REPUBLIC OF AZERBAIJAN

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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

RESEARCH ON GENETIC DIVERSITY AND COMBINATIVE VARIABILITY IN LOCAL AND INTRODUCED TOMATO VARIETIES OF AZERBAIJAN

Speciality: 2409.01 – Genetics

Field of science: Biology

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INTRODUCTION

Research relevance and degree of completion. Meeting the demand for quality and environmentally friendly food products is one of the most important issues in the country[1]. Vegetable crops play an important role in meeting people's food needs. Among vegetable crops, tomato (*Solanum lysopersicum* L.) ranks first in terms of production and consumption. From this point of view, proper and sustainable organization of the collection, study, protection, and efficient use of tomato genetic resources are extremely important. The technique of hybridizing suitable combinators (parental forms selected as donors) can be used to reveal the reserve potential of tomato, to replace low-yielding varieties with high-yielding varieties, and to improve the important quantitative and qualitative characteristics further.

In recent times, the application of biotechnology and genetic engineering achievements to the selection, the increase and the wide spread of heterosis hybrid production cause risk of the rapid loss of the genetic resources of traditional folk selection varieties of tomato. One of the important conditions is the genetic recombination of traits in new hybrid generations obtained by crossing parental forms that differ in certain characteristics, monitoring the patterns of variation occurring in them, and, as a result, obtaining recombinants containing valuable biomorphological indicators[1,2]. Therefore, the creation of "allele" collections, comprehensive research and selection of genotypes with high indicators, determination of suitable parental forms and their favorable combinations for obtaining hybrids with new qualities based on them, detection of heterosis effects due to various traits in hybrids are of particular importance[3].

Object and Subject of the Research. The object of the research consists of traditional, scientifically bred, and introduced tomato

[1] https://www.fao.org/faostat

[2] Ayenan M.A.T. (2022). Combining abilities and heterotic patterns for heat tolerant traits in tomatoes (*Solanum lycopersicum* L). Plant Breeding №. 4. 585-597.

[3] Basavaraj LB. V. D. (2016). Combining ability analysis for fruit yield and quality traits in tomato (*Solanum lycopersicum* L.). Green Farming. 7 (1): 26-30.

varieties and forms cultivated in Azerbaijan, while the subject focuses on the study of genetic diversity and combinative variability within these varieties.

Purpose and tasks of the research. The main objective of the research was to study the genetic diversity and combinative variability of local and introduced tomato samples cultivated in Azerbaijan.

To achieve this objective, the following tasks were set:

- Conduct a comprehensive evaluation of the biomorphological-quantitative and qualitative traits of local and introduced tomato samples cultivated in the Absheron region, based on international descriptors, and assess promising samples using multidimensional statistical programs.
- Select parent forms of tomato samples adaptive to Absheron conditions using biostatistical methods and perform topcross hybridization based on the "Line x Tester" scheme.
- Investigate combinative variability by assessing general combining ability (GCA) in the combinators and specific combining ability (SCA) in the hybrids of tomato samples, based on biomorphological indicators, and determine genetic similarity and trait correlations between genotypes.
- Evaluate the heterosis effect (HE) in hybrids derived from tomato combinations and select stable hybrid lines of agricultural significance.
- Determine the effectiveness of RAPD and ISSR microsatellite primers used in the study, assess the level of polymorphism in the genomes of tomato samples (combinators and hybrids), and determine genetic similarities and genetic distances between genotypes.

The main provisions presented to the defense of the dissertation:

1. Adaptability was high in the Masalli variety form (v/f), Leyla, Zafar, Sheker, Shahin, and Ilkin varieties among the presented 31 varieties and variety forms and among introduced varieties, adaptivity was high in Volgograd-5/95.
2. According to the Euclidean distance index of similarity on

20 biomorphological-quantitative and qualitative indicators, the closest genotypes of the tomato plant are Leyla, Shahin, Masalli (v/f), introduced varieties Garant and Tamara, and the most distant genotypes are Leyla and Utro.

3. High GCA was detected in F1 hybrids obtained from Masalli v/f × Utro and Tamara × Utro combinations, while high SCA was observed in F1 hybrids obtained from Leyla × Utro, Tamara × Ilkin və Tamara × Volgograd-5/95 combinations.

4. The lowest degree of gene effects of the combining ability was observed in combinations with Utro (5%), Garant (26%) and Tamara (30%) varieties, and the highest degree in combinations with Masalli v/f (75%), Volgograd-5/95 (65%), and Zafar (63%) varieties.

5. In hybrids obtained from local and foreign varieties, the heterosis effect and specific combining ability were 25% higher than in others.

6. RAPD analysis was considered effective in detecting differences between tomato genotypes.

7. According to the assessment based on ISSR primers, Tamara × Utro and Zafar × Ilkin genotypes are the most distant hybrids.

–**Scientific novelty of the research.** This study represents the first research in Azerbaijan investigating the combinative variability and genetic diversity of local and introduced tomato samples. Conducted in the Absheron region, it applies a combination of biomorphological, molecular, and biostatistical methods to valuable local and introduced parent forms and their resulting hybrids, highlighting the study's scientific novelty.

– For the first time, genetic diversity in the hybrids obtained from local and introduced tomato samples' combinators and combinations has been studied comparatively, using both biomorphological-quantitative and qualitative traits, and RAPD and ISSR microsatellite markers. The genetic structure of these samples was analyzed using multidimensional statistical methods.

– It was identified for the first time that the Masalli (s/f), Leyla, Zafer, Sheker, Shahin, Ilkin, and Volgograd-5/95 varieties, cultivated in the Absheron region, show greater adaptability to environmental factors. The primary adaptive morphogenetic traits to environmental

factors include main stem height, the number of clusters per plant, the number of flowers per cluster, the number of fruits per cluster, and juice yield.

– For the first time, it was found that the Masalli (s/f) × Utro and Tamara × Utro combinations exhibit high allele interactions, and Leyla × Utro, Tamara × Ilkin, and Tamara × Volgograd-5/95 samples show high non-allele gene interactions.

– The degree of combinative variability in hybrids was calculated for the first time based on combining ability values, with the highest values found in the maternal forms Masalli (s/f), Zafer, Sheker, and Shahin, and in the paternal form Volgograd-5/95.

– Shahin × Ilkin, Leyla × Utro, and Masalli (s/f) × Utro combinations were identified for the first time as productive hybrids with a heterosis effect, with high SCA values.

– For the first time, high allele density was observed in the hybrids Leyla × Utro, Tamara × Ilkin, and Tamara × Volgograd-5/95, indicating a high proportion of genes controlling cytoplasmic inheritance.

– Stable lines were selected from the hybrids obtained from Leyla × Ilkin, Leyla × Utro, Shahin × Ilkin, Masalli (s/f) × Utro, Sheker × Volgograd-5/95, and Zafer × Volgograd-5/95 tomato combinations.

– The soluble solids content in the combinators ranged from 4.17% to 7.27% and from 4.93% to 9.9% in hybrids. The highest value of this trait was recorded in the maternal form Zafer at 7.27% and in the hybrid Shahin × Utro at 9.9%.

