

DIVA-GIS APPROACH TO DIVERSITY ANALYSIS OF *Aegilops cylindrica* GERMPLASM

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Abstract

The object of the study was 152 specimens of *Aegilops cylindrica* Host. collected in 2024 from 17 districts in Bulgaria. DIVA-GIS approach was used to analyze the diversity. The descriptive analysis of the studied quantitative characteristics showed a wide range of variability among accessions. The Shannon diversity index exhibited a high level of diversity, with a maximum range of 1.109-2.0, 1.109-2.0, and 0.879-2.0, respectively, for the traits of plant height, spike length, and number of spikelets per spike. These findings indicate a notable responsiveness of these traits to the ecosystem. The grid maps generated for the diversity analysis of these characters indicated the occurrence of diverse accessions for plant height and for spike length from the municipality of Elin Pelin (district Sofia), and for number of spikelets per spike from the Kyustendil (district Kyustendil), Belitca and Bansko (district Blagoevgrad), Sevlievo (district Gabrovo), Karlovo (district Plovdiv), Mineralni bani (district Haskovo) and Elin Pelin (district Sofia) municipalities. The study would facilitate more effective management and utilization of *Aegilops cylindrica* Host. in the country.

Key words: *Aegilops cylindrica* Host., diversity, expedition, Diva-Gis.

INTRODUCTION

The generation, analysis and use of data on PGR is critical to their effective and rational management and can significantly increase the value of genetic resources. Many of these data are geo-referenced and can be analyzed using Geographical Information Systems (GIS) - and links can be made between them and other geo-referenced data from sources external to the plant genetic resources process (Garcia et al., 2017; Bagarinao, 2022).

The accurate determination and collection of plant material constitutes a pivotal step in the efficient conservation and utilization of available germplasm. DIVA-GIS is a methodology designed to support plant genetic resources and biodiversity communities in mapping the geographical distribution of species of interest (Hijmans et al., 2002). This analysis is imperative for identifying potential areas of biodiversity and collection gaps, which must be taken into consideration during the design of subsequent collection missions (Mujaju & Fatih, 2011; Suma et al., 2019; Reshmi et al., 2022). Projected distribution

maps for each taxon, based on climatic data, can identify gaps in *ex situ* conservation, both in regions where the species' seeds have not been collected, or where the species has not been sufficiently studied (Shehadeh et al., 2013; Garcia et al., 2017).

DIVA-GIS has been demonstrated to offer an efficacious means of evaluating diversity loss, thereby facilitating the formulation of effective strategies for the conservation and utilization of genetic resources in a perpetually evolving global context. GIS mapping has been successfully used over the years for assessing biodiversity and in identifying areas of high diversity (Chand et al., 2018)

The analysis of ecogeographic data has been demonstrated to be a valuable tool in the study of the habitat requirements of specific groups of plant species. Furthermore, this analysis has been shown to facilitate a more comprehensive understanding of the sympatric distributions of taxa within a given gene pool (Maxted et al., 2011, 2015).

The presence of genetic diversity within maintained or collected materials is of paramount importance to any crop

improvement program. A comprehensive understanding of the extent of genetic variability is essential for the judicious utilization of germplasm (Suma et al., 2019). Identification of potential donor(s) for yield, combined with desired quantitative and qualitative traits, is a prerequisite for the success of breeding programs. In order to ensure the preservation of allelic and genotypic variability within a species, it is imperative to collect a representative sample of genetically diverse populations. These populations should be studied and, when feasible, retained for the purpose of breeding improvement (Raina et al., 2013).

The aim of the study was to analyze the diversity of the collected jointed goatgrass germplasm in terms of some quantitative traits using DIVA-GIS approach.

MATERIALS AND METHODS

In the period June - August 2024, expeditions were carried out to identify the natural localities of the wild relative of wheat - *Aegilops cylindrica* Host. in 17 districts of the territory of the Republic of Bulgaria, as well as to collect seed material for storage in the National Genebank. A route method was used to conduct the expeditions, and seed material was collected from established and marked locations at the stage of full plant maturity using the method described by Porceddu and Damania (1994).

