Bangladesh J. Bot. **53**(4): 871-880, 2024 (December) DOI: https://doi.org/10.3329/bjb.v53i4.77265

POPULATION STRUCTURE ANALYSIS AND MOLECULAR CHARACTERIZATION OF DUCKWEED GENOTYPES USING iPBS AND ISSR MARKERS

OMER FARUK COSKUN* **AND DIDEM AYDIN[1]**

Department of Horticulture, Faculty of Agriculture, Hatay Mustafa Kemal University, Hatay, Türkiye

Keywords: Duckweed, Genetic characterization, ISSR, IPBS, *Lemna*, *Spirodela*

Abstract

Duckweed belongs to subfamily Lemnoideae, considered as potential minor vegetable for human consumption. Genetic diversity and molecular characterization of 67 duckweed genotypes grown in different ecosystems were determined using ISSR (Inter Simple Sequence Repeats) and iPBS (Inter-Primer Binding Site) markers. ISSR and iPBS methods identified 100% polymorphism, with genetic similarity coefficients between *Spirodela* and *Lemna* species ranging from 0.17 to 0.56. Structure analysis conducted according to K=6 calculation revealed that *L. minor* had the most homogeneous and diverse populations. Duckweed genotypes and species displayed a wide range of genetic diversity. Using various marker techniques, the genetic structure of duckweed was determined and characterized it as an alternative, sustainable, and cheap source of protein, vegetables, and biofuel. The results obtained from this study will be useful for understanding molecular mechanisms in future genetic improvements of duckweed genotypes and species.

Introduction

Lemnoideae is a subfamily of aquatic monocotyledonous flowering plants, known as duckweeds, water lentils, or water lenses (Cabrera *et al*. 2008), and its members have a rapid growth rate (Ziegler *et al*. 2015). *Landoltia*, *Lemna*, *Spirodela*, *Wolffia*, and *Wolffiella* are the five genera that comprise this subfamily. So far, 37 species have been identified among these genera. It contains 20-35% protein, 4-7% oil, and 4-10% starch (Appenroth *et al*. 2017). Duckweed's protein and other nutrients make it a valuable food source for humans (Acosta *et al*. 2021). In addition, they are relatively easy to cultivate and harvest. There are some species of duckweed that are becoming more common as minor vegetables (Beukelaar *et al*. 2019). Due to high starch content they are also considered valuable raw materials for bioethanol production (Tang *et al*. 2015).

Genetic variation plays a crucial role in product development and plant genetics. In order to identify varieties that produce higher yields and are more efficient, breeders need to understand genetic variation. There are various advantages of using molecular marker systems in biotechnology (Parveen *et al*. 2016). Molecular markers are used to detect polymorphisms among individuals in the population (El-Kholy *et al*. 2015, Tecirli *et al*. 2018, Coskun 2022, Coskun 2023). Furthermore, genetic modifications can be monitored to determine their effects on populations. A variety of marker techniques have been applied in genetic studies of plants including duckweeds (Rothwell *et al*. 2004, Wang and Messing 2011, Wani *et al*. 2014). There are several advantages of using ISSR markers to detect genetic variation, such as their technical simplicity, rapidity, and low cost. Low DNA rates and no prior knowledge of the sequence are the most significant advantages of this technique (Parveen *et al*. 2016). The ISSR technique has previously been used to study duckweed (Xue *et al*. 2012). It is also possible to determine genetic diversity using retrotransposon markers found throughout the eukaryotic genome. Following the

*Author for correspondence: <omerfaruk.coskun@mku.edu.tr>. [1]Department of Biology, Faculty of Science, Erciyes University, Kayseri, Türkiye.

outcomes of this method, researchers will be able to differentiate closely related species, as well as individuals within the same species. It is easy to apply, does not require sequence information, and is highly efficient in DNA fingerprinting and polymorphism identification (Kalendar and Schulman 2014). The aim of this study was to determine the genetic diversity and population structure of duckweed genotypes growing naturally in Türkiye's aquatic ecosystems.

Materials and Methods

There are 67 duckweed genotypes (44 genotypes of *Lemna minor*, 9 of *L. gibba*, 7 of *L. trisulca*, 3 of *L. turionifera*, and 4 of *Spirodela polyrhiza*) collected from different parts of Türkiye were used in this study (Table 1). A CTAB (2%) procedure was used to extract genomic DNA from freeze-dried young leaves.

Table 1. Coordinate information of 67 Duckweeds genotypes (Coskun *et al*. 2018).

