Title: Genetic Structure and Population Differentiation of *Chrysanthemum zawadzkii* in Its Isolated European Range

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Abstract

This study investigated the genetic structure and diversity of Zawadzki's chrysanthemum (*Chrysanthemum zawadzkii*), a relict and endemic species with an isolated population in the Pieniny Mountains, Central Europe. The primary objective was to determine whether this population is genetically homogenous or consists of distinct subpopulations. To achieve this, plant material was collected from 13 sites across the Pieniny Mts. We used DArT-seq markers, including SNP and SilicoDArT, along with statistical methods like PCA, DAPC, and sNMF, to analyze the genetic data. The results demonstrated clear genetic differentiation within the population. Populations from the Trzy Korony (TK) and Macelowa Góra (MA) sites were genetically distinct from the others, which formed a more cohesive group. This genetic structure was confirmed by both PCA and DAPC analyses, which showed a separation of individuals and suggested limited gene flow. The analysis also indicated that a high number of clusters for SNPs (K=6) and a lower number for SilicoDArT (K=2) best described the genetic structure, revealing both a primary genetic division and more subtle substructures. Overall, the findings suggest that the genetic diversity of the Pieniny population is shaped by a combination of geographic, environmental, and historical factors. The observed differentiation, particularly in high-elevation populations like TK, MA, HA, and SK, is crucial for conservation efforts, highlighting the need to protect these genetically distinct groups to preserve the species' biodiversity and gene pool.

Introduction

Isolated plant populations, particularly relict and endemic taxa, have long been a focal point of interest for systematists, ecologists, and geneticists (Tutin, 1976). Their role in preserving biodiversity is crucial, underscoring the urgent need for enhanced species protection and minimizing anthropogenic impacts on their natural habitats. Regions characterized by natural isolation, such as islands (Runemark, 1970) and mountain ranges (Pawłowski, 1970; Skalińska M., 1963; Zarzycki, 1976), provide ideal research systems for analyzing the dynamics and evolution of such populations. In Europe, the Pieniny Mountains, a small limestone range in the Carpathians, stand out as a particularly rich reservoir of endemic species.

The unique geology and topography of the Pieniny Mountains have created unique conditions for the development of small, often extremely sparse, plant populations with limited ranges. This area, unaffected by glaciation, became a refuge for ancient rock flora (Zarzycki, 1976). The history of floristic studies in the Pieniny Mountains dates to 1829 (Herbich, 1831; Knapp, 1872; Zawadzki, 1835), which led to a thorough understanding of the distribution of vascular plants in the region. Currently, we have detailed data on endemic and relict taxa, as well as estimates of the size of individual populations. The morphological, edaphic, and microclimatic diversity, as well as the specific geographic location and history of the Pieniny Mountains, contributed to the development of an exceptionally rich flora, encompassing over 1,100 species of vascular plants (Zarzycki, 1976).

The flora of the Pieniny Mountains is known for its high degree of endemism and relictness. It includes unique taxa at various ranks, such as *Taraxacum pieninicum* Pawł., *Erysimum pieninicum* Zapał., *Minuartia setacea* var. *pieninica* (Zapał.) Pawł., *Artemisia absinthium* var. *calcigena* Rehm (Kirschner et al., 2021; Konowalik et al., 2010; Konowalik & Kreitschitz, 2012; Zarzycki, 1976). Among these, we focus on Zawadzki's chrysanthemum (*Chrysanthemum zawadzkii* Herbich), a species with a unique biogeographic status for which the Pieniny Mountains also serve as the *locus classicus*.

The distribution of endemic and relict species in the Pieniny Mountains is highly concentrated in the Central Pieniny Mts, an area characterized by steep and nearly vertical rock faces, inaccessible to most other plant species. Cool microclimatic conditions in the peaks of the Trzy Korony Mountains, the Sobczański Gorge, the Dunajec River Gorge, and the Homole Gorge create a complex mosaic of microhabitats. This environmental structure favors species survival in the face of unfavorable climatic changes and competitive pressures (Zarzycki, 1976). Despite the existence of ancient migration routes connecting Pieniny Mts and Tatra Mts populations, contemporary anthropogenic activities and climate change contribute to habitat fragmentation and loss of connectivity between populations.

In the context of their broader distribution, populations from Pieniny Mts often exhibit reduced morphological and genetic variability, a characteristic of isolated populations. An example is the Zawadzki chrysanthemum, which occurs in the Pieniny

Mountains as a narrow-leafed and hexaploid species, while its Asian populations are characterized by significant morphological and karyological diversity, ranging from diploids to decaploids (Moon et al., 2023). Reduced genetic and morphological variability in Pieniny relict and endemic populations may be analogous to other isolated areas, such as the flora of Crete (Greuter, W., 1972), where a limited area was inhabited by a small number of individuals, which may have hindered further evolutionary development and narrowed the gene pool.

The Zawadzki's chrysanthemum is one of the most fascinating botanical curiosities of the Pieniny Mountains, with the only localities in Europe located in this range (Zarzycki, 1976). It occurs in difficult-to-access limestone rock grasslands. The Pieniny population, although covering an area of approximately 10 km², is described as very numerous (Zarzycki, 1976). This species, the only European representative of the genus *Chrysanthemum*, is also important from a breeding perspective due to its increased frost resistance (Jerzy, 2006). In Poland, *Chrysanthemum zawadzkii* is strictly protected and is included in the Polish Red Data Book of Plants and on the Polish Red List in the VU (vulnerable) category.

Considering the above, the key question remains whether the original colonization of the Pieniny Mountains by *C. zawadzkii* included diverse populations, only some of which survived subsequent environmental changes, or whether they were initially isolated individuals that subsequently spread over a wider area (Zarzycki, 1976). Despite the available reports on the morphology and genetics of *C. zawadzkii* in Asia, further detailed studies are necessary to better understand the morphological and ecological adaptations of this species in its European, Russian and Asian populations, especially in Poland, where knowledge in this area is still limited.

Materials and methods

Collection of Plant Material and DNA extraction

Plant material (fresh leaves) was collected from 13 separate *Chrysanthemum zawadzkii* sites in the Pieniny Mts. Randomly selected individuals were sampled from each site maintaining a distance between them to avoid sampling clones. The collected leaves were stored in silica gel for rapid drying and DNA stabilization. DNA was isolated using the NucleoSpin Plant II kit (Macherey-Nagel) according to the manufacturer’s protocol, with minor modifications, such as slightly longer incubation times and adjusted buffer solutions. Electrophoresis was performed to check the quality of DNA isolation and the 94 best samples were placed into a 96-well plate in a predetermined order. The plate was secured and sent for DArT-seq sequencing to Diversity Arrays Technology Pty Ltd. in Australia.