– For the first time, the total number of synthesized bands across 10 ISSR primers was 94, with 65 of these being polymorphic. The highest number of polymorphic DNA fragments was detected with the ISSR-855, ISSR-811, and ISSR-840 primers, with the respective band counts of 13 and 11. Thus, ISSR-2 was determined to be most effective in terms of polymorphism and genetic diversity (GD), while ISSR-855 and ISSR-818 were most effective in terms of the number of polymorphic bands.

– Based on ISSR markers, Tamara and Volgograd-5/95 among combinators and Zafer × Ilkin and Tamara × Utro among hybrids were

identified as the most genetically distant genotypes in the dendrogram reflecting GD.

The scientific and practical significance of the work. Determining the yield components among biomorphological-quantitative traits in hybrids obtained from the combinators and combinations of the studied tomato samples through multidimensional statistical methods is significant in the field of breeding. This approach allows for the forecasting and implementation of measures that will lead to increased yields. Identifying genetic diversity (GD) among the samples both in terms of biomorphological-quantitative traits and directly at the nuclear genome level, along with verifying GD among hybrids from the tomato sample combinators and combinations, serves to guide future breeding processes.

In this study, the optimization of research using molecular markers facilitates the development of durable tomato varieties. The ultimate aim of future studies in this direction is to enable the identification and mapping of valuable genes from important tomato samples, as well as the detection of allele and non-allele gene effects on quantitative and qualitative traits. Thus, uncovering the significant dependence between specific combining ability (SCA) and heterosis effect (HE) increases the potential for rapidly developing hybrids with heterosis effects. The assessment of GD in hybrids obtained from combinators and combinations through local × introduced schemes may allow for the use of these genotypes as donors or genetic sources in the selection of significant genotypes.

The results of this research can be used in higher educational institutions and for conducting practical work.

Approbation and application. The results of the dissertation were presented and discussed at the Republican Scientific Conference dedicated to the 80th anniversary of the Faculty of Biology of BSU on "Development prospects of experimental biology" (Baku, 2014), the XI International symposium on "New and unconventional plants and prospects of their use" (Russia, 2015), the VII International Scientific Conference of Young Scientists and Researchers on "Innovation Problems of Modern Biology" dedicated to the 94th

anniversary of the birth of the Great Leader Heydar Aliyev (Baku, 2016), the 12th International symposium on "New and non-traditional plants and prospects of their use" (Kazakhstan, 2016), the VII International Scientific Conference of Young Scientists and Researchers on "Innovation Problems of Modern Biology" dedicated to the 94th anniversary of the birth of the Great Leader Heydar Aliyev (Baku, 2017), International scientific conference of young researchers (Baku, 2017), the II International scientific conference of young researchers (Baku, 2018), the 7th Republican Scientific Conference dedicated to the 95th anniversary of the birth of the National Leader Heydar Aliyev on the topic "Actual problems of ecology and soil sciences in the 21st century" (Baku, 2018), the International Scientific Conference on "Actual problems of contemporary natural and economic sciences" (Ganja, 2018), the VI International Scientific-Practical Conference (Ukraine, 2020), the International Scientific Conference on “Theoretical and practical aspects of losses in the vegetable growing industry under modern conditions (Ukraine, 2021), the XII International forum "Garden Days in Biryulyovo: Contribution of Fundamental Science to Sustainable Development of Agriculture. Formation of Health and Quality of Life of the Population of the Russian Federation" (Moscow, 2022), the Republican scientific conference organized by the Faculty of Biology of BSU on "Achievements And Challenges In Biology” devoted to the 120th anniversary of professor Mirali Akhundov (Baku, 2022), the International Scientific Conference on "Innovative Studies of Contemporary Sciences" (Tokyo, 2024), as well as at the general meetings of the departments (Baku-2023) and the Scientific Seminar (Baku-2024) of the Institute of Genetic Resources of MSE AR.

26 scientific works reflecting the main provisions of the dissertation have been published, 12 of which are articles (6 of them were published in periodical scientific publications included in the international summarizing and indexing database).

The structure and volume of the dissertation. The dissertation is written in Azerbaijani and consists of a total of 179 pages, including an introduction, six chapters, a conclusion, results and recommendations, abbreviations, a list of references, and

appendices. The research references a total of 145 sources. The total volume of the dissertation is 210,441 characters (including the introduction - 19767; Chapter I - 50623; Chapter II - 18,955; Chapter III - 36,662; Chapter IV - 28,221; Chapter V - 16,517; conclusion - 9,338; results - 2,091; recommendations - 941. The dissertation includes 26 figures and 28 tables.

MAIN CONTENT OF THE WORK
CHAPTER I LITERATURE REVIEW

In the literature review, scientific works related to the dissertation topic are analyzed in a logical sequence, examining studies on the genetic diversity and combinative variability of the cultivated tomato species *Solanum lycopersicum* L., which is the focus of the research.

CHAPTER II
MATERIALS AND METHODS OF THE RESEARCH

2.1. Soil and climate conditions of the Absheron peninsula. The Absheron Research Base is located on the Absheron peninsula, at an altitude of 80 m above sea level, and is characterized by a dry subtropical climate, hot summer, hot dry autumn, and a calm and short winter. The total solar radiation varies between 120 and 135 kcal/cm during the year. Soils in Absheron are mostly gray-brown, brown, saline, or semi-desert type.

The soils of the Absheron peninsula are young, have different levels of salinization, carbonation, and mechanical composition[4]. Morphological-genetic characteristics, physicochemical properties of gray-brown soils prevailing in Absheron have been determined, systematics and nomenclature have been improved.

2.2. Materials used in the research. Research was conducted in

[4] Salayev, M.E. Azərbaycan torpaqlarının diaqnostikası və təsnifatı / M.E.Salayev, H.Ə.Əliyev. Moskva: -1991. 240 s.

2014-2019 on 31 local and introduced tomato varieties, variety forms, and 21 hybrid plants obtained from them.

Samples obtained from the National Genbank of the Institute of Genetic Resources of the MSE AR, the collection of the "Vegetables and Melon Plants" department of the same institute, as well as the samples obtained from the Scientific Research Institute of Horticulture of the MSE AR, the Lankaran Zonal Experimental Station and the Belarusian State University were used in the study. The varieties -Sheker, Utro, Volgograd-5/95, Ilkin, Shahin, Leyla, Zafar, S-258, Novichok, Gafgaz, Vatan-1, Titan, Voronezh, Ilyas, Azerbaijan, Rubin, Avart, To 79\35, Chirok, Zorka, CC1\1, Pi 99\3A, Me 78\56, Tamara, Garant, Irma, and Berkut as well as variety forms- Masalli v/f, Hersegovina v/f, Lankaran v/f, and Ordubad v/f were evaluated based on biomorphological as well as complex indicators of economic importance. Six local (Leyla, Shahin, Zafar, Shekar, Ilkin and Masalli v/f) and four introduced (Garant, Tamara, Utro, Volgograd-5/95) varieties were crossed using the top cross method. Hybrids obtained from the combinations Leyla × Volgograd-5/95, Leyla × Ilkin, Leyla × Utro, Shahin × Volgograd -5/95, Shahin × İlkin, Shahin × Utro, Masalli v/f × Volgograd -5/95, Masalli v/f × Ilkin, Masalli v/f × Utro, Sheker × Utro, Sheker × Volgograd - 5/95, Sheker × Ilkin, Garant × Volgograd -5/95, Garant × İlkin, Garant × Utro, Tamara × Volgograd - 5/95, Tamara × Ilkin, Tamara × Utro, Zafar × Volgograd -5/95, Zafar × Ilkin, Zafar × Utro were studied comparatively.