Sl. No.	Botanical name	Coordinate	Sl. No.	Botanical name	Coordinate
1	*Lemna minor*	38^0 40’ 41.10” N 35^{0}18’ 24.59” E	35	*L. minor*	38^0 08’21. 5” N 30^0 46’ 21.7” E
2	*L. minor*	38^0 40’ 05.41” N 35^{0}17’ 16.07” E	36	*L. minor*	40^0 54’ 19.20” N 35^0 59’ 23.32” E
3	*L. minor*	38^0 14’ 20.72” N 35^{0}11’ 47.12” E	37	*L. minor*	42^0 01’ 30.10” N 35^0 04’ 55.41” E
4	*L. minor*	38^0 13’ 56.78” N 35^{0}11’ 41.08” E	38	*L. minor*	41^0 38’ 22.89” N 36^0 05’ 24.66” E
5	*L. minor*	38^0 13’ 45.20” N 35^{0}12’ 13.09” E	39	*L. minor*	41^0 40’ 06.91” N 36^0 01’ 45.97” E
6	*L. minor*	39^0 55’ 04.76” N 32^{0}59’ 56.66” E	40	*L. minor*	41^0 13’ 57.37” N 36^0 27’ 18.25” E
7	*L. minor*	40^0 24’ 40.29” N 32^{0}54’ 49.92” E	41	*L. minor*	36^0 17’ 51.98” N 30^0 08’ 44.92” E
8	*L. minor*	40^0 21’ 43.19” N 32^{0}26’ 04.18” E	42	*L. minor*	36^0 18’ 23.15” N 30^0 08’ 23.25” E
9	*L. minor*	40^0 46’ 28.20” N 32^{0}01’ 42.32” E	43	*L. minor*	36^0 19’ 06.65” N 30^0 15’ 55.06” E
10	*L. minor*	40^0 46’ 32.64” N 32^{0}02’ 07.75” E	44	*L. minor*	36^0 40’ 55.79” N 29^0 38’ 48.16” E
11	*L. minor*	40^0 45’ 05.27” N 32^{0}02’ 25.70” E	45	*L. gibba*	38^0 40’ 41.10” N 35^0 18’ 24.59” E
12	*L. minor*	40^0 46’ 57.23” N 33^{0}00’ 35.42” E	46	*L. gibba*	38^0 14’ 20.72” N 35^0 11’ 47.12” E
13	*L. minor*	40^0 44’ 37.69” N 32^{0}14’ 01.72” E	47	*L. gibba*	40^0 11’ 16.08” N 33^0 02’ 16.84” E
14	*L. minor*	40^0 19’ 18.16” N 32^{0}29’ 01.89” E	48	*L. gibba*	40^0 29’ 24.15” N 32^0 38’ 57.80” E
15	*L. minor*	41^0 19’ 09.99” N 32^{0}27’ 55.38” E	49	*L. gibba*	40^0 18’ 55.68” N 32^0 28’ 06.36” E
16	*L. minor*	41^0 19’ 09.99” N 32^{0}27’ 55.38” E	50	*L. gibba*	40^0 45’ 19.69” N 32^0 02’ 12.76” E
17	*L. minor*	41^0 12’ 42.56” N 37^{0}01’ 20.57” E	51	*L. gibba*	42^0 01’ 59.42” N 35^0 03’ 17.21” E
18	*L. minor*	41^0 14’ 13.51” N 36^{0}42’ 09.85” E	52	*L. gibba*	41^0 13’ 57.37” N 36^0 27’ 18.25” E
19	*L. minor*	40^0 45’ 19.69” N 32^{0}02’ 12.76” E	53	*L. gibba*	36^0 40’ 16.21” N 29^0 39’ 11.70” E
20	*L. minor*	40^0 45’ 19.69” N 32^{0}02’ 12.76” E	54	*L. trisulca*	38^0 46’ 23.67” N 35^0 17’ 52.37” E
21	*L. minor*	40^0 36’ 42.94” N 31^{0}17’ 09.52” E	55	*L. trisulca*	37^0 58’ 57.1” N 30^0 47’ 06.7” E

