

UDC 631.528.6: 635.521

PREDICTION OF THE BREEDING VALUE OF LEAF LETTUCE MUTANT LINES ACCORDING TO THE CORRELATION BETWEEN THE MANIFESTATION OF QUALITATIVE AND QUANTITATIVE TRAITS

S. I. Kondratenko ^{1*}, O. P. Samovol ¹, S. M. Kormosh ²,
I. M. Mytenko ¹, O. V. Poznyak ³, L.V. Chaban ³

¹ *Institute of Vegetable and Melon Growing, of the National Academy of Agrarian Sciences of Ukraine, 1, Instytutska Str., Seleksiynе village, Kharkiv district, Kharkiv region, Ukraine, 62478*

² *Transcarpathian State Agricultural Research Station of the National Academy of Agrarian Sciences of Ukraine, 17, Svobody Ave., V. Bakta village, Berehiv district, Transcarpathian region, Ukraine, 90252*

³ *Research station “Mayak” of the Institute of Vegetable and Melon Growing, of the National Academy of Agrarian Sciences of Ukraine, 39, Nezalezhnosti Str., Kruty village, Chernihiv region, Ukraine, 16645
E-mail: shtirlitsmail@gmail.com *, ovoch.iob@gmail.com, korsveta1967@ukr.net, mytenko.iob@gmail.com, dsmayak@ukr.net*

ORCID: <https://orcid.org/0000-0002-8859-1604>, <https://orcid.org/0000-0002-6798-7378>,
<https://orcid.org/0000-0002-1694-8280>, <https://orcid.org/0000-0001-8571-937X>,
<https://orcid.org/0000-0002-6659-3238>, <https://orcid.org/0000-0002-0854-3443>

Received / Received / Accepted July 19, 2023

Aim. To establish the effect of mutagenic factors on the formation of qualitative traits, defining the phenotype of the leaf blade, and to investigate the correlation between the manifestation of these factors and the variation of quantitative traits of breeding value in mutant lines of leaf lettuce. **Methods.** Non-parametric statistical and criteria of difference for biological specimens, computational and analytical calculations, correlation analysis. **Results.** The comparative analysis was conducted on the qualitative characteristics of the original leaf blade form of leaf lettuce, Zhnych variety, alongside 14 mutant lines derived from it through the application of mutagenic agents, including dimethyl sulfate (DMS) and its derivatives (D₃MU, DMU-10A, and DMU-9) in two varying concentrations. Notably, the derivatives of DMS exhibited superior mutagenic efficiency, leading to a four to fivefold increase in the number of mutant plant forms compared to the reference compound DMS. Among the derivatives, D₃MU proved the most effective, generating five mutant lines. In contrast, DMU-10A and DMU-9 yielded four mutant lines, while DMS produced only one mutant line. All mutant lines exhibited distinct variations from the initial form across 17 qualitative characteristics determining leaf blade phenotype. The analysis of Spearman’s rank correlation coefficient (r_s) demonstrated weak to moderate correlation ($r_s = -0.08...0.62$) between the original form and mutant lines. It was determined that three lettuce lines, namely B-7, B-22, and B-38, exhibited the most significant deviations from the initial form in term of leaf blade qualitative traits. Furthermore, it was established that non-parametric indicators of qualitative traits expression exhibited moderate or high correlation ($r_s = 0.57...0.74$) with an essential quantitative trait – the duration from the emergence of mass shoots to plant stem development in mutant lines. **Conclusions.** The identified correlations between quantitative and qualitative traits provide the opportunity to predictably select mutant genotypes with extended growth period during the early stages of lettuce plant ontogenesis in future breeding work.

Key words: leaf lettuce, leaf blade, qualitative characteristics, quantitative characteristics, mutagenesis.

DOI:

INTRODUCTION

In the recent decade, the production of different species of lettuce (*Lactuca sativa* L.) has gathered a

considerable momentum (Gandra et al, 2018; Kovácsné and Takácsné, 2022; Medina-Lozano et al, 2021; Shcherbyna and Yurlakova, 2019). Among all this

species diversity, particular popularity on the market is noted for leaf lettuce (*Lactuca sativa* var. *secalina* L.). The main requirements for modern varieties of this species are excellent taste qualities, original form and color of the leaf, high performance and biological value, and resistance to stress factors of production (Arnold et al, 2019; Damerum et al, 2020; Ferreira et al, 2020). The conventional breeding scheme of creating new leaf lettuce varieties envisages artificial hybridization with further individual, group, and mass selections. The flowers of leaf lettuce are facultatively pollinated, self-pollination takes place still in the buds via pollen tubes (Acquaah, 2012; Hassan et al, 2021; Patella et al, 2019). Therefore, the current technique of artificial hybridization is somewhat complicated; it is conditioned by the structure and size of the flower, adjusted to self-pollination, and the biological specificities of blossoming in this species of plants (Batista and De Souza, 2017; Han et al, 2019; Křístková et al, 2008). To expand the varietal diversity of leaf lettuce, it is reasonable to use alternative, more efficient methods of creating the initial material in the breeding practice. One of these is induced mutagenesis, which can be used to change only one or several traits of the initial form, which then can be stabilized in rather a short period of time due to inbreeding in several generations (Mou, 2011). Mutations can affect the evolution and improvement of lettuce (*Lactuca sativa* L.). First of all, this is a way of creating genetic variability that can be used in physiological or genetic studies (Delia et al, 2013; Cheng et al, 2018; Gurdon et al, 2019). The use of mutagenesis is also very efficient for the breeding of different lettuce species, especially for the purpose of improving a number of economically valuable traits – resistance to diseases, raising the yield and acquiring new useful qualitative traits, which cannot be obtained by conventional crossing, because the latter enables only the recombination of the traits, which already are present in paternal components (Ohki and Hatashita, 2012). Induced mutation-assisted breeding is considered non-transgenic; it is more acceptable for consumers and meets practically no opposition from the defenders of the environment, government, producers, and broader public opinion. Although the mutagenesis process may be uncomplicated, it still requires some practice and experience to determine the accurate dose of the mutagen, which ensures a high frequency of mutations and a low mortality rate. Due to the accidental nature of mutagenesis, a large population of samples that mutated should be detected so that they could be practically useful for the breeding process. A large area of the ex-

perimental plot may be required to grow the plants of generation M_1 and to obtain enough seeds to breed the generation M_2 (Franco et al, 2015; Marcu et al, 2013). There are five main types of lettuce: leaf lettuce (var. *secalina*), cabbage lettuce (var. *capitata*), romaine lettuce (var. *longifolia*), lettuce stem (var. *angustana jr-ish*), half a head of lettuce (var. *acefala* Alef.), which belong to the group of green (rare) crops. The inbred nature of lettuce governs its relatively limited genetic variability compared to cross-pollinated crops. Such mutagenic agents as X-rays, ultraviolet radiation, neutrons, protons, alpha-, beta-, and gamma-rays, or such chemical mutagens as ethyl methanesulfonate (EMS) and dimethyl sulfate (DMS) are used in breeding for a slight increase in the incidence of mutations, to obtain mutants with contrastive phenotypes as compared to the initial forms or as a source of valuable and useful traits (Begum and Dasgupta, 2010; Kodym et al, 2012; Kodym and Afza, 2003; Mir et al, 2021). Many foreign researchers study the mutagenesis of leaf lettuce, which allows for solving the problems of improving specific characteristics of this green crop (Huo et al, 2016; Sorel et al, 2014). It was proven that the highest effect of the mutagenic factors is achieved when they are used to treat seeds (Franco et al, 2015; Marcu et al, 2013).