2.3. Research methods. Both classical and modern methods were used in the research. The collection of tomato varieties and variety forms was carried out according to the methodology "Collection and passportization of genetic resources of vegetables and melons."[5]

Field trials on the study of tomato genotypes were carried out according to "Methodology of field trials with vegetables and melon plants"[6].

The "Descriptor for the tomato plant" developed by the

[5] Z.İ. Əkpərov və b. (2012). Tərəvəz və Bostan bitkiləri genetik ehtiyatlarının toplanması və pasportlaşdırılması. AMEA Genetik Ehtiyatlar İnstitutunun Elmi Əsərləri. IV cild.s.167-175.

[6] Clemans Van de Wiel and etc. (2010)Traditional breeding methods. https://edepot.wur.nl/141713/p.1-66.

International Institute of Biodiversity was used in determining and evaluating the biomorphological characteristics of tomato plants[7].

Statistical indicators of general and specific combining ability in hybrids obtained from 21 combinations were performed according to the methodology presented by Liu, Zenbirg, and colleagues[8].

Heterosis effect, the productivity of the parental forms was calculated in three ways: according to the average indicators of the productivity components (dominance), the superior parental indicators (superior dominance) and the average value of the control form (standard heterosis)[9].

Statistical calculations[10,11] were interpreted using ready-made statistical packages of the R programming language.

In the course of molecular-genetic studies, according to "New varieties of tomato, morphological aspects, molecular characterization with RAPD and ISSR markers", the seeds of research materials were germinated and DNA extraction was carried out[12]

CHAPTER III
DISCUSSION OF THE RESEARCH MORPHOGENETIC ANALYSIS OF TOMATO COMBINATORS AND HYBRIDS

3.1. Study of Quantitative and Qualitative Traits of Tomato

[7] https://pdf.usaid.gov/pdf_docs/Pnach864.pdf

[8] Liu, Zengbing, Jingbin Jiang, Ai Ren, Xiangyang Xu, He Zhang, Tingting Zhao, Xiuming Jiang, Yaoguang Sun, Jingfu Li, and Huanhuan Yang. (2021). "Heterosis and Combining Ability Analysis of Fruit Yield, Early Maturity, and Quality in Tomato" Agronomy 11, no. 4: 807.

[9] Madhavi Y, Reddy RVSK, Thirupathi reddy M, Sudheer Kumar S, Bhave MHV. Exploitation of heterosis and combining ability for yield, quality and processing in tomato (Solanum lycopersicum L.) Ph.D. thesis, Dr. Y.S.R. Horticultural University, Andhra Pradesh, 2013.

[10] https://myaseen208.com/ agricolae/ reference/ lineXtester. html

[11] https://www.tutorialspoint.com/r/index.htm

[12] Bădulescu, A., Popescu, C. F., Dumitru, A. M., & Sumedrea, D. I. (2020). New varieties of tomato - morphological aspects and molecular characterisation with RAPD and SSR markers. Notulae Scientia Biologicae, 12(4), 818–828.

Genotypes under Absheron Conditions. In our research, certain traits and characteristics of tomato plants were observed to vary depending on environmental conditions, and the inheritance patterns were studied. Varieties with superior traits were selected, and new hybrids were developed based on them. The study of the variation rates of local and introduced tomato varieties and forms under Absheron conditions reveals that local varieties such as Masalli s/f, Zafer, Sheker, Shahin, Leyla, and Ilkin, along with the introduced Volgograd-5/95 variety, were more adaptive compared to others, showing a higher level of morphological, physiological, and biological compatibility with the environment.

Upon analyzing the adaptive effects of environmental factors on morphogenetic traits, it was found that traits such as main stem height, the number of flowers per cluster, the number of fruits per cluster, and juice yield stood out with superior adaptive indicators. In terms of the values of phenotypic dispersion components, 5 out of the 20 studied traits (20%) in both adaptive and non-adaptive samples were less sensitive to macroclimatic conditions, while 15 traits (80%) were more sensitive. This is explained by the higher number of additive dominant genes that determine the development of the same traits.

3.1.1. Morphogenetic Analysis of Selected Tomato Combinators. Figure 3.1.1 illustrates the correlation and clustering heat map of selected tomato genotypes. The cluster analysis, based on Euclidean distance indices calculated using Ward's method, and the determination of correlations between these biomorphological traits were conducted using the R programming language. The heat map shows that the samples are grouped into two main clusters and four subgroups. Among the samples, Ilkin and Tamara varieties are the most genetically distant, with an inter-group genetic distance index of 0.25 to 0.75 units.

In the intensity map, the tomato genotypes were assessed considering both the length of the vegetative period and biomorphological characteristics. The early-maturing group comprises 42%, the mid-maturing group 33%, and the late-maturing group 25% of the studied samples. Among the genotypes studied, the shortest biological maturation period was observed in the varieties

Azerbaijan, Voronezh, Shahin, Utro, and Tamara (Figure 3.1.2).

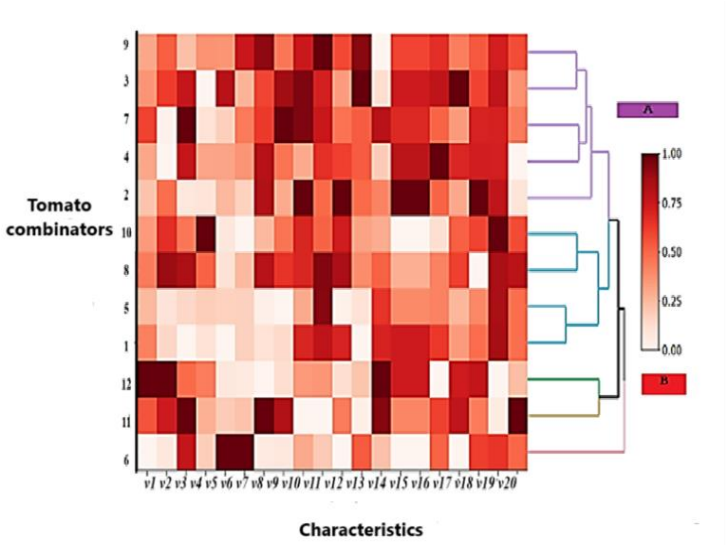

Figure 3.1.1. Correlation and clustering intensity heat map of selected tomato genotypes

3.2. Obtaining intraspecies hybrids of tomato. In the Absheron region, hybridization was carried out between 6 local and 4 introduced tomato genotypes selected as promising parental forms using artificial pollination based on the "Line × Tester" scheme (7 maternal forms × 3 paternal forms), resulting in the creation of 21 hybrid combinations. These hybrids were comparatively studied with respect to their quantitative and qualitative traits against their parental forms.