Genetic analyses

To assess the genetic variability of the studied individuals, the RStudio (Posit team, 2025) was used. The high-throughput genotyping method DArT-seq generates two types of markers: SNP (single nucleotide polymorphisms) and SilicoDArT (dominant sequence presence/absence markers). The data were first filtered using the dartR package (Gruber et al., 2018). Genetic differentiation analyses employed principal component analysis (PCA) and discriminant analysis of principal components (DAPC) (Jombart, 2008; Jombart & Ahmed, 2011), which enabled the detection of distinct genetic groups. The LEA package and the sparse non-negative matrix factorization (sNMF) method (Frichot & François, 2015) were used to visualize clusters. The Evanno method (Evanno et al., 2005) was used to determine the optimal number of genetic clusters (K), which was calculated based on the change in K (delta K). Ancestry matrices were also constructed to visualize the proportions of each individual's genetic assignment to individual clusters. The analysis of genetic diversity and population structure was supplemented with selected population statistics, calculated separately for SNP markers and SilicoDArT. The analyzed parameters included: Ho (observed heterozygosity), Hs (mean heterozygosity within the population), Ht (total heterozygosity), Dst/Dstp (difference in heterozygosity between populations), Fst/Fstp (population differentiation index), Fis (inbreeding index within the population), Dest (a measure of genetic differentiation independent of heterozygosity), Htp (total heterozygosity expected under the assumption of random distribution).

Results

Genetic analyses

Despite the relatively low variance explanation values for individual axes (PC1 2.27%, PC2 2.15%, and PC3 2.08%), the PCA results clearly show the genetic structure (Fig. 2). The first axis separates individuals from the TK population (purple triangles), and the second axis additionally divides individuals from this population and separates individuals from the MA population (red stars). This suggests significant genetic differentiation of the TK and MA populations relative to the other groups. The remaining individuals form a compact cloud, suggesting close kinship and low variability within them.

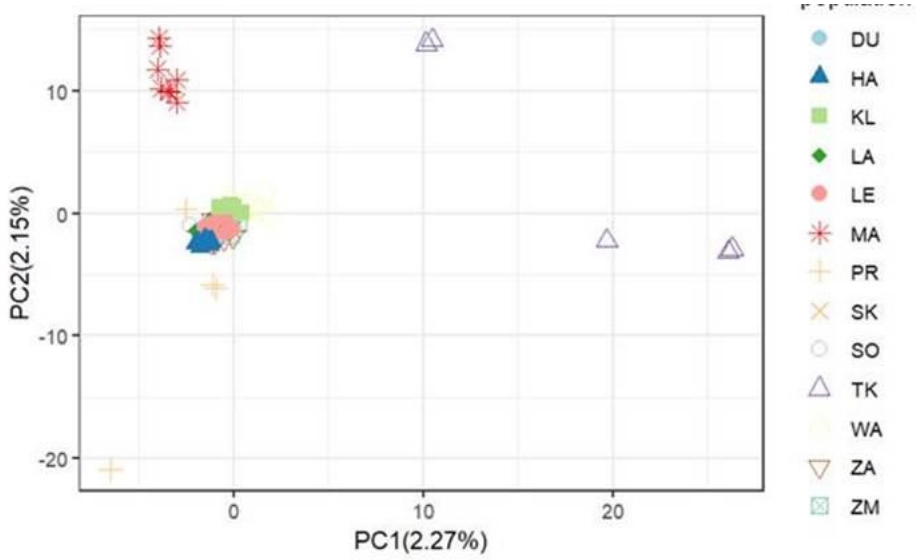

Figure 2. Distribution of *Chrysanthemum zawadzkii* individuals in the space defined by two principal components: PC1 (2.27%) and PC2 (2.15%) calculated based on SNP. Each point corresponds to a single individual. Colors and symbols indicate affiliation to a given population. The population abbreviations used correspond to the Table 1 and denote, respectively: HA - Haligowskie Skały/Aksamitka, ZA - Zawiesy, WA - Wąwóz Sobczański, TK - Trzy Korony, MA - Macelowa Góra, LE - Leśny Potok, PR - Przełęcz/blue trail, KL - Klasztorna Góra, ZM - Zamkowa Góra/Pieniński Castle, SO - Sokolica, SK - Sokola Perć, SK - Dunajec near the Trzy Korony Mountain Shelter/Stajnia nad Przełomem, LA - Las za Leśnym Potok.

Thanks to the PC3 component, we can see an additional dimension of genetic differentiation between populations, although it is less pronounced than in the case of the first two axes (Fig. 3). We can observe a weak separation of individuals from the PR population (orange crosses). The remaining populations, however, form a compact point cloud in the middle of the graph; this indicates little differentiation between them, although individuals from individual populations can be observed grouped together, e.g., LE, HA. The repeatability of the spatial patterns between the PC1-PC2 and PC1-PC3 graphs confirms the stability and regularity of the structure of the studied populations.

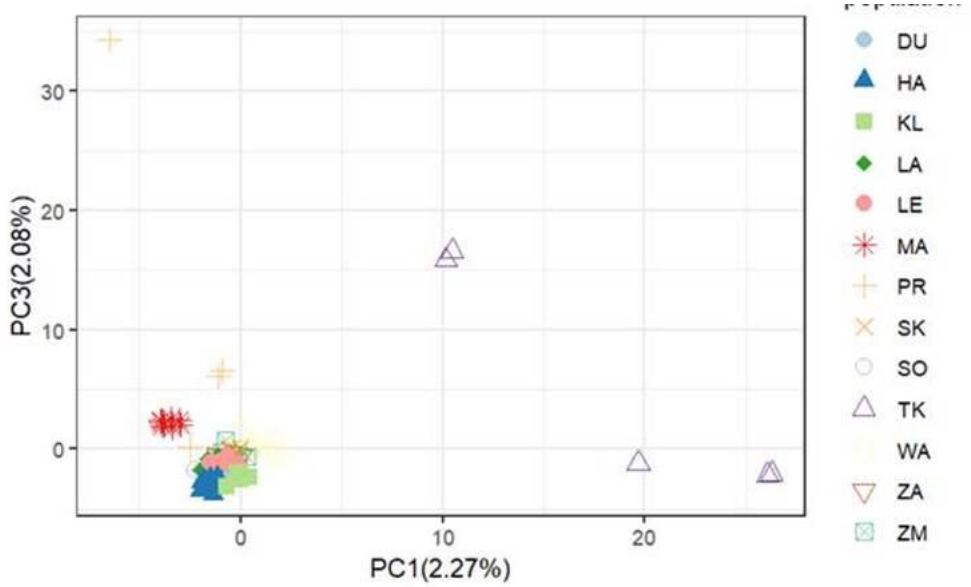

Figure 3. Distribution of *Chrysanthemum zawadzkii* individuals in the space defined by the components PC1 (2.27%) and PC3 (2.08%), calculated based on SNPs. Populations are labeled according to the legend as in the previous graph (RStudio).

DAPC analysis shows the genetic structure between individuals from individual populations (Fig. 4). Individual axes have a higher degree of explained variance (LD1 - 29.01%, LD2 - 17.02%). We can observe a stronger separation of individuals here than in the PCA graph. Individuals from several groups, such as TK, MA, ZA, and KL, form distinct, compact clusters – this indicates genetic differentiation. However, populations such as ZM, LE, and DU partially overlap, which may indicate gene flow or common ancestry. The LD1 axis explains the majority of the variation between groups and separates the TK population. The LD2 axis further separates the remaining groups. These results confirm the existence of several distinct genetic lineages within the studied populations.

