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RESEARCH ARTICLE

Genetic diversity analysis of lesser burnet (*Sanguisorba minor* Scop.) genotypes collected from Erzurum Province, Türkiye Using inter-simple sequence repeat (ISSR) markers

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Abstract This research was conducted over a three-year period from 2021 to 2023, utilizing the ISSR marker system to determine the kinship relationships among populations obtained from seeds of lesser burnet (*Sanguisorba minor* Scop.) collected from the natural pasture areas of the Erzurum region. The study revealed genetic diversity among 42 burnet genotypes, including 40 genotypes and 2 control cultivars (Bünyan 80 and Altınova). The work with ISSR primers yielded PIC values ranging from 0.24 to 0.34, with an average of 0.30. The highest PIC value (0.34) was obtained from the UBC811 primer, while the lowest value (0.24) was from the UBC812 primer. Among the ISSR primers, UBC811 showed the highest Na, Ne, I, and h values with 2, 1.63, 0.54, and 0.37, respectively, whereas the UBC812 primer displayed the lowest Na, Ne, I, and h values with 2, 1.40, 0.42, and 0.26, respectively. When the results were evaluated as a whole, it was determined that there was no relationship among the collected lesser burnet (*S. minor* Scop.) populations solely in terms of geographical isolation. Additionally, we recorded that almost all of the 40 collected genotypes significantly differed from the commercial cultivars used as controls, indicating potential for the development of new cultivars.

Keywords Burnet · Population · ISSR · Characterization

Introduction

Sanguisorba minor Scop., also known as small or lesser burnet, is a perennial plant belonging to the Rosaceae family (Andrabi et al. 2012). This species exhibits a wide distribution in Türkiye as well as in Europe (Kendir 1999; Karkanis et al. 2019). Due to its biologically active compounds, it has significant uses in traditional medicine (Ceccanti et al. 2019; Karkanis et al. 2019). Moreover, because of its prolonged presence in pasture areas, lesser burnet serves as an important food source for wild and domestic animals (Kendir 1999; Erkovan et al. 2016). The burnet plant shows early growth in spring (Kozov 1965) and continues its development during mid-summer and autumn, producing green fodder when many

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other forage plants are in dormancy (Anonymous 1951; Kozov 1965). The plant, which is resistant to cold and drought, stands out as an alternative forage crop for artificial pasture establishments. Compared to alfalfa and sainfoin under arid conditions, burnet is a relatively higher-yielding plant, producing 250–300 kg/da of dry hay under dry conditions and 700–1500 kg/da under irrigated conditions (Owoahene 1991). Burnet does not cause bloating problems in animals (Meissner and Paulsmeier 1995) and has a crude protein content ranging from 5 to 18% (Meisser and Paulsmeier 1995; Acar et al. 1999). Additionally, burnet, which has a strong root system, is an important plant for protecting soil against water and soil erosion (Kendir 1999).

Due to the collection of most medicinal plant species from nature and their widespread use, some species are facing endangerment or extinction (Erayman et al. 2014). Therefore, the conservation of medicinal plants is of great importance. Analyzing the genetic diversity in natural populations is crucial for plant breeding programs. Moreover, since natural populations are significant sources of variation, conserving this variation plays a vital role in the evolutionary process (Shooshtari et al. 2024).