A productive organ of leaf lettuce is a rosette of leaves. Depending on the variety genotype, plants are in a saleable condition for about 10–15 days. Four to five days after the formation of cotyledons, the first real leaves are formed, making up a rosette. Then, the intensity of growth and development is enhanced considerably. The leaves of leaf lettuce are notable for vividly manifested phenotypic diversity. All the leaves are simple sessile, non-dissected or dissected, lanceolate. In terms of form, they are kidney-shaped, rounded, elliptical, obovate, lancet, or pinnately compound. The edges of the leaves are solid, toothed, or curly. The size of the leaf varies greatly; its surface can be from smooth to blistered, with a thick vein extending in the middle. The consistency of the leaves can be from fleshy-tender, crispy, oily to rough. The color of the leaves varies from light green, green to red. The color of the leaf blade is presented in a range of different shades, namely, green, dark green, dark grayish green, light green, yellowish green, pale yellowish green, brownish, red-brown, sometimes with sorrel pigmentation (Křístková et al, 2008). The morphogenesis and growth of leaf lettuce leaves have complicated regulation mechanisms. In addition to genetics, the environment of development can also have a consi-

derable impact on the change in the leaf structure, its color, and venation. The described and noticeable traits of the most common varieties of leaf lettuce are very important for the identification of the phenotype and analysis of the phenotype-genotype association. It is impossible to find two of the same leaves in the entire world, but the leaves of different lettuce varieties may have higher similarity (Du et al, 2022). Obviously, the statistical traits of the whole leaf are not sufficient for the detailed description of differences between varieties. Different components of leaves perform specific physiological functions and may be quantitatively described and interpreted using their observed properties. A leaf of leaf lettuce may be visually divided into different functional regions, such as a petiole and veins for mechanical support and transportation of nutrients; other regions are required for photosynthesis (Leschuk et al, 2017). At present, the current structural phenotyping of the leaves of leaf lettuce does not allow for accurate differentiation of its components. Some traits of leaf components can be measured manually, but it requires much time and effort and adds a subjective researcher's error during measurements. Several studies were conducted to investigate the phenotyping of leaf lettuce leaves based on their digital images for computer-assisted processing of the visualization results for structural components (Du et al, 2022; Bauer et al, 2019). The suggested experimental approach lays an effective strategy of quantitative analysis of the fine structure and components of the detached leaf of leaf lettuce. However, at present, these methods of analysis are still under development and require better systematization of the obtained data. Further progress in solving this issue will facilitate more detailed segregation of numerous traits for the classification and identification of leaves and provide a more accurate computational basis for its functional analysis (Du et al, 2022). The main structure of a proper leaf is established at the early stage of its development. Leaf morphogenesis involves the formation of a leaf blade, its venation, the forms of the edges, and color (Bar and Ori, 2014).

One of the breeding process stages is the selection of derivative sources, based on the complex of traits, inherent to genotypes. Their manifestation of species populations is conditioned by the specificities of the origin of species, variability, and stability of the origin of physiological functions of plants due to the interaction of a complicated system: a population – an ecological environment. The classification traits are divided into morphologically quantitative and qualitative ones. The qualitative traits are notable for the presence of clearly

visible alternative manifestations, for instance, the high and low habitus of plants, broad and narrow leaves, long or rounded spikes, etc. The quantitative traits are all those that can be measured, counted, and weighed. These traits demonstrate continuous variability, thus, the value of the trait varies to a different degree. In addition, quantitative traits have different regularities of their inheritance as compared to qualitative traits and respond to environmental conditions (Ceccarelli and Grando S, 2020; Ewing et al, 2019). As for leaf lettuce, attention is paid to the morphology of young real leaves, as the qualitative traits, defining this morphology, are stable, genetically fixed, and display almost no variation under the impact of environmental factors. The forms of the leaves, the venation of the leaf blade, and the waviness of the leaf edge allowed for identification of the first herbarium specimen of lettuce (Guo et al, 2023).

According to the UPOV classification, each plant has 40–50 characteristic traits, which should be known to each researcher-breeder (the International Union for the Protection of New Varieties of Plants, 2021). Taking the abovementioned into consideration, the authors of the article improved the classifying method to conduct the examination of plant species for differences, homogeneity, and stability of lettuce (*Lactuca sativa* L.) (Kondratenko et al, 2019). The classifying method presents economically valuable and morphological traits of the plants from the species of lettuce *Lactuca sativa* L. and its varieties: leaf lettuce (var. *secalina*), cabbage lettuce (var. *capitata*), romaine lettuce (var. *longifolia*), lettuce stem (var. *angustana jrish*), half a head of lettuce (var. *acefala* Alef.), which belong to the group of green (rare) crops. The classifying method is the basis for the examination of newly-created varieties and hybrids F₁ based on determining the difference, homogeneity, and stability of the manifestation of their traits, which is the main requirement while conducting scientific and technical evaluation in the system of state crop variety testing. According to the current genetic and breeding practice, the gradation of qualitative traits, defining the morphology of a leaf blade of leaf lettuce, is presented by some degrees of manifestation in the certain corresponding reference species (Kondratenko et al, 2019). To evaluate the difference and homogeneity, the elaborated classifying method used codes (points for the score) for the traits (from 1 to 9), required for computational processing of the data. The combination of these codes constitutes a coded formula of the species or a specimen, valuable for breeding, and is used to form a group of similar genotypes.

It should be noted that the score is widely used for registrations and observations in the breeding practice. It is substantiated by the need to avoid labor-consuming procedures of registering the values of traits in physical measurement units using specific equipment. The elaboration of the corresponding scales for the classification of physical values of traits (classifiers) into classes and the allocation of the corresponding score to them facilitates the use of a considerable scope of single-dimensional estimates in the registration process and their registration using points (Křístková et al, 2008; Mousavi et al, 2012; Basnet et al, 2014). When numerous traits should be registered, the application of scores for estimation is the only way to come to the comprehensive evaluation of the breeding material by the difference in morphogenetic processes under individual development and phenotype of traits, reflecting them. It provides a solution to the compromise between the accuracy of registering a single trait and the possibility of a deeper description of the breeding material in terms of differences in growth, development, and, finally, in epigenetic processes, which are the object of adaptive breeding. At the same time, the score allows for comparing the traits and properties that are actually impossible to evaluate in some physical measurement units. These are the traits with non-physical measurement units, which characterize some difference, integral by its form, including the morphotype, and integral specificities of phytocenosis as of a specific stage of ontogenesis. The scores of these traits are impossible to express in physical units using the corresponding classifying scales. The example may be found in the score by the degree of manifestation of 39 morphological traits, the results of which are attached to the application to transfer a species of leaf lettuce to the State Variety Testing institution (Kondratenko et al, 2019). The difference in the breeding material by the association of traits, registered in scores, can be evaluated using non-parametric criteria of differences (MacFarland and Yates, 2016). These criteria can be especially used while analyzing the differences in the breeding material by the association of traits, the combination of which reflects the corresponding integral properties in the manifestation of macroscopic variability. For instance, it is valid for the specifics of forming the vegetative sphere or a life form in general by a particular scope of traits, reflecting them.

The aim of the study was to establish the effect of mutagenic factors on the formation of qualitative traits, determining the phenotype of the leaf blade, and to investigate the correlation between the manifestation of

these factors and the variation of quantitative traits of breeding value in mutant lines of leaf lettuce.

MATERIALS AND METHODS

The field studies were conducted in 2015–2019 at the experimental facility of the Research Station “Mayak” of the Institute of Vegetable and Melon Growing of the NAAS, located in Baklanovo village within Nizhyn district of Chernihiv region, Ukraine. The local environment closely resembles the northern Forest-Steppe region of Ukraine, characterized by a moderately warm and temperate climate. The focus of our research was directed towards leaf lettuce (*Lactuca sativa* L. var. *secalina*). Specifically, we investigated mutant lines of leaf lettuce, which were of the fourth generation (M_4). These mutant lines were generated through chemical mutagenesis and derived from the Zhnych variety (K-046), which was bred at the Research station “Mayak” of the Institute of Vegetable and Melon Growing of the NAAS.

To obtain these mutant leaf lettuce plants, we conducted a pre-sowing treatment on air-dried seeds according to the scheme outlined in **Table 1**. This treatment involved the utilization of biologically active substances with mutagenic properties, including the reference compound dimethyl sulfate (DMS) and its chemical analogs, namely D_3MU , DMU-10A and DMU-9, synthesized at the experimental facility of the Institute of Bioorganic Chemistry and Petrochemistry of the NAS of Ukraine. These preparations were novel in the context of their application to leaf lettuce, and the objective was to determine their effectiveness, relative to DMS, the reference mutagen, in generating valuable mutant forms of leaf lettuce for breeding objectives.

The seeds of leaf lettuce were enclosed within cheesecloth pouches and treated with aqueous solutions of the abovementioned biologically active substances in the active concentrations of 0.1 and 0.05 % for 18 h prior to sowing in the field conditions (Table 1). The seeds,

Table 1. The scheme of the experiment in expanding genotypic variability of leaf lettuce by the method of chemical mutagenesis, 2015

Mutagenic preparations	Concentrations of biologically active substances of mutagenic effect
Control	Treatment of seeds with distilled water
DMS, reference	0.05 %; 0.1 %
D_3MU	0.05 %; 0.1 %
DMU-10A	0.05 %; 0.1 %
DMU-9	0.05 %; 0.1 %

soaked in distilled water, served as the control. Each experimental specimen of leaf lettuce was sown in the registration plots of 4.2 sq.m. with a sowing rate of 0.9 g/plot. The seeds were sown in four repeats within a single row, the a plot length of 5 m, a row spacing width of 70 cm, and the seed burial depth of 1 cm.

