



# A case study on lentil to demonstrate the value of using historic data stored in genebanks to guide the selection of resources for research and development projects

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**Abstract:** Plant genetic resources are essential for sustainable agriculture and a secure and stable global food supply. One of the most important pulses and an integral part of a healthy diet is lentil (*Lens culinaris* Medik.). The National Centre for Plant Genetic Resources of Ukraine (NCPGRU), based in Kharkiv, manages a lentil collection of 1,140 accessions. In 2019, the first exchange of 37 lentil accessions between NCPGRU and a French research team took place. This exchange was accompanied by the transfer of phenotyping data for multiple traits. Considering that data collected in different environments provide important information on trait stability, the lentil accessions were phenotyped under new conditions through field research. This research allowed a comparative analysis of agroclimatic conditions for lentil cultivation in Ukraine (Kharkiv region) and France (Bourgogne-Franche-Comté region). The possibility of using genebank information to guide plant material selection for research and development projects was assessed. As a result, six lentil genotypes that performed well in different environments were selected. This allowed the identification of genotypes with the highest yield potential: UD0600086, UD0600145, UD0600437, UD0600530, UD0600550 and UD0600638. Genotypes recommended for use in breeding to produce high-yielding, relatively stable lentil varieties were successfully selected in the application case of this study, confirming that the information in the trait database of the NCPGRU Genebank is an important resource for predicting the characteristics of lentil accessions. Of the eight accessions selected by GGE biplot analysis using field research, five were predicted to be more promising by previous genebank data.

**Keywords:** lentil (*Lens culinaris* Medik), pulses, genetic resources, genebank trait database, breeding material

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## Introduction

Sustainable agriculture and global food security depend on the availability of well-described plant genetic resources. However, differences in the methods used to evaluate and describe germplasm held in genebanks in different countries, and in the accessibility of data, are a major obstacle, leading to the underutilization

of these resources and negatively impacting future prospects (Egan *et al*, 2022). A better understanding of the stability and potential of measured traits can be achieved by considering data from different experiments, thereby improving the prospects of using genetic resources in novel breeding programmes (Cristobal *et al*, 2014). Consequently, genetic resource collections can become more utilized through enhanced cooperation and sharing not only seeds but also the accumulated knowledge gained over many years of resource regeneration and/or research (Guerra-García *et al*, 2021).

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Lentil (*Lens culinaris* Medik.) is one of the most important pulse crops and is an essential component of a balanced diet. Historically, the primary producers of lentils have been Asian countries and Canada. In Europe, lentils account for only 4% of the total area devoted to pulses. EU countries produce significantly fewer lentils than required to meet domestic consumption needs (TerresUnivia, 2021). France and Spain are the main producers and consumers of lentils within the European Union. The area harvested for lentils has doubled between 2010 and 2018 (Kaale et al, 2023). The expansion of lentil production in Europe depends on the development of novel, advanced, high-yielding and nutritionally improved varieties (Rajpal et al, 2023). The initial step in this process is a comprehensive review and analysis of the available genetic resources in genebanks to identify suitable sources for selecting and crossing in order to breed elite lines. The creation of special medium-core and mini-core collections allowed for more detailed study of traits, systematization of accumulated material and information, and generally better management of research (Bisht et al, 1998; Díez et al, 2018). However, starting this process from scratch is long and costly. It is, therefore, more expedient to utilize the material and information available in genebanks to accelerate the preliminary phase. Some genebanks, such as the Indian National Gene Bank (ICAR), which currently hosts 2,324 lentil accessions, have initiated this process. This genebank has characterized the entire collection for dozens of agromorphological traits to identify the most suitable accessions for use in breeding programmes (Tripathi et al, 2022).

The lentil collection of the National Plant Genetic Resources Centre of Ukraine (NCPGRU) in Kharkiv was established in 1993 and currently comprises 1,140 accessions, which were collected through interbank exchanges, independent expeditions and collaboration with breeders (Kobyzeva et al, 2011; Kir'yan et al, 2014; Vus et al, 2020c). All genotypes were subjected to a comprehensive study over three years, during which their main phenotypic, morphological and agronomic characteristics were outlined. Based on the collected data, a basic collection, an educational collection and a reference basic set were created and registered (Bezuhla and Kobyzeva, 2021). Accessions that successfully completed the research cycle and exhibited specific traits were included into a specialized trait-oriented database. This database served as the source of information for the first exchange of lentil seed material between French researchers and NCPGRU, which took place in 2019. This exchange facilitated evaluating the performance of selected samples under different climatic conditions. This also enabled the assessment of the potential of using additional data from the donor genebank for a more comprehensive analysis of the genotypes. Developing methods for comparing accessions despite the differences in cultivation and research techniques,

as well as the soil and climatic characteristics of the regions, was particularly significant.

Information on accessions from the NCPGRU database collected during the period from 1993 to 2016 was processed for a preliminary evaluation and recommendation of genotype characteristics and a preliminary forecast of their use in breeding programmes. The results of field studies conducted in Ukraine (2019) and France (2021) were used to assess the accuracy of the provided forecasts, clarify the outcomes of the research, and refine the methodological approaches employed in such studies.

The aim of this research was to analyze data from the NCPGRU traits database to identify the most promising sources for breeding. The results were evaluated through trials conducted in two ecogeographical locations. The methods used took into account the specificity of each trial in terms of experimental design and choice of standards.

## Materials and methods

### Plant material

A total of 37 lentil (*Lens culinaris* Medik.) accessions from NCPGRU were included in this study, 29 of them belong to the subsp. *microsperma* (100-seed weight < 4.5g), while the rest belongs to subsp. *macrosperma* (100-seed weight > 4.5g). The accessions under study were from 19 countries (Table 1).

Transferring of lentil accessions was accompanied by SMTA agreement, according to the terms of the International Treaty on Plant Genetic Resources for Food and Agriculture (FAO, 2009).

For the list of 37 lentil accessions, which were transferred to the genebank of France (INRAE, Dijon), a preliminary evaluation was carried out according to the characteristic database of the genebank of Ukraine. Preliminary recommendations for their use were provided, together with a comparative assessment of their resistance to stress factors and potential productivity in comparison with standard accessions, based on phenotypic data during historic regenerations (1993-2016). In order to verify the applicability of the given prediction based on preliminary data from the genebank of Ukraine, two studies were conducted in Ukraine (2019) and in France (2021).

