of seed production can lead to free cross-breeding and the emergence of new forms (Andrello, et al., 2016). Therefore, we have singled out different varietal populations common in Armenia, the appearances of which were prominently different.

To assess the bio-economic values of the variety it is important to study its vegetation duration and the transition period of individual phases. The results of the phenological observations for the first vegetation year of the studied beet populations are introduced in Table 1.

Table 1. The transition periods of phenophases in the studied populations of the regionalized beetroot varieties (the I vegetation year)*

Populations	Seeding	Germination	Leaf rosette formation				Root crop maturation	Vegetation duration, day
			1 true leaf	6 true leaves	12 true leaves	18-20 true leaves		
Table beet								
Aparan	05.04	14.04	02.05	06.06	18.06	08.07	10.08	118
Aramus	05.04	12.04	28.04	26.05	13.06	28.06	02.08	112
Martuni	05.04	15.04	01.05	08.06	19.06	08.07	10.08	117
Ejmiatsin	05.04	13.04	26.04	27.05	11.06	30.06	02.08	111
Artik	05.04	14.04	01.05	08.06	17.06	10.07	10.08	118
Abovyan	05.04	12.04	28.04	27.05	14.06	01.07	02.08	112
Vardenis	05.04	13.04	30.04	03.06	15.06	04.07	10.08	119
Sugar beet								
Hrazdan	05.04	14.04	29.04	01.06	15.06	07.07	20.09	159
Artik	05.04	15.04	02.05	04.06	17.06	09.07	20.09	158
Fodder beet								
Sevan	05.04	14.04	29.04	01.06	15.06	02.07	25.09	164
Shirak	05.04	15.04	02.05	04.06	17.06	09.07	25.09	163

*Composed by the author.

During the first year of life the beet plant usually forms a rosette consisting of 60-90 leaves, anyhow, throughout the whole vegetation period the death of leaves and emergence of the new ones are alternating each other, and hence, the leaf rosette can be loaded with up to 18-20 leaves at a time. The table data show, that the seed germination of the investigated populations was registered mainly in rather close terms with maximum difference of 3 days. Emergence of first true leaves in the leaf rosette was observed within the period of April 26 – to May 2. The table beet population grown in Ejmiatsin was also distinguished by the early formation of the first true leaf. The whole process of leaf rosette formation lasted 61-70 days. Maturation of root crop was recorded about a month after the complete formation of leaf rosette. The 7 studied populations of table beet ripened in 2 periods with an interval of 8 days. Populations of Aramus, Ejmiatsin and Abovyan stood out for their precocity, the vegetation duration of which was 111-112 days. The populations of Aparan, Martuni, Artik and Vardenis were distinguished by 117-119 days of vegetation duration.

The maturation of sugar and fodder beets was delayed by about 30-35 days. There wasn't any significant difference in the duration of vegetation periods among the population varieties.

As we know, quantitative changes occur during individual development periods, which are expressed in the form of growth. This is an irreversible increase in the mass and volume of the plant. During the plants growth and development some qualitative changes take place at a certain age period, which are manifested through the emergence of new organs and well pronounced properties. In the first vegetation year the biometric indices and yield capacity of the studied populations were also determined (Tables 2, 3).

Among the populations of table beet varieties, mainly 2 types of leaves (lamina) were found: oblong and oblong-cordate. Populations with oblong leaves mainly had a smooth surface, while those with oblong-cordate leaves – slightly rough surface. In the populations of sugar beet the leaf lamina was cordate with twisted surface and in fodder beet it was oblong oppositely ovate and slightly wavy.

It is noteworthy that regarding the vegetation period and leaf lamina form the populations of table beet are grouped in 2 categories. The leaf stalks of table beet populations had averagely 15-17 cm length, leaf blades were with 17-19 cm length and 12-14 cm width. The mentioned indices were a bit higher in sugar and fodder beets. There weren't any significant differences between the populations.

