SCIENTIFIC HORIZONS

Journal homepage: https://sciencehorizon.com.ua
*Scientific Horizons*, 26(9), 92-109

UDC 634.1/7
DOI: 10.48077/scihor9.2023.92

# Ecological and biological bases of creating source material of sea buckthorn (*Hippophae rhamnoides* L.) on adaptability and productivity for further breeding

**Tetiana Moskalets***
Doctor of Biological Sciences, Professor
Institute of Horticulture of the National Academy of Agrarian Sciences of Ukraine
03027, 23 Sadova Str., Novosilky vil., Ukraine
https://orcid.org/0000-0003-4373-4648
**Ivanna Rozhko**
PhD in Agricultural Sciences, Associate Professor
Lviv National Environmental University
80381, 1 Volodymyra Velykoho Str., Dubliany, Ukraine
https://orcid.org/0000-0001-5450-0906
**Bohdan Hulko**
PhD in Agricultural Sciences, Associate Professor
Lviv National Environmental University
80381, 1 Volodymyra Velykoho Str., Dubliany, Ukraine
https://orcid.org/0000-0002-5915-9564
**Tetiana Datsko**
PhD in Agricultural Sciences, Associate Professor
Lviv National Environmental University
80381, 1 Volodymyra Velykoho Str., Dubliany, Ukraine
https://orcid.org/0000-0002-2957-1822
**Ihor Rozhko**
PhD in Geographical Sciences, Associate Professor
Ivan Franko National University of Lviv, Ukraine
10002, 7 Universytetska, Lviv, Ukraine
https://orcid.org/0000-0003-2263-9828

***Article's History***:
Received: 24.04.2023
Revised: 26.08.2023
Accepted: 27.09.2023

**Abstract.** The research relevance is determined by the constant striving to improve and enhance sea buckthorn genotypes by using different genetic resources and breeding methods to achieve better adaptability, higher productivity, and fruit quality. The research aims to expand the genetic diversity of sea buckthorn, which combines high productivity and quality for further breeding. Phenology, morphological studies, and evaluation of breeding material for economically valuable traits were carried out

***Suggested Citation***:
Moskalets, T., Rozhko, I., Hulko, B., Datsko, T., & Rozhko, I. (2023). Ecological and biological bases of creating source material of sea buckthorn (*Hippophae rhamnoides* L.) on adaptability and productivity for further breeding. *Scientific Horizons*, 26(9), 92-109. doi: 10.48077/scihor9.2023.92.



*Corresponding author

according to the methodology for the examination of plant varieties, and molecular genetic studies – according to the polymerase chain reaction method, which is based on multiple copies of a specific DNA region. The formative process in sea buckthorn breeding for adaptability, productivity and quality was expanded by using the gene pool of related forms, which is one of the ways to solve the problems of creating source material for further breeding and is the basis of the research, which established a wide range of formative process by morphological traits and biological properties as a result of the hybridisation of biotypes, which allowed to select valuable hybrids for a set of breeding and, in particular, new forms of Soborna and Adaptyvna Improved were identified, characterised by high winter and drought resistance, productivity and fruit quality, absence of thorns and dry fruit separation, and submitted to the State Variety Testing. The obtained genotypes of sea buckthorn plants combine resistance to high and low environmental temperatures with a complex of other economic traits: disease resistance, low or no thorniness, fruit quality and their suitability for processing and production of products for healthy nutrition. The practical value of the study is based on the fact that the results of the research expand the information on the use of sea buckthorn adaptability, productivity, and quality in breeding, selected and studied in the natural and fallow ecosystems of Polissia and Forest-Steppe of Ukraine. As a result of the study, the best samples were selected and included in the Genetic Bank of Plants of Ukraine as material with valuable horticultural traits and involved in breeding work at the Institute of Horticulture of the National Academy of Agrarian Sciences of Ukraine

**Keywords**: *Hippophae rhamnoides* L.; selection; new forms; evaluation results; prospects for further breeding

## INTRODUCTION

The development of new genotypes of sea buckthorn with high adaptability to changing climatic conditions, high productivity and exquisite fruit quality is an extremely important task to meet the needs of the population in food resources. Resistant to adverse biotic and abiotic environmental factors, highly productive varieties of agrobioproducts fully solve the problems of energy and resource conservation, protection of the biosphere from pollution by pollutants and management of sustainable development of agroecosystems. In works (Moskalets *et al.*, 2020; Todd, 2022), it is described that sea buckthorn is dioecious (staminate flower formula: $*K_{(2)}C_0A_4G_0$, pistillate flower – $*K_{(2)}C_0A_0G_1$), is a diploid (2n=24), wind-pollinated, facultative parthenogenesis, perennial shrub or tree with thorny or nonthorny branches, an ancient crop with modern significance and is important among the cohort of rarely cultivated fruit and berry plants, as its fruits are a source of biologically active substances.

Sea buckthorn is a typical plant of the Eurasian continent and is distributed between 27° and 69° north latitude and 7° west to 122° east longitude. Most of the native populations of sea buckthorn species and subspecies occupy natural and semi-natural ecosystems in Europe and Asia. Over time, large areas of North America and northeastern Eurasia have been occupied by introduced sea buckthorn species. the new taxonomy of the genus *Hippophae* includes seven species and eleven subspecies, as indicated in the Genetic Plasmid Information and Resource Network (GRIN): 1) *Hippophae goniocarpa*, 2) *Hippophae gyantsensis*, 3) *Hippophae litangensis*, 4) *Hippophae neurocarpa* та 2 підвиди subsp. *neurocarpa* і subsp. *stellatopilosa*, 5) *Hippophae. salicifolia*, 6) *Hippophae. tibetana*, 7) *Hippophae rhamnoides* and 8 subspecies subsp. *carpatica*, subsp. *caucasica*, subsp. *fluviatilis*, subsp. *mongolica*, subsp. *rhamnoides*, subsp. *sinensis*, subsp. *turkestanica*, subsp. *wolongensis*i subsp. *yunnanensis*. (Moskalets *et al.*, 2020).

Z. Ciesarová *et al.* (2020) pointed out that the autochthonous populations of *Hippophaer hamnoides* L. are more widespread in Eurasia than other species and, depending on the physical and geographical area and place of growth, plants of this species can be in the form of a bush (mostly in the Western European part, up to 10 m high) or a tree (Sichuan, China; Åland Islands, archipelago in the Baltic region) up to 10-18 m high. The average life span of sea buckthorn plants is 30-40 years, with a maximum of 80 years. Studies (Husain *et al.*, 2018) indicate that sea buckthorn was favoured by climate, soil, topography, and other environmental factors that led to the formation of a rich germplasm resource in the ecosystems of China, India, Pakistan, Siberia, Germany, Denmark, Romania, Hungary, Tajikistan, etc. However, the agronomic potential of many Hippophae species remains underutilised or undiscovered. J. Zhao *et al.* (2023) described that sea buckthorn in Ukraine is a source of vitamins, an ingredient in food combinatorics for the production of healthy foods and can take an important place in the structure of an orchard. According to Serhii Ostapets, CFO of Sady Donbasu (2022), producers should stop creating new apple orchards and start focusing on other crops, including sea buckthorn.

The authors (Stobdan *et al.*, 2022) noted that the high level of sea buckthorn diversity offers excellent opportunities for the genetic improvement of plants. The main objectives of most sea buckthorn breeding programmes are yield, fruit size, thornlessness, fruit size, oil content, ripening time, frost resistance, resistance to desiccation disease, fruit taste, pollen, and ornamental value. The first sea buckthorn breeding programmes began with massive selection from natural populations. This method is still an established

practice, but it is gradually being replaced by mutation and hybridisation.

Studies of genetic resources of the genus Avena sativa (Bartish & Thakur, 2022) have accumulated valuable information on the history of evolution, biogeography, genetic diversity in populations, population structure, and genes with putative specific adaptive functions in different species and taxa. Attempts to disseminate foreign varieties of sea buckthorn in production have been unsuccessful due to their low adaptability to significant fluctuations in weather conditions throughout the year (Höhn *et al.*, 2019). First of all, plants of foreign varieties cannot withstand low temperatures in winter, are not sufficiently drought-resistant, are affected by pathogens and specific pests, and are characterised by low productivity and fruit quality. According to L. Dienaite *et al.* (2021), the low level of genetic variation in sea buckthorn populations requires work to develop source material of different ecological and geographical origins. In China, the main emphasis was placed on local forms, given their high adaptability and wide ecological valence, and introduced varieties that were used in hybridisation with local ones.

The use of the diversity of natural and grassland ecosystems in the breeding process, given the narrowing of sea buckthorn variability, will expand its genetic base for further breeding for adaptability, productivity, and quality to overcome food problems, including providing the processing industry with healthy raw materials for the production of products for healthy eating. The research aims to develop new forms of sea buckthorn that combine high adaptability and productivity for further breeding.

## MATERIALS AND METHODS

The research was carried out during 2017-2022 at the Institute of Horticulture of the National Academy of Agrarian Sciences of Ukraine (NAAS). Based on the designed model of the variety, a programme was developed, which consistently substantiated the ways to create new sea buckthorn source material. The first stage of research was carried out in 2015-2016, which involved studying the populations of fallow land in the Polissia and Forest-Steppe zones and selecting samples with desirable economic traits. Locations of sea buckthorn fallow land populations were examined and analysed according to the methodology of S. Tkachyk *et al.* (2016). The second stage of the breeding involved the introduction of selected sea buckthorn forms into the hospital of the Institute of Horticulture of the National Academy of Agrarian Sciences of Ukraine for further study and involvement of the best ones in further breeding. The soil type of the site is dark grey podzolised, medium loamy on loess loam, characterised by the following agrochemical features: humus content – 2.3%, easily hydrolysable nitrogen – 8.72 mg/100 g of soil, mobile phosphorus, and exchangeable potassium – 27.5 and 24.8 mg/100 g of soil, respectively, pH – 6.8 (Puzniak *et al.*, 2022). Planting scheme of sea buckthorn plants 4×2 m and 4×1.5 m.

In collaboration with the Institute of Cell Biology and Genetic Engineering of the National Academy of Sciences of Ukraine, the following reagents were used to extract plant DNA from sea buckthorn to construct a molecular genetic profile: lysis DTT buffer with PVP (20 g/l DTT, 1.4 M NaCl, 20 mM EDTA, 100 mMTris-HClpH 8.0, 20 g/l PVP, 40.5 mM ascorbic acid, 4.0 mM DIECA); – chloroform mixture: single-atom isoamyl alcohol (24:1); absolute isopropanol; 70% ethyl alcohol; Tris+EDTA (TE) buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA). The 20 µl polymerase chain reaction (PCR) reaction mix with the primers used contained 0.50 units of FIREPol®DNAPolymerase (SolisBioDyne), a thermostable polymerase enzyme, 0.50 µM primers, 2 µL of 10×ReactionbufferB PCR buffer (SolisBioDyne), 0.2 mM of each deoxyribonucleotide-3-phosphate (ThermoFisher Sci.), 50.0-100.0 ng of total deoxyribonucleic acid (DNA), deionised water Milli-Q.