During the growth and development of mutant plants, phenological observations were diligently conducted. Any plants exhibited modified morphological and physiological characteristics were registered, and subsequent generations were checked for the inheritance of these changes. It is essential to clarify that only alterations in plant traits that were consistently passed down in subsequent generations were regarded as mutations. Detailed records of all types of mutations were maintained at every stage of growth and development from generations M_1 – M_5 .

In adherence to methodological guidelines, each experimental variant involved the treatment of 1 gram of seeds (Hvostova, 1965). To study the impact of chemical mutagens on the plants of the first generation (M_1), these seeds were sown in the field conditions in four repeats. Phenological observations were made throughout of the lifecycle of these cultivated plants, complemented by biometric measurements conducted during both the vegetative and reproductive phases of development. Mutations were visually identified in plants at critical stages of growth and development.

The registration process involved documenting mutations characterized by deviations in the leaf blade shape and color, stem and branches structure, variations in the degree of expression of quantitative traits, and differences in the timing of phenological phases compared to the initial form (Zhnych variety). The findings of these studies were instrumental in isolating mutant genotypes in which mutational variability had led to improvements in economically valuable traits.

In 2015, we obtained the seeds of mutant generation M_1 . In 2017–2019, we studied the mutant progeny of generations M_3 – M_5 under field conditions by the complex of quantitative and qualitative traits and the phenology of growth and development of plants. While testing the reference preparation, DMS, in the active concentration of 0.1 %, we did not succeed in obtaining mutant plants of leaf lettuce, Zhnych variety, as the pre-sowing treatment of seeds with this preparation made them inviable and under field conditions they had 100 % mortality rate.

The visual analysis of the mutant forms of leaf lettuce determined their difference from the initial variety

genotype by the association of qualitative traits, defining the phenotype of the real leaf. For instance, the effect of mutagenic factors was seen in the phenotypic differences of the leaf blade, defining its morphology, venation, form of the edge, and color. To estimate the manifestation degree of qualitative traits for this organ of mutant genotypes, the Methodology of conducting the examination of leaf lettuce (*Lactuca sativa* L.) varieties for differences, homogeneity, and stability, developed by the Ukrainian Institute of Plant Variety Examination, which suggests the corresponding points for the manifestation degree regarding 17 qualitative traits of the leaf to determine the approbation traits of varieties for DHS-testing (Kondratenko et al, 2019), was used as the estimation basis. Among the investigated qualitative traits, there are seven, defining the morphology and venation of the leaf blade (“the dissection of the edge of the leaf blade”, “the general shape of the leaf blade”, “the shape of the tip of the leaf blade”, “glossiness on the upper side of the leaf blade”, “blistering of the leaf blade”, “blisters according to the size of the leaf blade”, “venation of the leaf blade”), four traits defining the form of the edge of the leaf blade (“the waviness of the edge of the leaf blade”, “the dissection of the edge of the tip of the leaf blade”, “the depth of the dissection of the edge of the tip of the leaf blade”, “the degree of dissection of the edge of the tip of the leaf blade”), six traits defining the color of the leaf blade (“the shade of green coloring of the leaf blade”, “the intensity of coloring of the outer leaves”, “anthocyanin coloring of the leaf blade”, “the intensity of anthocyanin coloring of the leaf blade”, “the distribution of anthocyanin coloring of the leaf blade”, “the type of spread of anthocyanin coloring of the leaf blade”). The difference in the material by the association of traits, registered in scores, can be evaluated using non-parametric criteria of differences. The following statistical indices, suggested in respective scientific and methodological journals, were used in the work to conduct the variation analysis of qualitative traits, defining the phenotype of the leaf blade (Litun et al, 2009; MacFarland and Yates, 2016): Spearman’s rank correlation coefficient, r_s ; Friedman’s analysis of variance; Kendall’s concordance coefficient, W .

For each mutant specimen of leaf lettuce, the index of normalized values was determined by calculating the ratio between the score, corresponding to the manifestation degree of some qualitative trait, and the average value of the score, defining all the manifestations of the same qualitative trait of the leaf blade according to the classifying catalog (Kondratenko et al, 2019). This

experimental approach is widely used in the breeding practice to bring the manifestation of the investigated qualitative or quantitative traits to one measurement unit (Litun et al, 2009; Wen et al, 2023).

The statistical analysis of experimental data was conducted using STATISTICA program (StatSoft, Inc. (2014). STATISTICA, version 12. www.statsoft.com).

RESULTS

Due to the studies in mutational breeding, we created 14 mutant lines, which are derivatives of leaf lettuce, Zhnych variety, valuable for breeding purposes (**Table 2**).

The analysis of the qualitative traits of mutant lines, derivatives of this variety, was conducted in 2018 with generation M₄. At that time, all the linear specimen were in the nursery of the competition variety testing after three years of selecting for the stable manifestation of both qualitative and quantitative traits. The results of the study of linear material by the complex of quantitative traits were presented by the authors of this article in another publication (Kondratenko et al, 2020). The analysis of mutant lines revealed their morphological differences from the initial form in

terms of morphology, venation, form of the edge, and color of the leaf blade. According to the analysis, conducted for the manifestation of 17 qualitative traits of the leaf blade in the combined population of leaf lettuce genotypes, consisting of both mutant lines and the initial form, it was determined that one trait had 6 degrees of manifestation, one trait – 5 degrees of manifestation, 3 traits – 4 degrees of manifestation, 7 traits – 3 degrees of manifestation and 5 traits – 2 degrees of manifestation.

Due to the effect of mutagenic factors, the highest variability in terms of manifestation was noted for the traits “the shape of the leaf” and “leaf blisters according to the size”. The smallest changes were noted for the following qualitative traits: “the venation of the leaf blade”; “the dissection of the edge of the tip of the leaf blade”; “anthocyanin coloring of the leaf blade”; “the distribution of the anthocyanin coloring of the leaf blade”; “the type of the distribution of anthocyanin coloring of the leaf blade”. The group of 7 traits, defining the morphology of the leaf blade and its venation, had 26 degrees of manifestation. The group of 6 traits, defining the coloring of the leaf blade, had 16 degrees of manifestation. The group of 4 traits, defining the shape

Table 2. Mutant genotypes of leaf lettuce, generation M₁, obtained after the effect of mutagenic factors of different nature, 2015

Name of preparation	Applied concentration, %	Name of mutant line
<i>Name of mutant line Initial form – Zhnych variety (k-046)</i>		
DMS (reference)	0.05	B-38 (k-0296)
	0.1	No mutant plants were obtained due to 100 % mortality rate of the seeds after the pre-sowing treatment of the seeds with DMS preparation
D ₃ MU	0.1	B-2 (k-0262) B-4 (k-0264) B-6 (k-0266)
	0.05	B-7 (k-0267) B-8 (k-0268)
DMU-10A	0.1	B-11 (k-0271) B-14 (k-0274)
	0.05	B-16 (k-0275) B-18 (k-0277)
DMU-9	0.1	B-21 (k-0280) B-22 (k-0281) B-23 (k-0282)
	0.0	B-24 (k-0283)

of the edge of the leaf blade, had 12 degrees of manifestation.

One of the requirements to the application of non-parametric criteria to the entire range of traits is the need to bring their values to one measurement unit, which can be ranked. Different intervals of score variation were used in the elaborated classifying method to describe specific qualitative traits, which manifest the phenotype of the leaf blade: {1; 2}; {1; 3}; {1; 4}; {1; 7}; {3; 7}; {1; 9}. The absence of the phenotypic manifestation of the trait was marked as a zero score. To bring the scores of points to one measurement unit, we used the calculation of the index of normalized values. The registration

of the association of qualitative traits, manifesting the phenotype of the leaf blade in mutant lines not by their initial score of points but by the calculated indexes of normalized values, is presented in **Table 3**.