Table 2. Morphological indices of the leaves in the studied populations of the regionalized beetroot varieties*

Population	Leaf lamina/blade shape/surface	Leaf stalk length, cm	Leaf lamina length/ width, cm	Leaf lamina coloring		Total leaf surface, cm^2		
				At the start of rosette formation	At the harvest stage	At the stage of 6 leaves	At the stage of 12 leaves	At the stage of 18-20 leaves
Table beet								
Aparan	oblong/ slightly wavy	16	19/12	dark green	green-purple	1270	2395	3918
Aramus	oblong-cordate/ smooth	17	17/14	dark green	green-reddish	1152	2325	3511
Martuni	oblong/slightly wavy	15	18/12	dark green	green-purple	1170	2160	3420
Ejmiatsin	oblong-cordate/ smooth	16	17/13	dark green	green-reddish	1220	2439	3205
Artik	oblong/ slightly wavy	15	18/13	dark green	green-purple	1210	2325	3842
Abovyan	oblong-cordate/ smooth	15	17/14	dark green	green-reddish	1384	2489	3624
Vardenis	oblong/ slightly wavy	16	18/13	dark green	green-purple	1290	2380	3900
Sugar beet								
Hrazdan	cordate/ curled	18	18/15	green	green-yellowish	1298	2580	3775
Artik	cordate/ curled	17	19/15	green	green-yellowish	1300	2610	3924
Fodder beet								
Sevan	oblong, oppositely ovate / slightly wavy	23	20/14	dark green	dark green	1530	3075	4890
Shirak	oblong, oppositely ovate /slightly wavy	24	21/15	dark green	dark green	1740	3485	5220

Table 3. The biometric indices and yield capacity of the first year plants in the studied populations of the regionalized beetroot varieties*

Populations	Actual plant number per 1 ha, n	Average weight, g (at harvesting stage)			Root crop			Root crop yield, c/ha
		Total plant	including		diameter, cm	height, cm	index	
			Tops	Root crop				
Table beet								
Aparan	133330 (45x15 cm)	587	247	340	12.0	9.4	0.8	453
Aramus		454	219	235	13.2	7.6	0.6	313
Martuni		580	230	350	10.6	10.3	0.9	467
Ejmiatsin		445	225	220	12.8	5.6	0.4	293
Artik		584	254	330	11.1	10.8	0.9	440
Abovyan		461	216	245	13.5	6.3	0.5	393
Vardenis		585	285	300	11.5	9.0	0.8	400
Sugar beet								
Hrazdan	80000 (45x25 cm)	1203	353	850	13,0	17.0	1.8	680
Artik		1265	345	920	12.5	18.5	1.9	736
Fodder beet								
Sevan	60000 (50x30 cm)	1525	325	1200	16,0	32.5	2.3	720
Shirak		1612	332	1280	17.0	31.2	2.4	768

*Composed by the author.

The main differences in the coloring of leaf lamina were observed at the harvesting stage. The total leaf surface was calculated at the 3rd stage of vegetation. The table beet of Aparan, Artik and Vardenis populations were distinguished by a large leaf surface at the most intense foliation stage. The difference of leaf surfaces among the sugar beet populations is about 150 cm^2 and in fodder beet – about 330 cm^2.

The seeding of table beet populations was carried out with 45x15 cm planting pattern, that of sugar beet with 45x25 cm, and fodder beet with 50x30 cm planting patterns. The actual number of plants per 1 ha land area was calculated taking into account the actual number of plants in the experimental bed (Table 3).

The plant weighing was conducted at the harvesting stage. The largest root crops (350 g) were developed in the Martuni population. Aparan, Artik and Vardenis populations stay behind by 10-50 g. The populations of Aramus, Ejmiatsin and Abovyan developed much smaller root crops (220-245 g). The same regularity per populations was observed regarding the top mass. The sugar and fodder beets obviously develop larger root crops. The mass differences between the studied populations made 70-80 g, which is not considered as a significant difference. The characteristic index of the root crop appearance is the root crop index, that is the ratio of height and diameter. The closer the mentioned index to 1 is, the more roundish the root crop is, while the smaller from 1, the flatter its appearance is. The root crops with the index higher than 1 are conical. The studied population of the table beet were combined into 2 groups: those with roundish (0.8-0.9) and flat (0.4-0.6) root crops.

In the sugar and fodder beets conical root crops were developed, besides, in the fodder beet the mentioned property was more pronounced. Considering the actual plant number and the mass of a plant root crop the biological yield of the root crop was calculated. The table beet of Martuni and Aparan populations stood out by their highest yield capacity. The lowest yield capacity was recorded in the Ejmiatsin population, which is probably related not only to the varietal characteristics, but also to the circumstance that Ejmiatsin is not considered as a favorable climatic zone for beet breeding, which can cause to low yield production. The yield capacity difference between the sugar beet populations makes 56 c, and the maximum yield was registered in the Artik population. The fodder beet populations have provided rather close-to-each-other indices.