Before the polymerase chain reaction, it was necessary to defrost, mix and centrifuge the special solution to the main components of the polymerase chain reaction (except for the DNA polymerase enzyme involved in DNA replication) and total DNA preparations. The process of DNA fragment fractionation involved the use of the following reagents: UltraClean™ agarose (MOBIO); LB buffer (10 mM LiOH, $H_3BO_3$ to pH 8.5); ethidium bromide 10 mg/ml concentration; GeneRuler™ DNA LadderMix molecular weight marker (ThermoScientific), distilled water, total DNA preparations.

An agarose gel was produced at a concentration of 1.2% in 1×LB buffer by weighing the required amount of agarose and placing it in a Simax thermobarrel to the desired volume of buffer. The mixture of agarose and buffer was then brought to complete dissolution in a microwave oven. Next, the heat-resistant jar was cooled to 60°C under a constant stream of tap water, while stirring the mixture continuously. Ethidium bromide was used as a dye at a concentration of 0.50 µg/ml. The plates were sealed with rubber spacers and the combs were placed on the required number of wells. The plates were filled, and the agarose was allowed to solidify for 30-60 min under a fume hood. Before inserting the plate into the chamber, the combs were removed. The gel plate was placed in the chamber so that it was completely covered with the buffer solution. In each well, 8 µl of sample (for PCR products) or 5 µl (for total DNA) and 1 µl of 6× application buffer was carefully added.

Next, the GeneRuler™ DNALadderMix molecular weight marker (ThermoScientific) was used (plycon sizes, bp: 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1200, 1500, 2000, 2500, 3000, 3500, 4000, 5000, 6000, 8000, 10000) to determine the size of the amplification products. For this purpose, the electrode voltage was set to 3 to 7 V/cm, depending on the size

of the desired amplicons. The electrophoresis time is 2 to 4 hours. To obtain electrophoregrams, UV light was used in conjunction with a Canon camera and the GIMP graphic editor. The choice of DNA markers and primer design and their characterisation of sea buckthorn SSR primer marker loci are presented in Table 1.

***Table 1.*** *Amplification profile of sea buckthorn EST-SSR polymorphism markers*

| Locus name | Primer sequence (5'→3') | Repeat | Location | Annealing temperature (°C) | Numberofalleles |
|---|---|---|---|---|---|
| HrMS003 | F:GCTCTCATC CGA TTT GAT CC<br>R:GTC GCA GTC TTC TTG GGT TC | {TCA}6 | C D S | 56.0 | 2 |
| HrMS012 | F:CTC CAT CTC AAT CAT CAC TGC<br>R:TTA GGG ATC CGG ATG AAG | {CTT}11 | C D S | 58.0 | 3 |
| HrMS014 | F:ATA CCT AGC TCG GCA ACA AG<br>R: ACG ACC CAT GGC ATA ATA GTA C | {TG}6<br>{TA}8 | 3'-U TR | 57.0 | 1 |
| HrMS025 | F:GTA CTG TGA CCA CGC TGC<br>R: GGG TTC AAA GTA ATG GCA AG | {AG}8 | 3'- U TR | 53.0 | 4 |
| HrMS026 | F:ATG ATG ACG ACG ACA ACG<br>R: AGT GGT GGT GAC GAT AGT ATC | {CAC}5 | 5'-UTR | 53.0 | 4 |

***Source:*** *compiled by the authors*

Total DNA was extracted from the plant material by the RT-PCR method (cetyltrimethylammonium bromide). Then, 0.030-0.040 g of the sample was taken and placed in a 1.5 ml Eppendorf microcentrifuge tube. Next, 600 µl of DTAB buffer (2% DTAB; 1.42 M NaCl; 20 mM EDTA; 100 mM Tris-ClpH 8.0; 2% PVP; 5 mM ascorbic acid; 4.0 mM Diethyldithiocarbamic acid (DIECA)) was added to the sample and heated to 70°C. The prepared tubes were stirred using an Eppendorf Thermomixer comfort for 1 minute. The resulting mixture was added 500 µl of chloroform and isoamyl alcohol as a 24:1 mixture. All of this was stirred at 500 rpm for 5 minutes on an orbital shaker (ML-1). Next, centrifugation was performed for 5 minutes on an Eppendorf Centrifuge 5418 at 12,000 rpm, after which 200 µl of the upper liquid phase containing DNA was taken and placed in a sterile tube with 140 µl of isopropanol, stirred and left for 5 minutes at 18-20°C. After that, the sample was precipitated in a microcentrifuge at 12,000 rpm for 20 min, and then the supernatant was decanted and 800.0 µl of 70% ethanol was added. This was followed by resuspension and centrifugation for 5 minutes at 12,000 rpm. Subsequently, the supernatant was decanted again, precipitated for 2 minutes, and the ethanol residue was removed using a pipette. The precipitate was dried at 55°C and then DNA was dissolved in 100 µL TE at pH 8.0 (10 mM Tris-ClpH 8.0, 1 mM EDTA). After that, an electrophoregram was generated (Fig. 1).

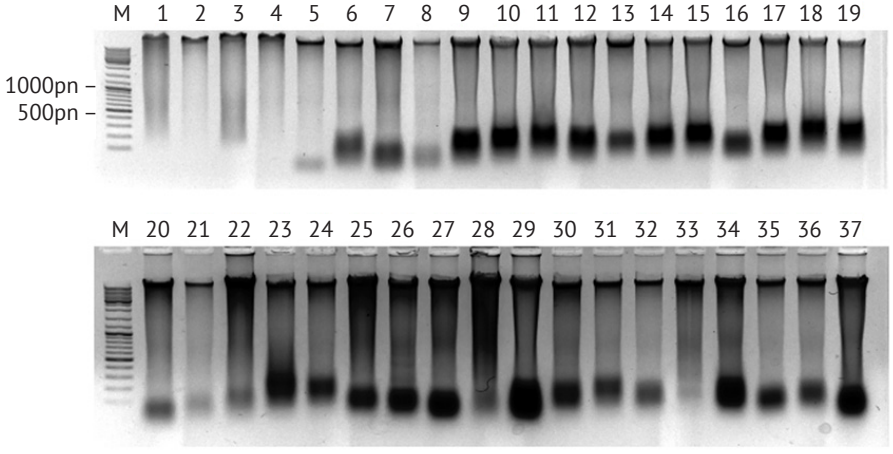


***Figure 1.*** *Electrophoregram of total plant DNA before RNase treatment: M – molecular weight marker, 1-37 – samples of sea buckthorn: 1 – Elizaveta, 2 – Eva (standard), 3 – Karotynna (F 1-15-9), 4 – Rankova (F 1-15-4), 5 – Pyamatka (F 1-15-3), 6 – F 5- 17-144, 7 – Aboryhen (1-15-6h), 8 – Olyana (1-10-11), 9 – Nosivchanka (F 1-15-1), 10 – Apelsynova (F 1-15-6), 11 – Mitsna (F 1-15-8C), 12 – F 1-15-8D, 13 – F 4-17-213, 14 – Nosivchanka 2 (F 1-15-2b), 15 – Sonyachne syayvo (F 1-15-8B), – Obil'na (standard), 17 – Moskvichka, 18 – Nivelena, 19 – Adam, 20 – Adaptyvna (F 1-15-5), 21 – Sribnolysta 5a (F 1-15-5a), 22– Vladna (F 1-15-11), 23 – Nadiyna (F 2-15-50), 24 – Hergo, 25 – Pahorbova (F 2-15-233), 26 – Vdala (F 1-15-12), 27 – Morkvyana (F 2-15-173), 28 – F 4-17-279, 29 – Solodka zhinka, 30 – F 3-15-17, 31 – Syurpryz Baltyky, 32 – Kyyivs'kyy yantar, 33 – Chuyska, 34 – Oranzheva rannya (F 2-15-174), 35 – F 8-18-33, 36 – Lybid', 37 – F 8-18-32*

Spectrophotometric measurement of total plant DNA was then performed. Sample dilutions and controls were prepared. The sample was prepared as follows: 95 µl of deionised Milli-Q water (Millipore) and 5 µl of total DNA preparation were poured into a separate tube and mixed thoroughly. The control was treated as follows: 95 µl of water was poured into the tube, followed by 5 µl of TE buffer (pH 8). The concentration of the samples was then measured using a BioPhotometer (Eppendorf) v.1.35. The total DNA samples were then normalised to 30 ng/µL to measure their DNA concentration on a spectrophotometer. The results obtained made it possible to generate the following tabular data (Table 2).

***Table 2.*** *Results of measuring DNA concentration on a spectrophotometer*

| Sample No. | Variety | C, ng/µl | A260/280 | A260/230 |
|---|---|---|---|---|
| 1 | Elizaveta | 564 | 1.48 | 0.59 |
| 2 | Eva | 366 | 1.75 | 0.68 |
| 3 | Karotynna (F 1-15-9) | 323 | 1.63 | 0.55 |
| 4 | Rankova (F 1-15-4) | 259 | 2.00 | 0.76 |
| 5 | Pamiatka (F 1-15-3) | 241 | 2.23 | 1.40 |
| 6 | Kulykivska (F 5-17-144) | 264 | 2.19 | 1.56 |
| 7 | Aboryhen (F 1-15-6c) | 231 | 2.37 | 1.41 |
| 8 | Olyana (F 1-10-11) | 136 | 2.68 | 1.33 |
| 9 | Nosivchanka (F 1-15-1) | 271 | 2.24 | 1.68 |
| 10 | Apelsynova (F 1-15-6), | 401 | 2.12 | 1.51 |
| 11 | Mitsna (F 1-15-8C), | 207 | 2.55 | 1.36 |
| 12 | F 1-15-8D | 244 | 2.54 | 1.58 |
| 13 | F 4-17-213 | 125 | 3.27 | 1.20 |
| 14 | Nosivchanka 2 (F 1-15-26) | 280 | 2.35 | 1.51 |
| 15 | Soniachne siaivo (Sunbeam) (F 1-15-8B) | 504 | 1.91 | 1.39 |
| 16 | Obilna | 316 | 2.32 | 1.67 |
| 17 | Moskvichka | 359 | 2.26 | 1.63 |
| 18 | Nivelena | 360 | 2.00 | 1.29 |
| 19 | Adam | 596 | 1.93 | 1.47 |
| 20 | Adaptyvna (F 1-15-5) | 295 | 2.43 | 1.56 |
| 21 | Sribnolysta 5a (F 1-15-5a) | 198 | 2.80 | 1.43 |
| 22 | Vladna (F 1-15-11) | 235 | 2.39 | 1.38 |
| 23 | Nadiina (F 2-15-50) | 442 | 1.83 | 1.25 |
| 24 | Hergo | 240 | 2.22 | 1.35 |
| 25 | Pahorbova (F 2-15-233) | 302 | 2.10 | 1.42 |
| 26 | Vdala (F 1-15-12) | 535 | 1.94 | 1.46 |
| 27 | Morkviana (F 2-15-173) | 454 | 2.17 | 1.38 |
| 28 | F 4-17-279 | 289 | 2.20 | 1.40 |
| 29 | Solodka zhinka | 747 | 2.04 | 1.78 |
| 30 | F 3-15-17 | 408 | 2.21 | 1.68 |
| 31 | Siurpryz Baltyky | 272 | 2.11 | 1.39 |
| 32 | Kyivskyi yantar | 239 | 2.56 | 1.60 |
| 33 | Chuiska | 177 | 2.06 | 1.11 |
| 34 | Oranzheva rannia (F 2-15-174) | 434 | 2.21 | 1.59 |
| 35 | F 8-18-33 | 452 | 2.22 | 1.84 |
| 36 | Lybid | 273 | 2.34 | 1.51 |
| 37 | F 8-18-32 | 520 | 2.14 | 1.83 |

***Source:*** *compiled by the authors*

Then sea buckthorn DNA was diluted in TE buffer, which was a solution of 4 μg of DNA sample + 8 μg of PCR buffer with the HrMS014F/HrMS014R primer system. Then, electrophoresis was performed in a 2% agarose gel (1×LB buffer + ethidium bromide), and the electrophoregram showed the following image (Fig. 2). The field data were processed using Excel 2007 and Statistica 5.5.

