Plant and Soil Science

Journal homepage: https://agriculturalscience.com.ua/en
Plant and Soil Science, 12(1), 102-111
Received xx.xx.2020 Revised xx.xx.2021 Accepted xx.xx.2021

UDC [635.262"324":631.526.3]:631.528(477.5)

DOI: 10.31548/agr2021.01.0102

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Productivity of Winter Garlic in the Left-Bank Forest-Steppe of Ukraine and Molecular Genetic Polymorphism of the Studied Varieties by ISSR Loci

Abstract. In Ukraine, the areas allocated to garlic cultivation are expanding and as of 2020 amounted to 1,100 hectares, which is driven by high profitability and steady demand in both domestic and foreign markets. At the same time, the evaluation of current and new varieties of winter garlic is important for further yield increase in this valuable crop. The purpose of the study is to evaluate the genetic diversity of the studied varieties of winter garlic by ISSR loci and determine the most productive ones in the conditions of the Left-Bank Forest-Steppe of Ukraine. Winter garlic varieties Dyushes, Lyubasha and Ugorskyi (line 20-16) were used for the research. The largest marketable

Suggested Citation:

Yaroviy, G.I., Filimonova, O.I., Romanov, O.V., & Hordiienko, I.M., (2021). Productivity of Winter Garlic in the Left-Bank Forest-Steppe of Ukraine and Molecular Genetic Polymorphism of the Studied Varieties by ISSR Loci. *Plant and Soil Science*, 12(1), 102-111. doi: 10.31548/agr2021.01.102.

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yield (14.1 t/ha) was obtained by growing winter garlic of the Lyubasha variety. The Ugorskyi variety (line 20-16) provided a yield of 12.9 t/ha, the Dyushes variety 9.4 t/ha. The formation of the yield of winter garlic by 12.3% depended on the characteristics of the variety, the impact of the growing season made up 87.3%. The variety of winter garlic Ugorskyi (line 20-16) with *As* = 73.8% was agronomically stable (As > 70%). Based on the above studies, allelic formulas (molecular genetic passports) of winter garlic varieties Ugorskyi (Line 20-16), Lyubasha and Dyushes were formed. The studied varieties of winter garlic contained several amplicons unique within the group under investigation. In the Dyushes variety, the unique loci were UBC812725 and UBC 812902, and in the Lyubasha variety UBC 812791 and UBC 842702. The 20-16 line had the most unique loci: UBC 812460, UBC812997, UBC826682, UBC8341283, and UBC846920. The calculated Nei-Li similarity coefficients indicate a close genetic distance between studied varieties of winter garlic. The varieties Lyubasha and Dyushes are genetically closer to one another compared to Ugorskyi (line 20-16)

Keywords: garlic, variety, Lyubasha, Dyushes, Ugorskyi, yield, polymorphism

RELEVANCE

Garlic (*Allium sativum* L.) is an annual, herbaceous, cold-resistant plant that is distributed throughout the Eastern Hemisphere, but most of all in Asia Minor and Central Asia (Kovarovič *et al.*, 2019) Almost 10 million tons of garlic are grown annually. The world's largest producers are China and South Korea (Etana, 2018), as well as Egypt, India, Turkey and Spain (Malik Geetika, 2017).

Due to its valuable properties, garlic is widely used for food and medicinal purposes (Shah Faraz, 2018). In Ukraine, the increase in production of this crop is mainly due to an expansion of the crop cultivation areas. The cause of the weak increase in winter garlic yields is its high conservatism and poor adaptability to growing conditions due to the vegetative type of reproduction, which results in a limited production area for the varieties created so far. For this reason, local varieties of garlic, which are well adapted to the ecological conditions of the region, are grown in Ukraine and other countries. Evaluation of existing and new varieties of winter garlic is important for further increase of production of this valuable crop both for the Left Bank Forest-Steppe zone and for the whole of Ukraine.

ANALYSIS OF RECENT RESEARCH AND PUBLICATIONS

In Ukraine, the area of garlic cultivation amounted to 1,100 hectares as of 2020. Its primary production (about 49% of the crops) is concentrated in Vinnytsia, Chernivtsi, Zaporizhzhia and Donetsk Oblasts. The yields and total harvests of seed and food garlic in the country are still low – 8.6 tons per hectare (Mogylna *et al.*, 2018).

