Black Sea Journal of Agriculture
doi: 10.47115/bsagriculture.1390066

Open Access Journal
e-ISSN: 2618 – 6578

Research Article
Volume 7 - Issue 1: 57-68 / January 2024

MORPHOLOGICAL AND GENETIC DIVERSITY OF *Schoenoplectiella mucronata* (L.) J. JUNG & H. K. CHOI (RICEFIELD BULRUSH) IN RICE

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Abstract: Since the beginning of rice cultivation, weed control has been a problem in Türkiye as well as in many other countries. Rice has both an important cultural plant and limited production for Türkiye. There are significant yield losses due to weeds and therefore weed control has an important place in rice agriculture. Species belonging to the genus *Scirpus* in rice production areas have recently become an important problem in rice cultivation areas of Türkiye as well as in rice cultivation areas of many other countries. In order to determine the morphological and genetic diversity of *Scirpus mucronata*, which is a problem in rice cultivation areas in Türkiye, 62 populations collected from the rice production areas of the Marmara and Black Sea Regions were evaluated over 8 ISSR primers and 12 morphological parameters. In the ISSR study, observed and expected heterozygosity levels ranged from 0.192 to 0.970 and from 0.136 to 0.566, respectively. In the morphological and molecular analyses performed, differences were detected in some quantitative characters between the examined populations. While morphological similarities were found between the populations grown in different regions that could not be ignored, genetic diversity was found to be higher. Morphological and genetic relationships between populations were not found to be related to geographic distance. In the context of the results, it is important to focus on field management practices such as cultural methods, as well as good control of rice seed traffic and herbicide use. It should not be forgotten that these measures are important in terms of integrated weed management strategies.

Keywords: *Scirpus mucronata*, ISSR-PCR, Morphological diversity, Genetic difference

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Received: November 13, 2023
Accepted: December 15, 2023
Published: January 01, 2024

Cite as: Kaya Altop E. 2024. Morphological and genetic diversity of *Schoenoplectiella mucronata* (L.) J. Jung & H. K. Choi (Ricefield Bulrush) in rice. BSJ Agri, 7(1): 57-68.

1. Introduction

Rice, widely cultivated in tropical and temperate climate regions, is the only cereal crop grown in water, utilizing dissolved oxygen for its development. To meet the demand by the year 2050, rice yield needs to be increased by 50% worldwide. Looking at the rice production situation in our country, it is observed that 95% of the production is concentrated in the Marmara and Black Sea regions. The remaining 5% of production takes place in the Mediterranean and Southeastern Anatolia regions. When comparing yield per unit area, Türkiye exceeds the world average (FAO, 2020). However, despite this, the cultivated area is not sufficient to meet domestic consumption, leading to the annual import of around 200 thousand tons of rice.

Weeds pose one of the most challenging factors in rice cultivation areas, and if not addressed, they can cause more than 40% crop loss depending on cultivation systems, rice varieties, weed species, and their density (Busconi et al., 2012; Chauhan and Abugho, 2013). Due to the completely aquatic nature of rice production systems, we observe that a limited but significant number of weed species have adapted to this system. In countries with intensive rice cultivation areas, such as those in Asia, America, and Europe, weed species belonging to the genera *Echinochloa, Cyperus,* and *Scirpus* are identified as significant problems, posing challenges for their control (Talbert and Burgos, 2007; Mennan and Kaya-Altop, 2012). Similar to many developed countries, in Türkiye, weed control in rice cultivation heavily relies on herbicides due to high labor costs and a shortage of qualified personnel. Within the *Scirpus* genus, *Schoenoplectiella mucronata* (L.) J. Jung & H. K. Choi (Syn. *Scirpus mucronatus*) and *Scirpus maritimus* L. are two significant problematic species in Türkiye. The control of these weed species predominantly involves the use of ALS inhibitor herbicides.

The *Scirpus* genus comprises numerous aquatics, grass-like species commonly known as bulrush. With approximately 35 species, it is predominantly found in

temperate regions of the Northern Hemisphere, displaying the highest diversity in North America (12 species), followed by China (11 species) and Europe (5 species) (Liang and Tucker, 2010). These plants are frequently cultivated to prevent soil erosion and offer habitats for waterfowl and other wildlife. Typically characterized by clusters of small, brown spikelets, the Scirpus genus can be identified through specific features. The rachilla, a diminutive axis of a spikelet, bears the florets. The achene, a small, dry, indehiscent one-seeded fruit, lacks a typical vascular tissue supply, usually having only a single trace. The culm, referring to the stem of any plant, plays a crucial role in these species. Additionally, the spikelet, a small or secondary spike with a varying number of reduced flowers, is subtended by one or two scale-like bracts. Bristles, resembling stiff, hair-like structures, and filaments, slender stalks supporting and holding pollen sacs (anthers), are integral components of these plants (Moore, 2014).

Ricefield Bulrush, is a keystone wetland plant species commonly found in rice and comprises a diverse group of wetland plants exhibiting remarkable adaptability to different ecological niches (Ge et al., 2023).