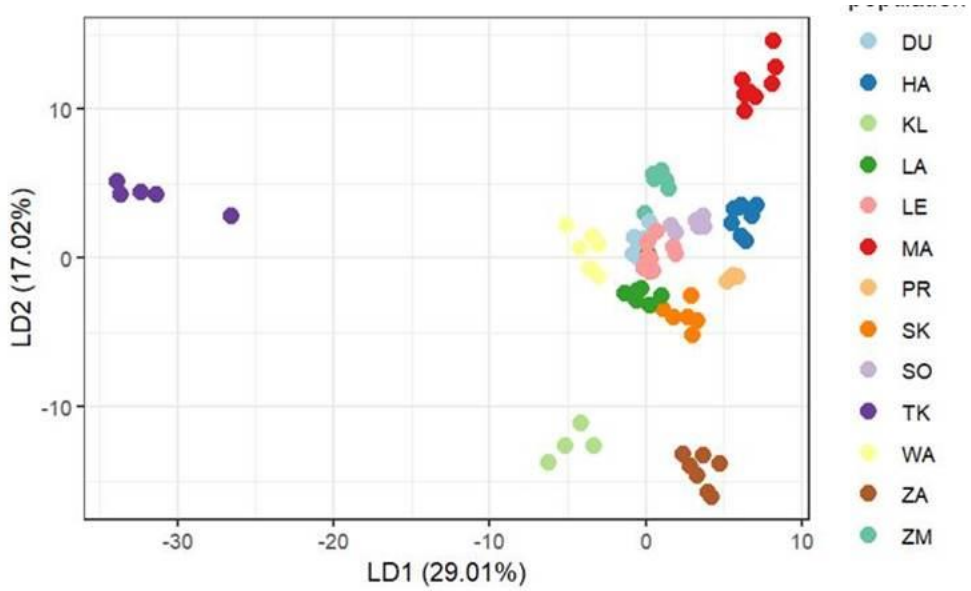

Figure 4. Distribution of *Chrysanthemum zawadzkii* individuals in the discriminant space defined by the first two discriminant functions: LD1 (29.01%) and LD2 (17.02%) calculated based on SNP. Each point corresponds to a single individual, and the colors in the legend indicate population affiliation (Table 1) (RStudio).

In the LD1-LD3 graph (Fig. 5), the LD1 axis strongly separates the TK (purple) population from the others. The LD3 axis further separates groups, particularly individuals from the HA, KL, and DU populations, which relatively form distinct clusters. The partially overlapping LE, SO, and SK populations may indicate genetic relatedness or common ancestry. The LD1-LD3 system confirms both clear and subtle differences in genetic structure in the studied *Chrysanthemum zawadzkii* populations.

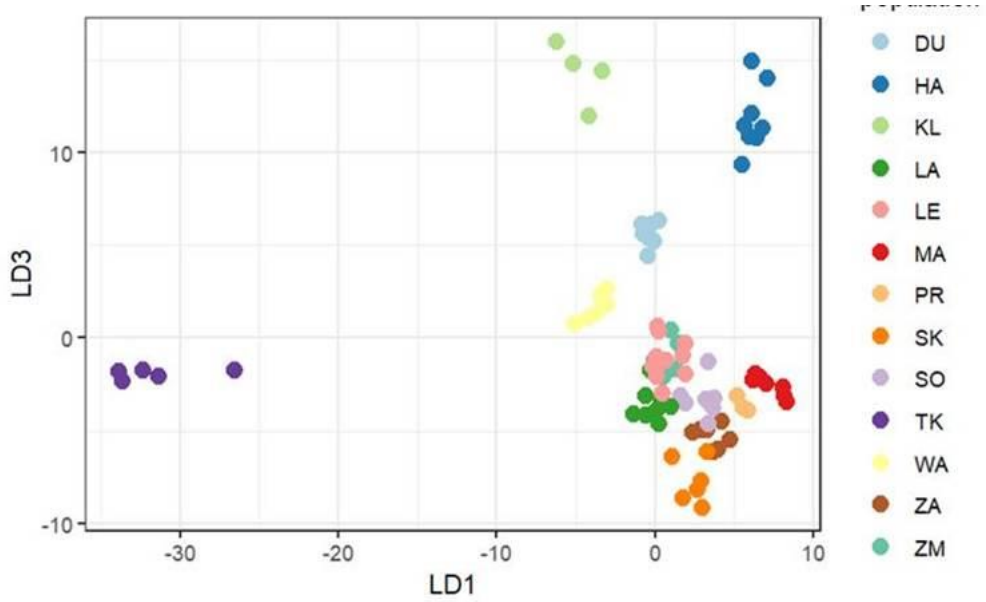

Figure 5. Distribution of *Chrysanthemum zawadzkii* individuals in the space defined by the first (LD1) and third (LD3) discriminant functions based on SNP data. Each point is an individual, and the colors in the legend indicate membership in a given population.

In the case of the SilicoDArT marker analysis, it can be seen that the vast majority of individuals form a compact point cloud, indicating a high degree of genetic similarity between individuals (Fig. 6). The first axis distinguishes one individual from the PR population, while individuals from the TK population (purple) are distinguished by separation along the PC2 axis.

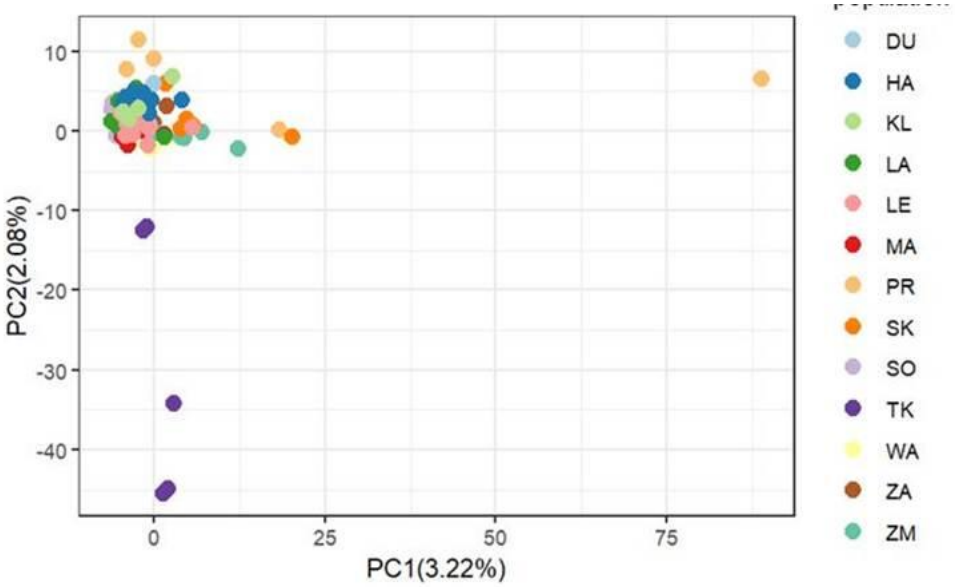

Figure 6. Distribution of *Chrysanthemum zawadzkii* individuals in the space defined by the PC1 (3.22%) and PC2 (2.08%) components calculated based on SNPs. Colors indicate population membership according to the legend, and each point represents a single individual.