After harvesting, the root crops were stored in order to continue the studies in the second year of vegetation. In the second year the planting of all options were implemented with 70x70 cm planting pattern. Here again phenological observations were carried out and the number of days required for seed formation and maturation was registered (Table 4). After root crop planting the leaf rosette per populations was developed in the same period with 1-2 days of differences and lasted about 17-19 days. Flower stems started to develop about 35-40 days after the rosette formation, whereas the mass development was recorded about a month thereafter.

Parallel to the flower stems formation, blossoming process starts, which in the table beet lasted about a month, while in the sugar and fodder beets this period was shorter – about 20 days. The first tops started to ripen 30-40 days after the start of blossoming, whereas the populations of sugar and fodder beet started to ripen much earlier. The mass harvesting of the table beet tops was implemented 130-138 days after planting, that of sugar beet – after 151 days, and the fodder beet – after 152-153 days. It is noteworthy that the populations of the table beet are combined into 2 groups for the second year vegetation indices as it was in the case of the first year results. In the second vegetation year plant measurements and weighing was also implemented (Table 5). In the populations of the table beet 4-5 flower-bearing stems were mainly developed, in the sugar and fodder beets this number was 5. Deviations in the branching number per the species and varieties haven't been recorded; all stems averagely developed 3 branches.

The height of flower-bearing stems fluctuated within the range of 96-121 cm in the table beet populations. The average height of stems in the sugar and fodder beets fluctuated within 114-117 and 125-127 cm, respectively. Here the difference per populations are not significant. The number of developed tops on 1 flower-bearing stem and the weight of tops developed in one plant has been also calculated. As to the numerical calculations, the highest number of tops has been recorded in Aramus population (405 n), but on the whole, the discrepancies in numbers were not significant. In the sugar and fodder beets 444-451 and 448-449 tops have been developed, respectively. Regarding the tops size of table beet populations (per the weight of 1000 tops) those of Vardenis, Aramus and Abovyan were distinguished, anyhow the other populations don't so much stay behind the mentioned ones. There weren't any significant differences between the sugar and fodder beets.

Along with the identification of morphological indices, the nature of inheritence of a number of bio-economic properties is also very important. To this end it is necessary to determine the degree of similarities for the above mentioned 2 groups regarding their genetic systems.

Table 4. The transition periods of phenophases in the studied populations of the regionalized beetroot varieties (II year of vegetation)*

Populations	Planting period	Leaf rosette formation	Emergence of 18-20 leaves in the leaf rosette	Flower stems development	Emergence of the secondary and tertiary flower stems	Mass inflorescence emergence	Blossoming		Maturation of beet tops		Vegetation duration, day
							Start	End	The first ones	Mass	
Table beet											
Aparan	27.03	15.04	20.05	27.05	12.06	28.06	12.06	12.07	23.07	10.08	136
Aramus	27.03	13.04	08.05	18.05	06.06	21.06	11.06	13.07	25.07	04.08	130
Martuni	27.03	15.04	19.05	29.05	14.06	28.06	12.06	13.07	25.07	12.08	138
Ejmiatsin	27.03	13.04	07.05	18.05	06.06	20.06	11.06	11.07	25.07	02.08	128
Artik	27.03	15.04	19.05	29.05	13.06	29.06	13.06	13.07	25.07	12.08	138
Abovyan	27.03	14.04	08.05	19.05	06.06	21.06	11.06	14.07	25.07	04.08	130
Vardenis	27.03	14.04	18.05	27.05	12.06	27.06	13.06	14.07	24.07	10.08	136
Sugar beet											
Hrazdan	27.03	14.04	06.05	18.05	04.06	22.06	03.07	22.07	02.08	25.08	151
Artik	27.03	13.04	07.05	18.05	06.06	20.06	01.07	21.07	02.08	25.08	151
Fodder beet											
Sevan	27.03	13.04	07.05	18.05	06.06	20.06	01.07	21.07	02.08	27.08	153
Shirak	27.03	13.04	07.05	16.05	06.06	19.06	01.07	21.07	01.08	26.08	152