There are about 600 cultivated varieties of garlic in the world (Anonymus, 2018). Most of them originated from a few basic types that grew in different environments and acquired unique characteristics over the centuries. At the same time, varieties play an extremely important role in increasing and stabilising garlic production. Their optimal selection can significantly increase gross production, improve product quality, and reduce the risk of plant damage by pests and diseases (Pinto *et al.*, 2000; Kyryk, Pikovskyi, Azaiki, 2012; Kyryk, Pikovskyi, Azaiki, 2016). For areas with more favourable natural conditions, it is important to introduce varieties with the appropriate genetic characteristic that can form a high level of yield and product quality.

The purpose of the research is to evaluate the genetic diversity of the studied varieties of winter garlic by ISSR loci and to determine the most productive cultivars within the conditions of the Left-Bank Forest-Steppe of Ukraine.

MATERIALS AND METHODS

The research was conducted during 2016-2020 in the experimental field of the Department of Fruit and Vegetable Growing and Storage of the V.V. Dokuchayev Kharkiv National Agrarian University, as well as at the Laboratory of Agrochemistry and Analytical Measurements of the Institute of Vegetable and Melon Growing of the NAAS of Ukraine. Winter garlic varieties Dyushes, Lyubasha and Ugorskyi (line 20-16) were used for the research. Field studies were conducted in accordance with generally accepted methods (era methodology, 2001). The experiment included 2 biometric measurements. The first measurement was carried out during the bulb formation period, and the second during the winter garlic harvest. The harvesting of garlic was carried out once. Each plot brought in a harvest that was weighed and counted.

The study of garlic DNA polymorphism using the polymerase chain reaction (PCR) method was carried out on the basis of the testing laboratory "AGROGEN NOVO" in 2020.

DNA was extracted by the solid-phase method using the Diatom DNA Prep100 DNA extraction kit. A lysis reagent with guanidine chloride was used to solubilize the cellular debris and denature cellular nucleases. In the presence of a lysis reagent, DNA was sorbed onto a silica sorbent and washed from proteins and salts with an alcohol solution. DNA extraction was performed according to the protocol described in the instructions for the commercial kit "Diatom DNA Prep100" (http://www.galartdiag.ru/files/diatom_dna_prep_100.pdf). The purity of the isolated DNA concentration was determined using a Shimadzu UV-1280 spectrophotometer (Japan) at a wavelength of 260 nm.

ISSR (Intersimple Sequence Repeats) primers. The DNA polymorphism of garlic varieties was studied using primers for intermicrosatellite sequences developed at the University of British Columbia (UBC, Canada) (Table 1).

Table 1. Nucleotide sequences of ISSR primers

Primer	Nucleotide sequence 5'-3'	Primer	Nucleotide sequence 5'-3'
UBC 803	$(AT)_8C$	UBC 825	$(AC)_8T$
UBC 804	$(TA)_8A$	UBC 826	$(AC)_8C$
UBC 807	$(AG)_8T$	UBC 834	$(AG)_8YT$
UBC 810	$(GA)_8T$	UBC 842	$(GA)_8YG$
UBC 812	$(GA)_8A$	UBC 846	$(CA)_8RT$

Note: Y = pYrimidine (C or T); R = puRine (A or G)

DNA amplification was performed using GenePaktm PCR core kits (Isogen, Russia). In tubes from these kits containing lyophilised dry reaction mixtures for PCR, 20 ng of extracted DNA, 0.2 µM primer was added, and then the reaction mixture was brought to 20 µL with the solvent from the PCR kits.

PCR was performed in a TP4-PCR-01-"Tertsik" thermocycler (Russia) under the following conditions: initial denaturation of DNA at 94°C for 5 min; 40 amplification cycles under the following conditions for each cycle: denaturation at 94°C – 40 s, hybridisation – 45 s – at 50°C (for primers UBC 803, UBC 804, UBC 807, UBC 810, UBC 812 and UBC 825), 52°C (for primers UBC 826, UBC 834 and UBC 846) or 54°C (for primer UBC 842), elongation at 72°C – 2 min; final elongation at 72°C – 7 min.