Figure 1 illustrates the complete data set, showing the accessions and the years in which they were evaluated.

### Field trials

The field trials in Ukraine were carried out at the Plant Production Institute of named after V. Ya. Yuriev, Kharkiv, Ukraine (PPI NAAS) at the Laboratory of Genetic Resources of Grain Legumes - Elitne vilage, Kharkiv district, Kharkiv region (49°59'31"N, 36°26'55"E; 95m above sea level). A 4-year crop rotation was applied with winter wheat being systematically grown as the preceding crop for lentil. The experiments were conducted according to the *Methodical rec-*

**Table 1.** List of lentil accessions used in this study. The accession ID has been provided here to facilitate reference to the accessions. Genebank ID, accession name, country of origin and subspecies information are provided according to the National Plant Genetic Resources Centre of Ukraine (NCPGRU) database. The accessions were grouped in four clusters, for more details on clustering see Figure 4.

Accession ID	Genebank ID	Accession name	Country of origin	Subspecies	Cluster
1	UD0600006	CF 17-5	Morocco	<i>microsperma</i>	3
2	UD0600007	MEL M 5	France	<i>microsperma</i>	1
3	UD0600028	-	Ethiopia	<i>microsperma</i>	3
4	UD0600036	Krasnohads'ka 49	Ukraine	<i>macrosperma</i>	2
5	UD0600052	Stepova 244	Ukraine	<i>microsperma</i>	4
6	UD0600065	Giza 9	Egypt	<i>microsperma</i>	1
7	UD0600084	Anicia	France	<i>microsperma</i>	4
8	UD0600086	MEL C 4	Morocco	<i>microsperma</i>	4
9	UD0600090	Dieu printemp foncé	France	<i>microsperma</i>	1
10	UD0600091	Du Puy	France	<i>microsperma</i>	4
11	UD0600092	Spatz Albinzée	France	<i>microsperma</i>	1
12	UD0600095	Ge IC-P	Hungary	<i>microsperma</i>	1
13	UD0600103	-	France	<i>macrosperma</i>	1
14	UD0600112	Krasnohads'ka 250	Ukraine	<i>macrosperma</i>	2
15	UD0600119	-	France	<i>macrosperma</i>	1
16	UD0600131	Gornostepnaia	Armenia	<i>macrosperma</i>	1
17	UD0600145	Code 35	Morocco	<i>microsperma</i>	4
18	UD0600163	-	France	<i>macrosperma</i>	2
19	UD0600203	Flip 86-38L	Syria	<i>microsperma</i>	3
20	UD0600248	-	Syria	<i>macrosperma</i>	1
21	UD0600347	ILL 481	Lebanon	<i>microsperma</i>	3
22	UD0600403	Nadejda	Bulgaria	<i>microsperma</i>	4
23	UD0600423	CDC Sunrise	Canada	<i>microsperma</i>	1
24	UD0600437	-	Israel	<i>microsperma</i>	4
25	UD0600443	-	Iran	<i>microsperma</i>	4
26	UD0600444	-	Ethiopia	<i>microsperma</i>	1
27	UD0600468	-	France	<i>macrosperma</i>	2
28	UD0600490	-	Afghanistan	<i>microsperma</i>	1
29	UD0600496	-	Mexico	<i>microsperma</i>	2
30	UD0600521	-	China	<i>microsperma</i>	1
31	UD0600530	Slovyanka	Russia	<i>microsperma</i>	2
32	UD0600550	-	Bulgaria	<i>microsperma</i>	4
33	UD0600563	-	Ethiopia	<i>microsperma</i>	4
34	UD0600614	Precoz	Brazil	<i>microsperma</i>	1
35	UD0600638	Eston	Canada	<i>microsperma</i>	4
36	UD0600686	-	France	<i>microsperma</i>	2
37	UD0601021	Beluga	Israel	<i>microsperma</i>	1

ommendations for studying the genetic resources of grain legumes (Kobyzeva *et al.*, 2016). The area of each experimental plot was 1m<sup>2</sup>, and the sowing design was 20cm × 10cm, 6 rows of 10 plants each, or 60 plants per plot. The seeds were hand sown. Weeds were removed manually. In accordance with the methodology of genetic resources studies, and considering that no replicates were included, a block of standards sown every 20 plots was systematically added for the correct evaluation of the accessions in the field. For lentil evaluation in NCPGRU, the three historically used standard accessions were included in each standard block, namely Stepova

244 (UD0600052), Krasnohads'ka 49 (UD0600036) and Krasnohads'ka 250 (UD0600112).

Field trials in France were conducted at the Epoisses Experimental Unit (U2E), managed by INRAE, in Bretenière, France (05°05'57"N, 47°14'11"E; 210m above sea level). Agroecological management practices were applied. Weeds were removed manually. Seeds were sown mechanically and the experimental plot consisted of three rows of 1m each with 20 seeds per row, or 60 plants per plot. Three replications per accession with Anicia (UD0600084) and Beluga (UD0601021) were used as standard accessions.





data collected between 1993 and 2016 can provide valuable information on the potential of the accessions to be used for research and breeding, including outside Ukraine.

### Comparison of weather conditions in trials in Ukraine (1993–2019) and France (2021)

The sowing dates of lentil accessions in Kharkiv, Ukraine varied each year depending on weather conditions. In general, sowing took place between the end of March and the beginning of May. Plants matured between the end of July and the beginning of August and the average length of the vegetation period was 84 days. Average daily temperatures during the lentil growing season in Ukraine in the years of our observations (1993–2016) ranged from 15.6 to 21.3°C. The average sum of temperatures during the lentil period of vegetation was 1,661.3°C, and the average sum of precipitation during the same period was 176.8mm (Figure 2).

Weather conditions in 2019, when the research trial was conducted in Ukraine, were characterized by high precipitation at the beginning of the growing season and intermittent showers during flowering and maturity. The end of the growing season featured a prolonged period of drought and high temperatures, which led to rapid desiccation of the plants and had a negative impact on seed filling.