Genetic diversity can be determined by various methods, including morphological and molecular markers. Molecular markers are known as the most powerful and reliable biotechnological tools used in determining variations within the genome (Güngör et al. 2022; Shooshtari et al. 2024). After restriction fragment length polymorphisms (RFLP), the first marker developed by Botstein et al. (1980), markers such as random amplified polymorphic DNA (RAPD; Williams et al. 1990), amplified fragment length polymorphisms (AFLP; Vos et al. 1995), simple sequence repeats (SSR) and short tandem repeats (STR; (Litt et al. 1989; Tautz 1989; Schlotterӧer et al. 1991), inter-simple sequence repeats (ISSR; Zietkiewicz et al. 1994), and single nucleotide polymorphisms (SNP; Wang et al. 1998) have been developed over the years due to advancing technology. Recently, molecular markers such as start codon targetedSCoT) polymorphisms (Collard and Mackill 2009), inter primer binding site (iPBS; Kalendar et al. 2010), retrotransposon-microsatellite amplified polymorphisms (REMAP), inter-retrotransposon amplified polymorphisms (IRAP), CAAT box- derived polymorphisms (CBDP), and conserved DNA-derived polymorphisms (CDDP) have also been developed. Among these, ISSR marker technology is a PCR-based DNA marker technique that can determine more inter- and intra-population polymorphisms by utilizing the advantages of SSR and RAPD (Zhou et al. 2020; Marval and Gaur 2020). This technique has several advantages such as being dominant, highly reproducible, highly polymorphic, and requiring low amounts and quality of DNA (Mazher et al. 2024; Shooshtari et al. 2024). It is specific because it uses longer primers based on SSRs (Hamdan 2024).

In general, literature investigationss have focused on the secondary metabolites contained in the lesser burnet species and their medicinal uses, and it has been determined that research on kinship relationships has not been conducted. However, Tausch et al. 2017 conducted a study using a molecular marker system (AFLP) to determine the genetic kinship degrees of lesser burnet genotypes distributed in Europe for the first time. The aim of this second study, which used a molecular marker system to determine the kinship relationships of lesser burnet genotypes, was to identify the genetic differences and relationships among lesser burnet genotypes collected from different regions of Erzurum province using the ISSR marker technique.

Materials and methods

Plant materials

We collected a total of 40 genotypes of lesser burnet (*S. minor* Scop.) from 20 different locations in Erzurum, located in northeastern Türkiye, as plant materials for this study. We also used two cultivated cultivars, Altnova from the Field Crops Central Research Institute and certified Bünyan 80, as controls. Supplementary. Table 1 and Fig. 1 provide the genotype locations and some characteristics of the burnet seed collection sites.

Genomic DNA isolation and PCR amplification

The genomic DNA of each genotype used in the study was isolated from young leaves based on the CTAB method (Murray and Thompson 1980) with minor modifications. We quantified the genomic DNA using a nanodrop spectrophotometer (Nanodrop

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Table 1 The information of primers used in the study

Primer Name	Primer sequence (5'–3')
UBC807	AGAGAGAGAGAGAGAGT
UBC808	AGAGAGAGAGAGAGAGC
UBC809	AGAGAGAGAGAGAGAGG
UBC810	GAGAGAGAGAGAGAGAT
UBC811	GAGAGAGAGAGAGAGAC
UBC812	GAGAGAGAGAGAGAGAA
UBC817	CACACACACACACACAA
UBC825	ACACACACACACACACT
UBC841	GAGAGAGAGAGAGAGAY
UBC843	CTCTCTCTCTCTCTCTRA
UBC844	CTCTCTCTCTCTCTCTRC
UBC848	CACACACACACACACARG

Lite, Thermo Scientific, Wilmington, USA) at an $A_{260/280}$ nm ratio. We confirmed the quality of the DNA on 0.8% agarose gels. Table 1 provides detailed information on the 12 ISSR primers used for genotyping.

Polymerase Chain Reaction (PCR) analysis was conducted in a 20 µL reaction volume containing 30 ng of genomic DNA, 2.5 mM $MgCl_2$, 2 µM of each primer, 1 U of Taq polymerase, and 2 mM of dNTPs in a 10X reaction buffer. PCR amplifications were carried out using the SensoQuest LabCycler (SensoQuest GmbH, Goettingen, Germany) with an initial denaturation at 95 °C for 5 min, followed by 35 cycles of 30 s of denaturation at 94 °C, 30 s of annealing at 50–60 °C, and 1 min of extension at 72 °C, with a final extension at 72 °C for 10 min.

The amplified PCR products were electrophoresed on 1.5% (w/v) agarose gels containing 1X Tris–Borate-EDTA buffer, stained with 1 µg/mL ethidium bromide. The gels were run at 140 V for 80 min and visualized using a UV transilluminator (Gel Doc XR, Bio-Rad Laboratories, Hercules, USA).

