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Cultivation Of Winter Garlic Under Conditions Of The Left-Bank Forest-Steppe Of Ukraine And Molecular And Genetic Polymorphism Of Winter Garlic Varieties By Issr Loci

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ABSTRACT

In Ukraine, there is a tendency to increase the area of garlic, which is due to the high profitability of its production and constant demand in both domestic and foreign markets. The increase in the volume of this crop production occurs mainly due to an increase in the area and not due to an increase in cropping capacity. The reason for the slow growth in the cropping capacity of winter garlic is the limited possibilities of seed production of the crop and low adaptability to growing conditions, caused by the vegetative type of reproduction, which leads to a limited production area of the varieties which had been created by this time. For this reason, mainly local garlic varieties are grown in Ukraine and abroad, which are well adapted to the ecological conditions of the region. Based on this, for the further growth of garlic production, both for the Left-Bank Forest-Steppe zone and for the whole of Ukraine, the assessment of existing and new varieties of this crop is important.

The aim of conducted studies was to assess the genetic diversity of winter garlic varieties selected for research by ISSR loci and determine the most productive of them under conditions of the Left-Bank Forest-Steppe of Ukraine. The study was conducted with winter garlic varieties Duchess, Lubasha and Hungarian (line 20-16).

On average, during the three years of research, the highest level of commercial cropping capacity was observed in Lubasha variety (14.1 t/ha), in Hungarian variety 12.9 t/ha and 9.4 t/ha in Duchess variety (control variant). Formation of winter garlic cropping capacity by 12.3% depended on the peculiarities of the variety, the influence of growing season conditions was 87.3 %, the combined effect of AB factors was not significant, its share in cropping capacity variability was only 0.5 %. Agronomically stable was Hungarian winter garlic variety with As = 73.8 %.

In the studied varieties of winter garlic, several amplicons were found that are unique within the studied group. Duchess variety had unique loci UBC 812_{725} and UBC 812_{902}, while Lubasha variety had unique loci UBC 812_{791} and UBC 842_{702}. Hungarian variety (line 20-16) had a bigger number of unique loci: UBC 812_{460}, UBC 812_{997} UBC 826_{682}, UBC 834_{1283} and UBC 846_{920}

The calculated Nei-Li similarity coefficients indicate a significant genetic similarity of the studied winter garlic varieties. Lubasha and Duchess varieties are genetically closer to each other, compared to Hungarian variety.

Keywords: winter garlic, variety, molecular genetic polymorphism, cropping capacity, phylogeny.

INTRODUCTION

Almost 10 million tons of garlic are grown every year. The world's largest producers are China, Korea, India, the United States, Spain, Egypt and Turkey. Garlic is one of the most commonly used plants in many parts of India, Pakistan, and Bangladesh, and is popular in French, Spanish, Portuguese, and South Asian cuisine. The United Nations Food and Agriculture Organization (FAO) claims that garlic is currently one of five products whose demand is steadily growing by almost 8% annually. At the same time, prices in the world for it are also growing [1].

The area under garlic in Ukraine is small and as of 2020 it occupies 1,100 hectares. Its main production – about 49% of crops, is concentrated in Vinnytsia, Chernivtsi, Zaporizhzhia and Donetsk regions. The cropping capacity and gross yield of seed and commercial garlic in the country are still low – 8.6 t/ha [2].

There are about 600 cultivated varieties of garlic in the world, most of which originated from the few main types that grew in different conditions and developed their own characteristics over the centuries.

Varieties play an extremely important role in increasing and stabilizing garlic production. By selecting varieties, you can significantly increase gross production, improve product quality, and reduce the risk of plant damage by pests and diseases. For areas with more favorable natural conditions, it is important to introduce varieties with appropriate genetic characteristics that can form a high level of cropping capacity and product quality.

ANALYSIS OF LITERARY SOURCES, PROBLEM STATEMENT

Garlic Allium sativum (L.) – an annual, herbaceous, cold-resistant crop. It belongs to the bulbous family (Alliaceae), the botanical genus of onions – Allium (L.) [3–5]. The genus Allium has almost 600 species, which are distributed throughout the Eastern Hemisphere, but to the greatest extent in Asia Minor and Central Asia [6]. Due to its specific smell, high taste, canning and antiseptic qualities, garlic is widespread in many countries of the world.