Bioversity Multi-Crop passport descriptors (Alercia et al., 2012) including: institute code; temporary registration number for registration in the PHYTO 2000 database; international registration number in the EURISCO electronic database; taxonomic affiliation - genus, species; biological status; geographical coordinates - location (nearest locality), altitude, latitude, longitude were used to generate passport data of marked and collected material. A handheld geographical positioning system (GPS-Garmin 12) was used to record geographical coordinates. Additionally *in situ* assessment was carried out by analyzing the following quantitative traits viz. plant height, spike length and number of spikelets per spike,

and qualitative traits viz. color of the spike and the awns, hairness of the spike and the awns.

DIVA-GIS version 7.5.0 software elaborated by Hijmans et al. (2005) was utilised for the purpose of determining the spatial distribution. This was achieved by converting point data into a grid analysis using simple circular neighbourhood methods. The diversity analysis for quantitative traits viz. plant height, spike length and number of spikelets per spike was then calculated using the Shannon-Weaver diversity index (Hijmans et al., 2000; 2012; Shankar et al., 2023). For the analysis of country-level data, a grid of 90 × 90 km cells was utilised for the allocation of points to grid cells, with the objective of mapping the diversity of germplasm for the studied traits (Hijmans et al., 2000).

RESULTS AND DISCUSSIONS

A total of 152 accessions were collected from 136 localities, covering 17 districts of Bulgaria (Table 1, Figure 1). The recorded geographical coordinates of the marked sites vary widely, with latitude and longitude ranging from 41°23'-43°20'N and 22°32'-28°03', respectively. The altitudes at which the specimens were found ranged from 2 to 1185 metres above sea level (Table 1). The map for the distribution of accessions according to altitudinal range showed that most accessions had been collected at 0 to 554 m, following at 554 to 1110 m above sea level (Figure 2). Zaharieva et al. (2004) notes that *Aegilops cylindrica* Host. is found in nearly all ecological zones in Bulgaria, but is more common in the northern part of the country. In this study, the largest number of specimens was recorded in the Veliko Tarnovo district located in northern Bulgaria. A considerable number of accessions were also marked and collected in seven provinces (between 11 and 16 number of accessions). Expeditions have also confirmed that the species is most often to be found in uncultivated and highly disturbed areas, e.g. wastelands, roadsides, dry, sandy, grassy slopes, grasslands (Valchinova et al., 2019; Sanchez, 2022). The collection sites mapped are provided in Figure 1.

Table 1. *Aegilops cylindrica* Host. collected during several germplasm expeditions in 2024 on the territory of Bulgaria

District	No. of municipality	No. of habitats	No. of collected accessions	Geographical coordinates (range)		
				Latitude (N)	Longitude (E)	Altitude
Pazardzhik	3	12	15	42°07'-42°30'	24°07'-24°56'	227-570
Plovdiv	6	13	16	41°51'-42°48'	24°37'-25°54'	189-1185
Lovech	1	5	6	43°04'-43°08'	24°44'-24°53'	199-488
Gabrovo	2	4	5	42°46'-42°59'	25°5'-25°18'	207-950
Stara Zagora	6	16	16	42°24'-42°42'	24°46'-25°48'	237-552
Kardzhali	1	3	3	41°43'-41°47'	25°14'-25°21'	402-563
Blagoevgrad	6	12	16	42°02'-41°29'	23°29'-23°44'	244-306
Burgas	6	12	16	42°25'-42°62'	27°6'-27°15'	2-344
Smolyan	2	3	3	41°23'-42°30'	24°48'-25°05'	444-702
Haskovo	2	5	5	41°49'-42°00'	25°19'-25°23'	221-388
Yambol	1	1	1	42°32'	26°29'	146
Sofia	5	16	16	42°04'-42°43'	23°35'-24°01'	494-811
Veliko Tarnovo	4	19	19	42°40'-43°08'	25°18'-25°45'	50-326
Varna	2	2	2	42°58'-43°08'	27°18'-27°26'	103-111
Dobrich	1	1	1	43°20'	28°03'	5
Pleven	1	1	1	42°08'	24°15'	186
Kyustendil	3	11	11	42°13'-42°18'	22°32'-25°02'	454-938
17	52	136	152	41°23'-43°20'	22°32'-28°03'	2-1185