Data analysis

The TotalLab TL120 program analyzed the ISSR gels, classifying the bands observed on the gel as 1 (present) or 0 (absent) based on their polymorphic status, forming a matrix from which Jaccard and Davidson (1972) calculated genetic distances and similarities. Additionally, we used the online tool



Fig. 1 Map of the locations where lesser burnet genotypes were collected

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iMEC (Amiryousefi et al. 2018; https://irscope.shinyapps.io/iMEC/) to determine the polymorphism information content (PIC), marker index (MI), and resolving power (Rp) values for each amplified primer. The polymorphism ratios of the markers used in the study were calculated using the formula: polymorphism ratio (%) = (number of polymorphic bands / total number of bands) × 100. Cluster analysis was performed using the NTSYS-pc software package (Rohlf 1998), and dendrograms were drawn according to the UPGMA method. We calculated the observed number of alleles (Na), effective number of alleles (Ne), Shannon's information index (I), and Nei's genetic diversity (h) values from the ISSR primer data using GenAlEx software (Peakall and Smouse 2012). A model based on a clustering algorithm (STRUCTURE v.2.3.4), which genetically divides groups according to allele frequencies (Pritchard et al. 2000), determined the genetic structures of the genotypes used in the study. This value is expressed as the K value, and the STRUCTURE program was run by setting the number of populations (K value from 1 to 10). We performed 10 runs with a 100,000-iteration burn-in period and 100,000 Markov Chain Monte Carlo (MCMC) repetitions for each K value. In each repeated analysis, the admixture model was selected to reveal genetic clustering. The data obtained from STRUCTURE were evaluated using STRUCTURE HARVESTER (Evanno et al. 2005), and the Evanno method was applied to this online program (Earl and von Holdt 2012).

Results

We used a total of 12 ISSR markers to determine the polymorphisms in *S. minor* Scop. genotypes, which included 40 genotypes collected from different regions of Erzurum province in Türkiye and two cultivars (Altnova and Bünyan 80). The band profiles obtained from the 12 ISSR primers were used to study different genetic indicators, yielding a total of 287 bands ranging from 12 to 35 bands per primer, with an average of 23.92 bands per primer. The study determined all bands for 42 genotypes to be polymorphic, and Table 1 displays the band data obtained from the 12 ISSR primers.

Using 42 genotypes, a total of 2,945 bands were obtained from the primers, with an average of 245.42 bands per genotype. The highest number of bands (378) was obtained with the UBC841 primer, while the lowest number (112 bands) was obtained with the UBC848 primer. The number of bands per allele totaled 123.33 with an average of 10.28. The highest number of bands per allele (13.79) was obtained with the UBC811 primer, whereas the lowest number (7.17) was obtained with the UBC812 primer. The gel image belonging to UBC841 was given in Fig. 2.

As shown in Table 3, PIC values ranging from 0.24 to 0.34 were obtained in the study conducted with ISSR primers, with an average value of 0.30. The highest PIC value (0.34) was obtained from the UBC811 primer, while the lowest value (0.24) was from the UBC812 primer.

To assess the effectiveness of the ISSR markers in burnet genotypes, the marker index (MI) and resolving power (Rp) values were calculated for each primer, recording MI values ranging from 0.001 to 0.003. In the study, the highest Rp value (16.10) was obtained from the UBC810 primer, whereas the lowest value (5.33) was from the UBC848 primer (Table 2).