3.2.1. Comparative evaluation of quantitative and qualitative traits of hybrids and parental forms. The best hybrid combinations were found to be Masalli v/f × Utro, Sheker × Utro, Masalli v/f × Utro, Leyla × Utro, Zafar × Volgograd-5/95, and Tamara × Ilkin. The Tamara × Ilkin combination was superior for 9 out of 20 traits. The Tamara (Belarus) × Volgograd-5/95 (Russia) combination obtained from the crossing of introduced varieties was selected as a parental pair (combination) with superior non-additive

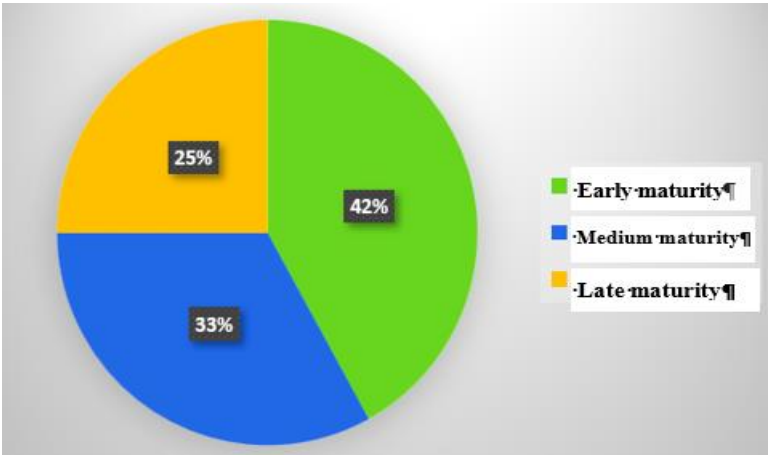

Figure 3.1.2. Distribution ratio among superior adaptive genotypes according to biological ripening period in Absheron

genes, showing higher heterogeneity compared to other introduced varieties (multiallelic) for 8 traits (main stem height, number of stems per plant, number of clusters per plant, number of fruits per plant, average fruit height, number of days to fruit maturity, sugar content and total acidity content). They showed high genetic variation.

CHAPTER IV. GENERAL AND SPECIFIC COMBINING ABILITY OF HYBRIDS

4.1. Evaluation of the general combining ability of combinatoric forms. The ease of controlling cross-pollination in tomato plants has allowed for the calculation of combining ability values, which are important for ongoing work in genetic improvement and increasing yield. The data obtained on the ratio of General Combining Ability (GCA) to Specific Combining Ability (SCA) is of great significance for the creation of F1 hybrid generations. The term GCA is used to determine the average performance of combinators in hybrids. Such genetic effects indicate the magnitude of additive gene (allelic gene) effects and demonstrate that a particular line possesses superior genes overall. The term SCA is used to identify cases where

the average performance of hybrids in certain combinations is better or worse than expected, considering the non-additive (non-allelic) gene effects and the degree of sensitivity to environmental factors.

The analysis of GCA showed that three out of twenty quantitative and qualitative traits (the height of the main stem, the number of clusters per plant, and the number of fruits per plant) had high additive gene effects with low sensitivity to environmental influences, exhibiting very little variability under Absheron conditions. When analyzing the parental forms for GCA, the maternal combinators Zəfər (20%), Leyla (35%), Şahin (40%), and Masallı s/f (45%) distinguished themselves with high performance, while the paternal combinators Utro (55%) and Volqaqrad-5/95 (40%) also showed notable high values (Figures 4.1 and 4.2).

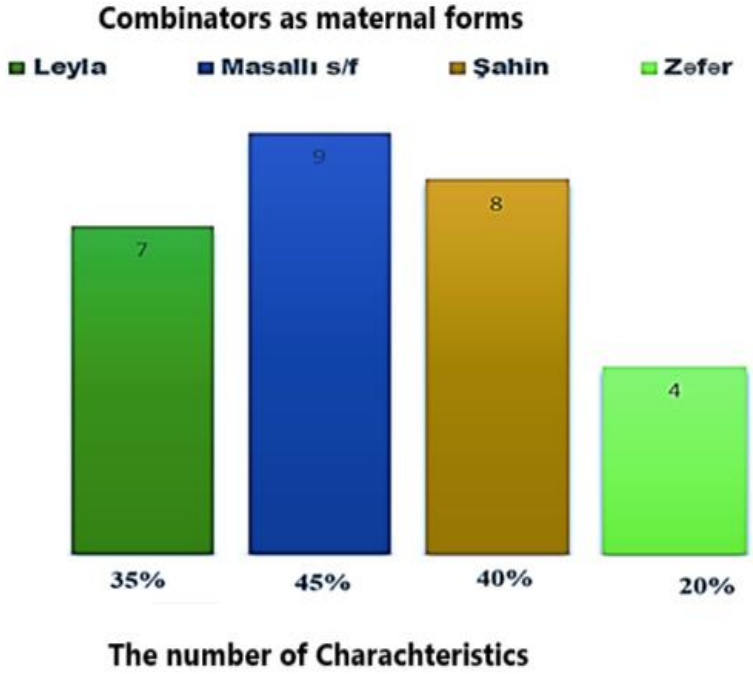

Figure 4.1. Maternal parents differentiated by general combining ability

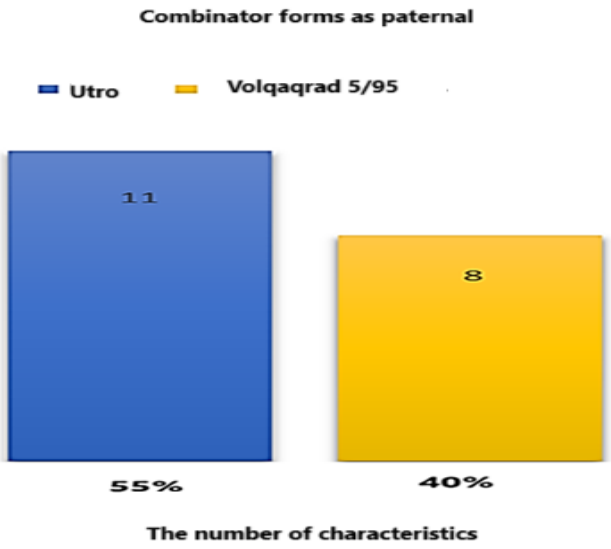

Figure 4.2. Paternal parents differentiated by spesific combining ability

It is known from the literature that the success of obtaining combinations in hybridization largely depends on the correct selection of parents. This allows for the selection of any desired trait in combinators that produce additive and non-additive gene effects, depending on the objective. Additionally, since not every trait and characteristic inherent to the parents is transmitted to the progeny, and because alleles and non-alleles passed on undergo recombination, leading to a reorganization of alleles, this results in the formation of divergent groups.

4.2. Analysis of the specific combining ability of hybrids. Nine combinations from the studied hybrids (Leyla × Utro; Garant × Ilkin; Garant × Volgograd-5/95; Shahin × Ilkin; Sheker × Utro; Tamara × Ilkin; Tamara × Utro; Tamara × Volgograd-5/95; Zafar × Volgograd-5/95) were selected. Based on them, it is possible to obtain positive results from selection for 11-13 traits, mainly traits that affect productivity.

Hybrids, which are superior in terms of productivity based on

the evaluation results of the general and specific combining ability are listed in the table below (Table 1).

Table 1.