In Bretenière, France (Bourgogne-Franche-Comté region), in 2021, sowing took place on 25 February and germination was observed on 10 March. The plants reached maturity at the beginning of July, with an average vegetation period of almost 119 days. The weather conditions in the region during this year were atypical, exhibiting a greater degree of dryness and heat than is typical for a multi-year period. The weather conditions that accompanied the study in France were characterized by a lower temperature (average sum 1,528.1°) and a significantly higher amount of rainfall (258.1mm) during the growing season, compared to most of the trials conducted in Ukraine (Figure 2).

Analysis of the lentil yield data collected in Kharkiv, Ukraine in the period ranging from 1993 to 2016 and 2019 showed a rather high variability due to a wide range of weather conditions over the study years (Figure 3). The years with high productivity were 2006 and 2015, while the years with the lowest seed production were 1996 and 2014. This allowed a comprehensive assessment of the stability of the performance of the lentil accessions.

### Analysis of Ukrainian genebank data

The database of the NCPGRU lentil collection includes results from multiple years of research in the field and in the laboratory. The analysis of variance for 37 lentil accessions from the genebank database in this study, which provided insights into the genetic diversity and variability, is presented in Table 2.

Considering ten agronomic traits with a significant coefficient of variation (ranging from 11.7 to 75.92%),

it was found that the duration of the growing season (11.7%) and the number of days to flowering (15.87%) were the most stable traits. Conversely, seed yield per plot exhibited the highest coefficient of variation (75.92%).

The generalization of the long-term data (1993–2016) of the genebank of Ukraine was carried out using principal component analysis (PCA) (Figure 4).

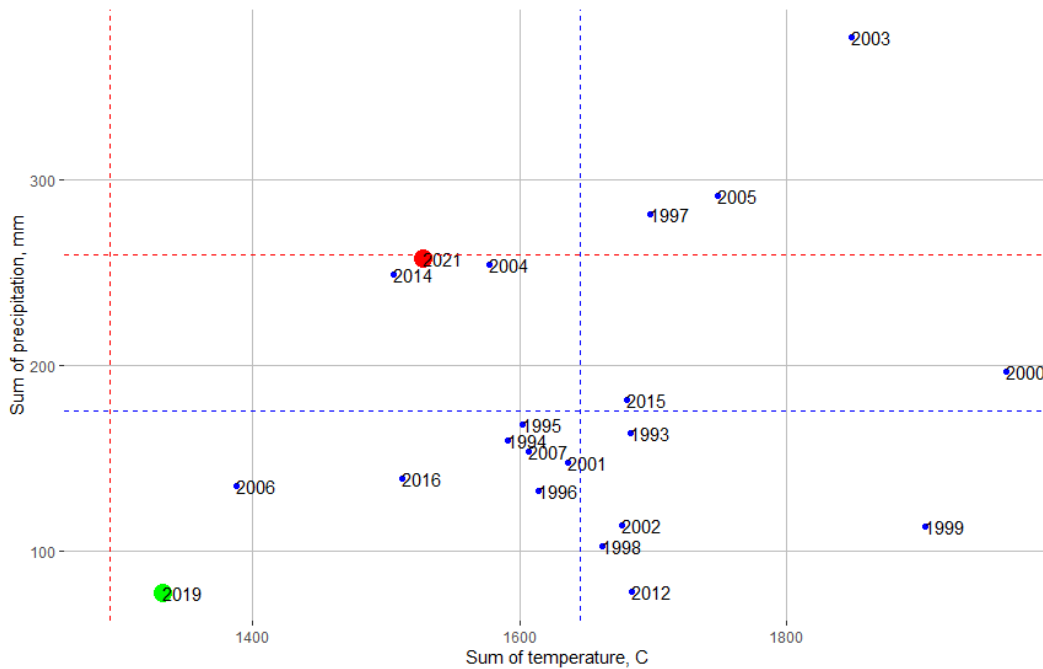
PCA of agronomic traits (seed yield per plant (P1), weight of 100 seeds (P2), seed yield per area (P3), number of seeds per plant (P6), plant height (M1), height of the lowest pod (M2), and relative yield to standards (CY)) and weather parameters including sum of effective temperatures (and sum of precipitations by different phenophases) revealed that the first two components accounted for over 50% of the total variance. A strong correlation was observed between yield components and weather conditions (both total and relative yield, weight and number of seeds, height of attachment of the lower pod). For the second axis, the most significant indicator was the duration of the growing season and the sum of temperatures experienced during this period.

The qualitative factor map (Figure 4B) illustrates that Dimension 1 opposes individuals located on the right of the graph, characterized by strongly positive coordinates on the axis to individuals characterized by strongly negative coordinates on the axis (left of the graph). This group, characterized by positive coordinates on the axis, showed high values for variables like seed yield per plant (P1), seed yield per area (P3), relative yield to standards (CY), number of seeds per plant (P6), plant height (M1), height of the lowest pod (M2), days from germination to full maturity (F5), sum of effective temperatures during the vegetation period (S\_T\_C), days to 50% of flowering (F8) and days to 50% of flowering (F2). The variables are ranked from the strongest to weakest.

A K-means cluster analysis was performed to identify groups of lentil accessions based on the similarity of their agronomic characteristics. The 37 accessions were grouped into four clusters with no direct relationship between cluster separation and the geographical origins of the accessions (Table 1, Figure 4). Cluster 1 and Cluster 4 were the largest with 15 and 10 total accessions, respectively. The average indicators for each cluster are summarized in Table 3.

Cluster 4 is the most promising for breeding. The accessions have a high mean yield (0.156kg/m<sup>2</sup>) and a high mean percentage yield compared to the standards (103.45%) and all belong to the subspecies *microsperma*. Three of them are from France. The complete characteristics of the lentil accessions from this cluster, according to the most important agronomic traits, are given in Table 4.