Additionally, the GeneAlEx 6.5b2 software was used to study population genetics based on the band

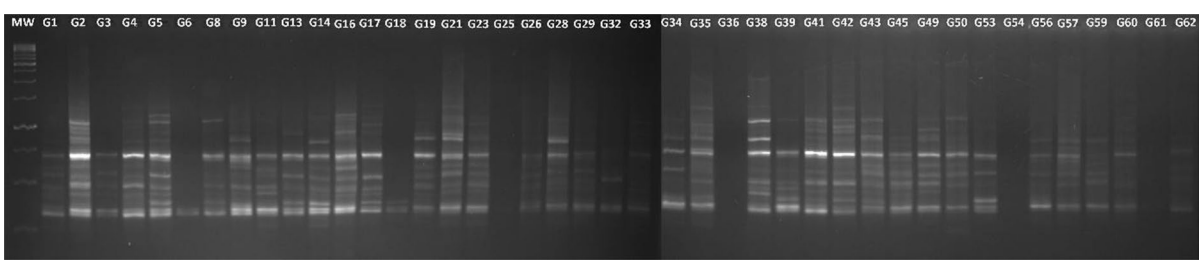

Fig. 2 The gel displays distinct banding patterns in 42 *Sanguisorba minor* Scop genotypes using the UBC841 inter-simple sequence repeat (ISSR) primer

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Table 2 Detailed band data of 12 inter-simple sequence repeat (ISSR) primers for *Sanguisorba minor* genotypes collected from different regions

Primer Name	Number of allels	Percentage of polymorphism (%)	PIC	Number of total bands	Bands per Locus	MI	Rp
UBC807	23	100	0.26	185	8.04	0.001	8.81
UBC808	18	100	0.27	149	8.28	0.001	7.10
UBC809	29	100	0.26	231	7.97	0.001	11.00
UBC810	35	100	0.29	338	9.66	0.002	16.10
UBC811	24	100	0.34	331	13.79	0.003	13.29
UBC812	23	100	0.24	165	7.17	0.001	7.86
UBC817	14	100	0.33	180	12.86	0.003	8.00
UBC825	30	100	0.31	334	11.13	0.002	14.48
UBC841	33	100	0.32	378	11.45	0.003	15.33
UBC843	21	100	0.33	258	12.29	0.003	11.43
UBC844	25	100	0.32	284	11.36	0.003	12.19
UBC848	12	100	0.29	112	9.33	0.002	5.33
Total	287		3.56	2945	123.33	0.025	130.90
Mean	**23.92**	**100**	**0.30**	**245.42**	**10.28**	**0.002**	**10.91**

PIC: Polymorphic Information Content, MI: Marker Index, Rp: Resolving power

data obtained from ISSR markers. We calculated the observed number of alleles (Na), effective number of alleles (Ne), Shannon's information index (I), and Nei's genetic diversity (h) values using the ISSR primer data. Among the ISSR primers, UBC811 showed the highest values for Na, Ne, I, and h with 2, 1.63, 0.54, and 0.37, respectively, whereas the UBC812 primer exhibited the lowest values for Na, Ne, I, and h with 2, 1.40, 0.42, and 0.26, respectively (Table 3).

Additionally, dendrogram and principal coordinate analyses were conducted using the ISSR data sets to determine the genetic relationships among the lesser burnet genotypes. According to the dendrogram analysis, three clusters were obtained: Cluster I, Cluster II, and Cluster III (Fig. 1). Cluster I includes genotypes G1, G3, G6, G11, and G29, while Cluster II comprises 34 lesser burnet genotypes. Cluster III contains genotypes G54, including the control genotypes G61 and G62. Cluster II identified genotypes G21 and G23 as the most similar genotypes.

Additionally, presents the distance matrix for 42 lesser burnet genotypes based on ISSR data. According to, the highest genetic distance ratio was found to be 0.64 between genotypes G21 and G23.