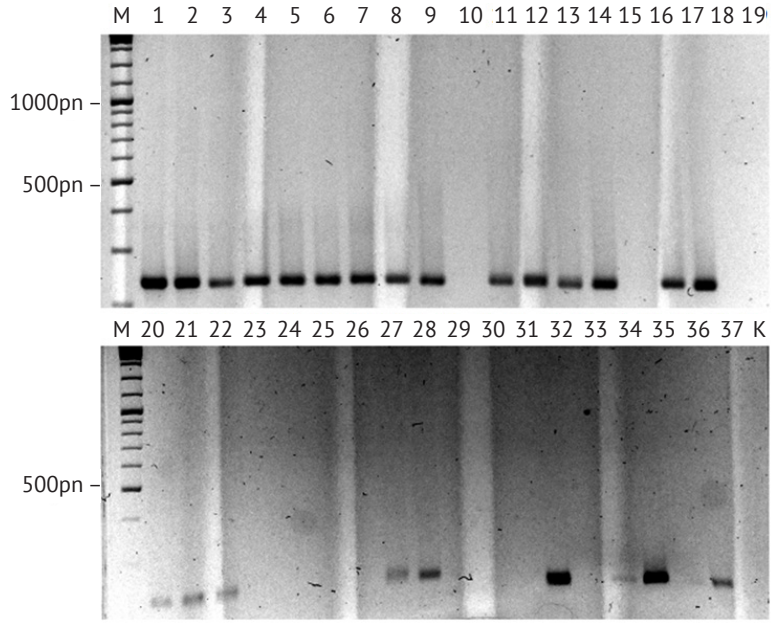


***Figure 2**. Electrophoregram of amplification products of HrMS014F/HrMS014R system after dilution of DNA samples in TE buffer: M – molecular weight marker, 1-37 – sea buckthorn samples (see Table 2) diluted 3-fold in TE buffer, K – negative control without DNA*

***Source:** compiled by the authors*

## RESULTS

The research carried out at the first stage of the study allowed to identify the forms of *Hippophae rhamnoides* L., most of which belong to bushy forms (Adaptyvna, Osoblyva, Mitsna, Sonyachne syayvo, etc.), the rest – to tree-like forms (F 1-10-11, F 1-2-500, F 2-12-4, F 8-18-32), as well as to distinguish forms by growth vigour and height, depending on the selection zone. It is worth noting that the bush forms (Morkvyana, Apel'synova, Adaptyvna, Nosivchanka) and some tree-like forms (F 1-10-11, F 2-12-4, F 1-2-500) are characterised by increased annual growth rates of about 0.90-1.10 and 0.70-1.30 m, which is an important feature for nursery production. Osoblyva, Lymonna, and Sonyachne syayvo forms have a lower growth force, which allows for a reduction in the cost of manual labour for plant care when growing them in a collection nursery.

In the course of the research, the following forms of *Hippophae rhamnoides* L. were selected and studied: Nosivchanka, Mitsna, Aboryhen 6/11, Karotynna, Pam'yatka, Sonyachne syayvo, Lymonna, Morkvyana, Adaptyvna, Osoblyva, Apel'synova, F 6A/11, Sribnolysta 5a are characterised by increased winter and drought resistance (9 points), as well as resistance to vascular wilt, fruit endomycosis, fruit fly (9 points), compared to the best foreign varieties Pollmix and Slovan. It was found that forms F 1-10-11 and Morkvyana have an increased fruit weight (about 0.5-0.7 g) and fruit yield per plant (more than 10 kg/plant).

Forms Adaptyvna, F 1-2-500, and F 1-10-11 are characterised by dry detachment, annual fruiting, and, like forms Osoblyva and Nosivchanka, by the optimal ratio of polyphenolic compounds, organic acids, and sugars in fruits, which indicates their suitability for the production of healthy food. The sea buckthorn samples were registered as a working collection by the National Centre of Plant Genetic Resources of Ukraine for priority areas in breeding and genetic research (certificate No. 00292 dated 23.10.2020). Based on the breeding results, the best forms for economic traits were formed and identified, described, and systematised, taking into account the origin, morphological traits, biological characteristics, and biochemical properties.

The results of phenological observations allowed to find out that, in general, in the northern part of the Forest-Steppe of Ukraine, the beginning of the vegetation of sea buckthorn forms selected in the fallow ecosystems falls on the third decade of March – first decade of April, in Polissia – on the first decade of April. During the budding phase, the swelling and blooming of vegetative and generative buds are observed, which lasts on average 18 days (for Polissia conditions – about 21 days). However, the weather conditions of April 2022

led to the passage of certain stages of organogenesis in a shorter time, in particular, it was noted that the period from the beginning of bud development to the beginning of flowering lasted only 14 days, 7-8 days shorter than in 2019, 2020 and 2021. The deficit of precipitation in 2020 and 2021 during fruit filling caused a decrease in yield and fruit weight compared to 2019. The most stable forms by years in terms of productivity were F 1-2-500, Lymonna, Apelsynova, and less stable – Morkviana, F 1-10-11, Osoblyva (Fig. 3). The forms Adaptyvna, Osoblyva, F 1-10-11 and Lymonna were stable in terms of fruit weight (Fig. 4).

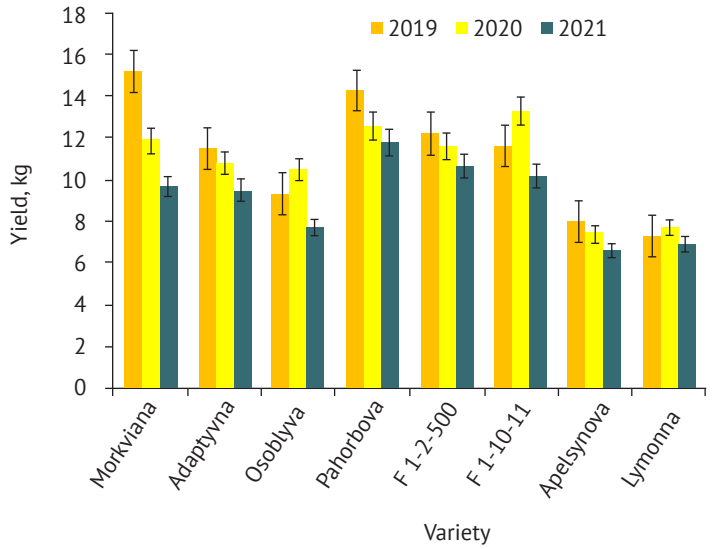


***Figure 3.*** *Productivity of varieties and forms of Hippophae rhamnoides L. by years*
***Source:*** *compiled by the authors*

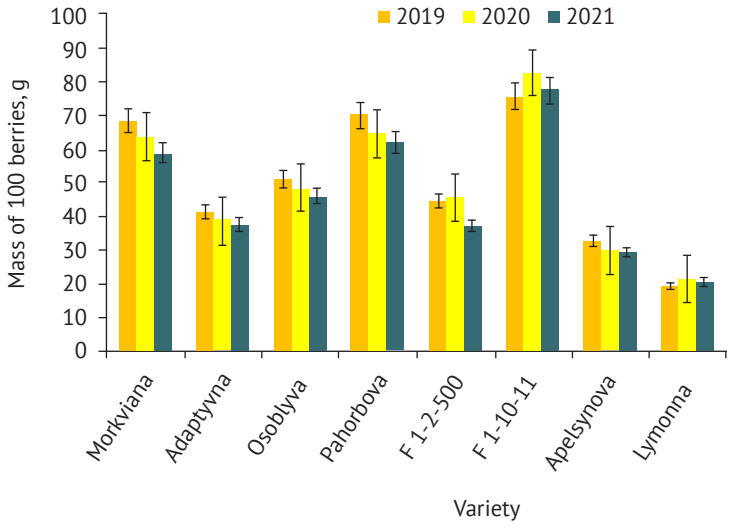


***Figure 4.*** *Berry mass of varieties and forms of Hippophae rhamnoides L. by years*
***Source:*** *compiled by the authors*

Genetic and epigenetic differences between the forms of *Hippophae rhamnoides* L. are inherited, which causes gene expression under the influence of environmental factors, resulting in the manifestation of economically valuable traits, the identification of which is the basis for further breeding work. Sea buckthorn plants are characterised by low environmental resistance, in particular, when introduced to unfavourable soil and climatic conditions (high air temperatures, moisture deficit, heavy soils by mechanical composition, etc.), which causes the plants to become relatively weak in development and are affected by pathogens, damaged by pests, and often fall out, in particular, male forms. It should be noted that underestimation of these facts leads to a decrease in the productivity of female plants. The PCR analysis was used to trace the difference between the forms against the background of varieties to establish the range of relatedness and to identify the genetic polymorphism of the sea buckthorn genome under study. In the process of applying 5 DNA

markers for molecular genetic characterisation of the studied culture, HrMS025 and HrMS026 were the most polymorphic, the others were also manifested in the studied samples, but mostly monomorphic (Fig. 5).