Hybrids with superior SCA for productivity

Combinations	SCA	GCA (maternal form)	GCA (paternal form)	GP (hybrid)	GM (maternal form)	GM (paternal form)
Leyla × Utro	13.26**	8.1**	0.3	551.7**	268.3	153.9
Masalli v/f × Utro	10.08**	-2.7*	0.3	412.8**	228.2	153.9
Garant × Ilkin	5.355*	-3.5*	-8	339.5*	215.2	209.4
Shahin × Ilkin	16.624**	10.6**	-8	594.2**	286.8	209.4
Tamara × Ilkin	5.387*	7.1*	-8	304.18*	145.8	209.4

SCA-specific combining ability, GCA-general combining ability, GP-general productivity (kg\ha), ($p < 0.05$*, $p < 0.001$**).

It should be noted that the negative value of the productivity indicators according to the GCA shows poor additive gene effects on the samples. This is also reflected in their average values, as the total productivity index (cwt/ha) is lower than that of hybrids. In such hybrids, non-additive gene effects have a higher value and non-allelic effects are larger.

As seen in Table 1, the specific combining ability for productivity in the selected tomato samples changes from 5.35% to 16.62% in hybrids, the general combining ability ranges between -2.7% and 10.6% for the maternal form, and from -8% to 0.3% for the paternal form, from 304.1(cwt/ha) to 594.2(cwt/ha) for total productivity, from 145.8 (cwt/ha) to 286.8 in maternal forms, between 153.9 (cwt/ha) to 209.4 cwt/ha in paternal forms. Among the hybrid combinations, the highest productivity was recorded in Shahin × Ilkin and Leyla × Utro, and the lowest in Tamara × Ilkin.

As seen in the table, the value of GP of Shahin × Ilkin hybrids is higher when compared to the maternal and paternal forms. This has also manifested itself in the GCA. Based on the literature data, a high

index in the hybrid is due to cytoplasmic inheritance. This regularity was observed in Leyla × Utro and Masalli v/f × Utro hybrids.

This character of hybrid combinations was also manifested in the specific combining ability and they were evaluated more highly than the general combining ability. The fact that the SCA is greater than the GCA is an indicator of the high non-additive effects of genes (genes dependent on environmental factors). The use of these results in selection can lead to an increase in productivity under favorable cultivation conditions.

CHAPTER V.
STUDY OF HETEROSIS IN TOMATO HYBRIDS

5.1. Evaluation of heterosis effect. In recent times, the widespread application of hybrid production in agricultural selection has been accelerated, and the improvement of methods for breeding heterozygous plants and growing hybrid plants has become one of the main tasks facing breeders. The selection of parents with high combining ability is one of the prerequisites in the heterosis selection program. Such research not only helps to select the best parents but also reveals the importance and nature of the genes involved in the reproduction of desirable traits. Studies on combining ability allow for a deeper understanding of the importance of first-generation F_1 heterosis hybrids and their commercial use.

The regularities of inheritance of valuable selection traits have been studied in our research on the selection of F_1 hybrids with heterosis effect between local and introduced varieties of tomato. A number of economically important traits were used in the descriptor for tomato developed by the International Biodiversity Institute. Their heterosis effect was evaluated in the first generation. The heterosis effect was calculated mainly in three ways: based on the average indicators of productivity and its components in parental forms (MF, dominance), the superior parental indicator (BF, superior dominance-heterobeltiosis) and the average value of the control form meeting the market requirements (SF, standard heterosis)[8]. The values of the

average heterosis effects changed as follows: the parental heterosis effects ranged between 28.1 and 161%, the heterosis effect of the superior parents changed from 6.5 to 105%, and the values for the standard heterosis ranged between 8.9 and 181% (Table 5.1).

According to the average productivity of varieties with superior parental indicators, the hybrids of combinations Leyla × Utro (105%), Masalli v/f × Utro (81%), Masalli v/f × Volgograd-5/95 (19%), Garant × Ilkin (58%), Garant × Volgograd-5/95 (13%),

Table 5.1.

Heterosis effect and depression of average yield in the next generation of hybrids (D, %)

Hybrids	MF(%)	BF(%)	SF (%)	D %
Leyla × Ilkin	39.3	24.0	57.7	-6.23
Leyla × Utro	161.4	105.0	161.8	5.45
Leyla ×Volgograd-5/95	28.1	30.0	65.3	-5.84
Masalli vf ×Ilkin	-21.5	-25.0	-18.4	-7.34
Masalli v\f × Utro	115.6	81.0	95.7	-9.13
Masalli v\f × Volgograd-5/95	-26.7	19.0	54.4	15
Garant × Ilkin	29.1	58.0	61.1	4.34
Garant × Utro	59.9	6.5	8.9	-6.14
Garant × Volgograd-5/95	24.3	13.0	48.2	1.72
Shahin × Ilkin	27.3	101.0	181.9	2.13
Shahin × Utro	139.6	17.0	58.8	8.5
Shahin ×Volgograd-5/95	51.9	33.0	80.0	-3.9
Sheker × Ilkin	35.0	5.0	17.5	6.4
Sheker × Utro	11.4	26.0	41.0	-1.2
Sheker × Volgograd-5/95	52.6	-12.0	62.9	-8.14
Tamara × Ilkin	34.5	22.0	44.3	-6.44
Tamara × Utro	71.4	45.0	5.9	-2.3
Tamara ×Volgograd-5/95	48.9	-10.0	17.5	-11.2
Zafar × Ilkin	17.6	4.4	24.2	-8.82
Zafar × Utro	13.9	27.0	50.8	7.23
Zafar × Volgograd-5/95	57.2	27.0	65.5	5.41

Shahin × Ilkin (101%), Shahin × Utro (17%), Sahin × Volgograd-5/95 (33%), Sheker × Volgograd- 5/95 (-12%), Tamara × Ilkin (22%), Tamara × Volgograd-5/95 (-10%), and Zafar × Volgograd-5/95 (27%) were distinguished by their high performance.

Hybrids with standard heterosis effect were related to the combinations Leyla × Utro (161.8%), Masalli v/f × Utro (95.7%), Masalli v/f × Volgograd-5/95 (54.4%), Garant × Ilkin (61.1%), Garant x Volgograd-5/95 (48.2%), Shahin × Ilkin (181.9%), Shahin × Utro (58.8%), Sahin × Volgograd-5/95 (80%), Sheker × Volgograd-5/95 (62.9%), Tamara × Ilkin (44.3%), Tamara × Volgograd-5/95 (17.5%), Zafar × Utro (50.8%), and Zafar × Volgograd-5/95 (65.5%). The fact that the SCA is greater than the GCA is an indication of higher non-additive gene effects in gene transmission. This regularity is also evident in the values of the heterosis effect. Thus, the higher the SCA, the more likely it is to get hybrids with a heterosis effect. In the future, breeders can use this information to increase the heterosis effect by obtaining hybrids with high productivity by influencing the environment.

5.2. Correlation and cluster-based kinship in hybrid combinations. The results of the cluster analysis of the combining ability of a number of complex biomorphological traits, can be used as a valuable resource for both the selection of the right combinators and the obtaining of new hybrids.