In addition, Cluster 2, which combines seven accessions of the *macrosperma* type, is important for further breeding processes. Cluster 2 includes two standard accessions from Ukraine and five landraces – three from France, one from Mexico and one from



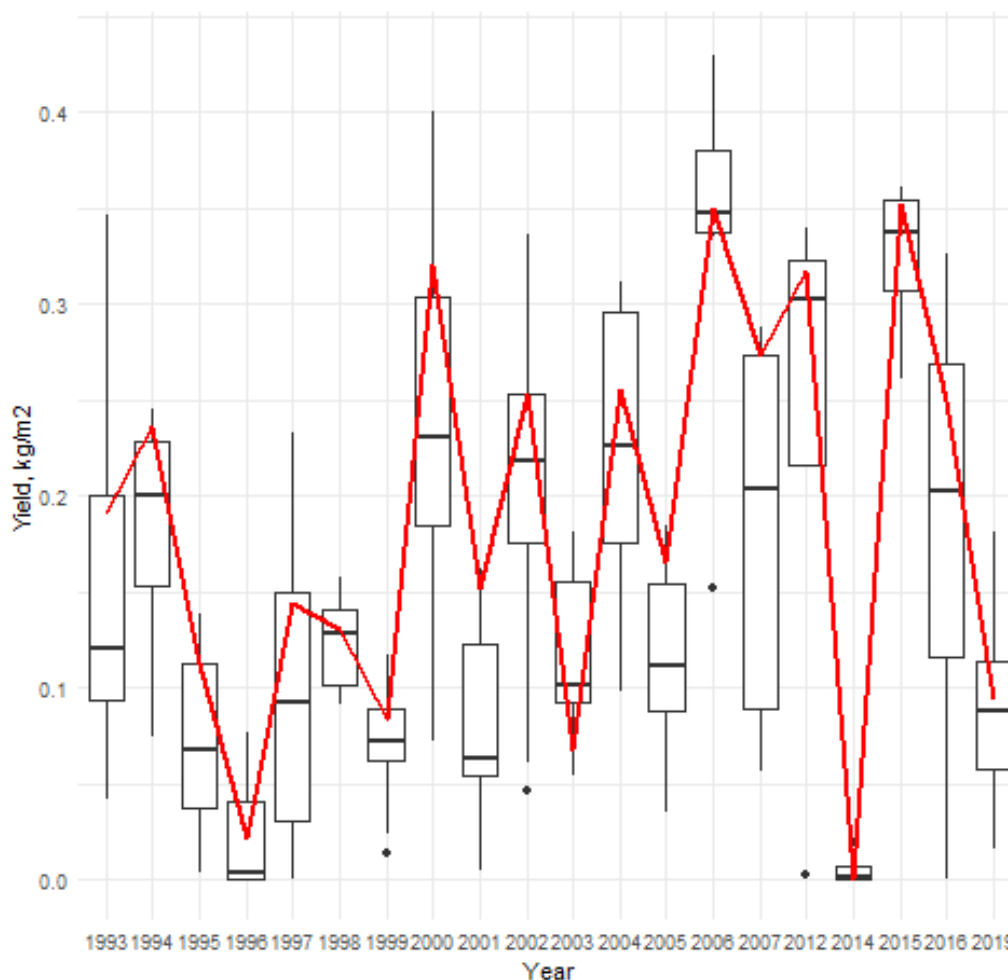
**Figure 2.** Sum of temperature (°C) and sum of precipitation (mm) during the lentil vegetative period in Ukraine (1993–2016 and 2019) and France (2021). The red dot refers to the year when the accessions provided by the National Plant Genetic Resources Centre of Ukraine (NCPGRU) were evaluated in the field in France. The green dot refers to the year when the maximum number of accessions evaluated in France were considered for seed multiplication and phenotyping in Ukraine. The dashed lines correspond to the average sum of temperature and sum of precipitation: blue for Kharkiv, Ukraine (1993–2016) and red for Bretenière, France (2011–2021).

**Table 2.** Analysis of variance for 37 lentil accessions from the National Plant Genetic Resources Centre of Ukraine (NCPGRU) database (1993–2016). SD, standard deviation; CV, coefficient of variation; \*\*\*, statistically significant at  $P < 0.001$ .

Traits	Min–max	Mean	SD	CV, %	Source of variation	
					Genotype	Year
Plant height (M1), cm	0–80	37.54	13.76	36.66	4.617***	11.491***
Height of the lowest pod (M2), cm	0–26	14.43	6.11	42.35	3.496***	8.842***
Number of seeds per plant (P6)	0–329	93.3	69.08	74.04	5.116***	6.319***
Weight of 100 seeds (P2), g	0–87.6	39.45	19.92	50.50	12.412***	6.156***
Seed yield per plant (P1), g	0–9.5	2.96	1.96	66.24	3.496***	8.842***
Seed yield per area (P3), kg/m <sup>2</sup>	0–0.43	0.137	0.104	0.076	4.978***	16.621***
Relative productivity (CY), %	0–457.45	145.44	110.42	75.92	4.978***	16.621***
Days from germination to 50% flowering (F2)	25–56	42.6	6.76	15.87	14.04***	29.13***
Days from 50% flowering to full maturity (F8)	13–66	41.38	8.49	20.51	2.479***	18.948***
Days from germination to full maturity (F5)	61–108	83.98	9.83	11.70	7.231***	26.837***

**Table 3.** Agronomic traits characterizing the four clusters of lentils (1993–2016) (means for cluster).

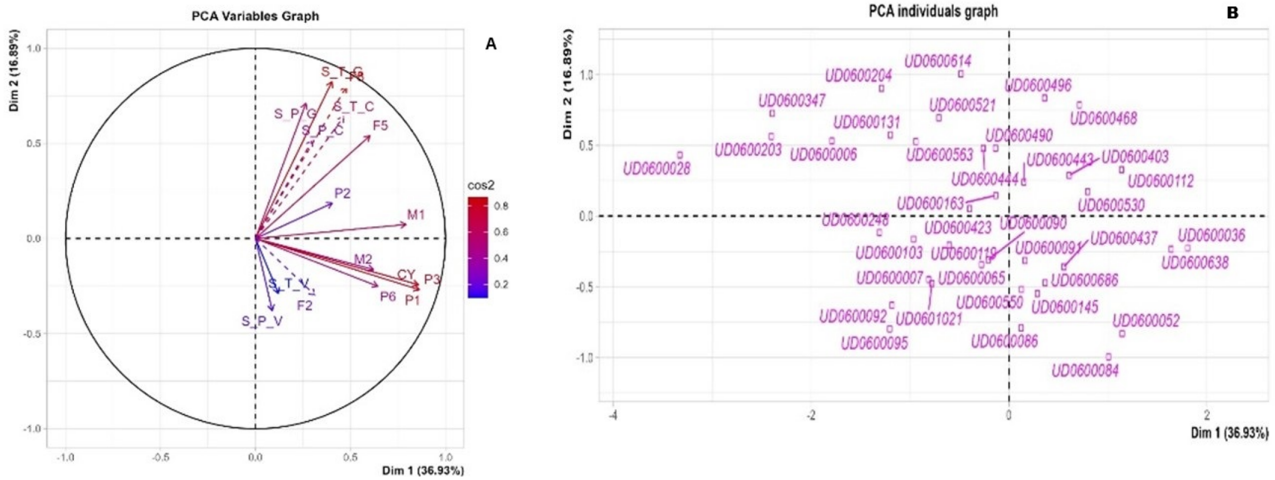
Cluster	Plant height, cm	Height of the lowest pod, cm	Number of seeds per plant	Weight of 100 seeds, g	Yield of seeds per plant, g	Yield per plot, kg/m <sup>2</sup>	Relative yield to standards, %
1	33.39	14.91	70.63	3.4	1.85	0.080	59.63
2	42.91	19.56	59.86	5.9	2.71	0.110	85.9
3	25.44	8.95	23.13	3.2	0.88	0.024	23.69
4	39.66	15.17	115.41	2.9	3.15	0.156	103.45