Amplification products were electrophoresed in a 1.5% ethidium bromide agarose gel for 1.5 h at 120 V. The Tris-EDTA-borate buffer system used in the study was 0.09 M Tris, 0.09 M H3PO3, 0.0031 M EDTA (pH 8.3). Visualisation of the spectra of amplified DNA regions was performed using a TCR-20 MS transilluminator (France) followed by photographing the gels. M combi (fragment sizes: 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, 1000, 1200, 1400, 1600, 1800, 2000 bp) was used as a marker for determining the size of amplicons.

The polymorphism of the spectra, as well as the presence/absence of amplification products, was assessed visually. Each amplification product was considered as a marker of the corresponding locus in the genomic DNA with a dominant type of inheritance.

Amplicon sizes were calculated using a demo version of the TotalLab TL120 software package (http://www.totallab.com).

The level of polymorphism for each primer was defined as the proportion of polymorphic loci of the total number of loci per primer, expressed as a percentage. The level of genetic diversity of varieties was assessed using the Nei-Li similarity coefficient (Dij), which was calculated using the Phylip-3.69 software package (Nei and Li, 1979). The matrix of Nei-Li similarity coefficients was used for cluster analysis by the Neighbor-joining method (NJ).

RESULTS

In 2017, according to the results of research, the yield of winter Garlic of the Lyubasha variety was 9.2 t/ha (Table. 2), which is 1.9 t/ha or 26.0% more than the control variant and is a significant difference (LSD_{05} = 0.65). The Ugorskyi variety had a yield of 10 t/ha, which is significantly different from the control variant and exceeded it by 3.2 t/ha or 43.8%. In 2018, the yield of winter garlic of the Lyubasha variety was 16.1 t/ha, which is significantly higher than the control variant. The difference between the yield of the control variant and the Lyubasha variety was 7.9 t/ha (LSD_{05} = 3.42). The yield of the Ugorskyi winter garlic variety was 13.4 t/ha, which is 5.2 t/ha more than the Dyushes variety, which is a significant difference.

On average, over the three years of research, a higher level of marketable yield was observed in the Lyubasha variety (14.1 t/ha), in the Ugorskyi variety 12.9 t/ha and 9.4 t/ha in the Dyushes variety (control variant) (Table 2).

The analysis of the weather conditions over the years of research shows that fluctuations in air temperature and uneven precipitation during the growing season largely caused fluctuations in the yield of winter garlic. In 2017, The HTC of the growing season of winter garlic was 0.69, which can be considered slightly arid. According to the results of this year's research, the yield of winter garlic averaged 9.0 t/ha. The vegetation period of 2018 has not been sufficiently moist (HTC = 0.38). Under such conditions, the average yield of winter garlic was

12.6 t/ha. The weather conditions of 2019 can be described as dry (HTC = 0.65). According to the study results, the yield of winter garlic under these conditions was 14.84 t/ha (Table 2).

The analysis of variance found that the formation of winter garlic yields by 12.3% depended on the characteristics of the variety (factor A), the influence of the growing season conditions (factor B) was 87.3%, the combined effect of factors AB – 0.5%, other factors (elements of cultivation technology, etc.). According to the research, only the Ugoskyi variety is stable: *As* = 83.3%. The coefficients of agronomic stability of the Lyubasha and Dyushes varieties are 69.4 and 55.2%, respectively (Table. 2). These varieties of winter shooting garlic are unstable. Thus, the value of these indicators confirmed that the adaptive capacity influenced the yield during the years of cultivation. Furthermore, the yield of underground bulbs of winter garlic showed significant dependence on both the variety and the weather conditions of the year.

Table 2. The yield of bulbs in winter arrow garlic varieties

Cultivar	Yield, t/ha			Average	Yield increase		Coefficient of stability, SF	Agronomic stability As
	2017	2018	2019		t/ha	%		
Dyushes (control)	7.3	8.2	12.7	9.4	0.0	0.0	1.74	55.2
Lyubasha	9.2	16.1	17.1	14.1	4.7	50.0	1.88	69.4
Ugorskyi (line 20-16)	10.5	13.4	14.7	12.9	3.5	37.2	1.40	83.3
LSD_{05}	0.65	3.42	2.84					

These data are also confirmed by the coefficient of agronomic stability (*As*), which was proposed by V.V. Hangildin. It characterises the resistance of varieties to adverse conditions of the growing season. A variety is considered stable if *As* is over 70%. Over the years, the yield level of the Dyushes variety ranged from 7.3 to 12.7 t/ha, which caused a lower phenotypic stability coefficient compared to other variants. These data are also confirmed by the coefficient of agronomic stability (*As*), which characterises the resistance of hybrids to unfavourable conditions of the growing season (Sych, 2005; morphological..., 2006).