To estimate the differences between mutant forms and the initial form – Zhnych variety, the work with qualitative traits involved the use of Friedman’s analysis of variance, Kendall’s concordance coefficient, W , and Spearman’s rank correlation coefficient (r_s). The breeding material was compared using the abovementioned criteria and the average values of the index of normalized values of traits in the experiment. The analysis of qualitative traits, defining the morphology of the

Table 3. Normalized values of qualitative traits for leaf blade in mutant lines and the initial form (Zhnych variety) of leaf lettuce (generation M₄, 2018)

Phenotypic trait	Mutant lines														
	Zhnych variety	B-2	B-4	B-6	B-7	B-8	B-11	B-14	B-16	B-18	B-21	B-22	B-23	B-24	B-38
<i>Differences in the morphology of the leaf blade and venation</i>															
Dissection of the edge	1.5	1	1.5	1.5	1	1.5	1.5	1.5	1.5	1.5	1.5	0.5	1.5	1.5	1
The shape of the leaf	1.62	1.85	1.15	1.85	0.69	1.85	0.23	0.46	1.62	1.85	1.62	0.23	1.15	1.62	0.69
The shape of the tip	1.5	1	0.5	1.5	1.5	0.5	0.5	1.5	1	0.5	0.5	1	0.5	0.5	1.5
Glossiness on the upper side	1.4	1.4	1	1.4	1	0.6	1	1	1	0.6	1.4	1.4	0.6	1	1
Blistering	0.67	0.22	0.22	0.67	1.11	0.22	0.67	0.22	0.67	0.67	0.22	2	0.22	0.22	1.11
Blisters according to the size	2.19	0.94	1.56	0.94	0.94	0.31	0.94	0.94	1.56	1.56	0.94	1.56	0	1.56	1.56
Venation	1.33	0.67	1.33	1.33	1.33	0.67	0.67	0.67	1.33	1.33	0.67	0.67	1.33	1.33	0.67
<i>Differences in the shape of the edge of the leaf blade</i>															
Waviness of the edge	1.17	1.17	1.5	1.5	1.17	1.17	1.17	0.83	1.17	0.83	1.5	0.5	1.5	1.17	0.5
Dissection of the edge of the tip	1.8	0.2	1.8	0.2	0.2	1.8	1.8	0.2	1.8	0.2	1.8	0.2	1.8	1.8	0.2
Depth of the dissection of the edge of the tip	1.12	0	1.12	0	0	1.12	1.12	0	1.12	0	1.12	0	1.87	1.12	0
Degree of the dissection of the edge of the tip	1.12	0	1.87	0	0	1.12	1.87	0	1.12	0	1.12	0	1.87	1.12	0
<i>Differences in the coloring of the leaf blade</i>															
Shade of green coloring	1.33	1.33	1.33	1.33	1.33	1.33	1	1.33	0.67	1	1	1.33	0.67	0.67	1
Intensity of coloring of the outer leaves	1.17	1.5	1.17	1.17	0.83	1.5	0.5	1.5	0.5	0.83	1.17	1.5	0.83	0.83	1.17
Anthocyanin coloring	1.13	1.13	1.13	1.13	1.13	1.13	1.13	1.13	1.13	1.13	1.13	1.13	1.13	1.13	0.88
Intensity of anthocyanin coloring	1	1.29	1	1	0.71	1.29	0.71	1.29	1	1	1.29	1.29	0.71	1	1
Distribution of anthocyanin coloring	1.33	1.33	0.67	0.67	0.67	1.33	0.67	1.33	0.67	0.67	1.33	1.33	0.67	0.67	1.33
Type of distribution of anthocyanin coloring	0.5	0.5	0.5	1.5	1.5	0.5	1.5	0.5	0.5	1.5	0.5	0.5	1.5	0.5	1.5

leaf blade in mutant forms, demonstrated their differences from the initial form (Table 4).

One of the criteria of differences by the integral manifestation of the block of traits in the investigated sampling of mutant forms is the comparison of the sum of ranks (S_i) and the experimental value of the criterion χ^2_{exp} (MacFarland and Yates, 2016). The range of variability for the index of “the sum of ranks” in our experiment (117.0...175.5) considerably exceeded χ^2_{exp} (13.63), which confirms significant differences between mutant lines and the initial form by the investigated association of qualitative traits (Table 4). The calculation of Kendall’s concordance coefficient ($W = 0.057$) proved insignificant concordance between morphogenetic processes, preceding the block of formed associated traits, and considerable specificity by them.

As for the entire investigated sampling of mutant lines and the initial form, a more manifested evaluation of differences is ensured by the index of “average index of normalized values” of Friedman analysis of variance and the value of Spearman’s rank correlation

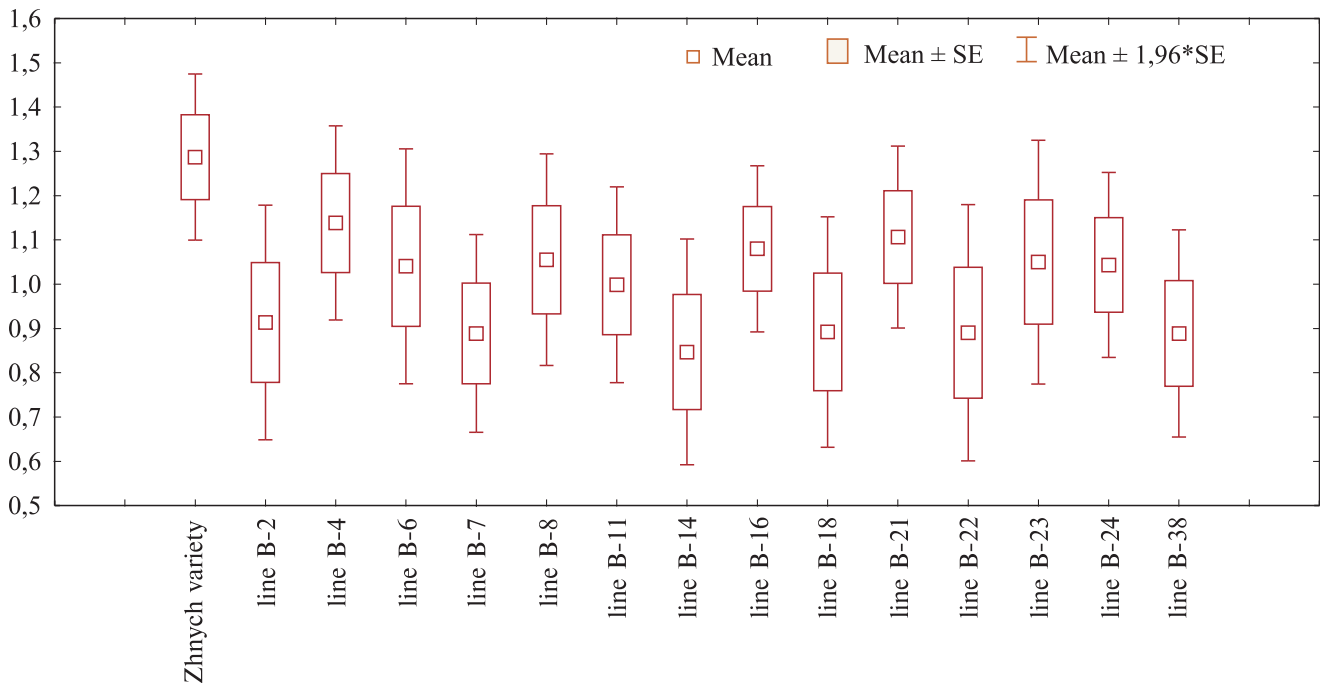
coefficient (r_s), which are in good agreement. According to the data in Table 4, the correlation between the initial form and mutant lines by the manifestation degree of 17 qualitative traits varied from -0.08 to 0.62 . By the degree of genetic affinity to the initial form, the investigated group of mutant lines can be conditionally divided into three groups. The first group is the lines with the highest affinity and average strength of the correlation ($\pm 0.3 < r_s < \pm 0.69$). This group consists of two lines B-16 ($r_s = 0.62$) and B-24 ($r_s = 0.58$), which showed statistically reliable association with the initial form (Table 4). In addition to these lines, this group also includes three following lines: B-4 ($r_s = 0.43$); B-8 ($r_s = 0.31$); B-21 ($r_s = 0.41$).

A weak correlation ($\pm 0.1 < r_s < \pm 0.29$) was noted for six lines: B-2 ($r_s = 0.27$); B-6 ($r_s = 0.25$); B-11 ($r_s = -0.18$); B-14 ($r_s = 0.25$); B-18 ($r_s = 0.20$); B-23 ($r_s = -0.22$). The most contrastive lines, with practically absent correlation in terms of manifesting the association of qualitative traits, defining the phenotype of the leaf blade, were three lines ($r_s \leq \pm 0.09$): B-7 ($r_s = -0.08$); B-22 ($r_s = 0.02$); B-38 ($r_s = 0.09$).