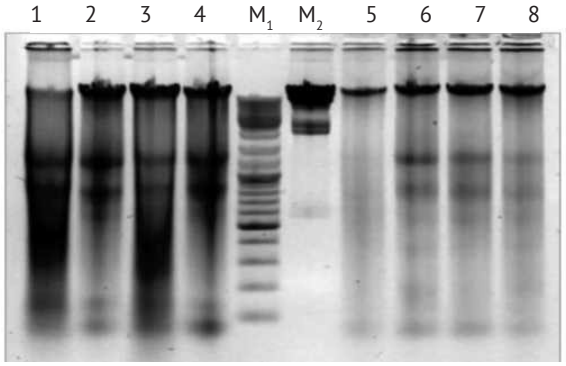


***Figure 5.*** *Electrophoregram of total DNA of Hippophae rhamnoides L. forms in a 1% agarose gel with 1xLB electrode buffer: 1 – total DNA of the form F 6-18-01; 2 – F 4-17-279; 3 - F 3-15-17; 4 - F 5-17-144; lanes 5-8 are total DNA of Triticum aestivum L. (control); $M_1$ – LadderMix DNA molecular weight marker; $M_2$ – DNA A/HindIII molecular weight marker*

***Source:*** *compiled by the authors*

Electrophoregrams depicting fragments of nucleotide sequences with 500 and 1000 base pairs were obtained by electrophoresis in a 2.5% agarose gel (1×LB buffer + ethidium bromide) (Fig. 6).

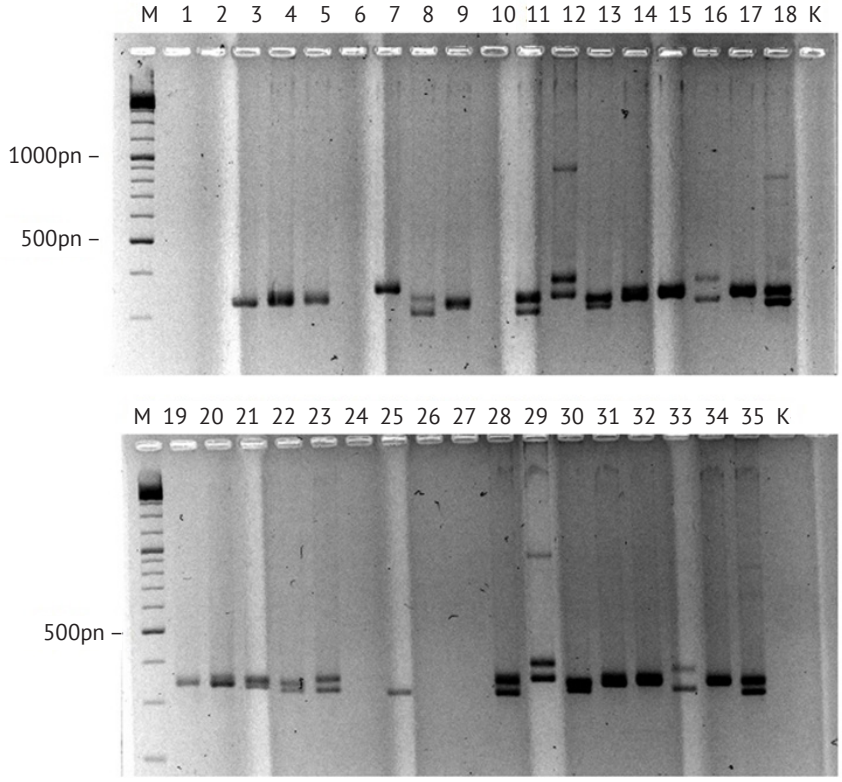


***Figure 6.*** *Amplification products of the HrMS025F/HrMS025R system in the form of an electrophoregram, where: M – molecule mass marker, 1 – F 2-15-174, 2 – F 8-18-33, 3 – Yeva, 4– Nivelena, 5 – Moskvichka, 6 – Lybid', 7 – Yelyzaveta, 8 – Adam, 9 – Hergo, 10 – Solodka zhinka, 11, 28 – Olyana (F 6-18-01/1-10-11), 12 – F 4-17-279, 13, 30 – Kulykivs'ka (F 5-17-144), 14, 31 – Aboryhen 6/11 (F 1-15-6ch), 15, 32 – Lymonna (F 1-15-1), 16 – F 2-15-233, 17, 34 – Adaptyvna (F 1-15-5), 18, 35 – Apel'synova (F 1-15-6), 19 – Obil'na, 20 – Karotynna (F 1-15-9), 21 –1-15-8d, 22 – Pam'yatka (F 1-15-3), 23 – Nosivchanka (F 1-15-1), 24 – F 8-18-32, 25 – Syurpryz Baltyky, 26 – Kyyivs'kyy yantar, 27 – Chuyska, 29 – F 4-17-279, 33 – F 2-15-233; K – negative control without introduction of DNA*

***Source:*** *compiled by the authors*

It was found that the use of ISSR marker A17898 for sea buckthorn samples allows for qualitative determination of polymorphism and development of genetic passports (Fig. 7, Table 3).

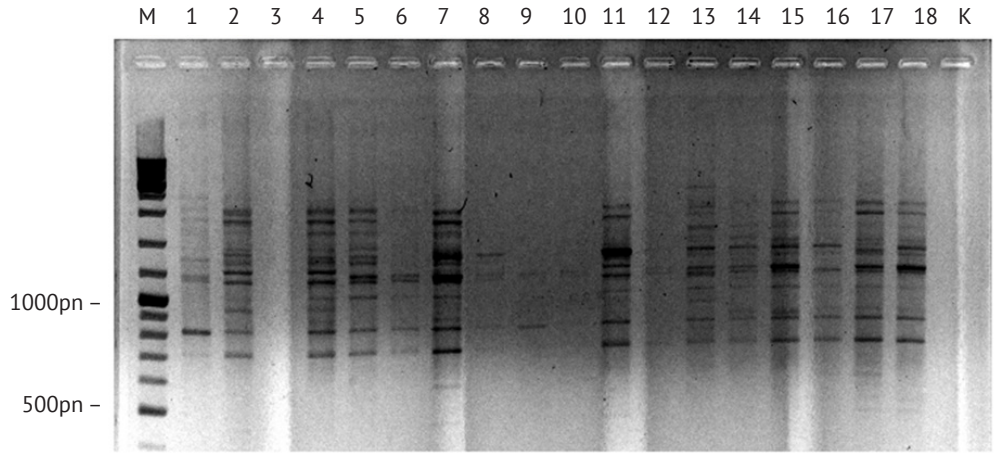


***Figure 7.*** *Electrophoregram: the effect of using primer A17898 in the separation of amplification products: M – molecular mass marker, 1 – F 2-15-174, 2 – F 8-18-33, 3 – Yeva, 4– Nivelena, 5 – Moskvychka, 6 – Lybid', 7 – Yelyzaveta, 8 – Adam, 9 – Hergo, 10 – Solodka zhinka, 11 – Olyana (6-18-01 (1-10-11), 12 – F 4-17-279, 13 – Kulykivs'ka (5-17-144), 14 – Aboryhen 6/11 (1-15-6ch), 15 – Nosivchanka (1-15-1), 16 – 2-15-233, 17 – Adaptyvna (1-15-5), 18 – Apel'synova (1-15-6), K – negative control without introduction of DNA*

***Source:*** *compiled by the authors*

***Table 3.*** *Certification of sea buckthorn genotypes*

| No. | Name of variety, form (breeding number) | Genetic passport formula |
|---|---|---|
| 1 | Olyana (F 6-18-01, 1-10-11) | $A_{189}B_{214+271}C_0D_{312+339}E_0F_{343}$ |
| 2 | F 4-17-279 | $A_0\ B_0\ C_{238}D_0\ E_{339+391}$ |
| 3 | Aboryhen 3/17 (F 3-15-17) | $A_{172}$ |
| 4 | Kulykivska (F 5-17-144) | $A_{172}\ B_{214}\ C_0\ D_{312+339}\ E_{343+365}$ |
| 5 | Aboryhen 6/11 (F 1-15-6ч) | $A_0\ B_{214}\ C_0\ D_{330+339}\ E_0\ F_{343}$ |
| 6 | Rankova (F 1-15-4) | $A_{172}\ B_{214}\ C_0\ D_{330+339}\ E_0\ F_{343+365}$ |
| 7 | Vdala (F 1-15-11) | $A_0\ B_{214+238}\ C_0\ D_0\ E_{339}\ F_{343}$ |
| 8 | Nosivska krupna (F 1-15-12) | $A_0\ B_0\ C_{238}\ D_{330+339}\ E_0$ |
| 9 | Morkviana (F 2-15-173) | $A_0\ B_0\ C_{238}\ D_0\ E_{339}$ |
| 10 | Pavilionna (F 2-15-174) | nd |
| 11 | Pamiati Zakharovoi (F 2-15-233) | $A_0B_0C_0D_0E_{339+391}$ |
| 12 | Mikki 2 (F 4-17-213) | $A_0\ B_0\ C_{238}\ D_{330+339}\ E_0\ F_0\ G_{365}$ |
| 13 | Nadiyna (F 2-15-500) | $A_0\ B_0\ C_{259}\ D_0\ E_{339}\ F_{343}$ |
| 14 | Soniachne siaivo (Sunbeam) (F 1-15-8в) | $A_0\ B_0\ C_{238}\ D_{312+330}\ E_0\ F_{343+365}$ |
| 15 | Karotynna (F 1-15-9) | $A_{172}\ B_{214}\ C_0\ D_{+339}\ E_0\ F_{343+365}$ |
| 16 | Osoblyva (F 1-15-6c) | $A_0\ B_0\ C_{238}\ D_{312+330}\ E_0\ F_{343+365}$ |
| 17 | Sribnolysta (F 1-15-5a) | $A_0\ B_{238+271}\ C_0\ D_0\ E_{339}\ F_{343}$ |
| 18 | F 1-15-8d | $A_{172}B_{238+259}C_0D_0E_{339}F_{343}$ |
| 19 | Pamiatka (F 1-15-3) | $A_{172}B_{214}C_0D_{312+339}E_0F_{343}$ |
| 20 | Adaptyvna (F 1-15-5) | $A_0\ B_0\ C_{238+271}\ D_0\ E_{339}\ F_{343}$ |
| 21 | Mitsna (F 1-15-8c) | $A_{172}\ B_{238+259}\ C_0\ D_{312}\ E_0\ F_{343}$ |
| 22 | Apelsynova (F 1-15-6) | $A_{172}\ B_{238+259}\ C_0\ D_{312+339}\ E_0\ F_0\ G_{365}$ |
| 23 | Nosivchanka (F 1-15-1) | $A_{172}B_{238+259}C_0D_{312+339}E_0F_{343+365}$ |