22	*L. minor*	40^0 36’ 42.94” N 31^{0}17’ 09.52” E	56	*L. trisulca*	40^0 36’ 33.04” N 31^0 17’ 31.99” E
23	*L. minor*	40^0 56’ 29.69” N 31^{0}44’ 50.44” E	57	*L. trisulca*	40^0 56’ 18.05” N 31^0 44’ 21.34” E
24	*L. minor*	40^0 56’ 17.68” N 31^{0}44’ 30.94” E	58	*L. trisulca*	41^0 49’ 57.71” N 36^0 11’ 12.69” E
25	*L. minor*	40^0 56’ 18.05” N 31^{0}44’ 21.34” E	59	*L. trisulca*	41^0 29’ 02.04” N 36^0 06’ 31.97” E
26	*L. minor*	40^0 56’ 17.50” N 31^{0}44’ 18.78” E	60	*L. trisulca*	41^0 40’ 06.91” N 36^0 01’ 45.97” E
27	*L. minor*	40^0 56’ 18.00” N 31^{0}44’ 30.40” E	61	*L.turionifera*	40^0 44’ 37.69” N 32^0 14’ 01.72” E
28	*L. minor*	40^0 56’ 34.40” N 31^{0}44’ 45.99” E	62	*L.turionifera*	40^0 16’ 46.18” N 28^0 02’ 52.27” E
29	*L. minor*	40^0 29’ 17.25” N 32^{0}39’ 03.78” E	63	*L.turionifera*	36^0 40’ 55.79” N 29^0 38’ 48.16” E
30	*L. minor*	40^0 29’ 24.15” N 32^{0}38’ 57.80” E	64	*Spirodela polyrhiza*	40^0 19’ 18.16” N 32^0 29’ 01.89” E
31	*L. minor*	40^0 47’ 36.15” N 35^{0}27’ 24.16” E	65	*S. polyrhiza*	38^0 40’ 05.41” N 35^0 17’ 16.07” E
32	*L. minor*	40^0 16’ 46.18” N 28^0 02’ 52.27” E	66	*S. polyrhiza*	38^0 14’ 20.72” N 35^0 11’ 47.12” E
33	*L. minor*	37^0 58’ 57.1” N 30^0 47’ 06.7” E	67	*S. polyrhiza*	40^0 45’ 19.69” N 32^0 02’ 12.76” E
34	*L. minor*	38^0 02’ 01.5” N 30^0 49’ 27.5” E			

Twenty ISSR primers were used to characterize 67 duckweed genotypes through molecular analysis. The next step was to analyze seventeen primers that showed suitable banding patterns. PCR reactions were carried out in a thermal cycler, within 15 µl total volume containing 2 µl genomic DNA (25 ng/µl), 1.5 µl 10X PCR buffer, 1 µl of each primer, 0.25 mM of each dNTP, 1.5 mM of $MgCl_2$, 0.1 µl of TAQ DNA polymerase (5U/µl) and 7.6 µl bidistilled water. The following PCR conditions were used for amplification: denaturation at 94°C for 2 min, 35 cycles 94°C for 45 sec, primer annealing temperatures for 1 min, 72°C for 2 min and a final elongation at 72°C for 7 min. PCR products were separated by 2% agarose gel in TBE 0.5X buffer at 110 volts for 6 hrs, and visualized under UV light in a gel documentation system (BIO-RAD). The molecular size of the amplified fragments was estimated by a 50 pb DNA ladder. In additionaly, DNA samples of 67 genotypes were analyzed using 12 iPBS primer combinations that produced clear, polymorphic, and reproducible bands. For amplification, each 15 µl of PCR components consisted of 1 µM of each primer, 0.25 mM of each dNTP, 1.5 µl of 10X PCR buffer, 1.5 mM of $MgCl_2$, 7.62 µl of bidistilled water, 5 U of Taq polymerase, and 20 ng of template DNA. PCR cycling consisted of initial denaturation at 95 °C for 4 min, 30 cycles of 15 s denaturation at 95 °C, 1 min for annealing at the primer-specific melting temperature and 1 min of extension at 68 °C, with a final extension at 72°C for 5 min. PCR products were separated on 2% agarose gel at 110 V for 6 hrs and visualized under UV light.

Amplifications were scored denoting the presence (1) or absence (0) of polymorphic markers. A DNA ladder was used to estimate bands' molecular weight. A binary matrix was generated for all gel bands. NTSYS-PC version 2.11 (Numerical Taxonomy Multivariate Analysis System, Exeter Software, Setauket, N.Y., USA) was applied to analyze the resultant data. Initially, Dice similarity index values among the genotypes were calculated and a 67 × 67 matrix presenting genetic similarities was generated. Genetic similarity matrices were clustered using the UPGMA method. Using Mantel's matrix correspondence test, dendrograms were found to be able to measure similarity matrixes. With this test, a cophenetic correlation coefficient (r) value was

obtained. Principal Component Analysis (PCA) was also conducted using this matrix in NTSYS software. Along with molecular genetic data, STRUCTURE 2.3.3 (Pritchard *et al*. 2000) software was exercised to assign individuals of populations into a generation or generations. In STRUCTURE method, number and length of Monte Carlo Markov Chain (MCMC) was identified as 10000. The software was run for five replications and K= 1-10.