Table 4. The results of the analysis of variance for the expression of qualitative traits defining leaf blade morphology in mutant forms derived from the Zhnych Variety of leaf lettuce

Mutant specimen	No of cat.	Results of Friedman’s analysis of variance *				Spearman’s rank correlation coefficient, r_s
		Average rank	Sum of ranks (Si)	Average index of normalized values (X_{med})	Average square deviation (σ_x)	
Zhnych variety	k-046	10.32	175.50	1.29	0.39	–
line B-2	k-262	7.97	135.50	0.91	0.56	0.27
line B-4	k-264	8.82	150.0	1.14	0.46	0.43
line B-6	k-266	9.12	155.0	1.04	0.56	0.25
line B-7	k-267	7.15	121.50	0.89	0.47	–0.08
line B-8	k-268	8.44	143.50	1.06	0.50	0.31
line B-11	k-271	7.12	121.0	1.0	0.47	–0.18
line B-14	k-274	7.38	125.50	0.85	0.54	0.25
line B-16	k-275	8.09	137.50	1.08	0.39	0.62
line B-18	k-277	7.09	120.50	0.89	0.55	0.20
line B-21	k-280	8.76	149.0	1.11	0.43	0.41
line B-22	k-281	7.76	132.0	0.89	0.61	0.02
line B-23	k-282	7.47	127.0	1.05	0.58	–0.22
line B-24	k-283	7.62	129.50	1.04	0.44	0.58
line B-38	k-296	6.88	117.0	0.89	0.49	0.09
Average		8.0	136.0	1.01	0.50	–

Note. * – The results of calculations for Kendall’s concordance coefficient (W): $W = 0.057$ and the calculations for the criterion of X^2 -square are as follows $\chi^2_{exp} = 13.63$ and $\chi^2_{Table} = 0.48$, i.e. $\chi^2_{exp} > \chi^2_{Table}$, thus the significance of the concordance coefficient W is considerable in 5 % of the significance level; in bold are Spearman’s rank correlation coefficients r_s , the reliability of which was proven at the significance level of $p < 0.05$.



The diagram of comparative difference in the mutant lines of leaf lettuce, derivatives of Zhnych variety, by genotypic average indices of the normalized values, defining the variability level of the associated traits, defining the morphology of the leaf blade (generation M_4)

The visualization of the data in Table 4 by the variability of “the average index of normalized values” for the entire sampling of the investigated genotypes of leaf lettuce is presented in the diagram (**Figure**). One of the specificities of mutant lines was the presence of lower values of this index as compared to the initial form. The explanation of this experimental fact from the genetic standpoint requires further studies. To determine the degree of the efficiency of mutagenic factors, we conducted their differentiation by the number of formed mutant linear genotypes, which had a different degree of difference from the initial form. The index of “the average index of normalized values” was considered to be the integral criterion for the formation of new degrees of the manifestation of qualitative traits, defining the phenotype of the leaf blade as compared to the initial form. All mutant lines of leaf lettuce were conditionally divided into three groups. The data about the differentiation of mutagenic factors by the efficiency degree were compiled in **Table 5**.

The obtained data demonstrate that the most efficient preparation of the mutagenic effect, among all the investigated ones, was found to be D_3 MU. Its application in the active concentration of 0.05 % resulted in creating two mutant lines (B-7 and B-8) and three more mutant lines (B-2, B-4, and B-6) were obtained using

the concentration of 0.1 %. The preparation D_3 MU was used to create the lines with a different affinity degree regarding the initial form – from the most contrastive line B-7 to the lines with a weak (B-2 and B-6) and moderate correlation (B-4 and B-8) by the manifestation of the association of qualitative traits, defining the phenotype of the leaf blade (Table 5).

The mutagenic effect of DMU-10A preparation was seen in creating four mutant lines – B-16 and B-18 while testing the concentration 0.05 % and B-11 and B-14 while testing the concentration of 0.1 %. Three lines, B-11, B-14, and B-18, had a weak correlation with the initial form ($\pm 0.1 < r_s < \pm 0.29$) by the manifestation of the association of qualitative traits. Line B-16 had the highest affinity with Zhnych variety among all the obtained mutant linear genotypes ($r_s = 0.62$).

The pre-sowing treatment of leaf lettuce seeds with the preparation DMU-9 in the active concentration of 0.05 % promoted the creation of one line (B-24), and three more lines were obtained using the concentration 0.1 % of this preparation – B-21, B-22, and B-23. The lines were distinguished by a different degree of affinity in terms of the investigated association of qualitative traits. Line B-22 ($r_s < \pm 0.09$) was most different from the initial form. We isolated line B-23 with a weak correlation and two lines B-21 and B-24 with a moderate correlation to Zhnych variety (Table 5). Due to the ef-

PREDICTION OF THE BREEDING VALUE OF LEAF LETTUCE MUTANT LINES

Table 5. The differentiation of mutagenic substances using non-parametric criteria to evaluate differences in mutant lines of leaf lettuce (Derivatives of Zhnych Variety, K-046) based on the association of traits defining the leaf blade phenotype

Name of preparation	Concentration of mutagenic substance for presowing treatment of leaf lettuce seeds	Mutant line	Average index of normalized values	Limits of variation for Spearman's rank correlation coefficient, r_s		
				$r_s < \pm 0.09$	$\pm 0.1 < r_s < \pm 0.29$	$\pm 0.3 < r_s < \pm 0.69$
<i>Initial form – Zhnych variety</i>			1.29	–	–	–
DMS (reference)	0.05 %	B-38	0.89	+	–	–
D ₃ MU	0.05 %	B-7	0.89	+	–	–
		B-8	1.06	–	–	+
DMU-10A	0.1 %	B-2	0.91	–	+	–
		B-4	1.14	–	–	+
	0.05 %	B-6	1.04	–	+	–
		B-16	1.08	–	–	+
DMU-9	0.1 %	B-18	0.89	–	+	–
		B-11	1.0	–	+	–
	0.05 %	B-14	0.85	–	+	–
		B-24	1.04	–	–	+
		B-21	1.11	–	–	+
0.1 %	B-22	0.89	+	–	–	
	B-23	1.05	–	+	–	

fect of the reference preparation DMS (concentration of 0.05 %), we succeeded in obtaining only one mutant line B-38, but it was rather contrastive as compared to the initial form ($r_s < \pm 0.09$).

Having determined the specificities of the manifestation of qualitative traits of the leaf blade, we conducted a correlation analysis between them and other economically valuable traits to determine a probable connection between the phenotype elements of mutant lines and their productivity potential. The correlation analysis involved a total of 9 quantitative traits, valuable for breeding: “plant rosette height”; “plant rosette diameter”, “the number of leaves on one plant”, “leaf length”, “leaf width”, “weight of one plant”, “crop capacity”, “the duration of the period from the appearance of mass seedlings to stemming”, “the duration of the period from the appearance of mass shoots to flowering”. The correlation analysis involved the following non-parametric indices of evaluating the qualitative traits of “average rank” and “average index of normalized values”. To determine more generalized, common, correlation we used integral data for the study years by all the linear genotypes and initial form, as a cumulative value. Thus, while forming the initial database to conduct the correlation analysis, we used the block of

comparative respective indices of qualitative and quantitative traits of mutant lines and added the corresponding data about the initial form – Zhnych variety. The formation of the database of qualitative and quantitative traits was done using the results of three years of studies (2017–2019), obtained for the mutant generations M₃–M₅. The data of the correlation analysis are compiled in **Table 6**.

The obtained results demonstrated a statistically reliable correlation between the pairs of separate quantitative and qualitative traits. For instance, the average, practically same negative correlation bond ($r_s = -0.39...-0.40$) was noted for two pairs of indices – “average rank” and “average index of normalized values”, which were compared to the quantitative trait of “plant rosette diameter”.

A moderate or strong correlation ($r_s = 0.57...0.74$) was found for the abovementioned non-parametric indices with a relevant phenological phase of the development of leaf lettuce plants – the duration of the period from the appearance of mass shoots to stemming (Table 6). This phenological phase is especially relevant for the breeding selection, as it defines the completion of the vegetative period of leaf lettuce plants and their transition to the reproductive phase of development. Mu-

Table 6. Spearman's rank correlation coefficient (r_s) between the manifestation degree of qualitative and quantitative traits in mutant lines and the initial form, Zhnych variety*

Quantitative traits Non-parametric statistical indices	Plant rosette height	Plant rosette di-iameter	Number of leaves on one plant	Leaf length	Leaf width	Weight of one plant	Crop capacity	Duration of the pe-riod from the ap-pearance of mass se-edlings to stem-ming	Duration of the period from the appearance of mass sho-ots to flowering
Average rank	-0.01	-0.40	0.03	-0.04	0.10	-0.17	-0.16	0.74	-0.27
Average index of normalized values	-0.06	-0.39	0.01	-0.24	0.16	-0.08	-0.08	0.57	-0.07

Note. * Spearman's rank correlation coefficients, r_s , the reliability of which was confirmed on the significance level of $p < 0.05$, are in bold.

tant lines with a longer vegetation period are of higher breeding value, since they also have a longer period of economic suitability for the collection of leaf mass for fresh green products. Different components of lettuce leaves perform specific physiological functions and may be quantitatively described and interpreted using their observed properties. For instance, the petiole and veins perform the function of mechanic support for the leaf as an integral organ, while other components are closely related to photosynthesis (Leschuk et al, 2017; Du et al, 2022). The changes in anthocyanin coloring of the leaf blade in mutant forms of lettuce demonstrate the changes in their biosynthesis, and thus a different photoperiodic reaction as compared to the initial form – Zhnych variety.