Garlic is rich in nutrients. Its bulbs accumulate up to 41.3% of dry matter; 6.7% of protein; 29.3% of carbohydrates; 7-25 mg % of vitamin C; 0.19 mg % of vitamin B_1 [7]. The caloric content of 1 kg of garlic cloves and single cloves is 1110-1327 calories. In addition, garlic contains 0.94 mg/kg of iodine [8]. Young leaves accumulate up to 15.9% of dry matter; 2.7% of protein; 10.4% of carbohydrates and 2.5 mg % of carotene [9]. The sharp taste and specific smell of garlic are due to the presence of sulfides in its bulbs and up to 0.1% of essential oil, which includes phytoncides that inhibit the development of microorganisms [10].

In Ukraine, the area under garlic is increasing, which is due to the high profitability of production and constant demand in both domestic and foreign markets. The increase in the volume of this crop production occurs mainly due to an increase in the area. The reason for the slow growth in the cropping capacity of winter garlic is the limited possibilities of seed production of the crop and low adaptability to growing conditions, caused by the vegetative type of reproduction, which leads to a limited production area of the varieties which had been created by this time. For this reason, mainly local garlic varieties are grown in Ukraine and abroad, which are well adapted to the ecological conditions of the region.

Based on this, for the further volume growth of garlic production, both for the Left-Bank Forest-Steppe zone and for the whole of Ukraine, the assessment of existing and new varieties of this crop is important.

PURPOSE OF RESEARCH

To assess the genetic diversity of the studied varieties of winter garlic by ISSR loci and determine the most productive of them under conditions of the Left-Bank Forest-Steppe of Ukraine.

MATERIALS AND METHODS OF RESEARCH

Winter garlic varieties Duchess, Lubasha and Hungarian (line 20-16) were used for research.

Fig.1. Winter garlic Duchess variety

Duchess variety is an early-ripening variety of winter garlic, bred by breeders of the Institute of VegetablesandMelons growingand registered in 2004. the advantages of the variety are large air bulbs, which, when sown for the winter, form a single-clove one weighing 5-8 g and about 5% of the bulbs are divided into cloves. A plant with a flower-stalk 90-120 cm high forms bulbs weighing 40-50 g and large nut-shaped air bulbs (weight of 1000 pieces – 180 g). It reproduces by cloves, air bulbs, and single-clove. The leaves are light green with a length of 47-48 cm and a width of 2.2-2.5 cm. On one plant, as a rule, 8-10 leaves are formed. Bulbs have a rounded-flattened shape, they are covered with 4-5 dry scales of white colour with purple stripes along the vessels. The bulb consists of 5-6 aligned, mostly large cloves covered with thick parchment light brown scales.

Lubasha winter garlic variety is large in size. Its average height is from 100 to 120 cm, and some specimens, judging by the description, reach 1.5 m. The leaves are characterized by a rich dark green colour and the presence of a light waxy coating. The length of one leave is approximately 40 cm, the width is about 20 mm. The high cropping capacity of the variety is caused by the size of the ripe head. Its weight usually ranges from 100 to 120 g. The colour of the outer scales of the head is usually white, sometimes with a pinkish tinge, purple veins are clearly visible on them. The colour of individual cloves is closer to cream, their number can be different: from 5 to 9.

Fig.2. Winter garlic Lubasha variety

Lubasha garlic has a lot of advantages, which include: frost resistance, resistance to dry conditions, good storeability. Judging by the description, it does not lose its taste and benefits even after 10 months from the moment of harvesting. The cropping capacity of this variety is 35 t/ha.

Hungarian (line 20-16) is large in size. Its average height is from 100 to 120 cm, and some specimens reach one and a half meters. The leaves are characterized by a rich dark green colour and the presence of a light waxy coating. The length of one leave is approximately 45 cm, the width is about 30-35 mm.