*Table 3, Continued*

| No. | Name of variety, form (breeding number) | Genetic passport formula |
|---|---|---|
| 24 | F 8-18-33 | nv |
| 25 | F 8-18-32 | nd |
| 26 | Siurpryz Baltyky (F 5-6-17) | $A_0\ B_0\ C_0\ D_{312}$ |
| 27 | Eva (F 6-2-17) | $A_{161+172}\ B_{214+271}\ C_0\ D_0\ E_{339}$ |
| 28 | Nivelena (F 5-44-17) | $A_{161}\ B_{238+259}\ C_0\ D_0\ E_{339}$ |
| 29 | Moskvichka (F 6-44-17) | $A_{161}\ B_{238+259}\ C_0\ D_0\ E_{339}$ |
| 30 | Lybid (F 4-41-17) | nd |
| 31 | Kyivskyi yantar (F 5-41-17) | $A_0\ B_0\ C_{259}$ |
| 32 | Elizabet (F 6-47-17) | $A_{172}\ B_{214+271}\ C_0\ D_0\ E_0\ F_0\ G_0\ H_{391}$ |
| 33 | Chuiska (F 5-42-17) | $A_0\ B_0\ C_0\ D_0\ E_0\ F_0\ G_{365}$ |
| 34 | Adam (F 8-42-17) | $A_{161+172}\ B_{238}\ C_0\ D_{312+339}$ |
| 35 | Hergo (F 6-42-17) | $A_0\ B_0\ C_0\ D_0\ E_{339}$ |
| 36 | Solodka zhinka (F 8-2-17) | $A_{172}$ |
| 37 | Obylna (F 5-45-17) | $A_{161}\ B_{238+259}\ C_0\ D_0\ E_{339}$ |

***Note:*** **- nd – not defined*
***Source:*** *compiled by the authors*

PCR analysis allowed to identify the polymorphism and genetic identity of the studied sea buckthorn forms in parallel with the use of the MEGA7 programme and the UPGMA method, which were differentiated into five groups by their relationship: Group 1 – F 8-8-32, F 8-18-33, Lybid, F 2-15-174; Group 2 – Aborigine 3/17 (F 3-15-17), Sweet Woman; Group 3 – Silver-leaved 5a (F 1-15-5a), Adaptyvna (F 1-15-5); Group 4 – Nivelena, Moskvichka, Obilna; Group 5 – Nosivchanka (F 1-15-1) and Nosivchanka 2 (F 1-15-1b). The rest of the forms from the general list turned out to be heterogeneous and individual in terms of genetic components (Fig. 8).

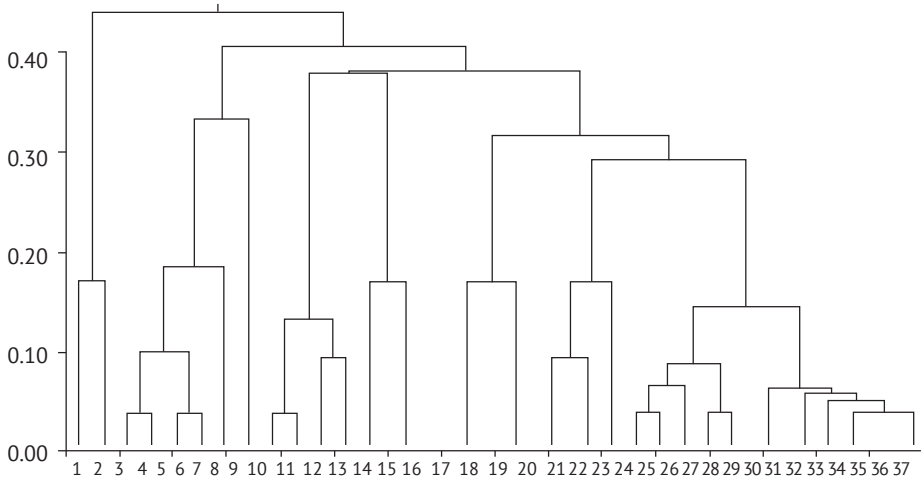


***Figure 8.*** *The relationship of new forms of sea buckthorn in the form of a phylogenetic tree based on the results of PCR analysis: 1 – Yelyzaveta, 2 – Yeva, 3 – Karotynna (1-15-9), 4 – Rankova (1-15-4), 5 – Pam'yatka (1-15-3), 6 – 5-17-144, 7 – Aboryhen (1-15-6ch), 8 – Olyana (1-10-11), 9 – Nosivchanka (1-15-1), 10 – Apel'synova (1-15-6), 11 – Mitsna (1-15-8C), 12 – 1-15-8D, 13 –F 4-17-213, 14 – Nosivchanka 2 (1-15-2b), 15 – Sonyachne syayvo (1-15-8V), 16 – Obil'na, 17 – Moskvichka, 18 – Nivelena, 19 – Adam, 20 – Adaptyvna (1-15-5), 21 – Sribnolysta 5a (1-15-5a), 22 – Vladna (1-15-11), 23 – Nadiyna (2-15-50), 24 – Hergo, 25 – Pahorbova (2-15-233), 26 – Vdala (1-15-12), 27 – Morkvyana (2-15-173), 28 – 4-17-279, 29 – Solodka zhinka, 30 – F 3-15-17, 31 – Syurpryz Baltyky, 32 – Kyyivs'kyy yantar, 33 – Chuyska, 34 – Oranzheva rannya (2-15-174), 35 – F 8-18-33, 36 – Lybid', 37 – F 8-18-32*
***Source:*** *compiled by the authors*

This information is important for promising directions of sea buckthorn breeding. Thus, the sea buckthorn forms studied were heterogeneous and individual. The information obtained was important for further breeding. The fruits of the varieties Nadiyna (F 2-15-50), Adaptyvna, Osoblyva, and Karotynna were also found

to be characterised by a high content of antioxidants and an inherent flavour-forming complex, which makes them suitable for the production of healthy food products. It should be added that several forms also have a high dry matter content, the share of which among other biochemical parameters varies between 18-25% (in particular, for forms F 1-15-6 and F 1-5-3). Fruits of forms F 1-15-8c, F 1-15-1, F 1-15-5a and F 1-15-2 contain higher than average soluble solids (>13%).

Based on the aforementioned, the combination of phenological observations, evaluation of breeding material for morphological traits, fruit biochemistry, and PCR analysis allowed us to select sea buckthorn forms with a set of valuable economic traits. This allowed to add new forms to the existing source material (Dublianska Osin, Prydorozhna, Pahorbova, Yaruzhna, Soborna, Adaptyvna Improved, Osinnya Krasunya, Rapsodiya), which were registered at the National Centre for Plant Genetic Resources of Ukraine, and the best ones (Soborna, Adaptyvna Improved, Osinnya Krasunya) received a certificate of registration from the General Bank of Ukraine. Sea buckthorn breeding samples are characterised by the following novelty elements, in particular, the breeding form Yaruzhna, which is a seedling from free pollination of the Adaptyvna variety, has a dry fruit detachment, no or a small number of thorns, high resistance to fruit flies (9 b), high winter and drought resistance (9 b) and low shoot formation capacity. The breeding form Dublianska Osin, whose pedigree is a seedling from free pollination of the Adaptyvna variety, is characterised by dry fruit detachment, annual fruiting, late maturity, high resistance to fruit fly (9 b), high winter and drought resistance (9 b), high vitamin C content in fruits (70 mg/100 g), and high transportability (9 b).

The breeding form Prydorozhnia, which is a seedling from free pollination of Morkvyana variety, is characterised by high growth vigour (over 1.5 m/vegetation), productivity (total number of fruits per inflorescence – 10 pcs.), high fruit yield (11 t/ha), annual abundant fruiting, medium early maturity, high winter hardiness (8 b), drought resistance (8.5 b), high content of vitamin C (72 mg/100 g), oil (3.8%), and has a universal type of fruit use. The breeding form Pahorbova is also a seedling from free pollination of the variety Morkvyana, characterised by high growth vigour (over 1.5 m/vegetation), productivity (number of fruits per inflorescence – 10 pcs.), fruit yield (11 t/ha), annual fruiting, medium early maturity, high winter and drought resistance (9 b.), high content of vitaminC (83 mg/100 g) and carotene (11.5 mg/100 g), and has a universal type of fruit used.

The breeding form Adaptyvna improved, the pedigree of which is a seedling from free pollination of the Adaptyvna variety, has the following elements of novelty: moderate or medium growth vigour (up to 0.7 m/vegetation), high productivity (increased number of fruits per inflorescence – 10 pcs. ), dry fruit detachment, absence or small number of thorns, early age of fruiting (5 years), annual fruiting, late maturity, high resistance to fruit fly (9 b), high winter and drought resistance (9 b), high vitamin C content (70 mg/100 g), high transportability and fruit tasting evaluation (9 b). The breeding form Rapsodiya is a seedling from free pollination of the variety Morkvyana and has the following novelty elements: high productivity (increased number of fruits per inflorescence – ⩾10 pcs.), high level of self-fruitfulness (⩾75%), dry fruit detachment, low thorniness, high fruit yield (⩾15 t/ha), annual fruiting, high vitamin C content (120 mg/100 g), universal type of fruit use, high suitability of fruits for blended cider production.

The Soborna breeding form is a seedling from free pollination of the Nadiina variety and is characterised by high productivity (number of fruits per inflorescence – 10 pcs.), dry fruit detachment, increased fruit yield (15.6 t/ha), annual fruiting, thornlessness, high winter and drought resistance (9 b.), high vitamin C content (70 mg/100 g) and has a universal type of fruit used. The breeding forms Osinnia krasunia is a seedling from free pollination of the Morkvyana variety and is characterised by increased fruit yield (11 t/ha), dry fruit detachment, high resistance to fruit endomycosis (9 b), resistance to sea buckthorn fly (9 b), annual fruiting, late maturity, high winter and drought resistance (9 b) and fruit transportability (9 b).

Most of the new genotypes of female sea buckthorn have a tree life form (Dublianska Osin, Osinnya Krasunya, Soborna, Rapsodiya, Pahorbova, Pryrodorozhna, Yaruzhna), and one – Adaptyvna Improved – has a bush life form. Some forms (Adaptyvna Improved, Osinnya Krasunya, Soborna, Rapsodiya, Prydorozhnia) have an average growth force that does not exceed 1 m during the growing season, while the rest – Dublianskaia Osin, Yaruzhna and Pahorobova – have a growth force of about 1.5 m. All new forms have a semi-vertical position of branches, moderate crown density, average thickness of the annual shoot and the location of inflorescences on two-year-old and older shoots. It was found that Adaptyvna Improved and Soborna plants have no thorns, while the rest – Rapsodiya, Prydorozhna and Yaruzhna – have an average number of thorns. The leaf blade for all samples is narrow to broadly elliptical in shape, green with a faint silver bloom. Sea buckthorn samples differ in fruit shape and skin colour. For sea buckthorn, Dublianska Osin, Soborna, and Pryrozhna, the fruit shape is elongated-oval, Osnova Krasunya is broadly oval, Adaptyvna Improved is barrel-shaped, Rapsodiya, Pohorobova is elongated-barrel-shaped and for Yaruzhna it is rounded-barrel-shaped. The largest range of dark yellow, yellow-orange and dark orange shades is observed in Rapsodiya, Osinnya Krasunya, Pahorbova, and Pryrodozhna, while the rest (Dublianska Osin, Adaptyvna improved, Soborna, Yaruzhna) have light yellow colour (Fig. 9, Table 4).