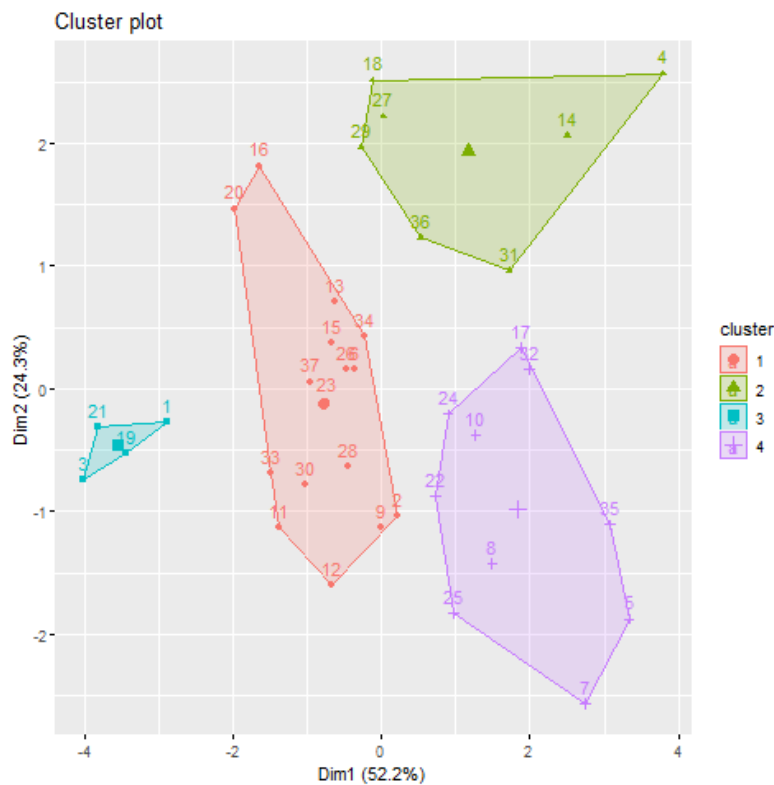
**Figure 3.** Boxplots showing seed yield ( $\text{kg}/\text{m}^2$ ) of lentil accessions per research year in Kharkiv, Ukraine. The red line represents the median of the yield of three standard accessions, Stepova 244 (UD0600052), Krasnohrad's'ka 49 (UD0600036) and Krasnohrad's'ka 250 (UD0600112), regularly sown and phenotyped in each year.

**Table 4.** Agronomical traits of ten *microsperma* lentil accessions composing Cluster 4 (National Centre for Plant Genetic Resources of Ukraine (NCPGRU), 1993–2016).

Accession ID	Plant height, cm	Height of the lowest pod, cm	Number of seeds per plant	Weight of 100 seeds, g	Yield of seeds per plant, g	Yield per plot, $\text{kg}/\text{m}^2$	Relative yield to standards, %
UD0600052	44.5	15	187	2.6	3.9	0.196	97.94
UD0600084	32.2	13.3	182.7	2.7	3.75	0.161	144.72
UD0600086	34.5	14.5	113.5	2.6	2.95	0.168	104.1
UD0600091	45.7	13.7	55.9	2.5	2.9	0.149	97.13
UD0600145	36.3	20	88	3.9	3.6	0.163	85.65
UD0600403	34.8	12.4	75	3.4	2.4	0.156	107.76
UD0600437	40.6	18.4	109	2.7	2.6	0.090	78.9
UD0600443	34.3	10.25	122	3	3	0.152	92.25
UD0600550	52.1	18.6	98	2.5	2.55	0.108	109.54
UD0600638	41.6	15.5	123.00	3.1	3.8	0.217	116.5



**Figure 4.** Principal component analysis of phenological, agronomic and meteorological parameters. A, Variables factor map; B, Qualitative factor map. Phenology traits include: F2, days to 50% of flowering; F5, days from germination to full maturity; F8, days from 50% of flowering to full maturity. Agronomic traits include: P1, seed yield per plant, g; P2, weight of 100 seeds, g; P3, seed yield per area, kg/m<sup>2</sup>; P6, number of seeds per plant; M1, plant height, cm; M2, height of the lowest pod, cm; CY, relative yield to standards. Weather parameters: S.T.C, sum of effective temperatures during the vegetation period; S.P.C, sum of precipitation during the vegetation period; S.T.G, sum of temperature during the pod filling period; S.P.G, sum of precipitation during the pod filling period; S.T.V, sum of temperature during the period from germination to the beginning of flowering, S.P.V, sum of precipitation during the period from germination to the beginning of flowering.



**Figure 5.** Cluster plot highlighting four groups of lentil accessions based on seven agronomical traits (1993–2016).



Russia. Table 5 shows the complete characteristics of the lentil accessions from this cluster.

### Comparative analysis of Ukrainian genebank data, and data from field experiments in Ukraine (2019) and France (2021)

A comparison of the duration of the phenophases in France and Ukraine, conducted in conjunction with the long-term observations of the genebank, showed that despite the significantly earlier sowing date in France, the growing season was not significantly longer, with an average of 118.72 days in France versus 105.24 days in Ukraine (2019). The experimental year in Ukraine (2019) was characterized by particularly unfavourable conditions for lentil cultivation, with a much shorter growing season than usual (63.94 days).