Table 5. The biometric indices of the second year plants in the studied populations of the regionalized beetroot varieties*

Populations	Number of plants per 1 ha, n	Number of a plant's flower-bearing stems, n	Average branching number of a flower-bearing stem, n	Average height of flower-bearing stems, cm	Average top number of 1 flower-bearing stem, n	Weight of 1 plant tops, g	Weight of 1000 tops, g
Table beet							
Aparan	20408 (70x70 cm)	4	3	105	387	42	27
Aramus		4	3	98	405	47	29
Martuni		5	3	121	394	53	27
Ejmiatsin		4	3	100	388	40	26
Artik		4	3	108	391	42	27
Abovyan		5	3	96	393	57	29
Vardenis		4	3	112	385	46	30
Sugar beet							
Hrazdan	20408 (70x70 cm)	5	3	117	444	75	34
Artik		5	3	114	451	79	35
Fodder beet							
Sevan	20408 (70x70 cm)	5	3	125	449	79	35
Shirak		5	3	127	448	83	37

*Composed by the author.

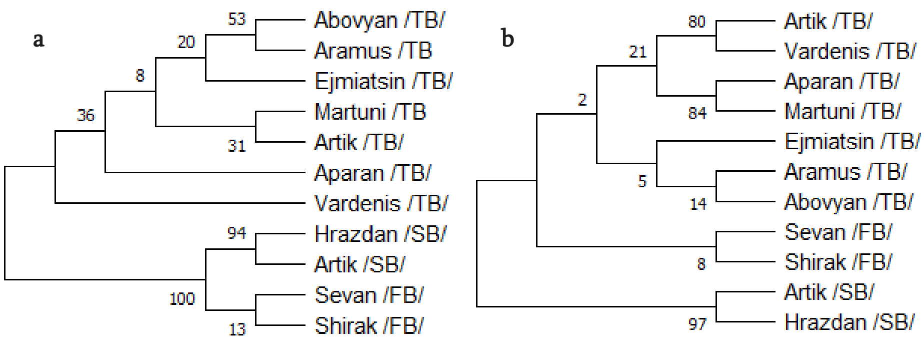

Diagram. Phylogenetic relationships among studied populations of table (TB), sugar (SB) and fodder (FB) beet cultivars according to core adh (a) and extra-core LF (b) genes.

The studies conducted on different varieties in the field of plant genetics prove that besides the hereditary characteristics, the quality of the obtained offspring is also related to the kinship ties between their parents (Zhang, et al., 2017, Wascher, et al., 2022). For this reason, a study on the phylogenetic relationships of the studied populations was also performed. Since in addition to the core genes there are also extra core genes in the plant cell, which can determine the hereditary nature of this or that trait, we have selected 1 core (adh) and 1 extra core, particularly chloroplast genes (LF) (Diagram).

The data of diagram make it obvious that the sugar and fodder beets are distinguished from the table beet both by core and extra core genes and that at the same time there are similarities between the populations. In case of table beet, formation of 2 groups is observed for both genes. The phylogenetic depiction once again confirms the results of the morphological studies, stating that 4 table beet populations belong to the Bordeaux 237 variety and 3 - to the Egyptian flat, and 2 sugar and fodder beet populations belong to 1 variety each, Belotserkovskaya single-seeded and Eckendorf Yellow, respectively.

Conclusion

Based on the morpho-biological and phylogenetic features of populations the following conclusions can be drawn:

1. Aramus, Abovyan and Ejmiatsin populations of table beet were developed from Egyptian flat, whereas Aparan, Martuni, Vardenis and Artik populations - from Bordeaux 237 varieties. The Hrazdan and Artik sugar beet populations were developed from the Belotserkovskaya single-seeded variety, and the Sevan and Shirak fodder beet populations were formed from the Eckendorf Yellow variety.

2. Each of the investigated populations has a certain advantage in terms of their economic and ecological properties: the Ejmiatsin population of the table beet is the most early-ripening variety, while the Martuni population is the most productive one. The populations of sugar and fodder beet varieties haven't demonstrated significant differences regarding their ecological and economic properties.

Based on the above stated conclusions and kinship ties disclosed between the populations it is recommended to use the afore mentioned populations which will provide high cross-breeding efficiency in order to produce early-ripening and high-yielding varieties in selection.

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