Results and Discussion

With the use of 17 ISSR primers 141 bands between 130 and 1520 bp were obtained . The number of total bands and polymorphic bands per primer varied between 5-13 (average 8.29) and the general polymorphism ratio was 100% (Table 2). On the other hand, 131 bands in the range of 100-1560 bp were obtained by using 12 iPBS primers. The total number of bands per primer varied from 6-16 (average 10.92) and the general polymorphism ratio of these bands was also 100% (Table 3). A total of 272 polymorphic bands were obtained using 29 primers of both marker techniques within the range of 100-1560 bp. A total of 5-16 polymorphic bands were detected per primer (average 9.38), and the general polymorphism ratio was 100%.

Table 2. Band profiles obtained by using ISSR primers.

Primer No.	Primer name	Total No. of bands	No. of polymorphic bands	Band sizes (bp)	Polymorphism rate (%)
2	CT8TG	13	13	210-1520	100
3	DBDACA7	7	7	340-1160	100
4	BDBCA7C	9	9	160-1440	100
5	HVHCA7T	9	9	280-1470	100
6	AG7YC	10	10	220-1100	100
7	GT8YA	5	5	400-1200	100
10	AG8T	9	9	130-1250	100
11	GACA4	8	8	300-1500	100
12	VHVGTG7	7	7	190-1250	100
13	CAC3GC	7	7	210-1510	100
14	CAC6	11	11	220-1200	100
15	AGC6G	6	6	400-1450	100
16	CA6AC	8	8	100-1050	100
17	GAA6	8	8	120-1510	100
18	GT6GG	9	9	210-1300	100
19	GA8YG	9	9	200-1480	100
20	TCC5RY	6	6	310-1250	100
Mean		8.29	8.29	-	100

Based on the Dice method, similarity index values among 67 duckweed genotypes were ranged from 0.17 to 0.92 (Table 4). Genetically, the closest genotypes were 3 - 4 and 34 - 35 (with a similarity index value of 0.92); the furthest genotypes were 8 - 64 (with a similarity index value of 0.17). The similarity index value of 44 genotypes belonging to *L. minor* species ranged from 0.41 to 0.92. Nine genotypes belonging to *L. gibba* species varied in similarity index values from 0.50 to 0.83. Similarity index values of 7 genotypes belonging to *L. trisulca* species varied between 0.34 - 0.84. A similarity index of 0.54 - 0.64 varied between three genotypes of *L. turionifera* species. Four genotypes of *S. polyrhiza* species showed similarity index values ranging from 0.29 to 0.80. Low similarity index values indicate high genetic variation among the present

genotypes. Genetic distances between the genus varied between 0.17 (genotypes 8 - 64) and 0.56 (genotypes 43 - 65). The genetic similarity distances were ordered as *L. minor – S. polyrhiza* (0.39) > *L. gibba – L. trisulca* (0.37) > *L. minor – L. trisulca* (0.35) > *L. minor – L. gibba* (0.34) > *L. gibba – S. polyrhiza* (0.33) > *L. minor – L. turionifera* (0.29) > *L. trisulca – S. polyrhiza* (0.28) > *L. trisulca – L. turionifera* (0.27) > *L. turionifera – S. polyrhiza* (0.24) > *L. gibba – L. turionifera* (0.14) (Table 4).

Table 3. Band profiles obtained by using iPBS primers.

Primer No	Primer name	Total No. of bands	No. of polymorphic bands	Band sizes (bp)	Polymorphism rate (%)
7	iPBS-2219	11	11	320-1520	100
10	iPBS -2222	11	11	330-1300	100
13	iPBS -2230	16	16	150-1480	100
15	iPBS -2237	11	11	200-1500	100
16	iPBS -2238	14	14	110-1490	100
20	iPBS -2246	10	10	360-1500	100
25	iPBS -2272	10	10	350-1560	100
27	iPBS -2375	11	11	180-1500	100
28	iPBS -2376	9	9	100-550	100
32	iPBS -2383	11	11	250-1200	100
35	iPBS -2388	6	6	550-1340	100
36	iPBS -2389	11	11	220-850	100
Mean		10.92	10.92	-	100

Table 4. Similarity coefficients determined among duckweed members.