The results of the field experiments in Ukraine (2019) and France (2021) are presented in Table 6. We found that the examined set of accessions (37 genotypes) showed a significant decrease in productivity, in both Ukraine (2019) and France (2021) compared to the characteristic indicators of the genebank for the evaluation period 1993–2016 (Table 2), most likely due to weather conditions.

The average seed yield per plot in France was almost half that in Ukraine (0.042kg/m<sup>2</sup> and 0.089kg/m<sup>2</sup> respectively), but this reflected the typical conditions of the year of the study and the design of the experiment, as can be seen from the fact that the relative yield to the standard is almost the same in both experiments (95.45% and 94.57%). The most stable trait in both experiments (Ukraine and France), as in the analysis of long-term data of NCPGRU, was the duration of the growing season. The most variable features were those representing yield components (Table 6).

The biplot analysis of the genotype×environment interaction and the ranking of genotypes revealed a notable discrepancy in environmental conditions between the long-term observations conducted by NCPGRU (1993–2016) and the experimental years in Ukraine (2019) and France (2021). This enabled a comprehensive evaluation of the selected accessions, with UD0600638 Eston (Canada) and UD0600052 Stepova 244 (Ukraine) identified as the most closely aligned with the 'ideal' genotype (Figure 5).

The most informative environment, as expected, turned out to be the conditional environment of genebank (information from the database of NCPGRU). The ranking of genotypes in relation to the 'ideal' genotype should be based on two criteria: average efficiency and stability across environments. The ideal genotype, represented by the point in the centre of the concentric circles, represents 'absolute stability' in the positive direction and has a vector length equal to the longest vectors of genotypes on the positive side of the highest average seed yield per plot. Consequently, genotypes that are closer to the ideal genotype are more desirable. By comparison with the ideal genotype,

six accessions were selected, located in the first three rounds of the concentric circles in the graph (Figure 5): UD0600638, UD0600052, UD0600145, UD0600403, UD0600084, UD0600086.

Significant differences in environmental conditions were analyzed by biplot analysis based on the indicator of relative yield to the standard (standard accessions were removed from the ranking). This approach gave a much larger range of variation in all three environments (Figure 6).

As a result, eight accessions were selected within the circles around the ideal genotype. Five of these accessions belong to Cluster 4 and one to Cluster 2 (Table 7).

### Discussion

The evaluation of genetic resources and the identification of the most promising parental lines is crucial in the development of new varieties (Sivaraj *et al*, 2022). The exchange of seed material and information on accessions and their characteristics is not always optimal due to the locations of genebanks in a wide range of climatic zones. It is known that environmental conditions have a significant influence on the yield of lentil genotypes (Khatun *et al*, 2022). The study of the lentil gene pool has a long history in Ukraine (Bezuhla and Kobyzeva, 2021), while it is a relatively new endeavour in France. It was therefore decided, upon seed material request, to accompany seeds with related phenotyping data to increase their utility use efficiency. The initial phase of the study involved gathering historic data on these accessions from NCPGRU, collected during previous regeneration cycles. It became evident that significant discrepancies in weather patterns and regional variations in agricultural technology and applied methods preclude a straightforward comparison of lentil productivity. Consequently, a comparison of the relative yield against the median of the standards included in field trials was conducted, accompanied by an assessment based on a set of characteristics.

The principal components analysis of the NCPGRU data revealed that PC1 was influenced by yield indicators, while PC2 was affected by weather conditions. The combination of temperature and precipitation exerted the most significant influence on the generative phase of lentil development, thereby determining the duration of the growing season in general (Maphosa *et al*, 2023). The characteristics of yield per plot (direct and relative to standards), the height of pods attachment, and the weight and number of seeds per plant exhibited the strongest correlation. This finding aligned with literature data (Hussain *et al*, 2022; Ilyas *et al*, 2024). Accessions situated in the lower right quadrant demonstrated a greater contribution in terms of yield, while those in the upper right quadrant exhibited a greater contribution in terms of the length of the growing season.

The results of the ANOVA indicated that the duration of phenophases, yield, plant height and the attachment of the lower pod bean exhibited a greater degree of

**Table 5.** Agronomic traits of seven *macrosperma* lentil accessions composing Cluster 2 (National Centre for Plant Genetic Resources of Ukraine (NCPGRU), 1993–2016).

Accession ID	Plant height, cm	Height of the lowest pod, cm	Number of seeds per plant	Weight of 100 seeds, g	Yield of seeds per plant, g	Yield per plot, kg/m <sup>2</sup>	Relative yield to standards, %
UD0600036	48.4	22.9	62.5	6.7	4.9	0.188	104.93
UD0600112	44	18.7	60.5	6.9	4.05	0.160	100
UD0600163	40	18	36	6.9	2.05	0.077	67.44
UD0600468	38.8	19.1	47.5	6.4	1.85	0.073	81.27
UD0600496	40	19.9	39.5	5.2	1.45	0.734	72.7
UD0600530	46.3	19.7	98.5	4.5	2.25	0.106	111.34
UD0600686	42.9	18.6	74.5	4.6	2.4	0.089	63.64

**Table 6.** Comparison of the main traits of lentils in experimental years in Ukraine (2019) and France (2021). SD, standard deviation; CV, coefficient of variation; \*\*\*, statistically significant at P < 0.001.

Traits	Ukraine				France			
	Min–max	Mean	SD	CV, %	Min–max	Mean	SD	CV, %
Plant height, cm	25–39	31.75	3.68	11.59	10–35	21.3	6.12	28.73
Height of the lowest pod, cm	8–23	15.86	3.26	20.55	5–23	12.58	3.97	31.56
Number of seeds per plant	9–117	48.61	30.69	63.14	1.3–128.77	49.9	27.85	55.81
Weight of 100 seeds, g	2.13–6.6	3.77	1.37	36.45	0–2.65	1.64	0.34	20.73
Yield of seeds per plant, g	0.3–3	1.54	0.76	49.35	0–2.6	0.83	0.49	59.04
Yield per plot, kg/m <sup>2</sup>	0.016–0.181	0.089	0.05	52.99	0–0.117	0.042	26.22	61.90
Relative yield, %	16.7–192.55	94.57	50.18	53.06	0–266.7	95.45	59.81	62.66
Days from germination to 50% flowering	13–44	39.47	5.22	13.23	68–90	83.88	3.45	4.11
Days from 50% flowering to full maturity	19–64	24.47	7.25	29.63	25–44	34.84	3.60	10.33
Days from germination to full maturity	63–77	63.94	2.32	3.63	110–128	118.72	4.25	3.58