To determine the genetic relationships among 42 different burnet genotypes, ISSR data sets were utilized, and principal coordinate analysis (PCoA) was performed (Fig. 3). The results of the PCoA analysis are consistent with the dendrogram findings. The PCoA analysis revealed that the burnet genotypes clustered into three distinct groups. The first cluster grouped genotypes G54, G61, and G62; the second cluster positioned genotypes G1, G3, G6, and G11;

Table 3 Population genetic structure calculated using GeneAlEx based on inter-simple sequence repeat (ISSR) primer data

Primer Name	Na	Ne	I	h
UBC807	2	1.45	0.45	0.29
UBC808	2	1.45	0.43	0.27
UBC809	2	1.44	0.44	0.28
UBC810	2	1.53	0.50	0.33
UBC811	2	1.63	0.54	0.37
UBC812	2	1.40	0.42	0.26
UBC817	2	1.64	0.54	0.36
UBC825	2	1.55	0.49	0.32
UBC841	2	1.54	0.50	0.33
UBC843	2	1.61	0.52	0.35
UBC844	2	1.55	0.49	0.32
UBC848	2	1.50	0.45	0.29

Observed number of alleles (Na), effective number of alleles (Ne; (Kimura and Crow 1964)), Shannon's information index (I; (Lewontin 1972)), and gene diversity (h; (Nei's 1973)) values

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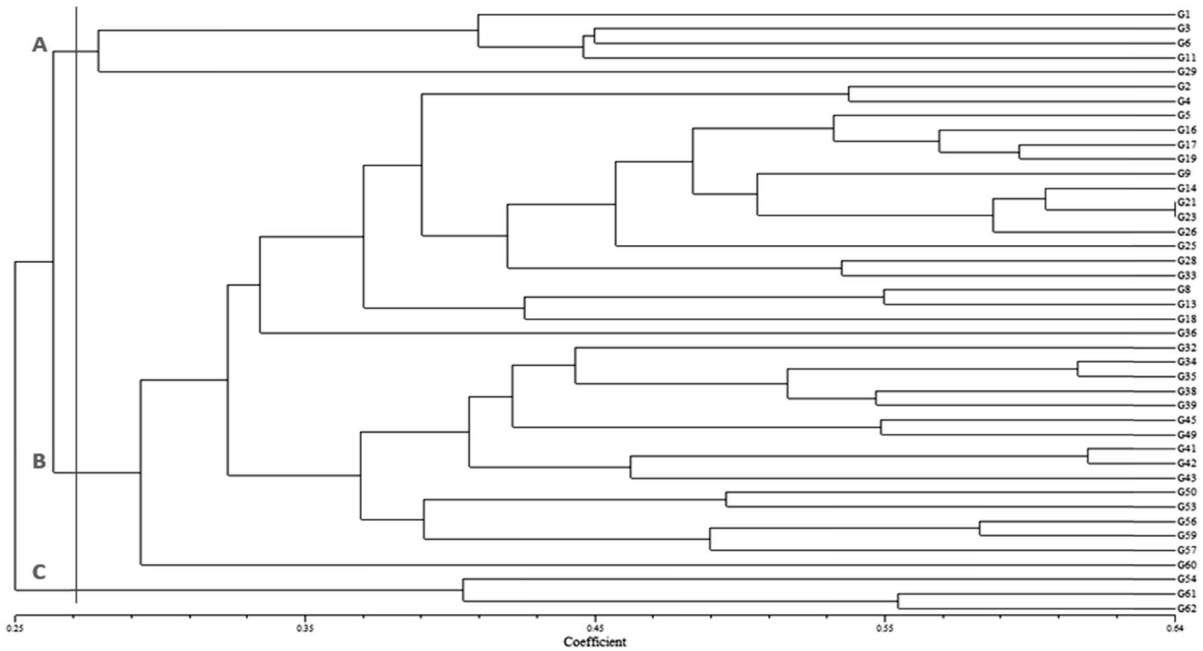

Fig. 3 Clustering analysis created using the unweighted pair group method with arithmetic mean (UPGMA) method with inter-simple sequence repeat (ISSR) markers

and the third cluster grouped the remaining genotypes. G61 and G62, the commercial cultivars, exclusively positioned themselves alongside G54. Genotypes G13, G34, and G35 were more distantly related to the other genotypes.