Group	Lemnoideae Members	Lowest	Highest
Intergenera	*Lemna- Spirodela*	0.17	0.56
Interspecies	*Lemna minor- Lemna gibba*	0.35	0.69
	Lemna minor- Lemna trisulca	0.29	0.64
	Lemna minor- Lemna turionifera	0.35	0.64
	Lemna minor- Spirodela polyrhiza	0.17	0.56
	Lemna gibba- Lemna trisulca	0.33	0.70
	Lemna gibba- Lemna turionifera	0.39	0.53
	Lemna gibba- Spirodela polyrhiza	0.22	0.55
	Lemna trisulca- Lemna turionifera	0.30	0.57
	Lemna trisulca- Spirodela polyrhiza	0.23	0.51
	Lemna turionifera- Spirodela polyrhiza	0.23	0.47
Intraspecies	*Lemna minor*	0.41	0.92
	Lemna gibba	0.50	0.83
	Lemna trisulca	0.34	0.84
	Lemna turionifera	0.54	0.64
	Spirodela polyrhiza	0.29	0.80

An UPGMA (Unweighted Pair Group Method Using Arithmetic Average) was used to create dendrograms using Dice similarity index values. Genetic similarity levels of 67 genotypes varied between 0.32 and 0.92 according to UPGMA dendrograms (Fig. 1). Based on the dendrogram of *L. minor* genotypes, genotypes 44, 32, 6, 40 and 31 branched independently. *L. gibba* genotypes were grouped into two main clusters, the first cluster consisted of genotypes 47, 48, and 53. According to the dendrogram of *L. trisulca* genotypes, genotype 57 was separated from other genotypes. In the dendrogram of *L. turionifera* genotypes, genotypes 61 and 63 were separated from genotype 62. In the dendrogram of *S. polyrhiza* genotypes, genotype 64 was separated from the others. These dendrograms grouped duckweed genotypes according to their species.

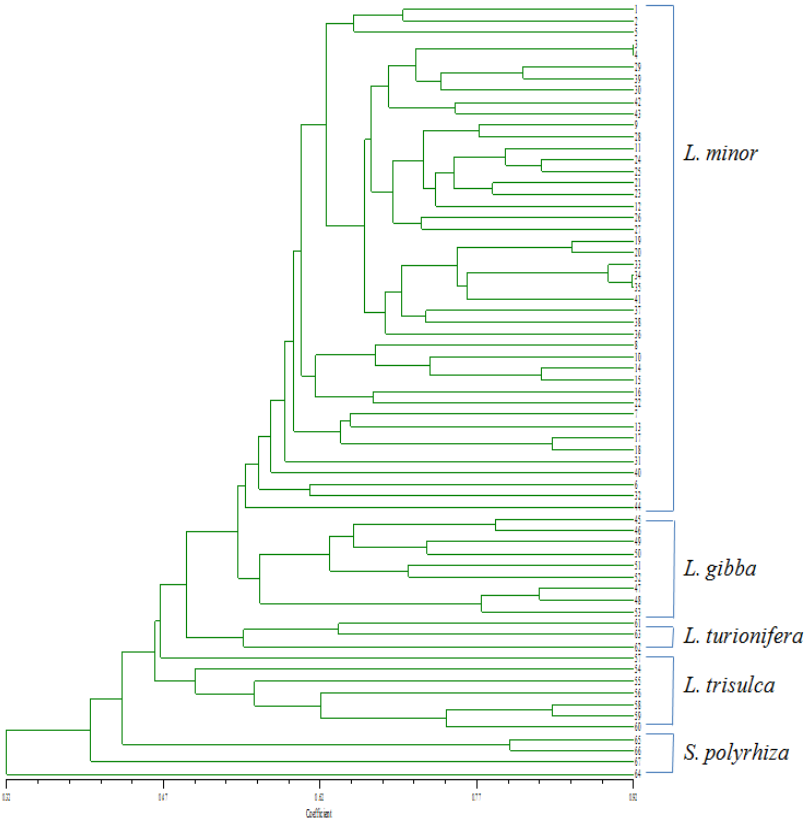

Fig. 1. UPGMA Dendrogram of duckweed genotypes.

A principal component analysis (PCA) was conducted to identify genetic variations within duckweed genotypes. With the help of NTSYS software, two- and three-dimensional scatter plots were prepared using the similarity matrix used in cluster analysis. The first three principal components explained 29.92% of the total variation. In terms of genetic variation among genotypes, present findings revealed three clusters. Among these three clusters, genotypes 44 and 64 were placed separately. Among the genotypes in cluster A were 54, 67, 32, 48, 47, 49, 53, 51, 52, 50, 55, 45, 46, 58, 59 and 60. Of these genotypes, the ones clustered quite close to each other within the same cluster had low genetic variation among them. A second cluster (B) included genotypes 65, 66, 62, 40, 61, 63, 6, 31, 43, 39, and 42. The rest of the genotypes were grouped

into a third cluster (Fig. 2). An analysis of principal components revealed that genotypes are distributed among species. A genotype's location also contributed to grouping the genotypes.