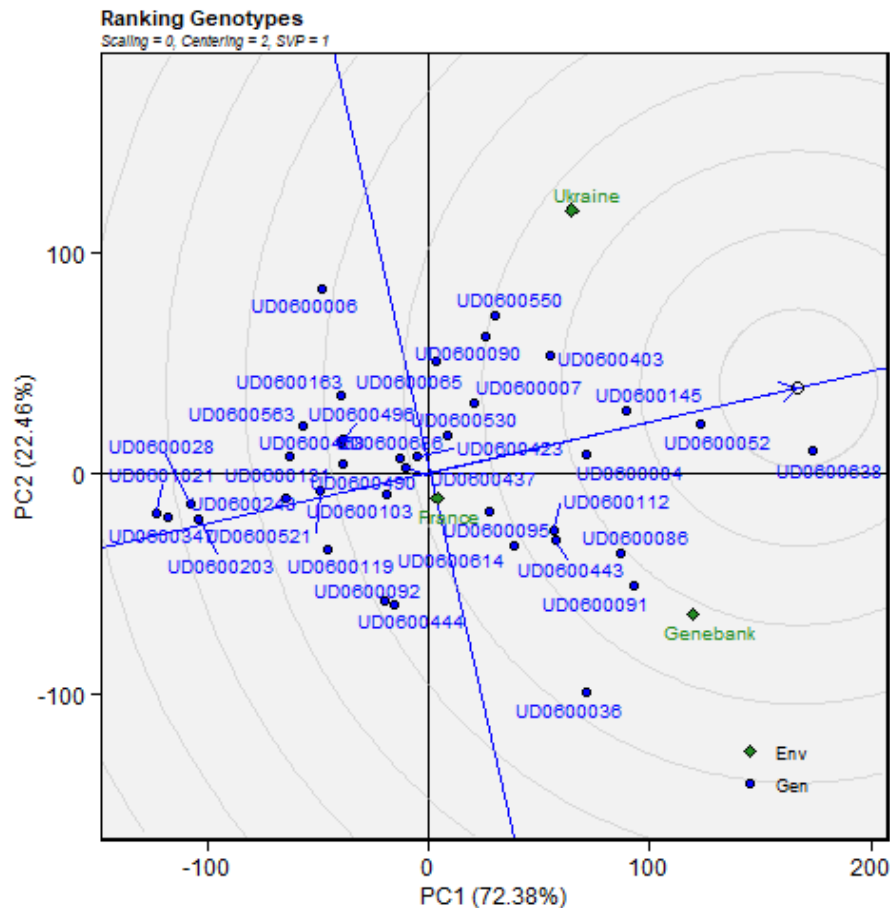
**Table 7.** Characteristics of the most promising lentil accessions revealed across trials conducted in Ukraine and France. \*, Accessions were not evaluated in France.

Accession ID	Seed yield per plot, kg/m <sup>2</sup>			Relative yield per plot from median of standards (CY), %			Cluster
	Genebank (median of years)	Ukraine (2019)	France (2021)	Genebank (median of years)	Ukraine (2019)	France (2021)	
UD0600006*	0.021	0.140	-	13.03	148.94	-	3
UD0600065*	0.082	0.136	-	42.71	144.68	-	1
UD0600086	0.197	0.099	0.034	102.87	105.32	76.72	4
UD0600145	0.168	0.158	0.044	71.04	168.09	99.29	4
UD0600437	0.092	0.088	0.047	64.17	93.62	106.75	4
UD0600530	0.102	0.110	0.047	105.66	117.02	106.16	2
UD0600550	0.096	0.167	0.031	177.66	78.63	69.92	4
UD0600638	0.252	0.181	0.030	111.43	192.50	68.38	4

variation in response to the prevailing conditions of the year (Lázaro et al, 2001). In contrast, seed size and time to 50% flowering were largely influenced by genotype (Bhartiya et al, 2015).

Cluster analysis allows the grouping of a large number of genotypes based on a list of characteristics and the selection of a group of accessions that meet the requirements of a specific breeding programme (Ahamed et al, 2014; Vus et al, 2020a; Zayed et al, 2022; Amit et al, 2023). The clustering of the 37 accessions from this study allowed the identification of a cluster of lines (Cluster 4; Table 3) as the most promising for breed-

ing with high and stable productivity. All accessions of this cluster were *microsperma* type and had a higher yield than accessions of *macrosperma* type, consistent with results from Mohammed et al (2019) and Preiti et al (2024) who suggested that this is caused by earliness of this type of lentils. Cluster 4 included well-known accessions such as UD0600638 Eston (Canada), the most popular Canadian variety. However, this one is also susceptible to Ascochyta blight (Sari et al, 2018). This cluster also contained accession UD0600052 Stepova 244 (Ukraine) – a reference for yield stability in Ukraine (Bezuhla and Kobyzeva, 2021), which has been