The graph depicting PC1-PC3 (Fig. 7) also shows a cluster of populations, indicating low genetic differentiation. Only the MA population (red) is separated along the PC3 axis, although most individuals are clustered within the point cloud. As in the previous graph, one individual from the PR population exceeds 75 units, which may indicate the presence of private alleles or a technical artifact. Despite the relatively small contribution of PC3 to explaining the total variance, the use of this component additionally reveals subtle differences in genetic structure. Both these and previous PCA results confirm the presence of common ancestry, genetic similarity, and differences within the studied populations.

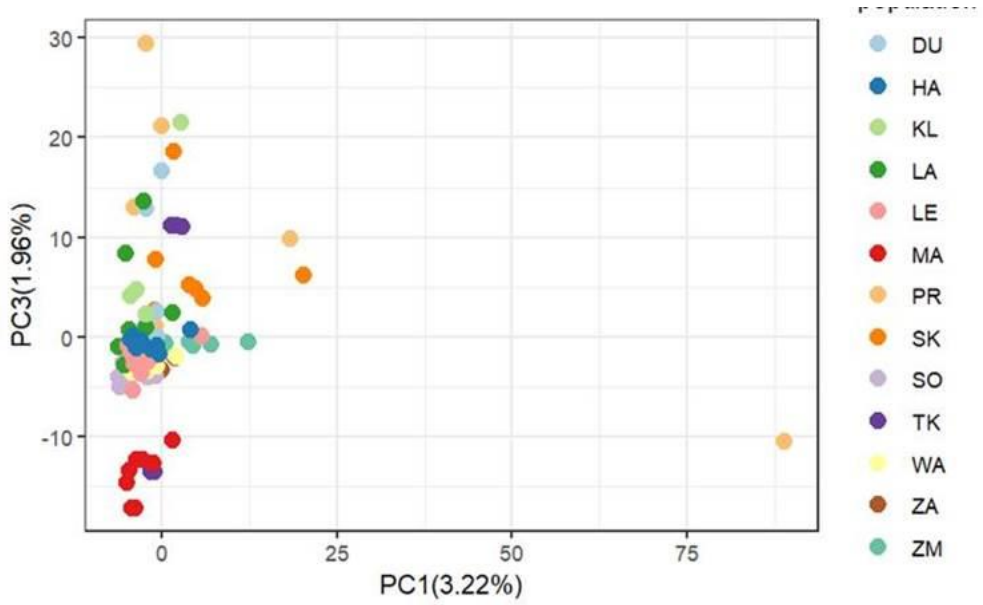

Figure 7. Plot of the spatial PCA analysis for the first PC1 (3.22%) and the third PC3 component (1.96%) calculated using SilicoDArT. Each point represents a single individual, and the color corresponds to the population affiliation according to the legend.

Analysis of the data from SilicoDArT markers using DAPC (Fig. 8) allows unambiguous separation of several genetically diverse populations. The LD1 axis separates the TK population (purple) to the right. LD2 separates the MA population (red) and slightly separates individuals from the ZA and SK populations. The remaining populations, such as KL, DU, LE, and WA, overlap, which may suggest common ancestry and gene flow. Recurring patterns confirm the existence of both clearly separated and more mixed genetic clusters within the *C. zawadzkii* populations.

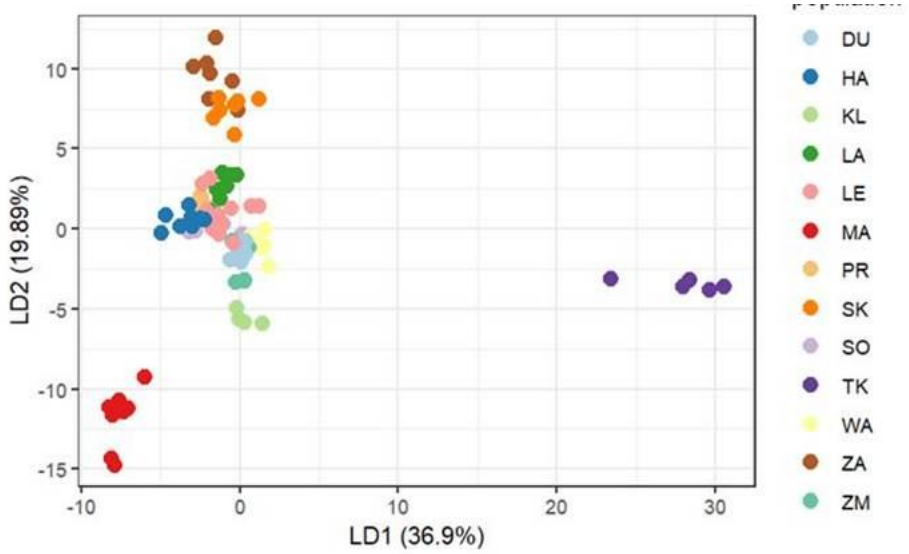

Figure 8. Distribution of individuals in the space defined by the first two discriminant functions: LD1 (36.9%) and LD2 (19.89%), calculated for SilicoDArT. Each point represents a single individual, and the colors in the legend indicate their affiliation to a particular population.

In the LD1-LD3 graph (Fig. 9), the first discriminant function LD1 also separates the TK population (purple) from the others, as in the previous analyses. The third LD3 axis, which explains 14.44% of the variation, allows for additional differentiation between the other populations. The HA, KL, DU, and WA populations form a cluster on the LD1 axis within the 2.5-12.5 range, indicating subtle genetic differences and their closer relationship to the other individuals. The MA population (red) remains separated in the lower part of the graph. The remaining populations partially overlap, and gradual changes in the point patterns are also visible, suggesting weak genetic structure. This analysis confirms the presence of repetitive genetic clusters and adds an additional spatial dimension to the previously identified population structure.

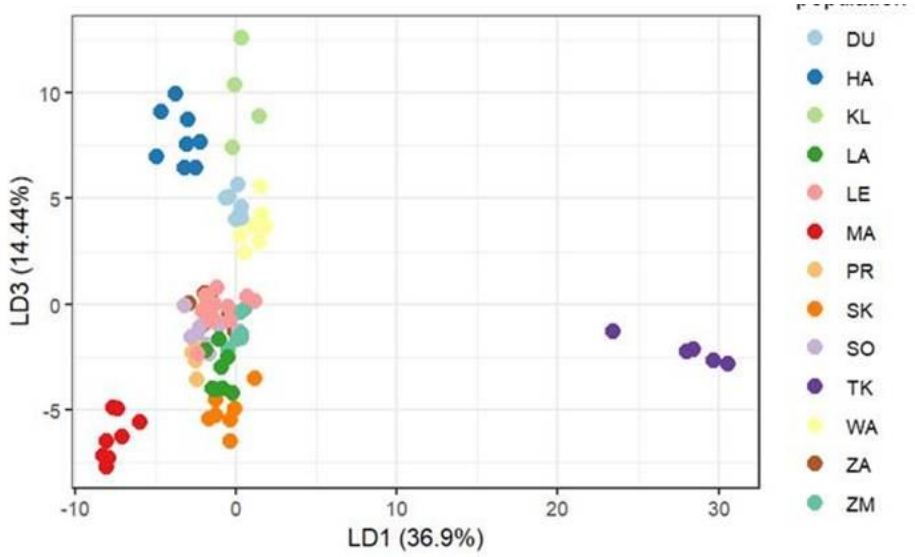

Figure 9. Distribution of *Chrysanthemum zawadzkii* individuals defined by the first and third discriminant functions: LD1 (36.9%) and LD3 (14.44%), calculated for SilicoDArT. Each point represents a different individual belonging to a specific population, color-coded according to the legend.