The STRUCTURE software determined the population structure based on ISSR molecular data. The STRUCTURE results indicated that the most probable number of subpopulations is K = 3 (Figs. 4 and 5). The first subpopulation is present in all burnet genotypes. All genotypes, except for genotype G43, contain the second subpopulation. The third subpopulation is present in all genotypes, except G1, G3, and G19. According to the results of the STRU CTURE analysis, genotypes G1, G3, G4, G6, G8, G11, G29, G33, G36, G54, G60, G61, and G62 are placed in the first group; genotypes G2, G5, G9, G13, G14, G16, G17, G18, G19, G21, G23, G25, G26, and G28 are in the second group; and the remaining genotypes are located in the third group.

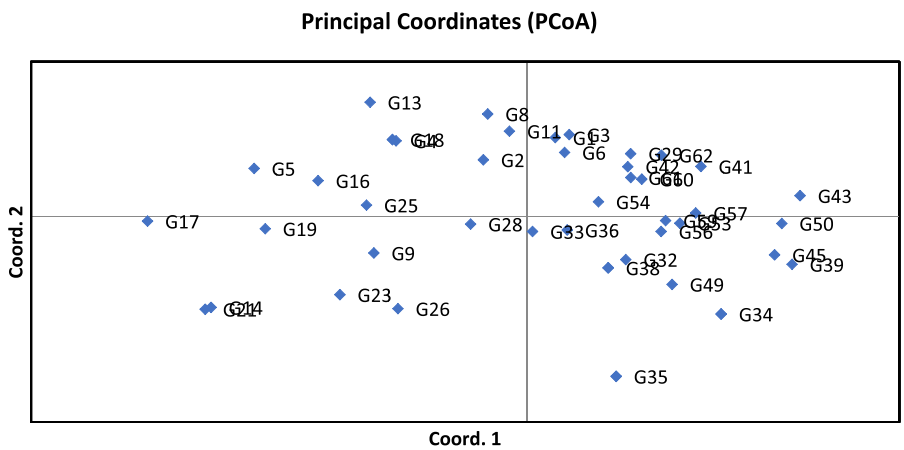

Fig. 4 Principal coordinate analysis (PCoA) of 42 lesser burnet genotypes based on 12 inter-simple sequence repeat (ISSR) markers

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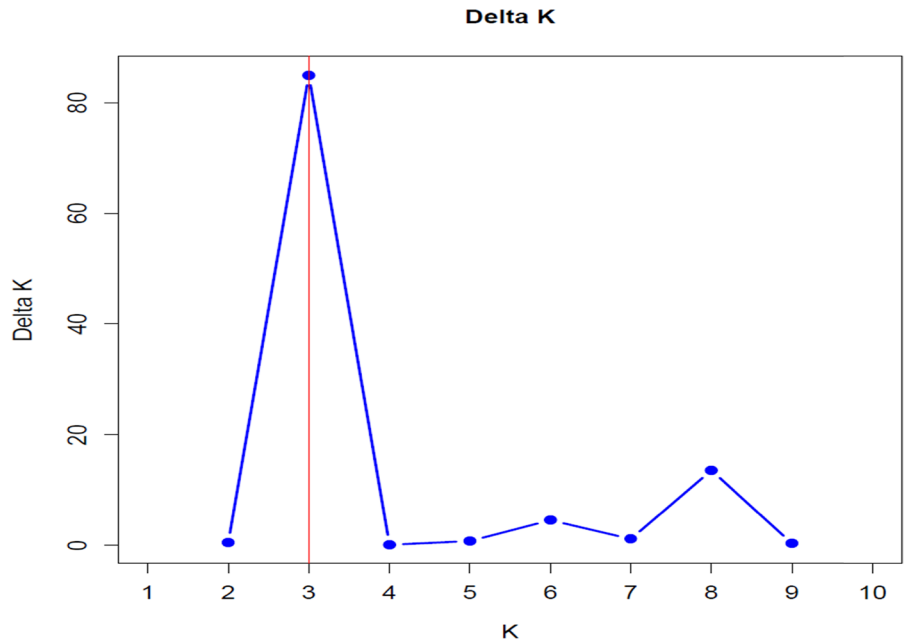