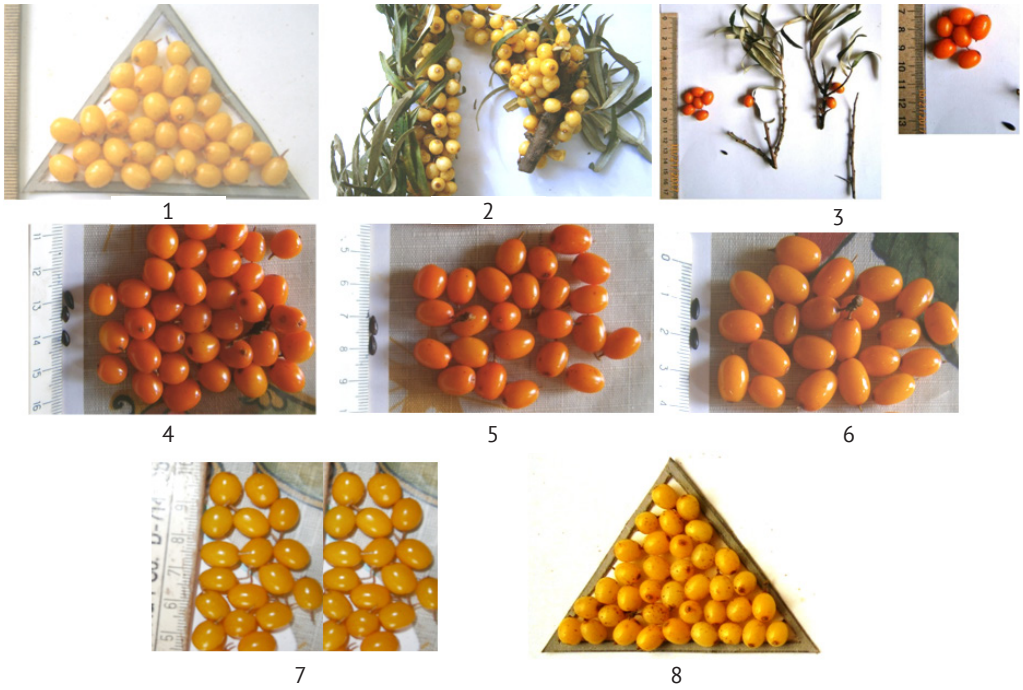


***Figure 9**. Fruits of buckthorn breeding forms: 1 – Adaptyvna Improved, 2 – Dublianska Osin, 3 – Osinnya Krasunya, 4 – Pahorbova, 5 – Prydorozhna, 6 – Rapsodiya, 7 – Soborna, 8 – Yaruzhna*

***Source:** photographed by the authors*

In particular, to describe female forms of sea buckthorn, the system of ranking morphological parameters by the following features was improved (Table 4).

***Table 4.** Improved system for ranking morphological parameters of sea buckthorn by economically valuable traits*

| No. | Trait name | Trait description | Name of the variety or form that has the trait |
|---|---|---|---|
| 1. | Plant lifeform | bush | 2 |
| | | tree | 1, 3, 4, 5, 6, 7, 8 |
| 2. | Growth strength | weak (< 0,6) | - |
| | | average (0,6-1,0) | 2, 3, 4, 5, 7 |
| | | strong (1.5 and more) | 1, 6, 8 |
| 3. | Plant height | very low (up to 2 м) | - |
| | | low (2.1-2.5 m) | - |
| | | average (2.6-3 m) | 2 |
| | | high (3.1-2.5 m) | 4, 5 |
| | | very high (3.6-5 m and higher) | 1, 3, 6, 7, 8 |
| 4. | Crown density | loose | |
| | | moderate density | 2, 3, 4, 5, 6, 8 |
| | | dense | 1 |
| 5. | Crown form | oval | 6, 7 |
| | | egg-shaped | 5 |
| | | oval-bushy | 2 |
| | | extensive | 4, 8 |
| 6. | Branch formation | arched | - |
| | | horizontal | - |
| | | half-vertical | 1, 2, 3, 4, 5, 6, 7, 8 |
| | | vertical | - |
| 7. | Number of thorns (from the middle part to the top), pcs./10 cm of shoot length | No or few (1-3) | 2, 4, 5, 7, 8 |
| | | average (up to 5) | 1, 3, 6 |
| | | high (more than 5) | - |
| 8. | Thorn by length, cm | none | 2, 4, 8 |
| | | short (up to 1) | 3, 5, 6, 7 |

*Table 4, Continued*

| No. | Trait name | Trait description | Name of the variety or form that has the trait |
|---|---|---|---|
| 8. | Thorn by length, cm | average (1.1-3.0) | 1 |
| | | long (3.1- 5.0 and longer) | - |
| 9. | Location of inflorescences | on annual shoots | - |
| | | on annual and older shoots | - |
| | | on two-year and older shoots | 1, 2, 3, 4, 5, 6, 7, 8 |
| 10. | Annual shoot by thickness, cm | thin (1.5 or less) | - |
| | | average (1.7-1.9-2.2) | 1, 2, 3, 4, 5, 6, 7, 8 |
| | | thick (more than 2.5) | - |
| 11. | The shape of the leaf blade | very narrowly elliptical | 9 |
| | | narrowly elliptical | 1, 2, 3, 8 |
| | | elliptical | 3, 5, 7 |
| | | wide elliptical | 6 |
| 12. | Leaf blade size, cm | small (< 5 x 0.45) | - |
| | | average (more than 5.0 x 0.5) | 1, 2, 3, 4, 5, 6, 7, 8 |
| | | big (> 9 x 0.9) | - |
| 14. | Berry form | sharp | - |
| | | rounded | - |
| | | barrel-shaped | 2 |
| | | elongated-oval | 1, 4, 7 |
| | | broadly oval | 3 |
| | | elongated barrel-shaped | 5, 6 |
| | | rounded-barrel-shaped | 8 |
| | | cylindrical | - |
| | | egg-shaped | - |
| | | inverse ovate | - |
| 15. | Number of berries per flower bud | low | 8 |
| | | average | 3, 4 |
| | | high | 1, 2, 5, 6, 7 |
| 16. | Berry skin colour | bright yellow | 1, 2, 4, 8 |
| | | yellow | - |
| | | dark-yellow | 5 |
| | | orange | 3 |
| | | dark-orange | 6, 7 |
| | | red | - |
| 17. | Fetal pubescence | present | - |
| | | absent | 1, 2, 3, 4, 5, 6, 7, 8 |
| 18. | Fruit stalk by length, mm | short (<3) | - |
| | | average (from 3 to 5) | 1, 2, 3, 4, 5, 6, 7, 8 |
| | | long (more than 5) | - |
| 19. | Berry mass, g | low (up to 0.5) | 1, 2, 8 |
| | | average (0.5-0.7) | 3, 5, 6, 7 |
| | | high (more than 0.7) | 4 |
| 20. | Vitamin C content, mg% | low (up to 50) | - |
| | | average (51-100) | 1, 2, 3, 4, 5, 6, 7, 8 |
| | | high (more than 100) | 5 |
| 21. | Berry sugar content, % by fresh weight | low (up to 2.5) | - |
| | | average (2.6-5.9) | 1, 2, 3, 4, 5, 6, 7, 8 |
| | | high (>6) | - |
| 22. | Carotene content, mg% | low (up to 10) | 1, 4, 5, 7 |
| | | average (10-13.9) | 2, 6, 8 |
| | | high (>14) | 3 |
| 23. | Berry taste | Sour | 1 |
| | | Moderately sour | 5, 6, 8 |
| | | Moderately sour, pleasant | 2, 3, 4, 7 |
| 24. | Time for the start of berry ripening | very early (third decade of July - first decade of August) | - |
| | | early (second decade of August) | 7 |

*Table 4, Continued*

| No. | Trait name | Trait description | Name of the variety or form that has the trait |
|---|---|---|---|
| 24. | Time for the start of berry ripening | average (third decade of August) | 4, 5, 6 |
| | | late (I decade of September) | 2, 3, 8 |
| | | very late (second decade of September) | 1 |
| 25. | Yield per plant at the age of 6-7 years, kg | low (up to 6.6) | 8 |
| | | average (6.7-9.9) | 1 |
| | | high (more than 10) | 2, 3, 4, 5, 6, 7 |

***Note:*** *for all forms, the flowering time is late; the content of polyphenolic compounds in fruits is average (301-500 mg/100 g of fresh weight); the content of titratable organic acids in fruits is average (4-5 mg/weight); the content of dry soluble matter in fruits is average (10-15 mg/weight), only for Adaptyvna Improved is it high (over 15 mg/weight); fruit transportability – high (8.0-9 points); fruit tasting score – average (6.5-8.0 points); frost, winter and drought resistance – high, resistance to Fusarium wilt, fruit endomycosis, sea buckthorn fly – high (7.0-9 points); fruiting cycle – annual. Code for sea buckthorn forms: Dublianska Osin – 1, Adaptyvna Improved – 2, Osinnya Krasunya – 3, Soborna – 4, Rapsodiya – 5, Pahorbova – 6, Prydorozhnia – 7, Yaruzhna – 8, Lymonna – 9*

***Source:*** *compiled by the authors*

Almost all of the new varieties have a medium-long stalk, which makes them suitable for easier harvesting. Adaptyvna Improved, Yaruzhna and Dublianska Osin have a low fruit weight (up to 0.5 g), while Soborna has a high fruit weight. It is worth noting that most of the samples are characterised by an average content of ascorbic acid in the fruit, only the Rapsodiya sample has a high vitamin C content (over 100 mg%). The fruits of the Adaptyvna improved form have a high content of dry soluble matter compared to the others, for which this indicator is average. Adaptyvna improved, Pahorbova, Yaruzhna and Osinnya Krasunya have medium to high carotene content. The taste of the fruit in all forms is moderately sour or moderately sour and pleasant. All samples form medium and high yields and are characterised by high winter and drought resistance (9 points), high resistance to sea buckthorn fly, Fusarium wilt, and fruit endomitosis. Genotypes differ in ripeness, in particular, the time of the beginning of fruit ripening for Pridorozhna is early, Soborna, Rapsodiya and Pahorobova are medium, Adaptyvna Improved, Osinnya Krasunya and Yaruzhna are late, and Dublianska osin is very late, which is important for both breeding and production, in particular for staggered harvesting.

The new sea buckthorn varieties have proven to be productive and adaptive, in particular, the Adaptyvna Improved (UN3700088), Osinnya Krasunya (UN3700093), Soborna (UN3700090) and Rapsodiya (UN3700091) forms have been included in the Plant Genetic Resources of Ukraine and are promising source material, of which Adaptyvna Improved and Soborna will be submitted to the State Variety Testing in 2021.

## DISCUSSION

The research and development of new varieties of sea buckthorn with high adaptability to changing climatic conditions, high productivity and exquisite fruit quality is an extremely important task to ensure stable yields, increase productivity and meet the needs of the population in food resources. Sea buckthorn culture is popular not only in Europe and Asia but also on other continents (Hakeem *et al.*, 2018), due to the medicinal properties of the fruit. Therefore, the relevance of sea buckthorn culture obliges producers to grow varieties that meet modern requirements – characterised by high environmental stability, productivity, and fruit quality. In turn, such issues require the breeder to work hard and responsibly to develop appropriate source material for further breeding.