SNP and SilicoDArT genetic markers show common patterns of population structure. This can be observed in the comparison of PCA results presented in Figure 10. Individuals from the TK (purple) and MA (red) populations are clearly separated from the rest, regardless of the marker type or axis arrangement used. This indicates strong genetic differentiation between these populations. PCA using codominant SNP markers shows better-defined clusters at higher resolution. However, in the case of SilicoDArT markers, despite weaker diversification, population groupings are still clearly visible. Despite differences in marker characteristics, both methods confirm the presence of genetic clusters and complement each other.

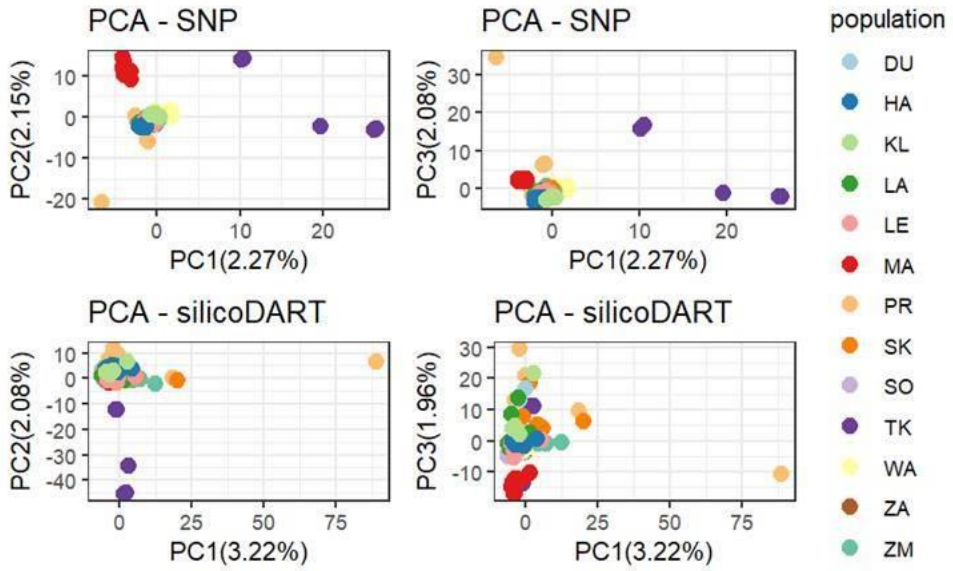

Figure 10. Comparison of PCA plots obtained using two types of markers: SNP (top row) and SilicoDArT (bottom row). The left column presents the PC1 and PC2 components, and the right column PC1 and

PC3. Each point corresponds to one individual belonging to a given population, marked with a color according to the legend (RStudio).

Similar relationships can be observed when comparing the DAPC results for SNP and SilicoDArT markers. Figure 11 shows that both marker types exhibit a similar population structure. The TK population (purple) clearly forms a separate group in all projections, indicating its genetic differentiation within the studied individuals. For the remaining populations, analysis using SNP markers shows that they form separate, yet closely related, groups. Weaker differentiation, which is also visible in analyses using SilicoDArT markers, showing overlap between individual groups. The LD1 axis explains the greatest intergroup variability (29.01% for SNPs and 36.9% for SilicoDArT) and represents the separation of the most distinct populations. The LD2 and LD3 axes show additional separation, particularly for groups such as MA, KL, HA, and SK.

These results confirm the repeatability of the grouping patterns and the consistency of both marker types in detecting the genetic structure of the *Chrysanthemum zawadzkii* population.

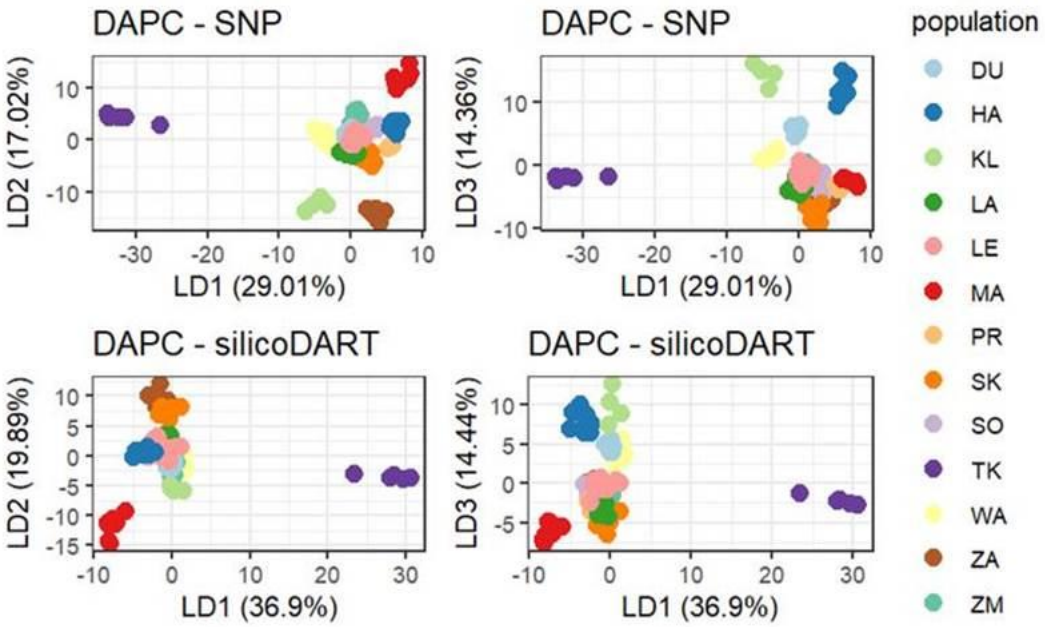

Figure 11. Combination of DAPC discriminant principal component analysis graphs obtained based on two types of markers: SNP (top row) and SilicoDArT (bottom row). The left column presents the LD1 and LD2 components, and the right column LD1 and LD3. Each point corresponds to one individual belonging to a given population, marked with a color according to the legend.

Cluster analysis

For SNP, the highest ΔK peak occurs at K = 6 (Fig. 12), indicating that six clusters best describe the genetic structure of the analyzed populations. This value

corresponds to the largest change in model fit and is interpreted as a biologically reasonable number of genetic groups. Although some differentiation is also observed for other values, these models do not reveal details within the population structure because they have lower ΔK. K=6 is considered the value that best reflects the actual genetic division in the dataset.