Fig. 5 Graph of delta K (ΔK) values obtained from STRUCTURE analysis of 42 lesser burnet genotypes

Genotypes G1 and G3, as well as G19 and G43, differed from the other genotypes in terms of their genetic structures, harboring characteristics of two different populations. The other genotypes contained genetic backgrounds from three populations. In the first group, genotypes G1 and G3 differed in genetic structure; in the second group, genotypes G14, G17, G19, and G21, along with G13, G18, and G25, exhibited differences; in the third group, genotypes G34 and G39, and G35, G38, and G42 showed variations. The analysis results revealed genetic diversity among the burnet genotypes collected from different regions of Erzurum, with all genotypes except G54 differing from the commercial cultivars G61 and G62.

In our study, we employed various clustering techniques, including UPGMA dendrogram, PCoA, and STRUCTURE analyses, to investigate the genetic diversity of *S. minor* genotypes using ISSR markers. Although different clustering methods may not always align due to differences in how they handle data, our results show consistency across these methods. Both PCoA and STRUCTURE analyses revealed three main genetic clusters. The UPGMA dendrogram also reflected these clusters, demonstrating the robustness of our results despite the potential limitations of small marker variables (Fig. 6).

Discussion

Lesser burnet (*Sanguisorba minor* Scop.) possesses antibacterial, antioxidant, anticancer, and

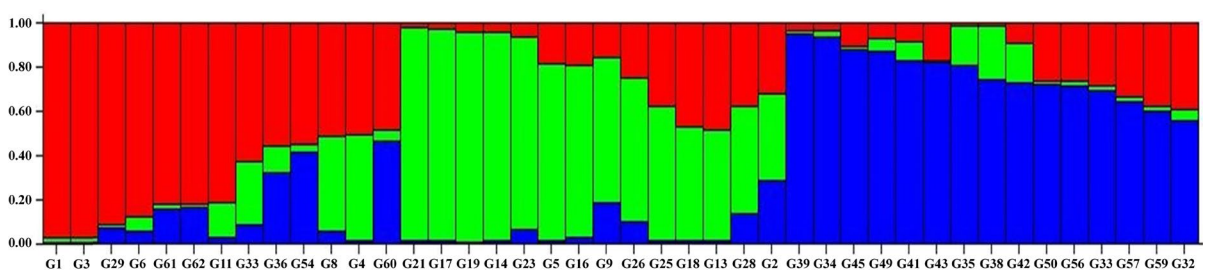

Fig. 6 Population structure analysis of 42 small meadow button genotypes for K = 3 groups obtained with Structure v2.3.4

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antiviral properties, making it a valuable plant that serves as an important resource for both the food and pharmaceutical industries (Tocai et al. 2023). Investigating the genetic diversity of such valuable plants provides significant contributions to various fields, including medicine, agriculture, ecology, and environmental sciences. In agriculture, examining the genetic diversity of plants can offer valuable insights to breeders, enabling them to make informed decisions in developing new cultivars that meet specific breeding objectives (Mohamed et al. 2024). Plant diversity also helps breeding programs by providing new genetic information that can be used to make crops that produce more food and plants that are better able to handle biological and environmental stresses by giving breeders access to a wider range of basic materials (Rao 2004; Cruz-Cruz et al. 2013).

Molecular marker approaches and genetic and physical mapping are utilized to assess genetic diversity (Alhasnawi et al. 2024). In this study, the genetic diversity among 42 lesser burnet cultivars, including 40 local and 2 commercial cultivars, was examined using ISSR technology. Tausch et al. (2017) found a polymorphism rate of 94.58% in their genetic diversity study of 190 lesser burnet cultivars using AFLP technology, which is quite close to the 100% polymorphism rate of the ISSR primers used in this study. Similarly, the Ne, I, and h values are consistent with those in the Tausch et al. (2017) study. This finding supports the use of ISSR markers as an easy, useful, repeatable, and good way to find out about safflower's genetic diversity and phylogenetic relationships. The PIC values ranging between 0.24 and 0.34 also demonstrate the discriminative power of ISSR markers.