Hybrid combinations 18,17, 20, 8, 7, 15, 12, 3,1, 21, 14, 6, 2 - Tamara × Volgograd-5/95, Tamara × Utro, Zafar × Utro, Garant × Utro, Garant × Shahin, Sheker × Volgograd-5/95, Shahin × Volgograd-5/95, Leyla × Volgograd-5/95, Leyla × Ilkin, Zafar × Volgograd-5/95, Sheker× Utro, Masalli v/f × Volgograd -5/95, Leyla × Utro located in this cluster were closer to each other. Genotypes No. 19, 11, 13 in the second cluster; Zafar × Ilkin and Shahin × Utro showed closer genetic similarity, while Sheker × Ilkin showed relatively close genetic similarity. The genotypes located in the third subcluster were different from the others, of which Tamara × Ilkin (16) and Masalli × Ilkin (4) were closer, Masalli v/f × Utro (5) and Sheker × Ilkin (10) were distant (Figure 5.2).

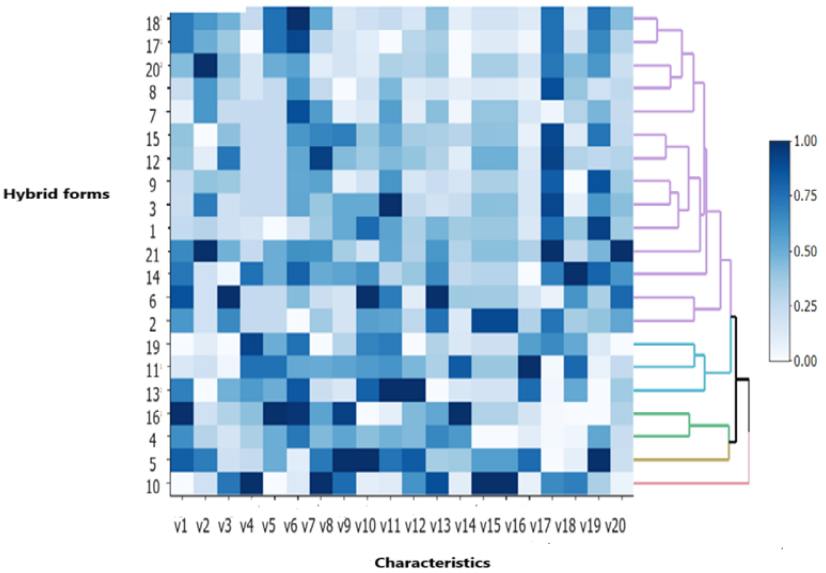

Figure 5.2. Dendrogram of the combining ability of hybrid forms based on correlation

The specific combining ability of distantly related genotypes has a greater value, and this can be explained by the accumulation of dominant and epistatic genes. Thus, based on the SCA, since Leyla × Utro, Tamara × Ilkin, Tamara × Volgograd-5/95 hybrids have additive genes, they were less sensitive to environmental factors and were identified as the best combinators. This is clear from the first figure. These data can be recommended for choosing a parent form in the creation of new varieties according to the purpose of the researcher in future selection processes.

CHAPTER VI.
MOLECULAR DIVERSITY ANALYSIS IN TOMATO

6.1. Evaluation of combinators (parental form selected as donor) and hybrids in tomato using ISSR primers

In this study, genetic diversity was investigated in 6 local and 4

introduced combinators of cultivated tomatoes (S. lycopersicum L.) and their hybrids, along with two control varieties, using 10 ISSR primers.

The analysis revealed a high level of polymorphism based on the obtained fragments. As shown in Table 6.1, the degree of polymorphism varied between 63% and 100%, with an average value of 71.7%.

Table 6.1.
Polymorphism and genetic diversity index observed with ISSR primers

Primers	Sequence (5-3)	Synthesized band	Polymorphic band	Polymorphism (%)	RP	PIC	EMR	MI	MRP	GM
IS-2	$(GA)_9C$	9	9	100	5.1	0.41	6.75	2.8	0.02	0.98
IS-3	$G(TG)_9$	7	4	57	7.46	0.41	4.1	1.6	0.02	0.85
IS-10	$(AG)_8G$	6	3	50	1.18	0.31	1.5	0.5	1.18	0.50
IS-811	$(GA)_8C$	9	6	66	5.7	0.33	6.8	2.2	0.02	0.96
IS-814	$(CT)_8A$	8	5	63	6.3	0.41	5.6	2.3	0.02	0.92
IS-816	$(CA)_8T$	6	4	67	1.74	0.32	2.7	0.9	0.44	0.64
IS-818	$(CA)_8G$	11	10	90	6.44	0.36	11	4.0	0.59	0.89
IS-840	$(GA)_8CT$	11	7	64	5.7	0.26	4.6	1.2	1.23	0.89
IS-855	(AC)8CTT	13	11	85	8.6	0.22	9.3	2.0	1.28	0.78
IS-848	$(CA)_8RG$	8	6	75	3.44	0.39	4.5	1.7	0.57	0.85
Total	-	94	65	-	-	-	-	-	-	-
Average value	-	9.4	6.5	71.7	5.2	0.22	5.7	1.9	0.53	0.83

RP represents the visualization ability, PIC stands for the polymorphism information content, EMR indicates the effective multiplex ratio, MI refers to the marker index, MRP signifies the average visualization ability, and GM denotes genetic diversity.

In our study of tomato combinators and their combinations, the

Genetic Diversity (GD) index for each ISSR locus ranged from 0.50 to 0.98. The high indicators of genetic diversity in the tomato genotypes we used are due to the reduction of similarities between the distant geographical hybridizations of the local and introduced varieties used both from different regions of Azerbaijan and from different regions of Russia and Belarus.

A total of 94 bands were synthesized for 10 ISSR primers, of which 65 were polymorphic and 29 were monomorphic. The number of amplified fragments per locus varied from 6 to 13. The largest number of amplicons was synthesized with primers ISSR-855 (13), ISSR-811, and ISSR-840 (11). Thus, the ISSR-2 primer was more effective in terms of polymorphism index and genetic diversity (GD), and ISSR-855 and ISSR-818 primers in terms of the number of polymorphic alleles in parental and hybrid forms of tomato.

Thus, the high efficiency of ISSR primers in the studied samples indicates that the combinators and their hybrids are genetically diverse. All molecular markers complement each other with biomorphological complex traits and allow the selection of different parental and geographically distant hybrid forms in breeding programs, as well as gathering detailed information about population diversity and genetic structure.

6.2. Cluster analysis based on the loci of ISSR marker of tomato combinators and hybrids.

Cluster analysis of the genetic distance between tomato genotypes was performed based on the Jaccard method, and phylogenetic diagnosis of the lineages reflecting the genetic kinship index of the samples was drawn up. These, in turn, consisted of groups and subgroups. Hybrids showed some degree of compatibility with their parents, collected in clusters and groups. Based on the ISSR primer, the Zafar × Ilkin and Tamara × Utro hybrids and the Tamara (Belarus) and Volgograd-5/95 (Russia) parental formes were selected as the most distant genotypes in the dendrogram showing the genetic kinship of the tomato samples (Figure 6.2).