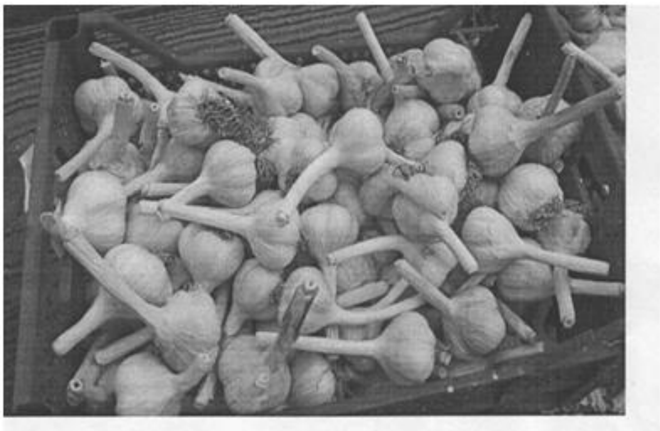

Fig.3. Winter garlic Hungarian variety

The high cropping capacity of the variety is due to the size of the ripe head. Its weight usually ranges from 90 to 120 g. The colour of the outer scales of the head is white. The colour of individual cloves is closer to cream, the number of cloves in the head is from 4 to 6 pieces.

Hungarian garlic (line 20-16) has a number of advantages: frost resistance, resistance to dry conditions, good storeability. It does not lose its taste and benefits for up to 10 months from the moment of harvesting.

The research was conducted in 2016-2020 in the experimental field of the Department of Fruit and Vegetable Growing and Storage of V. V. Dokuchaiev KhNAU, and in the laboratory of agrochemistry and analytical measurements of InstituteofVegetablesandMelons growingof NAASaccording to the "Methods of research in vegetable and melon growing" [11].

Field experience on growing winter garlic. Basic and pre-sowing tillage, as well as plant care and harvesting were carried out according to the recommendations for Forest-Steppe technologies of Ukraine. Garlic cloves were planted in the second decade of October. The method of placing plants is stripe sowing with a scheme of placement (20+45)×10. The density of plants is 220 thousand pieces/ha. The cloves were planted to a depth of 8-10 cm. The average weight of one clove is 10 g. the seeding rate is 2200 kg/ha.

Repetition in experiments is threefold. The area of the accounting plot is 10 m^2. Biometric observations were carried out twice a month, determining the dynamics of growth of vegetative mass, plant and yield mass. To do this, we took 10 plants from each variant. The mass of garlic, the mass of the 3rd leaf, the mass of all leaves, the mass of the bulb, the length of the 3rd leaf, the length of the entire plant, the length of the stem, the diameter of the bulb, the diameter of the stem, the number of leaves, the width of the 3rd leaf were determined. The area of the leaf was also determined.

The first accounting was carried out during the formation of the bulb, and the second – during the harvest of winter garlic. Garlic was harvested once. From each plot, the harvest was gathered, which was weighed, calculated in quantity.

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

The DNA was isolated by the solid-phase method using the “Diatom DNA Prep100” set for DNA isolation. For this purpose, a lysing reagent with guanidine chloride was used to solubize cellular debris and denaturate cellular nucleases. In the presence of a lysing reagent, DNA was sorbed on a silica sorbent, washed off from proteins and salts with an alcohol solution. DNA isolation was performed according to the protocol proposed in the instructions for the "Diatom DNA Prep100" commercial set (http://www.galartdiag.ru/files/diatom_dna_prep_100.pdf). The purity of the isolated DNA was determined using a Shimadzu UV-1280 spectrophotometer (Japan) at a wavelength of 260 nm.

ISSR (Intersimple Sequence Repeats, intermicrosatellite sequenced 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). 1).

DNA amplification was performed using GenePaktm PCR Core set (Isogen, Russia). 20 ng of isolated DNA and 0.2 µm of primer were added to test tubes from these sets, which contained freeze-dried dry reaction mixtures ready for PCR, and then the reaction mixture was adjusted to 20 µL with a solvent from the PCR sets.

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

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).

Electrophoresis of amplification products was performed in 1.5% agarose gel with ethidium bromide for 1.5 hours with a voltage of 120 V. A TRIS-EDTA-borate buffer system was used – 0.09 m Tris, 0.09 M H3VO3, 0.0031 M EDTA (ph – 8.3). Visualization of the spectra of amplified DNA areas was performed using a TSR-20 MS transilluminator (France), followed by gel photographing. As a marker for determining the size of amplicons we used M combi (fragment sizes: 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, 1000, 1200, 1400, 1600, 1800, 2000 nucleotide pairs).