As a result of molecular genetic analysis of winter garlic varieties Dyushes, Lyubasha and Ugorskyi (line 20-16) using 10 ISSR primers, 65 loci were identified, 23 of which were polymorphic. The number of detected amplicons varied depending on the primer and variety (Table 3). Thus, 48 out of 65 possible loci were detected in Line 20.16, and 57 loci each in varieties Lyubasha and Dyushes.

The studied loci were found to be low polymorphic. The polymorphism level averaged 28.6% and ranged from none for primers UBC 810 and UBC 825 to 58.3% for primer UBC 812.

Table 3. Molecular genetic polymorphism of garlic varieties revealed by ISSR analysis

Primer	The number of detected loci, pcs.	Polymorphism level, %	Amplicon size, minimum - maximum, bp.
UBC 803	no amplicons detected		
UBC 804	no amplicons detected		
UBC 807	8	25.0	354–1076
UBC 810	6	0.0	396–983
UBC 812	12	58.3	262–997
UBC 825	1	0.0	665
UBC 826	7	28.6	348–1020
UBC 834	11	36.4	206–1283
UBC 842	11	36.4	218–1265
UBC 846	9	44.4	336–1192
Total	65	On average 28.6	

In the studied garlic varieties, the amplicons in the electrophoretic spectra differed in number, size, and level of expression. Figures 1 and 2 show the electropherograms obtained.

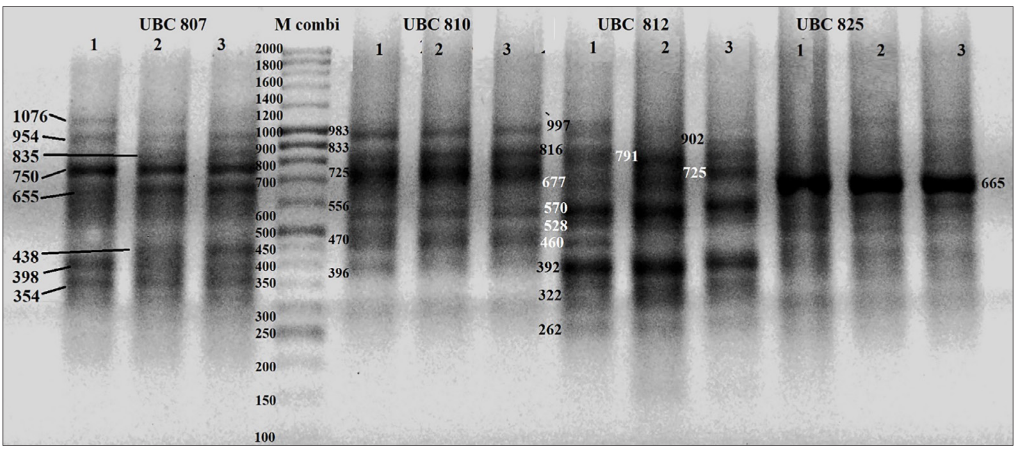

Figure 1. Electropherogram of garlic DNA amplification products with primers UBC 807, UBC 810, UBC 812, and UBC 825. M combi is a marker for determining the size of amplicons:
1 – Ugorskyi (Line 20-16), 2 – Lyubasha variety, 3 – Dyushes variety