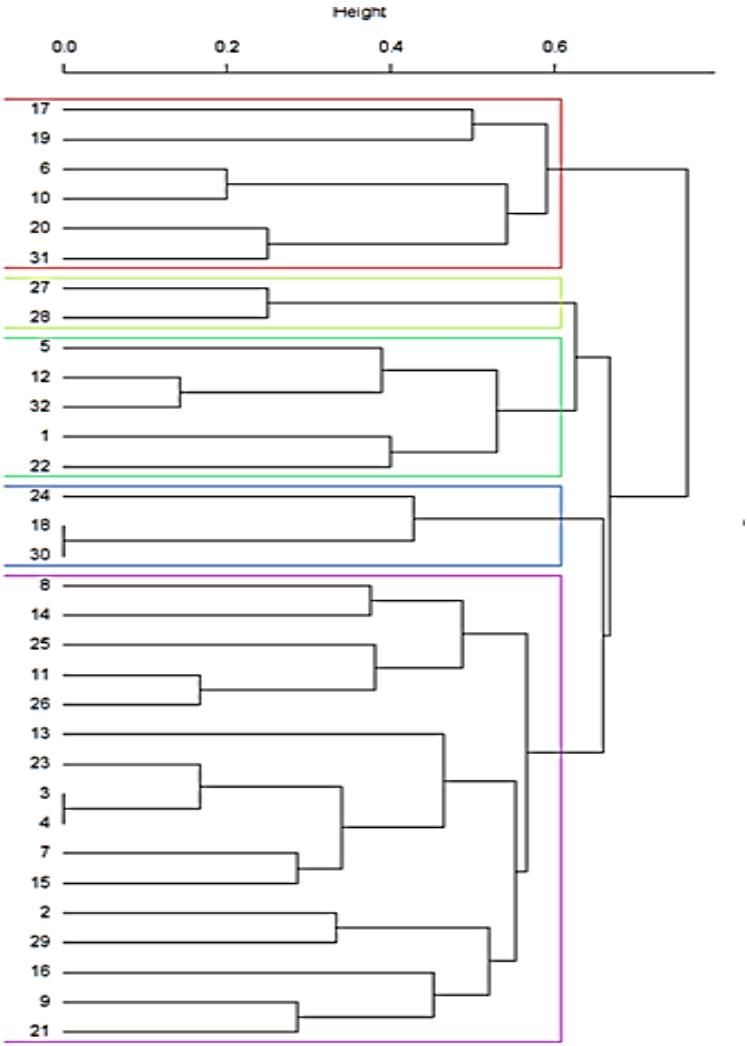

Figure 6.2. Dendrogram showing genetic kinship in tomato samples according to combinators and hybrids

6.3. Cluster analysis based on the loci of RAPD marker of tomato combinators and hybrids. The RAPD method was used to study genetic diversity on the basis of DNA markers at the molecular

level. Different RAPD primers were used in the analysis of the genetic diversity of 32 local and introduced tomato genotypes (Table 6.3). The average value of the determined polymorphism, taking into account all bands, was 73.2.

If we arrange the synthesized alleles from the largest to the smallest, 9 alleles were synthesized in both primers with motifs TGAGCGGACA (OPD-05) and GATGACCGCG (OPC-05). Although the number of synthesized alleles was the same, a different degree of polymorphism was observed, i.e., 33% and 67%, respectively, due to the small number of polymorphic bands.

Table 6.3.

Number of polymorphic bands and genetic diversity index of RAPD primers used

Primers	Sequence (5-3)	Number of synthesized alleles	Number of polymorphic alleles	Polymorphism (%)	GM
OPA-02	TGGCGAGCTG	7	6	86	0.91
OPA-03	AGTCAGCCAC	8	7	88	0.96
OPA-05	AGGGGTCTTG	6	5	83	0.94
OPA-10	GTGATCGCAG	5	4	80	0.85
OPD-02	GGACCAACC	7	4	57	0.67
OPD-05	TGAGCGGACA	9	3	33	0.68
OPC--05	GATGACCGCG	9	6	67	0.73
OPC-08	TGGACCGGTG	8	4	50	0.97
OPC-11	AAAGCTGCGG	6	6	100	0.88
OPB-10	CTGCTGGGAC	8	7	88	0.96
Total		73	52		
Average		7.3	5.2	73.2	0.86

Based on the Jaccard method, the phylogenetic diagnosis of the generations that reflected the genetic kinship index of the samples was drawn up. In the dendrogram created as a result of RAPD analysis, it was found that the most distant samples are Shahin and Masalli v/f × Utro. Genetically distant from the parents are Shahin and Masalli v/f, and the hybrids Shahin × Volgograd-5/95 and Masalli v/f × Utro.

6.4. Analysis of Genetic Divergence of Tomato Combinators and Hybrids Based on RAPD Loci.

In this study, 10 RAPD primers were used to successfully differentiate between local and introduced genotypes of tomatoes grown in Azerbaijan and their hybrids. The constructed dendrogram grouped the tomato genotypes into 8 main clusters (Figure 6.4). Among the parents, the most distant were identified as (Shahin and Masalli s/f), while the closest were (Qarant × Zafar and Zafar) with 9 and 25. Among the hybrids, the most distant were Shahin × Volgograd-5/95 and Masalli s/f × Utro, while the closest were Qarant × Ilkin and Tamara × Ilkin genotypes.

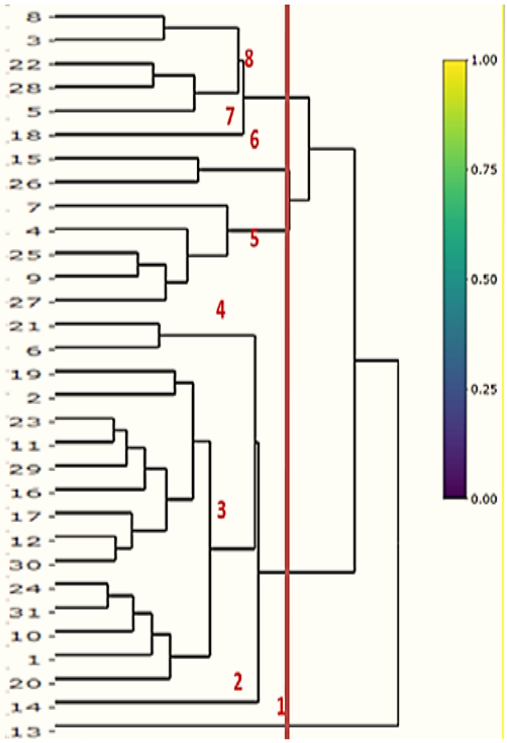

Figure 6.4. Dendrogram representing genetic relatedness of tomato combinators and hybrids based on RAPD primers

6.5. A comparative study of genetic diversity based on morphological quantification indicators and molecular markers.

The dendrograms obtained from the analysis of genetic diversity at the genome level using RAPD and ISSR markers were compared with the dendrograms constructed on the basis of the quantitative traits and the correlation analysis was performed using Ward's test. It was found that the results of genetic diversity studied through primers were completely different from those obtained by the morphological analysis. The correlation between morphological traits and the RAPD marker was estimated to be 0.072 and the correlation between morphological traits and the ISSR marker was 0.064. In the dendrogram, based on the morphological characteristics, the samples introduced from Belarus and Russia were located in subclusters close to each other, which was also observed in the results of these molecular analyses. Thus, Shahin × Ilkin and Masallı v/f × Ilkin hybrids, which did not show much difference in the 20 traits studied, were located in the same cluster in all three dendrograms, and the genetic distance index between them was very low. However, such compatibilities are a minority, and the groups created by molecular analyses are mostly composed of genotypes of mixed origin. Some morphologically similar combinators (parental form selected as donor) and hybrids of the same origin were located in different clusters according to the molecular analysis results.