Results processing and software. The polymorphism of the spectra, as well as the presence/absence of amplification products, were assessed visually. Each amplification product was considered as a marker of the corresponding locus in genomic DNA with the 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 from the total number of loci per primer, expressed as a percentage. The level of genetic diversity of varieties was estimated using the Nei–Li (Dij) similarity coefficient which was calculated using the Phylip-3.69 software package [12]. The Nei-Li similarity coefficient matrix was used for cluster analysis using the neighbor-joining method (NJ).

Research results and their discussion.Cropping capacity and quality of garlic products. In 2017, according to the results of research, the cropping capacity of winter garlic of the Lubasha variety was 9.2 t/ha, which is 1.9 t/ha or 26.0% more than the control variant and is a significant difference (SSD_{05} = 0.65). The Hungarian variety had the cropping capacity of 10.0 t/ha, which is 3.2 t/ha higher than in the control variant. In 2018, the cropping capacity of winter garlic of Lubasha variety was obtained at the level of 16.1 t/ha, which is 7.9 t/ha higher compared to the control for SSD_{05} – 3.42 t/ha. The cropping capacity of Hungarian winter garlic variety was 13.4 t/ha, which is 5.2 t/ha more than that of Duchess variety.

On average, during the three years of research, the highest level of commercial cropping capacity was observed in Lubasha variety (14.1 t/ha), in Hungarian variety 12.9 t/ha and 9.4 t/ha in Duchess variety (control) (table 2).

Table 2.Cropping capacityof bulbs of winter garlic varieties, t/ha

Variety	Cropping capacity				Yield growth		Stability factor, SF	Agronomic stability, As
	Year			Average	t/ha	%		
	2017	2018	2019					
Duchess (control)	7,3	8,2	12,7	9,4	–	–	1,74	55,2
Lubasha	9,2	16,1	17,1	14,1	4,7	50,0	1,88	69,4
Hungarian (Line 20-16)	10,5	13,4	14,7	12,9	3,5	37,2	1,40	83,3
SSD_{05}	0,65	3,42	2,84					

Analyzing the weather conditions in the years of research, it can be noted that fluctuations in air temperature and uneven precipitation during the growing season largely caused fluctuations in the cropping capacity of winter garlic. So, in 2017, the Hydrothermal Coefficient (HTC) of the growing season of winter garlic was 0.69, it can be considered slightly arid. According to the results of studies of this year, the average cropping capacity of winter garlic was 9.0 t/ha. During the growing season of winter garlic in 2018, there was a significant lack of precipitation (HTC = 0.38). Under such conditions, an average cropping capacity was obtained at the level of 12.6 t/ha. Weather conditions in 2019 can be described as dry (HTC = 0.65). According to the results of research under these conditions, the cropping capacity of winter garlic was obtained at the level of 14.6 t/ha.

Disperse analysis found that the formation of winter garlic cropping capacity by 12.3% depended on the peculiarities of the variety (factor A), the influence of growing season conditions (factor B) was 87.3%, the combined effect of AB factors was not significant, its share in cropping capacity variability was only 0.5%.

These data are also confirmed by the coefficient of agronomic stability (As), which was proposed by V.V. Khangildin. It characterizes the resistance of varieties to adverse conditions of the growing season. The variety is considered stable if the As is more than 70 %. The annual cropping capacity level of Duchess variety ranged from 7.3 to 12.7 t/ha, which provided a lower coefficient of phenotypic stability compared to other variants. These data are also confirmed by the agronomic stability coefficient (As), which characterizes the resistance of hybrids to adverse growing season conditions [13-16]. According to the results of research, only Hungarian variety is stable: As = 73.8 %.

The agronomic stability coefficients of Lubasha and Duchess varieties are 69.4 and 55.2%, respectively (table 2). These varieties of winter garlic are unstable. Consequently, the magnitude of these indicators confirmed that adaptive ability affected the cropping capacity during the growing years. In addition, based on the cropping capacity of underground bulbs of winter garlic, it should be noted that it depended on both the variety and the weather conditions of the year.