The average negative correlation between non-parametric statistical indices, defining the phenotype of the leaf blade, and the manifestation of the quantitative trait of “plant rosette diameter” demonstrated that the increase in mutational changes in the phenotype of the leaf blade resulted in the decrease in the degree of manifestation of the abovementioned quantitative trait. Thus, there was a change in the architectonics of mutant plants of leaf lettuce as compared to the initial form. At the same time, a weak or almost absent correlation with five quantitative traits, defining the productivity of mutant lines of leaf lettuce (“the number of leaves on one plant”, “leaf length”, “leaf width”, “the weight of one plant”, “crop capacity”) demonstrated their more complicated genetic organization and poor dependence on the phenotypic manifestation of the entire complex of the investigated qualitative traits.

DISCUSSION

Therefore, the testing of the domestically produced preparations of mutagenic effect, – D₃MU, DMU-10A, and DMU-9 – determined their high efficiency in creating a 4–5 times larger number of mutant forms of plants as compared to the reference preparation DMS (dimethyl sulfate). Their application facilitated the creation of 13 mutant lines, while only one mutant line was obtained using the reference preparation. While analyzing the mutant generation, it should be noted that the mutagenic effect of preparations was noted only in the partial change of the initial form genome – Zhnych variety. The most efficient preparation was found to be D₃MU, the application of which facilitated the creation of five mutant lines (B-2, B-4, B-6, B-7, and B-8). As compared to the initial form, the total number of changes in the leaf blade phenotype in the five abovementioned mutant lines was 56.5 % (Table 3). Out of these, the combination of seven qualitative changes, responsible for the leaf blade morphology and its venation, related to 74.3 % of changes. Four traits, responsible for the shape of the leaf blade edge, related to 60 % of changes, and six qualitative changes, responsible for the leaf blade coloring, related to 36.6 % of changes. As compared to the initial form, there were practically no phenotypic changes in the trait of “the shade of green coloring” (Table 3). The greatest mutational changes were noted for line B-7 with 12 out of 17 investigated differences in the manifestation degree of qualitative traits. Line B-2 had 11 differences, and lines B-4, B-6, and B-8 had 8 differences each, respectively.

The preparation DMU-10A was responsible for 4 mutant lines – B-11, B-14, B-16 and B-18. As compared to the initial form, the total number of changes in the leaf blade phenotype in the four abovementioned mutant lines was 60.3 % (Table 3). As for the more differentiated evaluation, the difference in the manifestation degree of the traits, responsible for the morphology of the leaf blade and its venation, was 64.3 %, those for the shape of the leaf blade edge – 56.3 %, those for the leaf blade coloring – 58.3 %. The greatest mutational changes were noted for line B-18 (12 out of 17 investigated differences in the manifestation degree of qualitative traits). Lines B-11 and B-14 had 11 differences from the initial form, line B-16 had six differences. As compared to the initial form, there were practically no phenotypic changes in the trait of “the dissection of the leaf blade edge” and “anthocyanin coloring” (Table 3).

Preparation DMU-9 facilitated the creation of four lines (B-21, B-22, B-23, B-24). According to the analysis of the abovementioned mutant lines by the leaf blade phenotype, the total number of their differences from the analogous organ of Zhnych variety plants was 55.9 % (Table 3). For instance, the block of qualitative traits, responsible for the morphology of the leaf blade and its venation, had 67.9 % differences from the same traits in the initial variety. The block of qualitative traits, defining the shape of the edge and the coloring of the leaf blade, had 50 % differences, respectively. The greatest mutational changes were noted for line B-23 (13 out of 17 investigated differences in the manifestation degree of qualitative traits). Line B-22 had 12 differences, and lines B-21 and B-24 had 7 differences each, respectively. As compared to the initial form, there were practically no phenotypic changes in the trait of “anthocyanin coloring” (Table 3).

The only mutant line B-38, obtained from the effect of the reference preparation DMS, had a high rate of changes in the leaf blade phenotype as compared to the initial form. 13 out of 17 qualitative traits had some differences from the initial Zhnych variety. Among the qualitative traits, defining the morphology of the leaf blade and its venation, only the trait of “the shape of the tip” had the manifestation degree, analogous to the initial form. All the rest were different. All four phenotypic traits, defining the shape of the edge of the leaf blade, had different manifestation degrees as compared to the initial form. Out of six qualitative traits, defining the coloring of the leaf blade, three traits – “the shade of green coloring”, “anthocyanin coloring”, and “the type of distribution of anthocyanin coloring” were

different in their manifestation degree from the initial form (Table 3).

The cumulative analysis of all 14 mutant lines of leaf lettuce demonstrated that the range of variation of the manifestation degrees of qualitative traits, defining the morphology of the leaf blade and its venation, was within 50–100 %. The variation from 50 to 71.4 % was noted for the manifestation degrees of the qualitative traits, defining the shape of the leaf blade edge, and the variation from 7.1 to 71.4 % – for the manifestation degrees of qualitative traits, defining the leaf blade coloring.

To analyze the differences between the initial form and mutant lines, in addition to the average index of normalized values, we used Spearman’s rank correlation coefficient to compare the complexes of values of this index by 17 investigated qualitative traits. There was great agreement between the evaluation of the difference, shown by the average index of normalized values from Friedman’s analysis of variance and Spearman’s rank correlation coefficient. For instance, the smaller number of differences was between the mutant line and the initial form, the stronger correlation was between them. The highest affinity to the initial Zhnych variety was noted for lines B-16 and B-24 ($r_s = 0.58...0.62$). Their differences by the leaf blade phenotype related to the smallest number of qualitative traits – 6–7 out of 17 under investigation. Lines B-2, B-11, B-14, B-18, B-22, B-23, and B-38, in which the differences from the initial form were in 11–13 qualitative traits, had a weak correlation or it was practically absent ($r_s = -0.22...0.27$).

The analysis of the correlation between qualitative and quantitative traits demonstrated the impact of phenotypic changes in the leaf blade morphology on the manifestation degree of such quantitative traits as “plant rosette diameter” and “the duration of the period from the appearance of mass seedlings to stemming”. There was a moderate negative correlation ($r_s \approx -0.40$) between the changes in qualitative traits and the size of plant rosette diameter of mutant line plants. A moderate or strong positive correlation ($r_s = 0.57...0.74$) was noted for such indices as “the average rank” and “the average index of normalized values” with a relevant phenological phase of development of leaf lettuce plants – the duration of the period from the appearance of mass seedlings to stemming (Table 6). Both quantitative traits are related to the functional role of leaves in the formation of architectonics and photoperiodic reaction of leaf lettuce plants.

CONCLUSIONS

Consequently, the evaluation of three novel mutagenic agents, namely D₃MU, DMU-10A, and DMU-9, has substantiated their remarkable efficacy in instigating mutational alterations within the leaf lettuce genome, particularly concerning leaf blade morphology, when compared to the reference compound, DMS. It is worth noting that certain authors of this study have been granted a utility model patent in Ukraine for this method of broadening the spectrum of genotypic variability in leaf lettuce via induced mutagenesis (Konratenko et al, 2023). The method of mutational breeding was successfully employed to yield 14 mutant lines of leaf lettuce, derived from the domestic Zhnych variety, thereby contributing valuable genetic resources for breeding endeavors.

Furthermore, it was ascertained that all examined mutagenic agents, namely D₃MU, DMU-10A, and DMU-9, consistently demonstrated their superior efficacy, generating a significantly higher number of mutant plant forms, approximately four to fivefold more, relative to the reference compound DMS. Depending on the mutagenic agent's impact, we conducted a comparative analysis of the distinctions between the initial form (Zhnych variety) and the 14 mutant lines across 17 qualitative traits, which define the leaf blade's phenotype, employing non-parametric statistical methods.

Subsequently, calculations of the Spearman's rank correlation coefficient (r_s) for the initial form and mutant lines, concerning the manifestation degree across these 17 qualitative traits that characterize the leaf blade phenotype, unveiled correlations ranging from weak to moderate ($r_s = -0.08...0.62$). Within the spectrum of differentiation, the most pronounced divergence from the initial form was observed in three specific lines, denoted as B-7, B-22, and B-38 ($r_s = -0.08...0.09$).