The results obtained from the analysis of genetic diversity at the genome level using RAPD and ISSR markers were compared and it was found that the genetic diversity studied by both methods was similar.

The correlation between the RAPD marker and ISSR determined by the Mantel test was 0.82. Some morphologically similar genotypes of the same origin were located in different clusters during molecular analysis, which can be explained by their genetic distance and mutations occurring as a result of geographical radiation in different regions of the same country.

Thus, while the most distant genotypes in the ISSR dendrogram are Shakar and Tamara and in the RAPD dendrogram, the Masalli v/f × Utro hybrid and the Sahin genotype, in the dendrogram of the two primers, Leyla × Volgograd-5/95, Zafar × Utro (hybridized with local and introduced scheme) hybrids had a more distant genetic index.

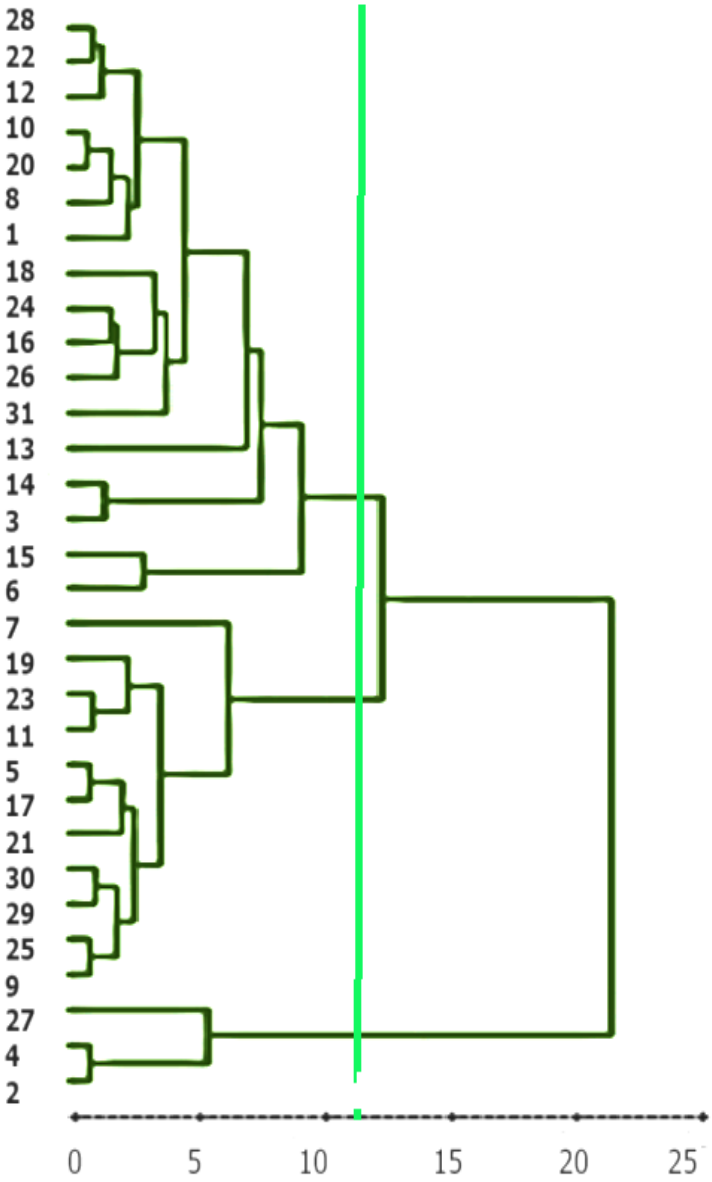

Figure 6. 4. Dendrogram showing genetic relatedness in tomato samples according to combinatorial and hybrids

CONCLUSIONS

Adaptability of Tomato Varieties: The local tomato varieties cultivated in the Absheron region—Masallı s/f, Leyla, Zəfər, Şəkər, Şahin, and İlkin—along with the introduced variety Volqaqrad-5/95, have been identified as adaptive to environmental influences.

Hybrid Combinations with High Combining Ability: Among the hybrids obtained from genetically distant tomato varieties, the combinations Masallı s/f × Utro and Tamara × Utro showed high general combining ability (GCA), while Leyla × Utro, Tamara × İlkin, and Tamara × Volqaqrad-5/95 exhibited high specific combining ability (SCA).

Combining Variability Analysis: For the first time, utilizing the "7 parental forms × 3 tester forms - Line × Tester" hybridization scheme, combining variability rates were determined as follows: Zəfər (20%), Leyla (35%), Şahin (40%), Masallı s/f (45%), Utro (55%), and Volqaqrad-5/95 (40%).

Heterosis Effect Assessment: In Azerbaijan, the heterosis effect was studied for the first time based on the evaluation of specific combining ability (SCA) in local and introduced tomato varieties. The yield indicators of the hybrids varied between 28.1% to 161% compared to the parents, from 6.5% to 105% compared to superior parents, and from 8.9% to 181% compared to standards.

High Yielding Genotypes: Hybrids derived from combinations Leyla × Utro, Tamara × İlkin, and Tamara × Volqaqrad-5/95 were evaluated as high-yielding genotypes based on SCA indicators and corresponding heterosis effects.

Soluble Dry Matter Content: The total soluble dry matter content in the tomato samples ranged from 4.17% to 7.27% for the parents and from 4.93% to 9.9% for the hybrids.

Genetic Similarity Analysis with ISSR Markers: Analysis using ISSR markers revealed the highest genetic diversity index (GO=0.82) in the hybrid combinations Zəfər × İlkin and Tamara × Utro. The most genetically distant parents observed were Tamara and Volqaqrad-5/95, with a genetic distance index of 0.64. In hybrids derived from local × introduced schemes, genetic distance indices of 1.0 were recorded for

Tamara × Utro and Zəfər × İlkin.

RAPD Marker Analysis: The highest genetic similarity (GO=0.78) was identified in the combinations Şahin × Volqaqrad-5/95 and Masallı s/f × Utro, while the lowest genetic diversity was noted in the combinations Qarant × Zəfər and Zəfər, indicating low genetic divergence.

PRACTICAL RECOMMENDATIONS

Cultivation of high-yielding varieties, it is recommended to cultivate the Masallı variety and Leyla, Zəfər, Şəkər, Şahin, İlkin, and Volqaqrad-5/95 varieties in agricultural settings. These varieties serve as valuable starting materials in tomato breeding programs. The combinations Leyla × İlkin, Leyla × Utro, Şahin × İlkin, Masallı s/f × Utro, Şəkər × Volqaqrad-5/95, and Zəfər × Volqaqrad-5/95 are suggested for application as stable hybrid lines.

Selection of parental forms for breeding for obtaining hybrids with heterosis effects, it is recommended to use local varieties Masallı s/f, Leyla, Şahin, Şəkər, and Zəfər as maternal parents, and the tester forms İlkin and Volqaqrad-5/95 as paternal combinators.

Improvement of traits in tomato varieties of the creation of heterotic hybrids can achieve the improvement of various traits in existing tomato varieties within individual or farmer enterprises.

Efficient use of molecular markers for RAPD primers OPA 02, OPA 03, OPB 10, and OPC 11, and for ISSR primers ISSR-2, ISSR-818, and ISSR-855, have proven to be effective in assessing DNA polymorphism between tomato hybrids and their parents.

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