2. MOLECULAR AND GENETIC POLYMORPHISM OF WINTER GARLIC VARIETIES

As a result of molecular and genetic analysis of winter garlic varieties Duchess, Lubasha and Hungarian (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). So, out of

65 possible ones, 48 loci were found in the line 20-16, and 57 loci were found in Lubasha and Duchess varieties.

Table 3.Molecular and genetic polymorphism of garlic varieties detected during ISSR analysis.

Primer	Number of detected loci, pieces	Level of polymorphism, %	Amplicon size, minimum-maximum, nucleotide pairs
UBC 803	no amplicons were detected		
UBC 804	no amplicons were 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	

The studied loci turned out to be low-polymorphic. The level of polymorphism averaged 28.6% and ranged from its absence for UBC 810 and UBC 825 primers to 58.3% for UBC 812 primer.

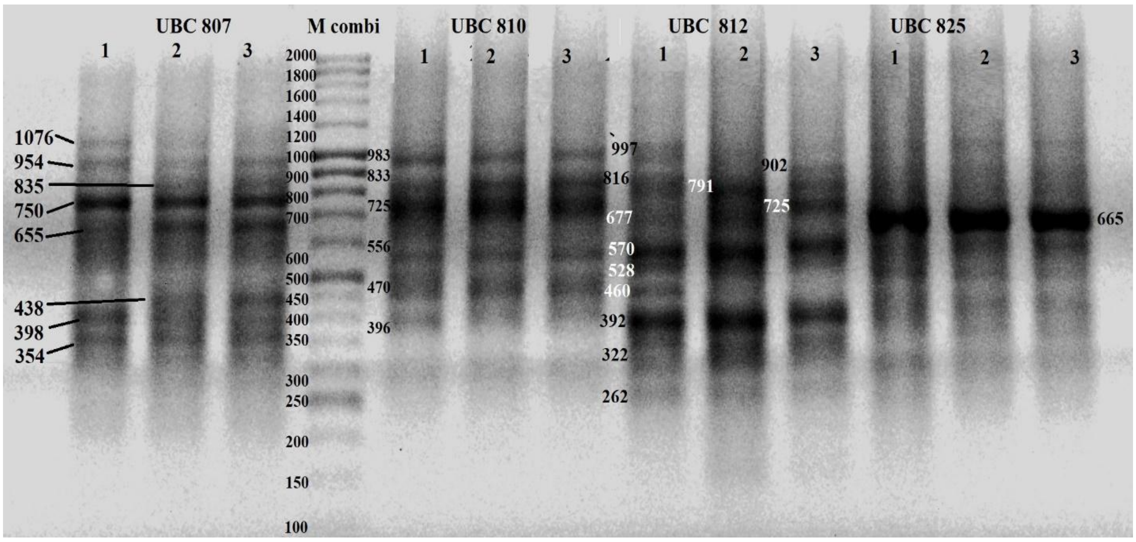

Fig. 4. Electrophoregram of garlic DNA amplification products with primers UBC 807, UBC 810, UBC 812 and UBC 825. m combi is a marker for determining the size of amplicons. 1 – Hungarian (line 20-16), 2 – Lubasha variety, 3 – Duchess variety.