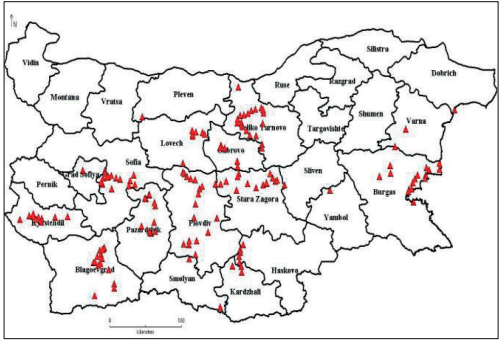


Figure 1. DIVA-GIS distribution mapping of collection sites of *Aegilops cylindrica* Host. in Bulgaria during 2024

Diva -GIS has been successfully applied to identify specific characteristics of samples collected from diversity-rich areas, thereby increasing the the likelihood of identifying ideal genotypes and saving the resources and time needed to evaluate large numbers of accessions. This facilitates faster use of germplasm in breeding programmes (Dar et al., 2016; Suma et al., 2019; Reshmi, 2022).

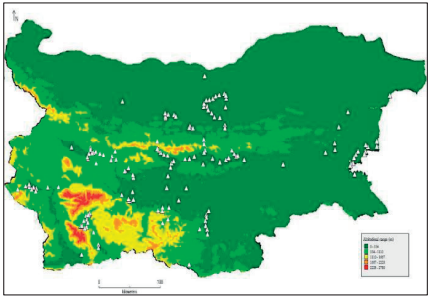


Figure 2. Distribution of collected accessions of *Aegilops cylindrica* Host. in Bulgaria according to altitude using DIVA-GIS

A wide range of variability was evident for the quantitative traits among the 152 accessions, as indicated by the wide range of Shannon diversity index for plant height (0.000-0.277 to 1.109-2.000), spike length (0.000-0.277 to 1.109-2.000) and spikelets per spike (0.000-0.220 to 0.879-2.000) (Figures 3-5). The grid map generated for the diversity analysis of the plant height showed that one grid cell (shown with red color) with the highest Shannon diversity index between 1.109 and 2.000 was located in the municipality of Elin Pelin (district Sofia), while 9 grid cells (orange color in the map) with high Shannon index between 0.832 and 1.109 were located in different provinces. Two grid cells were identified in Plovdiv (Sopot and Karlovo municipalities) and in Blagoevgrad districts (Belitza and Bansko municipalities), one in Kyustendil (Kyustendil municipality), Sofia (Elin Pelin municipality), Lovech (Lovech municipality), Gabrovo (Sevlievo municipality), Stara Zagora (Maglizh municipality) (Figure 3).

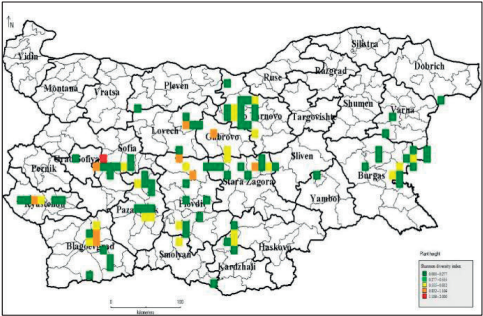


Figure 3. Diversity map of collected jointed goatgrass germplasm for the plant height in 90 x 90 km grid cells using the Shannon Diversity Index (H)

The diversity map for spike length revealed that the region with the highest diversity ($H=1.109-2.000$), was also in the municipality of Elin Pelin (district Sofia). Generated grid cells with high Shannon index ($0.832-1.09$) were in nine municipalities, respectively in 7 districts of the country - Kyustendil municipality (Kyustendil district), Bansko municipality (Blagoevgrad district), Panagyuriste municipality (Pazardzhik district), Karlovo, Sopot and Rodopi municipalities (Plovdiv district), Sevlievo municipality (Gabrovo district), Maglizh municipality (Stara Zagora district) and Mineralni bani municipality (Haskovo district) (Figure 4).