Clustering and principal component analysis (PCoA) are among the most effective ways to define relationships and diversity among different plant germplasms (Li et al. 2022; Kongjaimun et al. 2022). Cluster analysis revealed that adjacent entries exist in the same population or branches of the dendrogram. The dendrogram revealed the formation of three distinct clusters, each displaying a branching pattern distinct from the commercial cultivars, indicating the genetic diversity of the collected local plants. Additionally, Cluster II contains a larger number of genotypes compared to other clusters, which may be due to limiting the application area to the Erzurum region, as well as factors such as altitude, climate, or geographical distance among the genotypes selected to examine polymorphism (Mazher et al. 2024).

The low similarity rate observed among the genotypes included as plant material in this investigation may have several potential explanations. Factors encompass genetic diversity within the species, environmental adaptation, geographic isolation, reproductive barriers, foreign pollination, hybridization (Achleitner et al. 2004), human impact during selective breeding (LeRoy 2017; Bocianowski et al. 2024), phenotypic similarity, environmental influence (Levin and Kerster 1974; Vellend and Geber 2005), and the resolution of the employed molecular marker. The resolution of the marker employed in this investigation may have been inadequate to identify similarities or differences across meadow button genotypes. Consequently, markers with enhanced resolution may be crucial for identifying genetic distinctions among closely related genotypes exhibiting low similarity rates. Consequently, it is advisable to utilize a marker with higher resolution, such as SSR, rather than markers like RAPD, AFLP, and ISSR (Dilebo et al. 2024).

The results of UPGMA clustering, STRUCTURE, and PCoA analyses were consistent with each other, and all tests revealed the presence of three different populations. The genetic structure of the first subpopulation is present in all genotypes, and it is the dominant population. The other populations showed an almost equal distribution. Genotypes G1, G3, G19, and G43, differing from the others, harbored only two populations in their genetic structure. This is evidence of the genetic diversity among lesser burnet species in the Erzurum region. The geographical distribution of species leads to differences in genetic diversity and genetic structure due to variable habitat conditions and gene flow among species (Xiong et al. 2020; Shooshtari et al. 2024).

Moreover, although three groups were formed in the PCoA analysis results, no distinction occurred based on the geographical locations where the samples were collected. In ISSR research conducted on different plants, UPGMA, STRUCTURE, and PCoA analyses have been observed to exhibit a significant correlation with the geographical locations where the samples were collected. However, in our results, there is a weak correlation between the analyses performed and the geographical locations. A similar outcome was found in *Trifolium alexandrinum* (Mohamed

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et al. 2024). Several investigations that show many factors other than geographical isolation can affect genetic diversity in plant populations support our results. For example, research conducted with different marker systems also demonstrated a weak correlation between genetic relationships and geographical distribution according to UPGMA and STRUCTURE analyses (Wu et al. 2021). Additionally, Basheer-Salimia et al. (2013) and Hamdan et al. (2024) found a significant lack of consistency at certain points between plant traits, the geographical locations of seed collection, and the genetic similarities of the plants.

Conclusion

This study used ISSR markers to reveal the genetic diversity among 42 lesser burnet genotypes, providing a guide for future research on the species. The findings showed that the genetic diversity of lesser burnet populations in the Erzurum region is not just due to being geographically isolated. Even though the sample collection area was limited to the Erzurum region, there was still a lot of genetic diversity among the lesser burnet genotypes in this area. Additionally, 40 of the collected genotypes exhibit significant genetic differences from the existing commercial cultivars. According to these results, examining the genetic diversity of burnet will provide valuable information for breeders, helping them make informed decisions in developing new cultivars with desired traits. This approach can contribute to improving the quality and yield of burnet crops and provide benefits in many fields such as food, medicine, biology, and ecology.

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Author contributions Rufayi Karataş, Ayşe Gül Kasapoğlu, Mehmet Kerim Güllap Emre İlhan and, Murat Aydın conducted the experiment and wrote the paper. All authors read and approved the final manuscript.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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