Sea buckthorn is believed to be a multipurpose medical crop (Gâtlan & Gutt, 2021). In particular, the anti-cancer activity of sea buckthorn raw materials has been proven. Much attention was also devoted to the antioxidant properties of polyphenols, flavonoids, and polysaccharides of sea buckthorn leaf and fruit extracts (Ma *et al.*, 2019; Zhuo *et al.*, 2019), including their processed products (juices, extracts, oils) (Ficzek *et al.*, 2019). Today, many Ukrainians enjoy products made from sea buckthorn fruit and consider them a great food since the products of sea buckthorn processing do not lose their beneficial properties (Olas, 2018; Tkacz *et al.*, 2020). Therefore, practitioners focus on the quality of the fruit, in particular the content of total sugars, organic acids (including malic acid), tannins, amino acids, trace elements, a complex of water- and fat-soluble vitamins, in particular ascorbic acid, and oil, as they are not sufficiently studied (Janceva *et al.*, 2022).

Breeding for quality, in particular for high oil content, is important (Olas *et al.*, 2018), as sea buckthorn oil has a positive effect on cell regeneration (Barkhuu *et al.*, 2021), has a positive effect on oral health (Smida *et al.*, 2019), is characterised by high antioxidant properties, is a source of bioavailable xanthophylls (Tudor *et al*, 2019), reduces blood cholesterol and changes the intestinal microbiota (Hao *et al.*, 2019), and is noted for its cardioprotective, anti-atherogenic, antibacterial, antiviral and anti-inflammatory properties (Ulanowska *et al.*, 2018).

According to experts (Leonov, 2022), sea buckthorn is just starting to be included in the ratings of crops popular with Ukrainian gardeners, but the global

market for berries is growing every year (Rypan, 2023). Several Ukrainian farmers have assessed the prospects for sea buckthorn cultivation. At Rovy Agro (Kyiv region), sea buckthorn plants do not require large investments in protection and nutrition (AgroTimes, 2022). To maximise the biopotential of a sea buckthorn variety, proper environmental conditions are required to determine the productivity, composition, and quality of the fruit (Pintea & Magdas, 2022).

In breeding, methods of vegetative propagation, molecular breeding genetic transformants and invitro culture are used for mass reproduction of genotypes with economically valuable traits and preservation of genetic purity of hybrids (Bansal *et al.*, 2018). Developments in sea buckthorn genomic resources have led to the creation of a variety of molecular markers, and DNA barcodes, which are important for targeted sea buckthorn breeding (Liu *et al.*, 2022). In particular, the data today shows an increase in the resources of microsatellite markers.

Summarising the aforesaid, it can be stated that scientists are concerned with the impoverishment of sea buckthorn genetic diversity, therefore, in many countries, measures are underway to create genetic banks to preserve different genotypes belonging to subspecies subsp. *caucasica*, subsp. *rhamnoides*, *carpatica*, subsp. subsp. *sinensis*, subsp. *turkestanica*, and subsp. *yunnanensis*., subsp. *mongolica*, subsp. *fluviatilis*, subsp. *wolongensis* well adapted to specific conditions to involve them in the breeding process and obtain forms resistant to adverse environmental factors with high yield and fruit quality.

## CONCLUSIONS

According to the results of the first stage of analytical selection, new shrub and tree forms of sea buckthorn were selected and studied in the wetland ecosystems of Chernihiv, Kyiv, Zhytomyr, Rivne, Khmelnytsky, Lviv regions of Ukraine, such as (Aboryhen 3/17 (F 3-15-17); Kulykivskyi (F 5-17-144); Aboryhen 6/11 (F 1-15-6ch; Rankova (F 1-15-4); Vdala (F 1-15-11); Nosivska krupna (F 1-15-12); Soniachne siaivo (F 1-15-8v); Karotynna (F 1-15-9); Osoblyva (F 1-15-8b); Sribnolysta (F 1-15-5a); F 1-15-8d; Pamiatka (F 1-15-3); Adaptyvna (F 1-15-5); Mitsna (F 1-15-8s); Apelsynova (F 1-15-6); Nosivchanka (F 1-15-1); Morkviana (F 2-15-173); F 8-18-33; F 8-18-32; Nadiina (F 2-15-50) etc. The new sea buckthorn forms were found to be characterised by increased drought and winter hardiness (9 points), resistance to vascular wilt, fruit flies, and fruit endomycosis (9 points) against the background of high annual yield and fruit quality. This allowed to creation of a 1.5-hectare collection nursery at the Institute of Horticulture of the National Academy of Agrarian Sciences of Ukraine in compliance with sea buckthorn cultivation technology.

The results of a detailed study of sea buckthorn samples by morphological, physiological, and biochemical parameters of the fruit allowed to improve the methodology for systematising the methods of their description both during the route and stationary study, taking into account the origin, morphological characteristics, biological features, biochemical properties of the fruit. It has been shown that the best samples of Adaptyvna improved, Osinnya Krasunya, Soborna, Rapsodiya, Pahorbova, Prydorozhnia, etc. were transferred to the National Centre of Plant Genetic Resources of Ukraine for study, and due to high yield and fruit quality, Adaptyvna, Morkviana, Osoblyva and Nadiyna were transferred to the State Variety Testing, which resulted in certificates of registration of plant samples of the gene pool of Ukraine, plant genetic collection and certificates of State Registration of plant varieties suitable for distribution in Ukraine. For the first time in Ukraine, new sea buckthorn samples were genotyped in comparison with the best varieties, and the corresponding genetic passports were developed, which indicate genetic identity, which is important for breeding.

The second stage of research involved the involvement of sea buckthorn varieties Adaptyvna, Morkviana, Osoblyva, Nadiyna, etc. in breeding and the formation of 1.5-2 thousand hybrid nursery plants under controlled and uncontrolled pollination. The breeding work allowed us to study and select forms that are valuable in terms of a set of economic traits, such as Dublianska osin, Prydorozhna, Pahorbova, Yaruzhna, Osinnya Krasunya, Rapsodiya, etc. All samples were studied at the National Centre of Plant Genetic Resources of Ukraine, and the last four samples received a certificate of registration from the Ukrainian Plant Genebank. In 2021, the varieties Soborna and Adaptyvna Improved were submitted to the State Variety Testing. New high-yielding varieties of female sea buckthorn Adaptyvna, Osoblyva, Morkviana, Nadiina, Soborna, Adaptyvna Improved and pollinator varieties Obrii, Aboryhen adapted to the conditions of Polissia and Forest-Steppe of Ukraine have been proposed for production.

## ACKNOWLEDGEMENTS

We would like to take this opportunity to express our sincere gratitude to Vitalii Moskalets, a researcher at the Nosivka breeding and research station of the V.M. Remeslo Myronivka Institute of Wheat National Academy of Agrarian Sciences of Ukraine of the National Agrarian Academy of Sciences of Ukraine for his assistance in the study of fallow sea buckthorn plantations in the ecosystems of Chernihiv region, as well as scientific experts in horticulture Ivan Matlay, Zakhar Shusteruk and Vyacheslav Frantsko for their professional advice on biology, Bohdan Morgun, Doctor of Biological Sciences, Deputy Director for Scientific Work of the Institute of Cell Biology and Genetic Engineering of the National Academy of Sciences of Ukraine, for his assistance in the genetic certification of sea buckthorn forms under the bilateral agreement on scientific cooperation,

to the staff of the Institute of Horticulture of the National Academy of Agrarian Sciences of Ukraine for assistance in conducting agrotechnical activities in sea buckthorn nurseries, conducting biochemical analyses to determine the quality of the studied crop and processing of fruits to determine their suitability for the production of healthy food, as well as to the candidates of biological sciences Viktor Ryabchun and Olga Zadorozhna, head and leading presearcher of the National Centre of Plant Genetic Resources of Ukraine of the Plant Production Institute named after V.Ya. Yuriev of National Agrarian Academy of Sciences of Ukraine, for their work on thorough examination of applications and descriptions for sea buckthorn accessions, selection of the most valuable ones in the breeding and economic sense and their registration as accessions of the Genetic Bank of Ukraine.

## CONFLICT OF INTEREST

None.

## REFERENCES

[1] AgroTimes. (2022). Retrieved from https://agrotimes.ua/ovochi-sad/oblipyha-ne-potrebuye-velykyh-vkladen-u-zahyst-i-zhyvlennya/.

[2] Bansal, A., Salaria, M., Sharma, T., Stobdan, T., & Kant, A. (2018). Comparative de novo transcriptome analysis of male and female sea buckthorn. *Biotech*, 8(2), article number 96. doi: 10.1007/s13205-018-1122-5.

[3] Barkhuu, B., Lodonjav, M., Ganzorig, O., & Tumurtogoo, N. (2021). The physicochemical composition of sea buckthorn (*Hippophae rhamnoides* L.) oil and its treatment characteristics. *Atlantis Highlights in Chemistry and Pharmaceutical Sciences*, 2, 43-51. doi: 10.2991/ahcps.k.211004.008.

[4] Bartish, I.V., & Thakur, R. (2022). Genetic diversity, evolution, and biogeography of seabuckthorn. In P.C. Sharma (Ed.) *The sea buckthorn genome* (pp. 23-66). Cham: Springer. doi: 10.1007/978-3-031-11276-8_2.

[5] Ciesarová, Z., Murkovicb, M., Cejpekc, K., Kreps, F., Tobolková, B., Koplík, R., Belajová, E., Kukurová, K., Daško, Ľ., Panovská, Z., Revenco, D., & Burčová, Z. (2020). Why is seabuckthorn (*Hippophae rhamnoides* L.) So exceptional? A review. *Food Research International*, 133(6), article number 109170. doi: 10.1016/j.foodres.2020.109170.

[6] Dienaite, L., Baranauskiene, R., & Venskutonis, P.R. (2021). Lipophilic extracts isolated from European cranberry bush (*Viburnum opulus* L.) and sea buckthorn (*Hippophae rhamnoides* L.) berry pomace by supercritical $CO_2$ – promising bioactive ingredients for foods and nutraceuticals. *Food Chemistry*, 348(7), artile number 129047. doi: 10.1016/j.foodchem.2021.129047.

[7] Ficzek, G., Mátravölgyi, G., Furulyas, D., Rentsendavaa, C., Jócsák, I., Papp, D., Simon, G., Végvári, G., & Stéger-Máté M. (2019). Analysis of bioactive compounds of three sea buckthorn cultivars (*Hippophaë rhamnoides* L. 'Askola', 'Leikora', and 'Orangeveja') with HPLC and spectrophotometric methods. *European Journal of Horticultural Science*, 84(1), 31-38. doi: 10.17660/eJHS.2019/84.1.5.