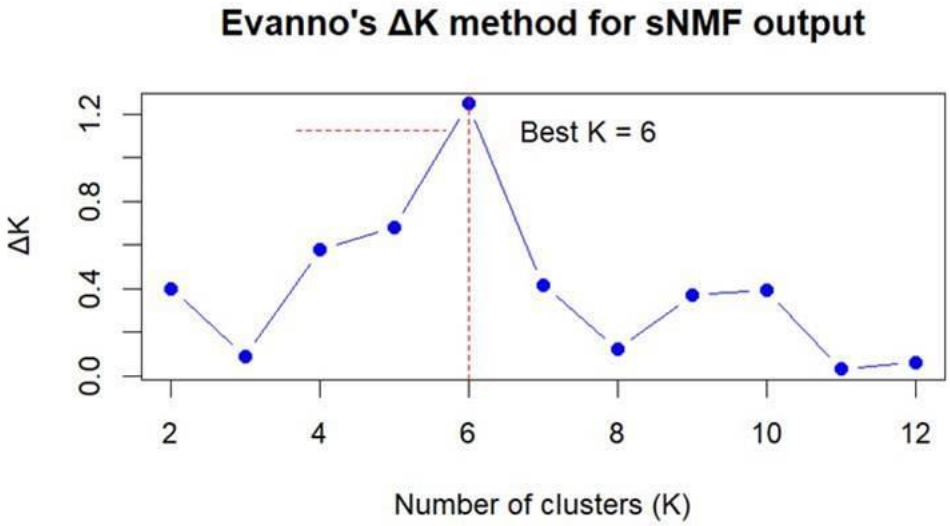

Figure 12. Results of the Evanno analysis to determine the number of genetic clusters (K) based on the ΔK statistic calculated from the sNMF model for SNPs. Each point corresponds to the ΔK value between subsequent K values.

For the SilicoDArT markers, a peak is observed for K = 2, indicating that the genetic structure of the *Chrysanthemum zawadzkii* population is best described by two main genetic clusters (Fig. 13). This model shows the most important statistically justified number of clusters in the dataset. Higher peaks are also observed for K = 4 and K = 6, but the ΔK values are lower here.

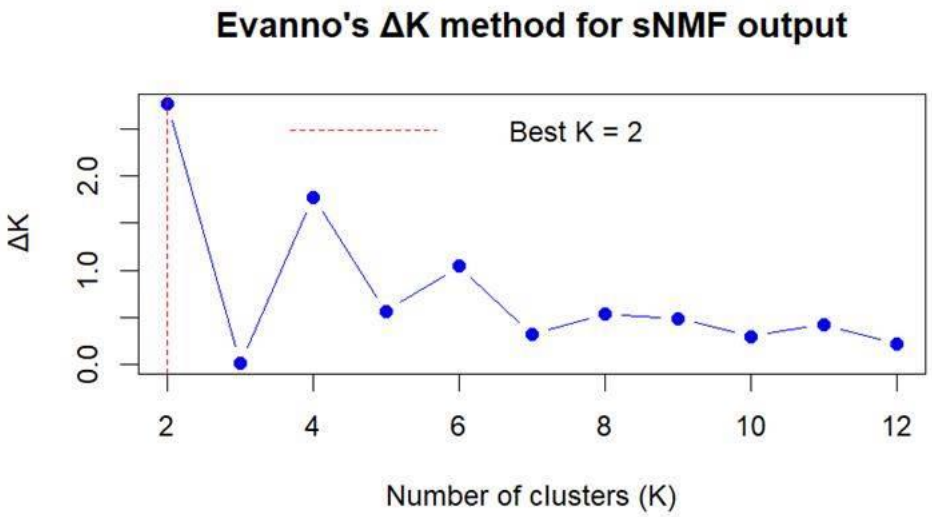

Figure 13. The graph shows ΔK values calculated using the Evanno method based on the results of sNMF clustering for SilicoDArT. Each point reflects the change in model probability with subsequent values of the number of clusters (K) (RStudio).

Because the ΔK values for SNP and SilicoDArT indicated different numbers of optimal clusters, we analyzed both the K=2 and K=6 clustering for both analyses. Figure 14, showing the results for SNP markers and K=2, shows a clear genetic division into two main clusters. Most individuals are assigned to cluster V2 (blue), while the group of individuals from the TK population is characterized by a high proportion of cluster V1 (red). Some individuals assigned to cluster V2 (blue) have a small proportion of the second cluster, which may indicate gene flow. The results confirm the existence of two main genetic lineages within the *Chrysanthemum zawadzkii* population. The observed pattern may result from geographic isolation, limited gene flow, or past divergence.

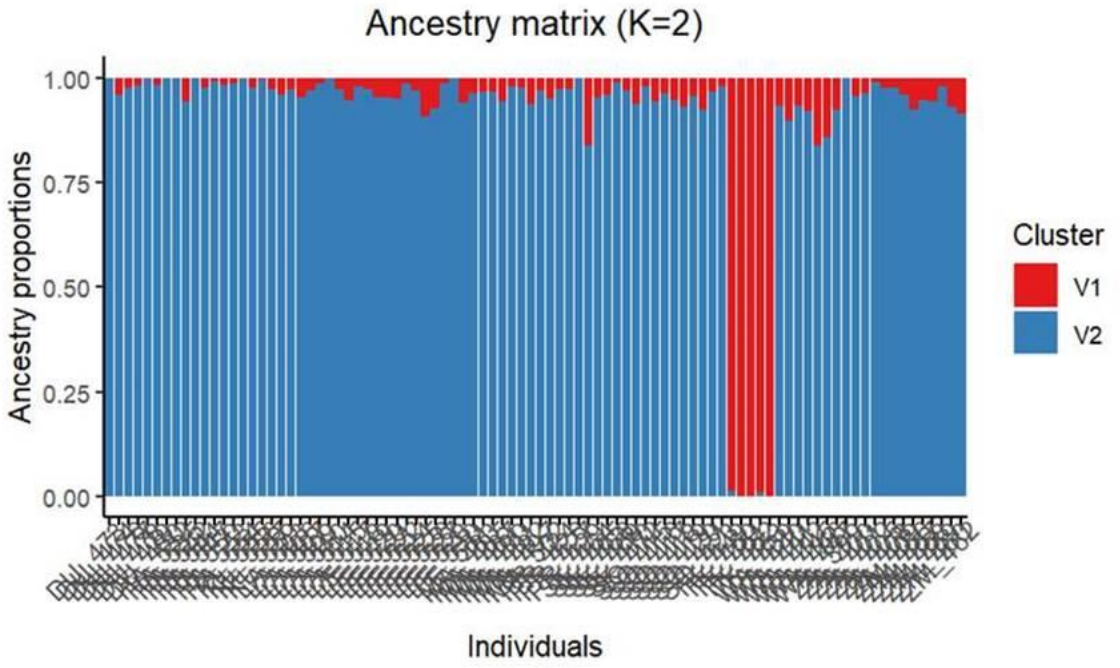

Figure 14. Bar graph showing ancestry proportions for each *Chrysanthemum zawadzkii* individual based on sNMF analysis for SNPs, assuming two clusters (K = 2). Vertical bars correspond to a single individual, and the colors: red (V1) and blue (V2), reflect the proportions of genetic affiliation to individual groups.

Figure 15, which shows the results for SilicoDArT markers, shows that the vast majority of individuals are almost entirely assigned to cluster V1 (red), indicating its dominant role in the genetic structure of the population. Only a few individuals indicate admixture from cluster V2 (blue), suggesting historical gene flow between populations or remnants of past differentiation. This structure reflects asymmetric ancestral contributions or directional gene flow in the evolutionary process of the *Chrysanthemum zawadzkii* population.