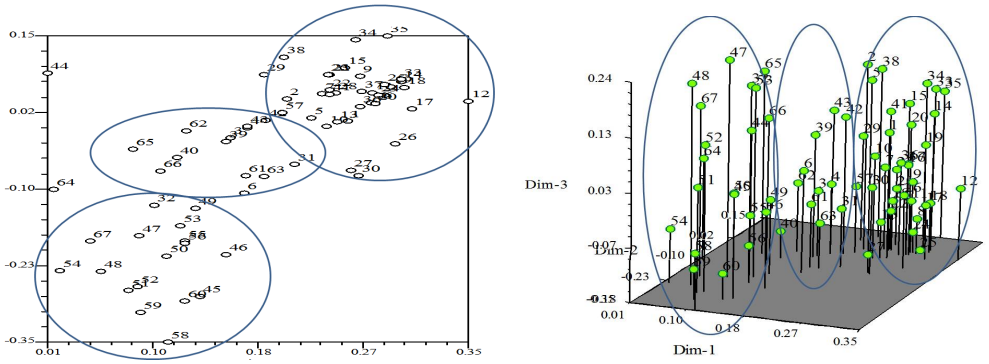

Fig. 2. Two- and three-dimensional PCA graphs of duckweed genotypes.

"Structure" analysis was exercised on 272 loci to identify the genotype population. A total of 29 different marker regions were analyzed on individuals, using K values of 5, 6, 7, 8, 9 and 10. Duckweed genotypes showed high levels of admixture based on structural analyses. Based on K=6 calculations, genotypes 3, 4, 14, 15, 24, 33, 34, and 35 were found to be the most homogeneous; genotypes 6, 30, 43, and 44 were found to be the most heterogeneous (Fig. 3).

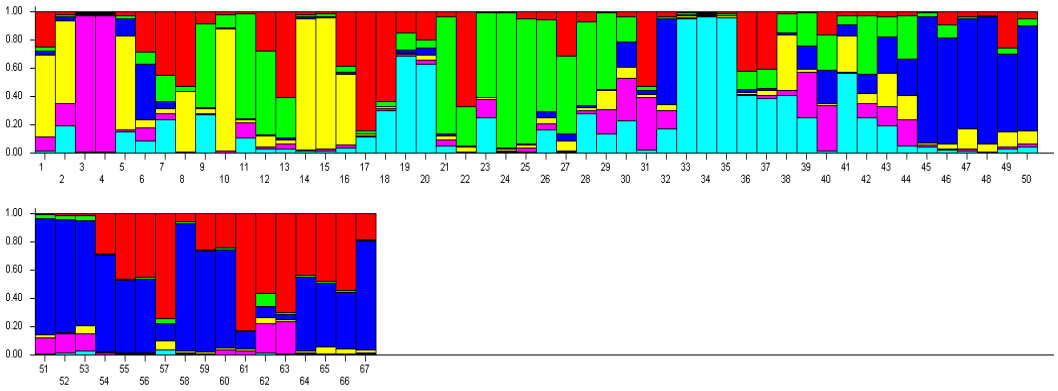

Fig. 3. Structure analysis according to K=6 value in duckweed genotypes.

With the use of two different marker systems, this study examined the genetic diversity of duckweed genotypes naturally grown in different regions of Türkiye. The use of retrotransposon primers for genetic characterization of duckweed was also identified for the first time. Duckweed genotypes were amplified with 29 primers, resulting in polymorphism ratios of 99% in *L. minor*, 93% in *L. gibba*, 97% in *L. trisulca*, 84% in *L. turionifera*, and 93% in *S. polyrhiza*. The present polymorphism ratios were greater than those obtained by RAPD in a previous study (83%) (Martirosyan *et al.* 2008). With the use of ISSR technique, Xue *et al*. (2012) reported 96.74% ratios in *Lemna* species. Unlike El-Kholy *et al*. (2015), the polymorphism ratio in *L. minor*

genotypes was greater than that in *L. gibba* genotypes. In the present study, there were more polymorphic bands due to the use of more *L. minor* genotypes.

There was a range of 0.17 to 0.92 in dice similarity index values among the entire duckweed genotypes. In addition to interspecific genetic differences, intraspecific genetic differences were also observed. There was a similar range of index values among *Lemna* genotypes and *Spirodela* genotypes was reported by Xue *et al*. (2012). In the present study, ISSR and iPBS markers were effectively used to separate duckweed genotypes. The primers were found to be suitable for genetic characterization of duckweeds at the intraspecific, interspecific, and intergenus levels.