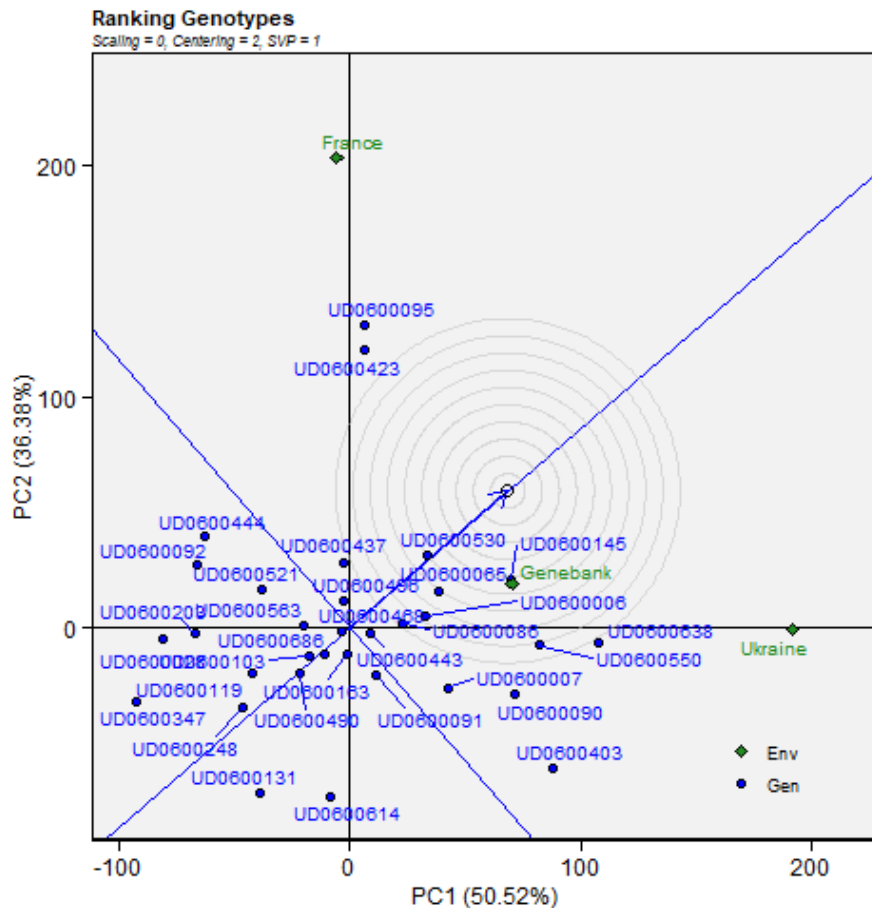
**Figure 6.** Biplot analysis of genotype-environment interaction by yield of seeds per plot. Ranking genotypes relative to the ‘ideal’ genotype (centre of the concentric circles). Green dots are environments and blue dots are genotypes.

used in many studies aiming at understanding drought tolerance (Vus *et al.*, 2020b) or long-term seed storage behaviour (Zadorozhna *et al.*, 2015). The Bulgarian variety Nadejda (UD0600403) is largely used in research by Bulgarian scientists. Its resistance to four of the seven pathotypes of *Ascochyta* blight known in the country has been established (Stanoeva and Koleva, 2017), but also the high sensitivity to changes in weather conditions was noted (Milev, 1999; Tonev *et al.*, 1999). This was also confirmed in our research: in France, the variety yielded significantly lower than the standard (13.52%), while in Ukraine it performed well (171.28%).

Large-seeded lentil genotypes in Cluster 2 (Table 4) included two Ukrainian reference accessions (UD0600036 and UD0600112) and UD0600530 Slovyanka, which has been identified as a source of the highest first pod (Kobyzeva *et al.*, 2012). This feature is an important character for mechanization in lentil, as it shows high variability depending on growth conditions and can cause significant yield losses (Gaad *et al.*, 2018; Kuzbakova *et al.*, 2022). Accession UD0600444 is part of a set of lentils with high seed nutritional value (Vus *et al.*, 2020a). It should be noted that some genotypes in this cluster, which have important breeding traits such as high weight of 100 seeds (UD0600163) or high number of seeds per plant (UD0600686), had a high

susceptibility to environmental changes and a low yield compared to the standard (27.42 and 32.6%, respectively), consistently with results from (Mohammed *et al.*, 2019).

The ranking of lentil accessions under different environmental conditions – using the genebank’s median data as one environment point, along with data from field trials conducted in Ukraine (2019) and France (2021) – was carried out using biplot analysis based on the feature ‘seed yield per plot’. It was noted that the years of research trials in Ukraine and France were significantly different from the long-term observations of NCPGRU. In France, a low variability of lentil accessions was observed, compared to Ukraine. According to the relationship to the ‘ideal’ genotype – represented by the centre of circles in the PCA – an ideal genotype should have both high mean performance and high stability across environments. Therefore, genotypes closer to the ideal are considered more desirable (Gedif and Yigzaw, 2014). Six lentil accessions located within the first three circles from the centre were selected as more promising for the three environments (Yan and Tinker, 2006; Khan *et al.*, 2021). Significantly, two of them were standards: UD0600052 (in Ukraine) and UD0600084 (in France), with a consistently high yield in the study regions. All of these accessions were also identified earlier as promising



**Figure 7.** Biplot analysis of genotype-environment interaction by relative yield (without standards accessions). Ranking genotypes relative to the 'ideal' genotype (centre of the concentric circles). Green dots are environments and blue dots are genotypes.

by results of multi-year observations in NCPGRU (Cluster 4). 'Stable' genotypes are desirable only when they have high mean performances.

It should also be noted that accessions UD0600095 and UD0600423 showed the highest level of productivity in the conditions of the year of research in France. It has been confirmed (Bezuhla and Kobyzeva, 2021) that UD0600423 CDC Sunrise was determined as a source of two valuable features: high protein content and low cooking time.

## Conclusions

The present study demonstrated how the valuable information stored in genebank databases is essential for supporting research and development, using an underutilised crop – lentil – as a key example. The performance of lentil accessions under different annual and agroclimatic conditions in Ukraine (Kharkiv region) allowed the identification of promising accessions that could be confirmed in parallel trials conducted in Ukraine and in France (Bourgogne-Franche-Comté region). Accessions that consistently outperformed the standards are recommended for use in breeding to create new lentil varieties.

## Authors contributions

Nadiia Vus: Conceptualization, data curation, analyses conduction, investigation, methodology, resources, provision of study materials, validation, verification of the overall result replications, visualization, prepared the figures and tables, writing (original draft, review and editing); Olha Bezuhla: supervision, data curation, resources, provision of study materials, validation; Hervé Houtin: resources, validation, provision of study materials, verification of the overall result replications; Florence Naudé: resources, validation, provision of study materials, verification of the overall result replications; Antonina Vasylenko: analysis conduction the analyses, methodology, validation, verification of the overall result replications, writing (review and editing); Anthony Klein: resources, validation, verification of the overall result replications, provision of study materials; Oleh Leonov: data curation, analysis conduction, validation, verification of the overall result replications; Nadim Tayeh: supervision, conceptualization, data curation, provision of study materials, formal analysis, investigation, resources, validation, visualization, writing (review and editing), acquisition of financial support.

All authors read and approved the final manuscript.



## Conflict of interest statement

The authors confirmed that no conflict of interest exists.

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