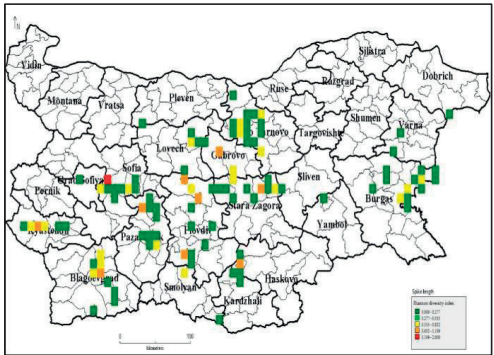


Figure 4. Diversity map of collected jointed goatgrass germplasm for the characteristic spike length in 90×90 km grid cells using the Shannon Diversity Index (H)

The diversity grid map generated for the number of spikelets per spike showed that nine grid cells with the highest Shannon diversity index between 0.897 and 2.000 were located in the following districts - Kyustendil (one cell in Kyustendil municipality), Sofia (two cells in Elin Pelin municipality), Blagoevgrad districts (one cell in Belitza and one cell in Bansko municipalities), Gabrovo (one cell in Sevlievo municipality), Plovdiv (one cell in Sopot and one cell in Karlovo municipality) and Haskovo (one cell in Mineralni bani municipality). Fifteen grid cells with Shannon index between 0.659 and 897 were deployed in nine districts, four cells in Veliko Tarnovo, two cells in each of the following provinces - Blagoevgrad and Plovdiv. The districts in which one orange cell was generated in Sofia, Pazardzhik, Plovdiv, Smolyan, in the border of Haskovo and Kardzhali, Stara Zagora and Burgas (Figure 5).

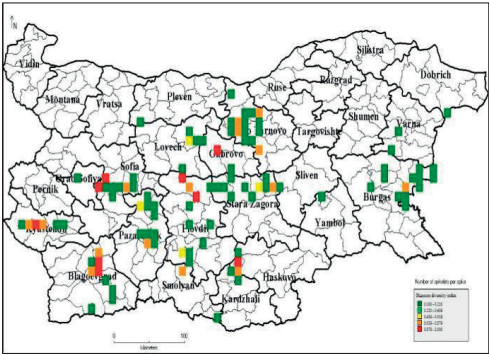


Figure 5. Diversity map of collected jointed goatgrass germplasm for the number of spikelets per spike in 90×90 km grid cells using the Shannon Diversity Index (H)

Awareness of the variation in morphology, its nature and extent, is vital to the choice of the appropriate genotypes for successful use in breeding programmes. The valuable resource of germplasm is dependent not only on the quantity of specimens, but also on the genetic variability among them (Kumar et al., 2013). The descriptive analysis of the studied quantitative characteristics showed a wide range of variability among the 152 accessions (Table 2). Plant height fluctuated between 16 and 80 cm. Spike length varied between 3.5 and 18 cm, while the number of spikelets per spike ranged between 4 and 10. The highest variability was found for plant height, where the calculated coefficient of variation (CV, %) was 30.336%.

Table 2. Descriptive statistical analysis of quantity characteristics for 152 accessions of *Aegilops cylindrica* Host

Traits	Min.	Max.	Mean	Standard deviation	Vari Ance	Coefficient of variation %
Plant height, cm	16	80	43.467	13.186	173.872	30.336
Spike length, cm	3.5	18	11.117	2.903	8.427	26.114
Number of spikelets per spike	4	10	6.563	1.268	1.608	19.32

The highest CV between 42% and 53% was recorded for accessions from Belica and Bansko municipality (Blagoevgrad province), Sevlievo municipality (Gabrovo province) and Polski Trambesh (Veliko Tarnovo province). The high CV of 31-42% was found in six municipalities filling in five districts (Figure 6).