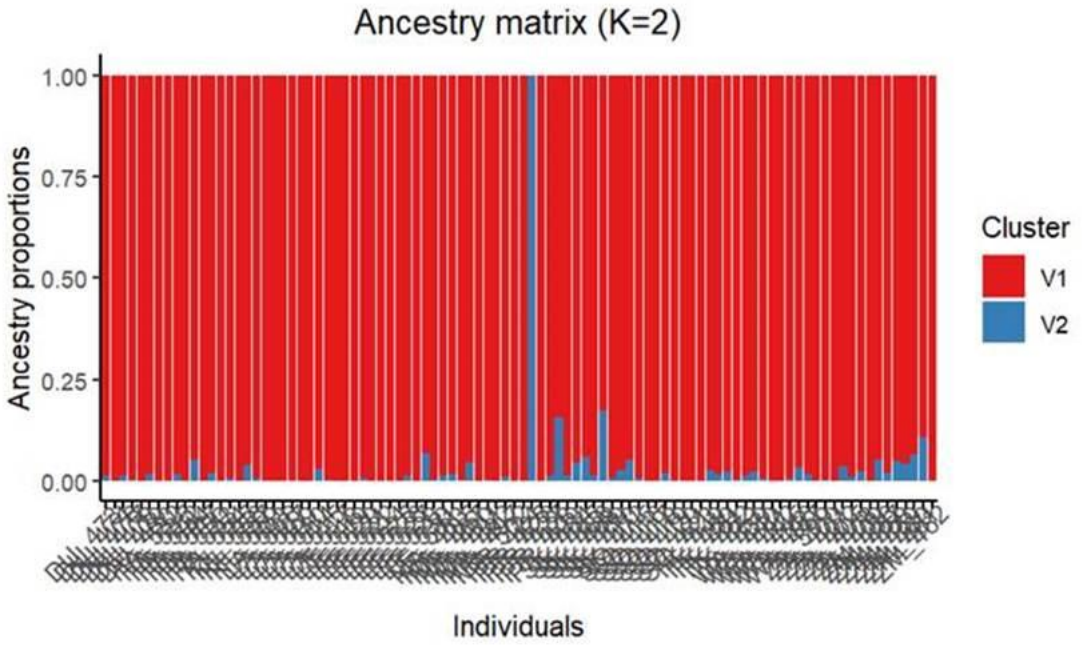

Figure 15. Bar graph showing the proportions of genetic affiliation of individual *Chrysanthemum zawadzkii* individuals obtained from sNMF analysis for SilicoDArT at K = 2. Each vertical bar represents one individual, and the colors (red – V1, blue – V2) indicate participation in a given genetic cluster.

The image in Fig. 16 obtained from SilicoDArT analysis shows a complex genetic structure involving six clusters. Cluster V5 (orange) dominates in a significant proportion of individuals. Clusters such as V3 (green) and V4 (purple) also have a significant contribution. The presence of bars composed of several colored segments indicates gene flow between populations. Compared to the K = 2 model, the structure at K = 6 shows a more detailed picture of differentiation, allowing for the detection of substructures and local genetic patterns. These results indicate that the genetic structure of the *Chrysanthemum zawadzkii* population is stratified – it includes both a main division into two genetic groups and further internal differentiation within them.

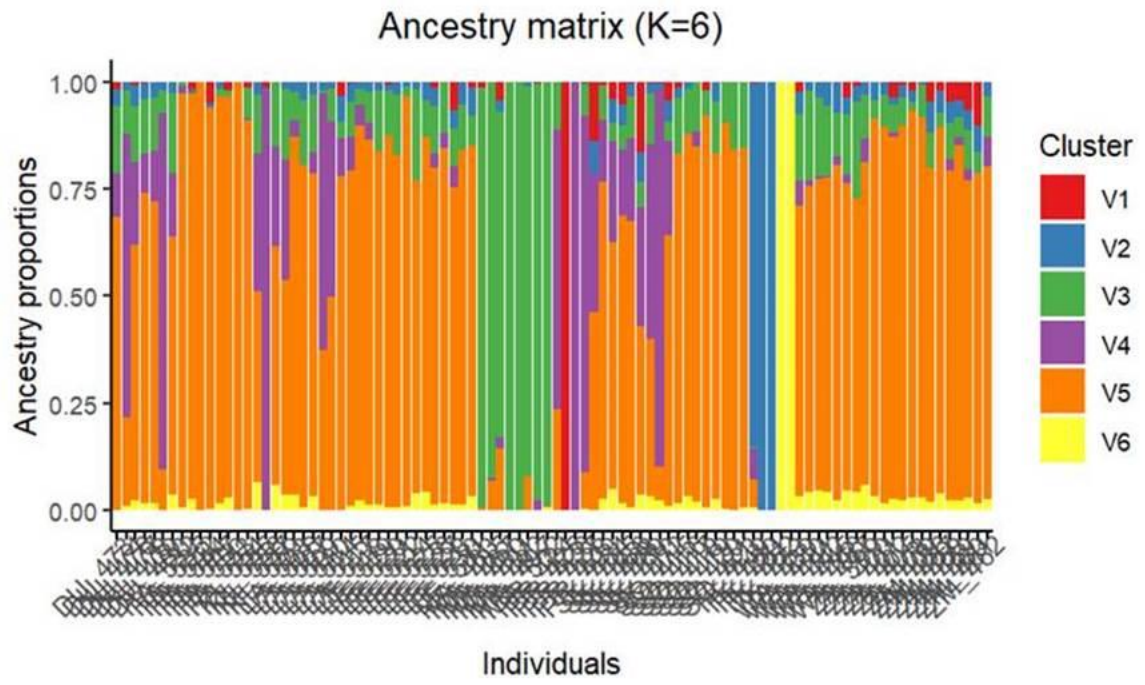

Figure 16. Distribution of ancestry proportions in *Chrysanthemum zawadzkii* individuals based on sNMF analysis for SilicoDArT, assuming six genetic clusters (K = 6). Each vertical bar represents a single individual, and the colors (V1–V6) indicate the proportion in the respective genetic groups.

The detailed genetic structure of the *Chrysanthemum zawadzkii* population for the SNP markers and K = 6 in Figure 17 shows that clusters V3 (green) and V1 (red) are dominant. The vast majority of individuals are a mixture of clusters (bars divided into different colors). This indicates common ancestry and gene flow between populations. Compared to the K = 2 model, the K = 6 model provides a more complete picture, revealing substructure within the main genetic lineages and ancient or ongoing hybridization. The results support the hypothesis of the coexistence of major distinct and related genetic lines.