[8] Gâtlan, A.M., & Gutt, G. (2021). Sea buckthorn in plant based diets. An analytical approach of sea buckthorn fruits composition: nutritional value, applications, and health benefits. *International Journal of Environmental Research and Public Health*, 18(17), article number 8986. doi: 10.3390/ijerph18178986.

[9] Hakeem, K.R., Ozturk, M., Altay, V., Letchamo, W., Musayev, M., & Nazim, M. (2018). An alternative potential natural genetic resource: Sea buckthorn [*Elaeagnus rhamnoides* (syn.: *Hippophae rhamnoides*)]. In *Global Perspectives on Underutilized Crops* (pp. 25-82). doi: 10.1007/978-3-319-77776-4_2.

[10] Hao, W., He, Z., Zhu, H., Liu, J., Kwek, E., Zhao, Y., Ma, K.Y., He, W.-S., & Chen, Z.Y. (2019). Sea buckthorn seed oil reduces blood cholesterol and modulates gut microbiota. *Food & Function*, 10(9), 5669-5681. doi: 10.1039/c9fo01232j.

[11] Höhn, M., Szelényi, M., & Halász, J. (2019). Low level of genetic variation and signs of isolation in the native Hungarian Sea buckthorn population compared to cultivated specimens. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 47(3), 699-705. doi: 10.15835/nbha47311333.

[12] Husain, M., Rathore, J.P., Rasool, A., Parrey, A.A., Vishwakarma, D.K., & Mahendar, K. (2018). Seabuckthorn: A multipurpose shrubs species in Ladakh cold desert. *Entomology*, 6, 1330-1337.

[13] Janceva, S., Andersone, A., Lauberte, L., Bikovens, O., Nikolajeva, V., Jashina, L., Zaharova, N., Telysheva, G., Senkovs, M., Rieksts, G., Ramata-Stunda, A., & Krasilnikova, J. (2022). Sea buckthorn (*Hippophae rhamnoides* L.) waste biomass after harvesting as a source of valuable biologically active compounds with nutraceutical and antibacterial potential. *Plants*, 11(5), article number 642. doi: 10.3390/plants11050642.

[14] Leonov, S. (2022). *Buckthorn for spring*. Retrieved from https://agrotimes.ua/interview/oblipyha-na-vesnu-oblipyhovyj-sad/.

[15] Liu, Y., Zhang, Y., Li, J., Xiang, L., Xiong, Ch., Chen, Sh., Xiao, P., & Sun, W. (2022). Identification of hippophae species using DNA barcoding. In P.C. Sharma (Eds.) *The Seabuckthorn Genome*. Cham: Springer. doi: 10.1007/978-3-031-11276-8_10.

[16] Ma, X., Moilanen, J., Laaksonen, O., Yang, W., Tenhu, E., & Yang, B. (2019). Phenolic compounds and antioxidant activities of tea-type infusions processed from sea buckthorn (*Hippophaë rhamnoides* L.) leaves. *Food Chemistry*, 272, 1-11. doi: 10.1016/j.foodchem.2018.08.006.

[17] Moskalets, V.V., Moskalets, T.Z., Hrynyk, I.V., & Lytovchenko, O.M. (2020). *Sea buckthorn as a selection-technological and consumer resource in modern fruit growing of Ukraine*. Kyiv: Agrarian Science.
[18] Olas, B. (2018). The beneficial health aspects of sea buckthorn (*Elaeagnus rhamnoides* (L.) A. Nelson) oil. *Ethnopharmacology*, 213, 183-190. doi: 10.1016/j.jep.2017.11.022.
[19] Pintea, A.M., & Magdas, D.A. (2022). Analytical techniques for the biochemical profiling in seabuckthorn. In P.C. Sharma (Eds.) *The Seabuckthorn Genome*. Cham: Springer. doi: 10.1007/978-3-031-11276-8_4.
[20] Puzniak, O., Hrynchyshyn, N., Datsko, T., Andruszczak, S., & Hulko, B. (2022). Consequences of the long-term fertilization system useon physical and microbiological soil statusinthe Western Polissia of Ukraine. *Agriculture*, 12(1), article number 1955. doi: 10.3390/agriculture12111955.
[21] Rypan, D. (2023). Establishing a sea buckthorn plantation: Requirements and rules. Recommendations of the manager of the nursery "Podil's'ki Sadzhantsi" Dmytro Rypan. *Berryfield*, 2(32), 90-91.
[22] Smida, I., Pentelescu, C., Pentelescu, O., Sweidan, A., Oliviero, N., Meuric, V., Martin, B., Colceriu, L., Bonnaure-Mallet M., & Tamanai-Shacoori, Z. (2019). Benefits of sea buckthorn (*Hippophae rhamnoides* L.) pulp oil-based mouthwash on oral health. *Applied Microbiology*, 126(5), 1594-1605. doi: 10.1111/jam.14210.
[23] Stobdan, T., Mishra, G.P., Yadav, A., & Chaurasia, O.P. (2022). Methods in sea buckthorn breeding. In P.C. Sharma (Eds.) *The Seabuckthorn Genome*. Cham: Springer. doi: 10.1007/978-3-031-11276-8_17.
[24] Tkachyk, S.O., Kienko, Z.B., Prysiazhnyuk, L.M., Shovgun, O.O., Ivanytska, O.P., & Pavlyuk, N.V. (2016). *Methodology for examination of varieties of groups of fruit, berry, nut and grape plants for distinction, homogeneity and stability*. Vinnytsia: FOP Korzun D.Yu.
[25] Tkacz, K., Wojdyło, A., Michalska-Ciechanowska, A., Turkiewicz, I.P., Lech, K., & Nowicka, P. (2020). Influence carrier agents, drying methods, storage time on physico-chemical properties and bioactive potential of encapsulated seabuckthorn juice powders. *Molecules*, 25(17), article number 3801. doi: 10.3390/molecules25173801.
[26] Todd, J. (2022). *Introduction to sea buckthorn*. Retrieved from https://www.ontario.ca/page/introduction-sea-buckthorn.
[27] Tudor, C., Bohn, T., Iddir, M., Dulf, F.V., Focşan, M., Rugină, D.O., & Pintea, A. (2019). Sea buckthorn oil as a valuable source of bioaccessible xanthophylls. *Nutrients*, 12(1), article number 76. doi: 10.3390/nu12010076.
[28] Ulanowska, K., Skalski, B., & Olas, B. (2018). Sea buckthorn (*Hippophae rhamnoides* L.) as a source of compounds with antitumor and radioprotective activity. *Postepy Higieny i Medycyny Doswiadczalnej*, 72, 240-252. doi: 10.5604/01.3001.0011.7481.
[29] Zhao, J., Zhang, Z., Zhou, H., Bai, Z., & Sun, K. (2023). The study on sea buckthorn (*Genus Hippophae* L.) fruit reveals cell division and cell expansion to promote morphogenesis. *Plants*, 12(5), article number 1005. doi: 10.3390/plants12051005.
[30] Zhuo, X., Tian, Y., Wei, Y., Deng, Y.-Y., Wu, Y., & Chen, T. (2019). Flavone of hippophae (H-flavone) lowers atherosclerotic risk factors via upregulation of the adipokine C1q/tumor necrosis factor-related protein 6 (CTRP6) in macrophages. *Bioscience Biotechnology and Biochemistry*, 83(10), 1-8. doi: 10.1080/09168451.2019.1634997.

# Еколого-біологічні основи створення вихідного матеріалу обліпихи крушиноподібної (*Hippophae rhamnoides* L.) на адаптивність і продуктивність для подальшої селекції

**Тетяна Захарівна Москалець**
Доктор біологічних наук, професор
Інститут садівництва Національної аграрної академії наук України
03027, вул. Садова, 23, с. Новосілки, Україна
https://orcid.org/0000-0003-4373-4648

**Іванна Семенівна Рожко**
Кандидат сільськогосподарських наук, доцент
Львівський національний університет природокористування
80381, вул. Володимира Великого, 1, м. Дубляни, Україна
https://orcid.org/0000-0001-5450-0906

**Богдан Іванович Гулько**
Кандидат сільськогосподарських наук, доцент
Львівський національний університет природокористування
80381, вул. Володимира Великого, 1, м. Дубляни, Україна
https://orcid.org/0000-0002-5915-9564

**Тетяна Миколаївна Дацко**
Кандидат сільськогосподарських наук, доцент
Львівський національний університет природокористування
80381, вул. Володимира Великого, 1, м. Дубляни, Україна
https://orcid.org/0000-0002-2957-1822

**Ігор Михайлович Рожко**
Кандидат географічних наук, доцент
Львівський національний університет ім. Івана Франка
79000, вул. Університетська, 1, м. Львів, Україна
https://orcid.org/0000-0003-2263-9828

**Анотація.** Актуальність досліджень полягає у постійному прагненні до поповнення і покращення українського генофонду обліпихи крушиноподібної шляхом використання методів аналітичної та синтетичної селекції для формування генотипів з високою адаптивністю, продуктивністю та якістю плодів. Мета роботи полягала в створенні і вивченні цінного за господарськими ознаками генетичного різноманіття обліпихи крушиноподібної для подальшої селекції. Фенологію, морфологічні дослідження, оцінку селекційного матеріалу за господарсько-цінними ознаками проводили згідно методики проведення експертизи сортів рослин, молекулярно-генетичні дослідження – згідно методу полімеразної ланцюгової реакції, що заснований на багаторазовому копіюванні певної ділянки ДНК. У результаті виконання досліджень розширено формотворчий процес у селекції обліпихи крушиноподібної на адаптивність, продуктивність і якість шляхом використання генофонду споріднених форм, що є одним із способів вирішення проблем зі створення вихідного матеріалу для подальшої селекції й на чому базується суть досліджень, в результаті яких встановлено широкий спектр формотворчого процесу за морфологічними ознаками та біологічними властивостями в результаті гібридизації біотипів, що дозволило відібрати цінні гібриди за комплексом селекційних і господарських ознак. Зокрема, виділено нові форми Соборна і Адаптивна поліпшена, що відзначаються високою зимо- і посухостійкістю, продуктивністю і якістю плодів, характеризуюься відсутністю колючок і сухим відривом плодів та передані на Державне сортовипробування. Інші отримані генотипи рослин обліпихи поєднують стійкість до високих і низьких температур довкілля з комплексом інших господарських ознак: стійкістю до хвороб, мало- або безколючковістю, якістю плодів і їх придатністю до переробки і виготовлення продуктів для здорового харчування. Практична цінність наукової статті ґрунтується на тому, що результати дослідження розширюють відомості про використання в селекції обліпихи крушиноподібноїна адаптивність, продуктивність і якість форм, відібраних і вивчених в умовах природних і перелогових екосистем Полісся і Лісостепу України. В результаті їх вивчення відібрано кращі зразки, які увійшли до Генетичного банку рослин України, як матеріал з цінними господарськими ознаками та які залучено до селекційної роботи в Інституті садівництва Національної академії аграрних наук України

**Ключові слова:** *Hippophae rhamnoides* L.; добір; нові форми; результати оцінювання; перспективи для подальшої селекції