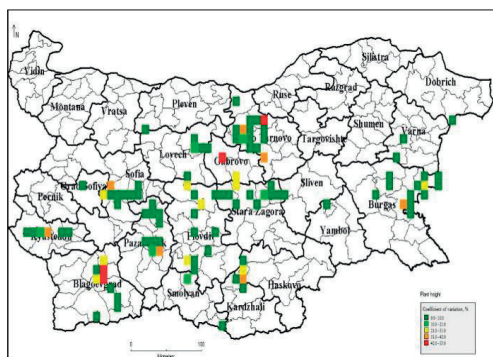


Figure 6. Grid map of coefficient of variation for plant height generated with DIVA-GIS

Germplasm accessions augmented from Sevlievo municipality (Gabrovo province) recorded the highest CV (54-69%) for spike length, from Chepelare municipality (Smolyan) and Karlovo municipality (Plovdiv) high CV (41-54%), while medium CV (27-41%) from one municipality in Blagoevgrad district (Figure 7).

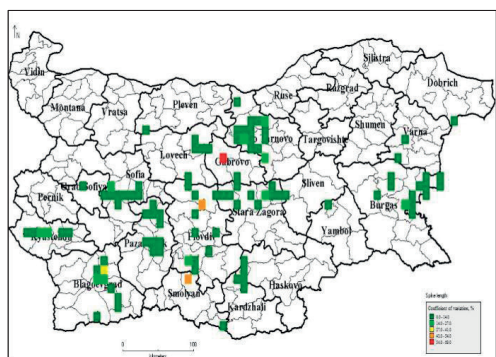


Figure 7. Grid map of coefficient of variation for spike length generated with DIVA-GIS

The accessions with the maximum CV (27-34%) recorded for the number of spikelets per spike originated from six municipalities, falling in five districts. The generated grid map identifies 76 grid cells with low CV (0-7%), indicating less variability for the accessions collected from these localities (Figure 8).

The present study revealed that diverse germplasm accessions of *Aegilops cylindrica* Host were dispersed all over the 17 districts, but regions where the diverse genotypes most likely to found were Sofia, Kyustendil,

Blagoevgrad, Gabrovo, Plovdiv, Stara Zagora, where high diversity indices and CV were recorded for the studied traits. These areas should be considered for future exploration programs to capture maximum diversity for these characters.

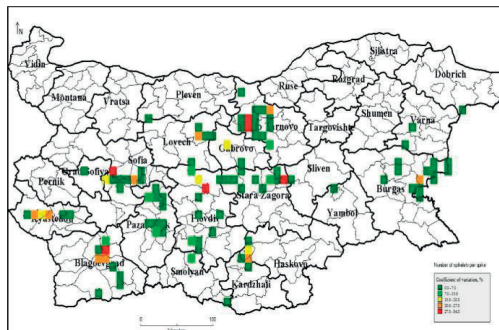


Figure 8. Grid map of coefficient of variation for number of spikelets per spike generated with DIVA-GIS

CONCLUSIONS

In this study, the diversity of jointed goatgrass germplasm collected in 2024 for three quantitative traits - plant height, spike length and number of spikelets per spike - was investigated using the DIVA-GIS tool, which helped to identify the regions with the greatest diversity for the traits studied. Future collection missions should focus on the following regions - Sofia, Plovdiv, Blagoevgrad, Kyustendil, Gabrovo, Stara Zagora - to capture maximum diversity for these and other traits. The study using DIVA-GIS should continue with the analysis of collected and preserved specimens of *Aegilops* in the National Collection to identify conservation gaps, prioritise areas for the establishment of genetic reserves. Further research is needed to predict distribution in other parts of the country and to assess loss of diversity as part of a future conservation strategy and sustainable use.

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