There was a range of genetic similarity between the *Lemna* and *Spirodela* species between 0.17 and 0.56. Genetic distances between these two species have been reported by Martirosyan *et al*. (2008) as 0.24 - 0.38 and by Martirosyan *et al*. (2009) as 0.12. In the present study, Greater range of genetic distance between species and greater interspecific differentiation between species was found. Compared to other genotypes, *L. gibba* and *L. trisulca* have the highest interspecific similarity (0.70). The presence of increasing interspecific similarity indicates the transfer of genes between species. There was the least similarity between *S. polyrhiza* and *L. minor* genotypes (0.17). Among the genotypes, *L. minor* and *S. polyrhiza* showed the highest similarity ratio (0.17-0.56), while *L. gibba* and *L. turionifera* showed the lowest (0.39-0.53). It was found that present genetic distances are greater than previously reported ones (Martirosyan *et al*. 2008, 2009). In this study, greater genetic diversity among duckweed genotypes both interspecifically and intraspecifically was found.

Based on UPGMA dendrograms, genetic similarity between whole genotypes was between 0.17 and 0.92. All duckweed species were grouped separately in this dendrogram. *S. polyrhiza* genotypes were the furthest from the other species' genotypes. A similar finding was reported by Rothwell *et al*. (2004) in regard to the closest duckweed species as *L. minor* and *L. gibba*. According to other researchers, these two species are the closest to one another (Martirosyan *et al*. 2008, Xue *et al*. 2012, Tang *et al*. 2014). In contrast to this findings, *L. gibba*, *L. trisulca*, and *L. turionifera* clustered closely together and *L. minor* clustered separately (Bog *et al*. 2010). The differences between this study and other studies may be due to the different methods used. In this study, *L. turionifera* clustered more closely with *L. minor* and *L. gibba* than the other species. Nevertheless, Tang *et al*. (2014) found that *L. trisulca* is the closest to *L. turionifera*. Additionally, geographical origins play an important role in intra-species groupings in this study. In this case, geographical isolation appears to have been a significant factor in the evolution of related species.

Using marker data, principal component analysis was used to generate graphs. There were three different clusters of genetic variation based on two and three-dimensional scatter plots. The PCA graphs revealed that genotypes 44 and 64 were clustered separately. It was observed that different species clustered differently in PCA graphs. Genotypes growing in the same or close areas are clustered in close positions on the graph. Duckweed evolution has been found to be largely affected by geographic isolation in previous studies (Xue *et al*. 2012). The genetic diversity of *Lemna* species varies by geographical region (Bog *et al*. 2010, Xue *et al*. 2012). There is a parallel between the present findings and those in the literature. In this study, as geographical locations got closer, genotype genetic similarity increased. Based on principal component analysis, species were effective in determining genotype distribution. The geographical origins of genotypes were also effective in grouping genotypes.

The *L. minor* genotype was identified to have the most homogeneous and diverse populations based on population structure. There was a high degree of homogeneity between populations from the same or close geographical areas. Increasing heterogencity was observed in single plant and further genotypes. Due to vegetative propagation, DNA transfer was not encountered in highly

homogeneous populations, and genotype structure became stable. The high homogencity populations were also found to grow for a longer period of time than other populations. As a result of long-term monitoring, these populations of *L. minor* in Sultan Marsh (Kayseri-Türkiye) might provide a good example of homogencity - long-term growth. Furthermore, environmental factors such as water quality may affect the genetic structure of duckweed populations.

In natural ecosystems, several factors determine the healthy growth of important plant species. The depletion of habitats, pollution of the environment, and anthropogenic effects have led to biodiversity reduction. It is essential to preserve genetic diversity and gene sources to maintain ecological balance, and further research is necessary to build up a profile of gene sources. The genetic characterization of duckweed genotypes could also be carried out using retrotransposon markers. The results showed a wide range of genetic diversity in the duckweed species and genotypes. Genetic analysis of duckweed will be an aid in its protection and breeding.

Acknowledgment

This study was funded by the Scientific Research Unit of Erciyes University (FDK-6650).

References

Acosta K, Appenroth KJ, Borisjuk L, Edelman M, Heinig U, Jansen MAK, Oyama T, Pasaribu B, Schubert I, Sorrels S, Sree KS, Xu S, Michael TP and Lam E 2021. Return of the Lemnaceae: Duckweed as a model plant system in the genomics and postgenomics era. Plant Cell. **33**: 3207-3234.

Appenroth KJ, Sree KS, Boehm V, Hammann S, Vetter W, Leiterer M and Jahreis G 2017. Nutritional value of duckweeds (Lemnaceae) as human food. Food Chem. **217**: 266-273.