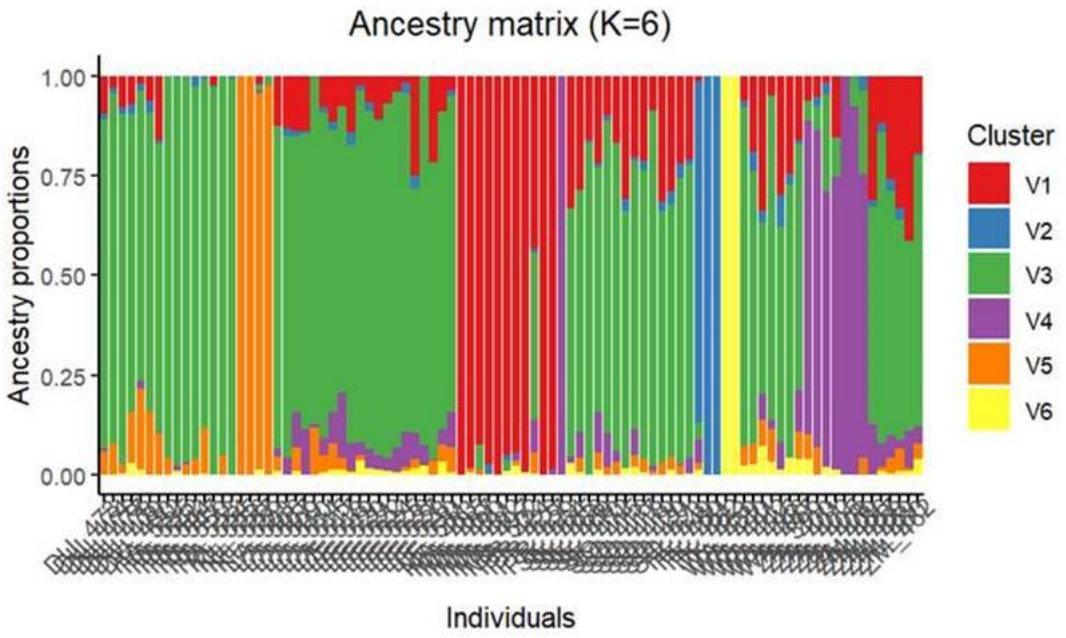

Figure 17. Distribution of genetic affiliation of individual *Chrysanthemum zawadzkii* specimens obtained on the basis of sNMF analysis for SNP, assuming K=6 clusters. Each bar represents a single specimen, and the colors (V1-V6) indicate the proportion of genes in individual genetic groups (RStudio).

Genetic parameters

Table 2 shows significant differences between the population parameters obtained for SNPs and SilicoDArT. The obtained Ho (heterozygosity) score is higher for SilicoDArT (0.49) compared to Ho (heterozygosity) for SNPs (0.04), which is due to the dominant nature of the SilicoDArT marker. Also, Hs (intra-population heterozygosity) and Ht (total heterozygosity) were higher for SilicoDArT (0.29 and 0.30) compared to the values obtained for SNP (0.17 and 0.20). However, the Fst and Fstp indices (differentiation between populations) reached higher values for SNP (0.16-0.17), and for SilicoDArT (0.01), suggesting a greater resolving power of SNPs in detecting population structure. The Fis index (intra-population inbreeding index) indicated an excess of heterozygosity for SilicoDArT (-0.68) and a deficiency of heterozygosity for SNP (0.74). These differences may be influenced by the nature of the markers used or the sample selection. Dst and Dest (difference in heterozygosity between populations) are higher for SNP (0.03 and 0.04) than for SilicoDArT (0.00 and 0.01), confirming the greater sensitivity of SNPs in detecting subtle differences. Furthermore, due to the codominant nature of SNP markers, they may better reflect genetic variation, unlike SilicoDArT markers, which are dominant.

Table 2. Comparison of SNP and SilicoDArT marker statistics.

	SilicoDArT	SNP

Ho	0.49	0.04
Hs	0.29	0.17
Ht	0.3	0.2
Dst	0	0.03
Htp	0.3	0.2
Dstp	0	0.03
Fst	0.01	0.16
Fstp	0.01	0.17
Fis	-0.68	0.74
Dest	0.01	0.04

Discussion

The obtained results indicate the existence of population differentiation, with populations such as TK and MA separated from the rest, despite a small percentage of explained variance (PC1 < 4%). The PC1-PC3 system revealed subtle but significant differences in population structure that are invisible to the PC1-PC2 system. In the case of the PC1-PC2 system, it is clear that only the TK and MA populations differ genetically from the others, while after applying the PC1-PC3 system, the HA and SK populations are additionally distinguished. This is due to the nature of the PC3 axis, which allows for the separation of individuals based on non-dominant factors determining diversity, such as historical genetic flow. Without considering the PC3 axis in the analyses, the additional genetic complexity between individuals would be omitted. The obtained results confirm previous studies showing high local diversity in the *Chrysanthemum zawadzkii* population (Moon et al., 2023; Zarzycki, 1976).

DAPC demonstrated better group separation, maximizing intergroup variance and revealing consistent separation of population units. Both SNPs and SilicoDArT for DAPC confirm the existence of population structures. TK and MA are completely

separated in the LD1-LD2 plots, indicating their genetic distinctness, lack of gene flow, and local adaptation (Figs. 2-11).

Genetic structure assessed using the sNMF model (Frichot & François, 2015) (Figs. 12-13). Based on the ΔK index, which indicated K=6 as the optimal partition for SNPs and K=2 for SilicoDArT, both possibilities were analyzed. The existence of two main genetic groups was demonstrated within the studied *C. zawadzkii* population. The peak at K=6 indicated the presence of genetic substructure, which may reflect historical division, local adaptation, and limited gene flow. Both types of markers separate individuals from the TK population from the rest, which are grouped into a single population. This demonstrates the differentiation of plants at the summit from other populations. It is clear that populations with different ecological requirements, inhabiting summits such as TK, HA, SK, or a remote site on MA, have different genotypes than the other populations (Zarzycki, 1976). These results are consistent with DAPC.

SilicoDArT markers were shown to be characterized by significantly higher observed heterozygosity (Ho=0.49) than SNP (Ho=0.04). Hs (within-population heterozygosity) and Ht (total heterozygosity) were also higher for the dominant SilicoDArT markers. Fst and Dest (genetic differentiation) indices were higher for the SNP markers (Fst = 0.16, Dest = 0.04) compared to SilicoDArT (Fst = 0.01, Dest = 0.01), indicating greater resolving power in detecting structure between populations. The Fis (inbreeding) index showed contrasting values; negative for SilicoDArT (Fis = –0.68) and positive for SNP (Fis = 0.74), indicating differences in heterozygosity levels and data structure resulting from different marker type properties (Table 2). It is likely that using other coefficients, better suited to dominant markers, would have produced more reliable results, but would have prevented comparisons between dominant and codominant markers.

The results indicate that population genetic diversity is influenced by landscape features (Wu et al., 2015; Zarzycki, 1976) and the distance between population sites (Durka et al., 2017, 2024). The genetic diversity of the TK, MA, HA, and SK populations suggests that genetic diversity is influenced by geographic, environmental, and historical factors (Cruzan & Hendrickson, 2020; Holderegger et al., 2010; Xu et al., 2017).

The obtained results provide significant information on the genetic diversity and population structure of *Chrysanthemum zawadzki*. This has taxonomic significance and is important for genetic resource conservation strategies. The observed high diversity in selected populations and the lack of homogeneity indicate the need to protect this relic, especially the TK, MA, HA, and SK populations. This is fundamental to preserve biodiversity, enrich the gene bank, and preserve the population structure. Furthermore, the use of advanced genetic analysis tools allows for the effective assessment of populations with complex evolutionary histories. Further research is needed, including both monitoring genetic changes over time and modeling potential

scenarios for population dispersal and isolation in the context of climate change and human impact.

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