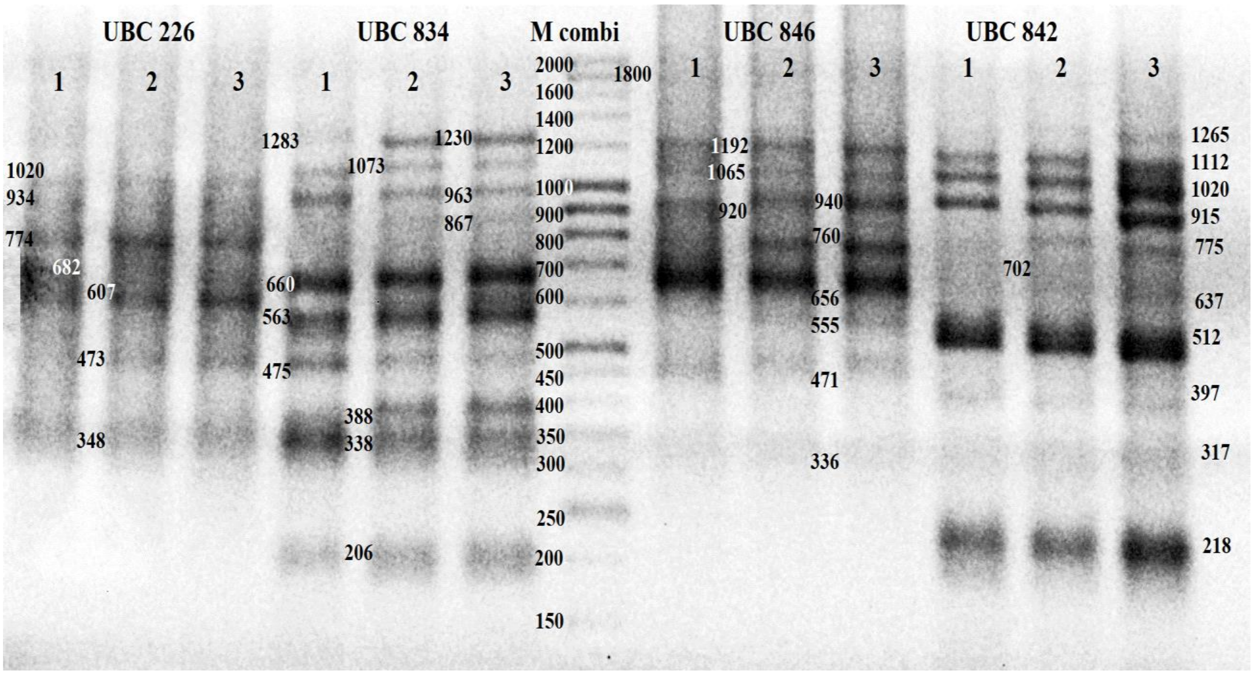

Fig. 4. Electrophoregram of garlic DNA amplification products with primers UBC 826, UBC 834, UBC 842 i UBC 846. m combi is a marker for determining the size of amplicons. 1 – Hungarian (line 20-16), 2 – Lubasha variety, 3 – Duchess variety.

In studied garlic varieties, amplicons on electrophoretic spectra differed in number, size, and level of manifestation. In Fig. 1 and 2 the obtained electrophoregrams are shown.

Amplification products could not be obtained using UBC 803 and UBC 804 primers. This may be due to the fact that microsatellites with sequences $(AT)_8C$ and $(TA)_8A$, respectively, are rare or do not occur in the genomes of the analyzed garlic varieties, or the distance between inverted repeats is too large for amplification in the mode we used, i.e. more than 2000 nucleotide pairs.

PASSPORT SYSTEMOF WINTER GARLIC VARIETIES BY ISSR LOCI

Identification of 65 loci of intermicrosatelitic repeats of winter garlic DNA allowed us to form allelic formulas of the studied varieties, which can be used as their molecular genetic passports. To develop these passports, we marked the loci in large Latin letters, next to which the size of the amplicon was indicated as a lower index. The loci were encrypted 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 obtained passports are shown in table 4.

Table 4.Molecular and genetic passports of winter garlic varieties

Varieties	Genetic passports
Hungarian (line 20-16)	$A_{354, 398, 655, 750, 954, 1076}$ $B_{396, 470, 556, 725, 833, 983}$ $C_{262, 322, 392, \mathbf{460}^{*}, 528, 570, 677, 816, \mathbf{997}}$ D_{665} $E_{348, 607, \mathbf{682}, 774, 934, 1020}$ $F_{206, 338, 475, 563, 660, 963, 1073, \mathbf{1283}}$ $G_{218, 317, 397, 512, 915, 1020, 1112}$ $H_{336, 471, 656, \mathbf{920}, 1065, 1192}$
Lubasha	$A_{354, 398, 438, 655, 750, 835, 954, 1076}$ $B_{396, 470, 556, 725, 833, 983}$ $C_{262, 322, 392, 528, 570, 677, \mathbf{791}}$ D_{665} $E_{348, 473, 607, 774, 934, 1020}$ $F_{206, 338, 388, 475, 563, 660, 867, 963, 1073, 1230}$ $G_{218, 317, 397, 512, 637, \mathbf{702}, 775, 915, 1020, 1112, 1265}$ $H_{336, 471, 555, 656, 760, 940, 1065, 1192}$
Duchess	$A_{354, 398, 438, 655, 750, 835, 954, 1076}$ $B_{396, 470, 556, 725, 833, 983}$ $C_{262, 322, 392, 528, 570, \mathbf{725}, 816, \mathbf{902}}$ 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 corresponding 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.