Additionally, it was established that moderate to high correlations ($r_s = 0.57...0.74$) were evident between non-parametric indicators that quantified the manifestation degree of qualitative traits and a vital quantitative trait – the duration from the appearance of mass shoots to the stemming of plants within the mutant lines.

The observed correlations between qualitative and quantitative traits offer a valuable tool for the early selection of mutant genotypes of leaf lettuce, which exhibit extended vegetation periods, thus facilitating further breeding efforts. Notably, at the level of the M4 mutant generation, the qualitative traits that define the

leaf blade phenotype are remarkably stable and genetically fixed, demonstrating minimal variation under the influence of environmental factors.

The pursuit of a more comprehensive understanding of phenotypic diversity within leaf lettuce necessitates ongoing refinement of methods for assessing its components. Shifting from score-based evaluations to quantitative assessments can provide enhanced accuracy and precision, thereby allowing for a more refined differentiation of these traits. Future investigations in the field of mutational breeding for leaf lettuce should prioritize the exploration of correlations between the manifestation degrees of qualitative traits, especially those related to leaf blade coloration, and the various phenological phases during the plant's development throughout the growing season.

In summary, our analysis of the results underscores the stability and genetic fixation of qualitative traits defining the leaf blade phenotype within mutant generations. We have successfully expanded the spectrum of genotypic variability in the morphological traits of the leaf blade of leaf lettuce through pre-sowing seed treatment with novel preparations, chemical analogs of DMS, surpassing the performance of the reference preparation. Ultimately, the outcomes of our mutational breeding efforts have yielded 14 mutant lines of leaf lettuce that hold significant value for future breeding initiatives.

Adherence to ethical principles. This article does not contain the results of any studies, involving animals as study objects.

Conflict of interests. The authors deny any conflict of interests.

Financing. This study was financed within the framework of implementing the task of level II, 18.00.01.14.F “To study the effect of induced mutagenesis to obtain mutations for the purpose of improving the gene fund of less common species of vegetables” (No. of state registration 0116U000046) ASR of the NAAS 18 “Vegetable and Melon Growing” for 2016–2020.

Прогноз селекційної цінності мутантних ліній салату листкового за кореляційною залежністю між проявом якісних і кількісних ознак

Кондратенко С. І. ^{1*}, Самовол О. П. ¹, Кормош С. М. ², Митенко І. М. ¹, Позняк О. В. ³, Чабан Л. В. ³

¹ Інститут овочівництва і баштанництва
Національної академії аграрних наук,
Вул. Інститутська, 1, с. Селекційне, Харківський р-н,

Харківська обл., Україна, 62478

² Закарпатська державна

сільськогосподарська дослідна станція

Національної академії аграрних наук,

Пр. Свободи, 17, с. В. Бакта Берегівський р-н,

Закарпатська обл., Україна, 90252

³ Дослідна станція “Маяк” Інституту овочівництва і ба-

штанництва Національної академії аграрних наук,

Вул. Незалежності, 39, с. Крути, Ніжинський р-н,

Чернігівська обл., Україна, 16645

E-mail: shtirlitsmail@gmail.com *, ovoch.iob@gmail.com,

korsveta1967@ukr.net, mytenko.iob@gmail.com,

dsmayak@ukr.net

orcid: <https://orcid.org/0000-0002-8859-1604>

<https://orcid.org/0000-0002-6798-7378>

<https://orcid.org/0000-0002-1694-8280>

<https://orcid.org/0000-0001-8571-937X>

<https://orcid.org/0000-0002-6659-3238>

<https://orcid.org/0000-0002-0854-3443>

Мета. Встановити дію мутагенних чинників на формування якісних ознак, які визначають фенотип листкової пластинки, та дослідити кореляційну залежність між їх проявом і варіацією селекційно-цінних кількісних ознак у мутантних ліній салату листового.

Методи. Непараметрична статистика та критерії відмінності для біологічних об'єктів, розрахунково-аналітичні обчислення, кореляційний аналіз. **Результати.** Проведено порівняльний аналіз якісних ознак фенотипів листкової пластинки вихідної форми салату листового сорту Жнич та створених на його основі 14 мутантних ліній, отриманих в результаті мутагенної дії диметилсульфату (ДМС) та його похідних (Д₃МУ, ДМУ-10А і ДМУ-9) у двох концентраціях. Встановлено вищу ефективність мутагенної дії похідних ДМС: кількість мутантних форм рослин, порівняно із еталонним препаратом, зростала у 4–5 разів. Найбільш ефективним препаратом виявився Д₃МУ за дії якого створено 5 мутантних ліній, за мутагенної дії препаратів ДМУ-10А і ДМУ-9 – 4 мутантних лінії, тоді як за дії еталонного препарату ДМС – лише одну. При цьому контрастні по відношенню до вихідної форми мутантні лінії було отримано за дії всіх препаратів. З'ясовано особливості мутагенної дії біологічно-активних речовин на розширення спектру генотипової мінливості салату листового за 17 якісними ознаками, які визначають фенотип листкової пластинки. Аналіз коефіцієнту рангової кореляції Спірмена (r_s) між вихідною формою і мутантними лініями показав наявність слабого або середньої сили кореляційного зв'язку ($r_s = -0,08...0,62$). Виявлено 3 найбільш контрастні з вихідною формою лінії салату за якісними ознаками листкової пластинки – В-7, В-22 і В-38. Встановлено, що середній або високий кореляційний зв'язок ($r_s = 0,57...0,74$) непараметричних показників рівня прояву якісних ознак із важливою кількісною характе-

ристикою – тривалістю періоду від появи масових сходів до стеблуння рослин мутантних ліній. **Висновки.** Встановлені кореляційні зв'язки між кількісними і якісними ознаками дозволяють проводити на ранніх етапах онтогенезу рослин салату листового добір мутантних генотипів із більш подовженим вегетаційним періодом порівняно із вихідною формою.

Ключові слова: салат листовий, листкова пластинка, якісні ознаки, кількісні ознаки, мутагенез.

REFERENCES

- Acquaah G (2012) Hybridization. In: (2nd ed) Principles of plant genetics and breeding, ch 7. John Wiley & Sons, Ltd 131–145 p. <https://doi.org/10.1002/9781118313718.ch7>
- Arnold PA, Kruuk LEB, Nicotra AB (2019) How to analyse plant phenotypic plasticity in response to a changing climate. *New Phytol* 222(3):1235–1241. <https://doi.org/10.1111/nph.15656>
- Bar M, Ori N (2014) Leaf development and morphogenesis. *Development*. 141(22):4219–4230. <https://doi.org/10.1242/dev.106195>
- Bauer A, Bostrom AG, Ball J, Applegate C, Cheng T, Laycock S, Rojas SM, Kirwan J, Zhou J (2019) Combining computer vision and deep learning to enable ultrascale aerial phenotyping and precision agriculture: a case study of lettuce production. *Hortic Res* 6(70). <https://doi.org/10.1038/s41438-019-0151-5>
- Batista MF, De Souza LA (2017) Flower structure in ten *Asteraceae* species: considerations about the importance of morpho-anatomical features at species and tribal level. *Braz J Bot* 40:265–279. <https://doi.org/10.1007/s40415-016-0312-9>
- Begum T, Dasgupta T (2010) A comparison of the effects of physical and chemical mutagens in sesame (*Sesamum indicum* L.). *Genet Mol Biol* 33(4):761–766. <https://doi.org/10.1590/S1415-47572010005000090>
- Basnet K, Adhikari NR, Pandey MP (2014). Multivariate analysis among the Nepalese and exotic mungbean (*Vigna Radiata* L. Wilczek) genotypes based on the qualitative parameters. *Universal J Agric Res* 2(5):147–153. <http://dx.doi.org/10.13189/ujar.2014.020502>
- Ceccarelli S, Grando S (2020) Evolutionary Plant Breeding as a Response to the Complexity of Climate Change. *iScience* 23(12):101815. <https://doi.org/10.1016/j.isci.2020.101815>
- Cheng J, Lu H, He X et al (2018) Enhancing growth rate of lettuce by mutating lettuce seeds with nuclear irradiation. *Carbon Resources Conversion* 1(1):55–60. <https://doi.org/10.1016/j.crcon.2018.05.001>
- Damerum A, Chapman MA, Taylor G (2020) Innovative breeding technologies in lettuce for improved post-harvest quality. *Postharvest Biol Technol* 168:111266. <https://doi.org/10.1016/j.postharvbio.2020.111266>
- Delia M, Cristea V, Daraban L (2013) Dose-dependent effects of gamma radiation on lettuce (*Lactuca sativa* var.