Beukelaar M, Zeinstra GG, Mes JJ and Fischer ARH 2019. The influence of meal context and information on duckweed acceptability of dutch consumers. Food Qual. Prefer. **71**: 76-86.

Bog M, Baumbach H, Schween U, Hellwig F, Landolt E and Appenroth KJ 2010. Genetic structure of the genus *Lemna* L. (Lemnaceae) as revealed by amplified fragment length polymorphism. Planta. **232**: 609-619.

Cabrera LI, Salazar GA, Chase MW, Mayo SJ, Bogner J and Davila P 2008. Phylogenetic relationships of aroids and duckweeds (Araceae) inferred from coding and noncoding plastid DNA. Am. J. Bot. **95**: 1153-1165.

Coskun OF 2022. Determination of genetic diversity in some pumpkin genotypes using SSR marker technique. Erzincan Uni. J. Sci. Technol. **15**(3): 942-952.

Coskun OF 2023. Molecular characterization, population structure analysis and association mapping of turkish parsley genotypes using iPBS markers. Hortic. **9-33**: 61-12.

Coskun OF, Aydin D, Akiska S, Ozel HB and Varol T 2018. Türkiye'de yayılış gösteren sumercimeğigil üyelerinin belirlenmesi. J. Bartin Facul. For. **20**(1): 145-151.

EL –Kholy A, Youssef M and Eid E 2015. Genetic diversity of *Lemna gibba* L. and *L. minor* L. populations in nile delta based on biochemical and ISSR markers. Egypt. J. Exp. Biol. (Bot.) **11**(1): 11-19.

Kalendar R and Schulman AH 2014. Transposon-based tagging: IRAP, REMAP, and iPBS. Methods Mol. Biol. **1115**: 233-255.

Martirosyan EV, Ryzhova NN, Kochieva EZ and Skryabin KG 2009. Analysis of chloroplast rpS16 intron sequences in Lemnaceae. Mol. Biol. **43**: 32-38.

Martirosyan EV, Ryzhova NN, Skryabin KG and Kochieva EZ 2008. RAPD analysis of genome polymorphism in the family Lemnaceae. Russ. J. Genet. **44**: 360-364.

Parveen S, Shahzad A and Yadav V 2016. Molecular markers and their application in plant biotechnology. *In:* A. Shahzad, S. Sharma and S. A. Siddiqui (Eds.), Biotechnological Strategies for the Conservation of Medicinal and Ornamental Climbers, 1st ed. Springer Inter Publishing, Cham (ZG) Switzerland, pp. 389-413.

Pritchard JK, Stephens M and Donnelly P 2000. Inference of population structure using multilocus genotype data. Genet. **155**: 945-959.

Rothwell GW, Van Atta MR, Ballard HE and Stockey RA 2004. Molecular phylogenetic relationships among Lemnaceae and Araceae using the chloroplast trnL-trnF intergenic spacer. Mol. Phylogenet. Evol. **30**(2): 378-85.

Tang J, Li Y, Ma J and Cheng JJ 2015. Survey of duckweed diversity in lake chao and total fatty acid, triacylglycerol, profiles of representative strains. Plant Biol. **17**: 1066-1072.

Tang J, Zhang F, Cui W and Ma J 2014. Genetic structure of duckweed population of Spirodela, *Landoltia* and *Lemna* from Lake Tai, China. Planta. **239**(6): 1299-1307.

Tecirli T, Dalda-Sekerci A, Coskun OF and Gulsen O. 2018. Morphological and molecular diversity among *Heliotropium greuteri* samples. Erciyes Univ. J. Inst. Sci. Technol. **34**: 1-7.

Wang W and Messing J 2011. High-throughput sequencing of three Lemnoideae (duckweeds) chloroplast genomes from total DNA. PLoS One. **6**: e24670.

Wani GA, Shah MA, Reshi ZA, Atangana AR and Khasa DP 2014. cpDNA microsatellite markers for *Lemna minor* (Araceae): phylogeographic implications. Appl. Plant Sci. **2**(7): 1300099.

Xue H, Xiao Y, Jin Y, Li X, Fang Y, Zhao H, Zhao Y and Guan J 2012. Genetic diversity and geographic differentiation analysis of duckweed using Inter-Simple Sequence Repeat markers. Mol. Biol. Rep. **39**: 547-554.

Ziegler P, Adelmann K, Zimmer S, Schmidt C and Appenroth KJ 2015. Relative *in vitro* growth rates of duckweeds (Lemnaceae) the most rapidly growing higher plants. Plant Biol. **17**: 33-41.

(Manuscript received on 10 March, 2024; revised on 27 October, 2024)