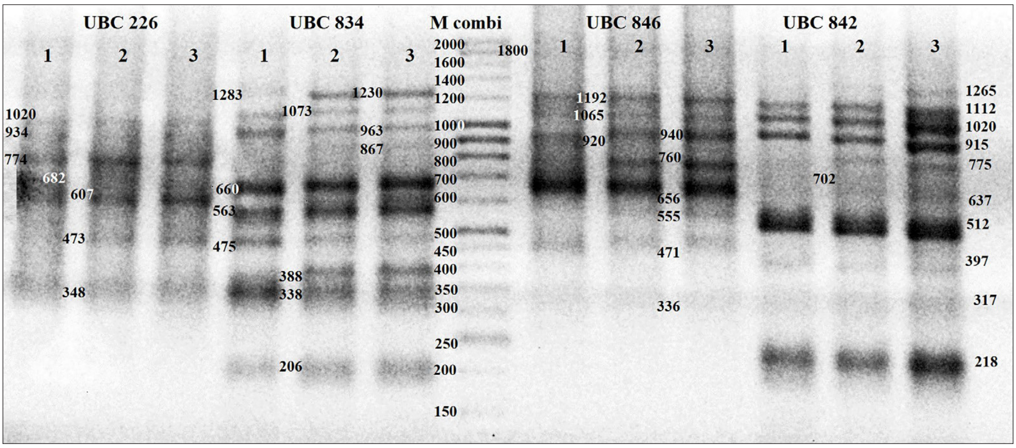

Figure 2. Electropherogram of garlic DNA amplification products with primers UBC 826, UBC 834, UBC 842 i UBC 846. M combi is a marker for determining the size of amplicons: 1 – Ugorskyi (Line 20-16), 2 – Lyubasha variety, 3 – Dyushes variety

The use of primers UBC 803 and UBC 804 failed to produce amplification products. This may be due to the fact that in the genomes of the analysed garlic varieties, microsatellites with sequences $(AT)_8C$ and $(TA)_8A$, respectively, are rare or absent, or the distance between inverted repeats is too large for amplification in the mode used here, i.e., more than 2000 bp.

The identification of 65 loci of intermicrosatellite DNA repeats in winter garlic made it possible to form allelic formulas of the studied varieties that can be used as their molecular genetic passports. To develop these passports, loci were labelled with capital Latin letters, next to which the size of the amplicon was indicated as a lower index. Loci were labelled as follows:

A – UBC 807	**E – UBC 826**
B – UBC 810	**F – UBC 834**
C – UBC 812	**G – UBC 842**
D – UBC 825	**H – UBC 846**

The resulting passports are shown in Table 4.

Table 4. Molecular genetic passports of winter garlic varieties

No.	Cultivar	Genetic passport
1	Ugorskyi (Line 2016)	$A_{354, 398, 655, 750, 954, 1076}$ $B_{396, 470, 556, 725, 833, 983}$ $C_{262, 322, 392, \mathbf{460}^{*}, 528, 570, 677, 816, \mathbf{997}}$ D_{665} $E_{348, 607, \mathbf{682}, 774, 934, 1020}$ $F_{206, 338, 475, 563, 660, 963, 1073, \mathbf{1283}}$ $G_{218, 317, 397, 512, 915, 1020, 1112}$ $H_{336, 471, 656, \mathbf{920}, 1065, 1192}$
2	Lyubasha	$A_{354, 398, 438, 655, 750, 835, 954, 1076}$ $B_{396, 470, 556, 725, 833, 983}$ $C_{262, 322, 392, 528, 570, 677, \mathbf{791}}$ D_{665} $E_{348, 473, 607, 774, 934, 1020}$ $F_{206, 338, 388, 475, 563, 660, 867, 963, 1073, 1230}$ $G_{218, 317, 397, 512, 637, \mathbf{702}, 775, 915, 1020, 1112, 1265}$ $H_{336, 471, 555, 656, 760, 940, 1065, 1192}$
3	Dyushes	$A_{354, 398, 438, 655, 750, 835, 954, 1076}$ $B_{396, 470, 556, 725, 833, 983}$ $C_{262, 322, 392, 528, 570, \mathbf{725}, 816, \mathbf{902}}$ D_{665} $E_{348, 473, 607, 774, 934, 1020}$ $F_{206, 338, 388, 475, 563, 660, 867, 963, 1073, 1230}$ $G_{218, 317, 397, 512, 637, 775, 915, 1020, 1112, 1265}$ $H_{336, 471, 555, 656, 760, 940, 1065, 1192}$

Note: * – amplicons unique to the respective variety are highlighted in bold. A – UBC 807; B – UBC 810; C – UBC 812; D – UBC 825; E – UBC 826; F – UBC 834; G – UBC 842; H – UBC 846

Notably, several amplicons unique within the studied group were detected in the winter garlic varieties presented in this study (Figs. 1 and 2, Table 3). In the Dyushes variety, the unique loci were UBC812$_{725}$ and UBC 812$_{902}$, and in the Lyubasha variety UBC 812$_{791}$ and UBC 842$_{702}$. Ugorskyi (line 20-16) had the most unique loci: UBC 812$_{460}$, UBC 812$_{997}$ UBC 826$_{682}$, UBC 834$_{1283}$ UBC 846$_{920}$. These plots can be used to develop more specific markers of the corresponding varieties.