- capitata*) seedlings. *Inter J Radiat Biol* 89(3):219–223. <https://doi.org/10.3109/09553002.2013.734946>
- Du J, Li B, Lu X et al (2022) Quantitative phenotyping and evaluation for lettuce leaves of multiple semantic components. *Plant Methods* 18:54. <https://doi.org/10.1186/s13007-022-00890-2>
- Ewing PM, Runck BC, Kono TYJ et al (2019) The home field advantage of modern plant breeding. *PLoS one* 14(12):e0227079. <https://doi.org/10.1371/journal.pone.0227079>
- Ferreira C, Lopes F, Costa R et al (2020) Microbiological and chemical quality of Portuguese lettuce – results of a case study. *Foods* 9(9):1274. <https://doi.org/10.3390/foods9091274>
- Franco CH, Santos VHM, Silva LP et al (2015). Mutagenic potential of lettuce grown from irradiated seeds. *Scientia Horticulturae* 182(5):27–30. <http://dx.doi.org/10.1016/j.scienta.2014.11.011>
- Gandra TKV, Alves PIC, Gandra EA (2018) Microbiological analysis of lettuce (*Lactuca sativa*) from conventional and organic cultivation commercialized at fairs in Brazil. *J Food Sci Technol* 3(2):288–294. <http://dx.doi.org/10.25177/JFST.3.2.4>
- Guo Z, Li B, Du J et al (2023) LettuceGDB: The community database for lettuce genetics and omics. *Plant Communications* 4(1):100425. <https://doi.org/10.1016/j.xplc.2022.100425>
- Gurdon C, Poulev A, Armas I et al (2019) Genetic and Phytochemical Characterization of Lettuce Flavonoid Biosynthesis Mutants. *Sci Rep* 9(1):3305. <https://doi.org/10.1038/s41598-019-39287-y>
- Han R, Truco MJ, Lavelle DO et al (2019) A composite analysis of flowering time regulation in lettuce. *Front Plant Sci* 2:632708. <https://doi.org/10.3389/fpls.2021.632708>
- Hassan MN, Mekkawy SA, Mahdy M et al (2021) Recent molecular and breeding strategies in lettuce (*Lactuca* spp.). *Genet Resour Crop Evol* 68:3055–3079. <https://doi.org/10.1007/s10722-021-01246-w>
- Huo H, Henry IM, Coppoolse ER et al (2016) Rapid identification of lettuce seed germination mutants by bulked segregant analysis and whole genome sequencing. *Plant J* 2016 88(3):345–360. <https://doi.org/10.1111/tpj.13267>
- Hvostova VV (1965) Metody primeneniya ioniziruyusih izluchenyi i drugih mutagennyih faktorov v selektsii rasteniy. In: *Radiatsiya i selektsiya rasteniy*. Moskva: Atomizdat, pp 39–50. (in Russian)
- International Union for the Protection of New Varieties of Plants (2021) List of Crops / Species. <https://www.upov.int/genie/species.xhtml>. Accessed 23 Aug 2023
- Kodym A, Afza R (2003) Physical and chemical mutagenesis. In: Grotewold E. (ed) *Plant Functional Genomics*. *Met Mol Biol* 236. Humana Press 189–203p. <https://doi.org/10.1385/1-59259-413-1:189>
- Kodym A, Afza R, Forster BP et al (2012) Methodology for physical and chemical mutagenic treatments. CABI Books. CABI International. <https://doi.org/10.1079/9781780640853.0169>
- Kondratenko SI, Krutko RV, Chaban LV et al (2020) Evaluation of yield potential of mutant lines of leaf lettuce under different climatic conditions of growing. *Scientific Reports of NULES of Ukraine* 2(84). <http://dx.doi.org/10.31548/dopovidi2020.02.005>
- Kondratenko S, Mohylna O, Khareba O et al (2019) *Metodyka klasyfikator provedennia ekspertyzy sortiv salatu posivnoho (Lactuca sativa L.) na vidmynnist, odnoridnist i stabilnist / 2nd ed., supplement. and finalized*. Vinnytsia: TVORY. – 64 p (in Ukrainian). <https://www.sops.gov.ua/2019>
- Kondratenko SI, Poznyak OV, Chaban LV et al (2023) The method of expanding the genotypic variability of seeding leaf (*Lactuca sativa* L. var. *secalina*) using the method of induced mutagenesis. UA Patent 153136, 25 May 2023. https://base.uipv.org/searchINV/search.php?action=view_details&IdClaim=285157
- Kovácsné MÁ, Takácsné HM (2022) Agronomic evaluation of different lettuce (*Lactuca sativa* L.) varieties under unheated plastic tunnel. *Inter J Horticult Sci* 28:50–56. <https://doi.org/10.31421/ijhs/28/2022/10314>
- Křístková E, Doležalová I, Lebeda A et al (2008) Description of morphological characters of lettuce (*Lactuca sativa* L.) genetic resources. *Hort. Sci. (Prague)* 35(3):113–129. <https://doi.org/10.17221/4/2008-HORTSCI>
- Leschuk N, Popova O, Shkapenko E et al (2017) Characteristics of the species diagnosis of the *Lactuca sativa* L. phenotype by the morphometric distances of leaf parameters. *Bulletin of Lviv National Environmental University. Series “Agronomy”* 21:101–110. (in Ukrainian). <http://visnuk.kl.com.ua/joom/images/docs/agro2017,%20%E2%84%9621.pdf>
- Litun PP, Kyrychenko VV, Petrenkova VP et al (2009) *Sistemnyi analiz v selektsii poliovykh kultur*. Navchalnyi posibnyk. Kharkiv: The Plant Production Institute named after V.YA. Yuriev. – 354 p. (in Ukrainian)
- MacFarland TW, Yates JM (2016) Friedman Two-way Analysis of Variance (ANOVA) by Ranks. In: *Introduction to Non-parametric Statistics for the Biological Sciences Using R*. Springer, Cham. https://doi.org/10.1007/978-3-319-30634-6_7
- Marcu D, Cristea V, Daraban L (2013) Dose-dependent effects of gamma radiation on lettuce (*Lactuca sativa* var. *capitata*) seedlings. *Int J Radiat Biol* 89(3):219–223. <https://doi.org/10.3109/09553002.2013.734946>
- Medina-Lozano I, Bertolín JR, Díaz A (2021) Nutritional value of commercial and traditional lettuce (*Lactuca sativa* L.) and wild relatives: Vitamin C and anthocyanin content. *Food Chemistry* 359:129864. <https://doi.org/10.1016/j.foodchem.2021.129864>
- Mir AS, Maria M, Muhammad S et al (2021) Potential of mutation breeding to sustain food security. *Intech Open*. <http://dx.doi.org/10.5772/intechopen.94087>
- Mou B (2011) Mutations in lettuce improvement. *Inter J Plant Genom* 2011:723518. <https://doi.org/10.1155/2011/723518>

- Mousavi S, Hassandokht M, Choukan R et al (2012) Assessment of qualitative and quantitative traits in commercial Iranian lettuce (*Lactuca sativa* L.) genotypes. *Ann Biol Res* 3(9):4352–4361. <https://www.scholarsresearchlibrary.com/articles/assessment-of-qualitative-and-quantitative-traits-in-commercial-iranian-lettuce-lactuca-sativa-l-genotypes.pdf>
- Ohki S, Hatashita M (2012) Mutation breeding by ion beam in lettuce (*Lactuca sativa* L.) using an *in vitro* regeneration system. *Acta Hort* 961:285–290. <https://doi.org/10.17660/ActaHortic.2012.961.37>
- Patella A, Palumbo F, Galla G et al (2019) The molecular determination of hybridity and homozygosity estimates in breeding populations of lettuce (*Lactuca sativa* L.). *Genes* 10(11):916. <https://doi.org/10.3390/genes10110916>
- Shcherbyna N, Yurlakova O (2019) Demand and supply in the market of horticulture. *Vegetable and Melon Growing* 60:294–298. <https://vegetables-journal.com/index.php/journal/article/view/255>
- Sorel M, Svanella-Dumas L, Candresse T et al (2014) Key mutations in the cylindrical inclusion involved in lettuce mosaic virus adaptation to eIF4E-mediated resistance in lettuce. *Molecular Plant-Microbe Interactions* 27(9):1014–1024. <http://dx.doi.org/10.1094/MPMI-04-14-0111-R>
- Wen P, Meng Yu, Gao C et al (2023) Field identification of drought tolerant wheat genotypes using canopy vegetation indices instead of plant physiological and biochemical traits, *Ecological Indicators*, 154: 110781. <https://doi.org/10.1016/j.ecolind.2023.110781>