It should be noted that several amplicons unique within the studied group were found in the varieties of winter garlic presented in this paper (see fig. 1 and 2, table 3). For example, Duchess variety had unique loci UBC 812_{725} and UBC 812_{902}, while Lubasha variety had unique loci UBC 812_{791} and UBC 842_{702}. Hungarian variety (line 20-16) had a bigger number of unique loci: UBC 812_{460}, UBC 812_{997} UBC 826_{682}, UBC 834_{1283} and UBC 846_{920}. These sites can be used to develop more specific markers of the corresponding varieties.

PHYLOGENY OF WINTER GARLIC VARIETIES

The Nei-Li similarity coefficients calculated by us indicate a significant genetic similarity of the studied garlic varieties. It should be noted that Lubasha and Duchess varieties were genetically closer to each other (*Dij* = 0.0009) compared to Hungarian one (line 20-16) –*Dij* between this line and each of the varieties was 0.0035. The obtained results are shown in table 6.

The genetic similarity of the studied varieties may be the result of using genetically uniform source material when creating these varieties.

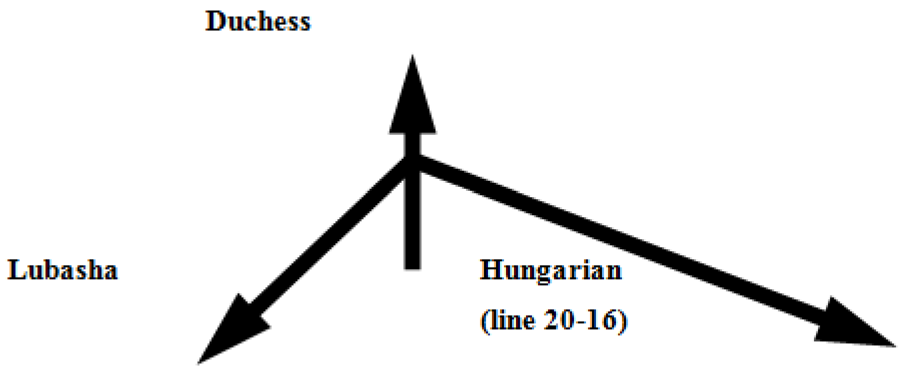

Fig. 6. The tree of phylogenetic relations of winter garlic varieties.

CONCLUSIONS

Based on the results of conducted studies the following conclusions should be drawn:

1. The highest level of commercial cropping capacity on average over the years – 14.1 t/ha, was obtained when growing winter garlic of Lubasha variety. At the same time, in 2018 and 2019, there was no significant difference in the cropping capacity between this variety and Hungarian variety, and in 2017, Hungarian variety formed a significantly higher cropping capacity – 10.5 t/ha, which is 1.3 t/ha more than Lubasha variety by SSD_{05} – 0.65 t/ha.

2. Agronomically stable (As > 70 %) was Hungarian winter garlic variety (line 20-16) with As = 73.8%.

3. Based on the obtained results, allelic formulas (molecular and genetic passports) of Hungarian winter garlic varieties (line 20-16), Lubasha, and Duchess were formed.

4. Several amplicons unique within the studied group were found in the studied garlic varieties. Duchess variety has unique loci UBC 812_{725} and UBC 812_{902}, while Lubasha variety has unique loci UBC 812_{791} and UBC 842_{702}. Line 20-16 had bigger number of unique loci: UBC 812_{460}, UBC 812_{997} UBC 826_{682}, UBC 834_{1283} and UBC 846_{920}.

5. The calculated Nei-Li similarity coefficients indicate a significant genetic similarity of the studied varieties of winter garlic. Lubasha and Duchess varieties are genetically closer to each other, compared to Hungarian variety.

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