The calculated Nei-Li similarity coefficients indicate a close genetic distance between studied varieties of garlic. It should be noted that the varieties Lyubasha and Duchesne were genetically closer to one another (*Dij* = 0.0009), compared to Hungarian (Line 20-16) – *Dij* between this line and each of the varieties was 0.0035. The genetic similarity of the studied varieties may be the result of the use of genetically uniform source material during the creation of these varieties.

CONCLUSIONS

The largest marketable yield (14.1 t/ha) was obtained by growing winter garlic of the Lyubasha variety. The Ugorskyi variety (line 20-16) provided a yield of 12.9 t/ha, the Dyushes variety 9.4 t/ha.

The formation of the yield of winter garlic by 12.3% depended on the characteristics of the variety, and the impact of the growing season made up 87.3%.

The variety of winter garlic Ugorskyi (line 20-16) with *As* = 73.8% was agronomically stable (*As* > 70%).

Based on the above studies, allelic formulas (molecular genetic passports) of winter garlic varieties Ugorskyi (Line 20-16), Lyubasha and Dyushes were formed.

The studied varieties of winter garlic contained several amplicons unique within the group under investigation. In the Dyushes variety, the unique loci were UBC 812725 and UBC 812$_{902}$, and in the Lyubasha variety, the loci were UBC 812$_{791}$ and UBC 842$_{702}$. The 20-16 line had the most unique loci: UBC 812$_{460}$, UBC 812$_{997}$, UBC 826$_{682}$, UBC 8341$_{283}$, and UBC 846$_{920}$.

The calculated Nei-Li similarity coefficients indicate a close genetic distance between studied varieties of winter garlic. The varieties Lyubasha and Dyushes are genetically closer to one another compared to Ugorskyi (line 20-16).

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Продуктивність часнику озимого в лівобережному лісостепу України та молекулярно-генетичний поліморфізм досліджуваних сортів за ISSR-локусами

Анотація. В Україні площі, відведені під вирощування часнику, розширюються і станом на 2020 рік склали 1100 га, що зумовлено високою рентабельністю та стійким попитом як на внутрішньому, так і на зовнішньому ринках. Водночас оцінка існуючих та нових сортів часнику озимого має важливе значення для подальшого підвищення врожайності цієї цінної культури. Мета роботи – оцінити генетичне різноманіття досліджуваних сортів часнику озимого за ISSR-локусами та визначити найбільш продуктивні з них в умовах Лівобережного Лісостепу України. Для досліджень використовували сорти часнику озимого Дюшес, Любаша та Угорський (лінія 20-16). Найбільшу товарну врожайність (14,1 т/га) було отримано при вирощуванні часнику озимого сорту Любаша. Сорт Угорський (лінія 20-16) забезпечив урожайність 12,9 т/га, сорт Дюшес – 9,4 т/га. Формування врожайності часнику озимого на 12,3% залежало від особливостей сорту, вплив вегетаційного періоду становив 87,3%. Сорт часнику озимого Угорський (лінія 20-16) з $As = 73{,}8\%$ був агрономічно стабільним ($As > 70\%$). На основі проведених досліджень сформовано алельні формули (молекулярно-генетичні паспорти) сортів часнику озимого Угорський (лінія 20-16), Любаша та Дюшес. Досліджувані сорти часнику озимого містили декілька ампліконів, унікальних у межах досліджуваної групи. У сорту Дюшес унікальними локусами виявилися UBC812725 та UBC 812902, а у сорту Любаша - UBC 812791 та UBC 842702. Найбільше унікальних локусів мала лінія 20-16: UBC 812460, UBC812997, UBC826682, UBC8341283 та UBC846920. Розраховані коефіцієнти подібності Nei-Li свідчать про близьку генетичну відстань між досліджуваними сортами озимого часнику. Сорти Любаша та Дюшес генетично ближчі один до одного порівняно з сортом Угорський (лінія 20-16)

Ключові слова: часник, сорт, Любаша, Дюшес, Угорський, урожайність, поліморфізм