With the advancement of molecular techniques in recent years, studies on the genetic diversity of weed populations have opened up new avenues in weed science (Huang et al., 2023). Genetic conservation efforts should prioritize preserving distinct genetic populations of *S. mucronata* to maintain genetic diversity, which may be critical for the species' adaptability to changing environmental conditions and serves as a valuable resource for understanding their evolutionary history. The use of molecular markers in detecting genetic diversity allows for the determination of not only genetic variation but also the degree of relatedness and morphological developments in both annual and perennial weed species (Mengistu et al., 2004). The broad morphological and genetic diversity exhibited by the species not only complicates control but also accelerates the formation of herbicide-resistant biotypes (Yabuno, 2001; Michishita and Yamaguchi, 2003). Species with high genetic diversity possess the ability to adapt, reproduce, and compete more successfully under changing environmental conditions over time and space (Birader, 2023). Genetic diversity studies have taken on a fundamental mission in determining how herbicides and environmental influences affect the dynamics of species, acquiring knowledge in combating significant weed species, and elucidating the reasons behind the variations that species may exhibit in response to herbicides (Sterling et al., 2004). The primary factor leading to high herbicide resistance in different rice fields has been identified as seeds carrying resistance genes. In this context, preventing seed production and dissemination in resistant biotypes constitute a fundamental objective in weed control strategies (Merotto et al., 2009).

It has been emphasized that spontaneous hybrid species can occur in species belonging to the Cyperaceae family, and this phenomenon is associated with polyploidy and asexual reproduction. It has also been highlighted that to explain this phenomenon, it is essential to evaluate both morphological and molecular differences together (Arriola and Ellstrand, 1996).

A variety of molecular methods are employed in calculating genetic diversity within weed populations. Inter simple sequence repeats (ISSR) typing offers significant advantages due to its relative simplicity, cost-effectiveness, rapid results, and the ability to analyze a large number of samples swiftly. ISSR markers have yielded promising results in obtaining data related to taxonomic relationships and genetic differences among weed species (Tayyar et al., 2003; Al Salameen et al., 2020). However, limited genetic diversity studies have been conducted on *S. mucronata.*

Understanding the genetic and morphological diversity within the genus *Scirpus* is crucial for its conservation within rice ecosystems, shedding light on its ecological adaptations and improving management strategies (Smith et al., 2020). This article delves into the intricate interplay between genetic and morphological variability in *S. mucronata*, a significant concern in Türkiye rice cultivation areas, aiming to investigate its impact on the existing resistance phenomenon. The observed high level of genetic diversity not only serves as a triggering factor in herbicide resistance but also contributes to the enhanced adaptability of resistant populations (Dixon et al., 2020). Despite the ecological importance of *Scirpus* species, the genetic and morphological diversity within the genus remains relatively understudied, emphasizing the need for further research in this area. In addition to genetic analyses, morphological traits, such as leaf architecture, stem morphology, and reproductive structures, play a pivotal role in comprehending the adaptive strategies of *Scirpus* species. Morphometric studies have been instrumental in elucidating phenotypic variability within and between populations (Thomson et al., 2000).

This study aims to fill this knowledge gap by providing an overview of the current state of research on the genetic and morphological aspects of *Scirpus* species and to provide guidance for a comprehensive interpretation to facilitate the determination of integrated management strategies against this species in Türkiye's rice fields.

2. Materials and Methods

2.1. Plant Material

A total of 62 populations were studied to determine the morphological and genetic diversity of *Schoenoplectiella mucronata* (Fam: Cyperaceae). The seed samples of the populations were collected from rice cultivation areas in the Marmara and Black Sea regions, including the provinces of Samsun, Balıkesir, Bursa, Edirne, Kastamonu, Kırklareli, Sinop, Tekirdağ, and Çorum during the 2016 vegetation period (Table 1).

Table 1. Geographic locations of the populations used to determine morphological and genetic diversity.

Number	Origin		Population	Coordinate	
1	Edirne	Havsa	EDI-S1	41° 25. 705'N	26° 48. 914'E
2			EDI-S2	41° 29. 246'N	26° 48. 811'E
3		İpsala	EDI-S3	40° 51. 748'N	26° 20. 546'E
4			EDI-S4	40° 52. 868'N	26° 23. 020'E
5			EDI-S5	40° 55. 921'N	26° 24. 520'E
6			EDI-S6	40° 56. 004'N	26° 24. 869'E
7			EDI-S7	40° 53. 652'N	26° 21. 898'E
8		Keşan	EDI-S8	40° 53. 353'N	26° 21. 493'E
9			EDI-S10	40° 53. 390'N	26° 21. 121'E
10			EDI-S11	40° 50. 381'N	26° 17. 704'E
11			EDI-S12	40° 44. 591'N	26° 25. 653'E
12			EDI-S13	40° 46. 678'N	26° 41. 873'E
13			EDI-S14	41° 05. 458'N	26° 22. 215'E
14		Meriç	EDI-S15	41° 06. 386'N	26° 20. 595'E
15			EDI-S16	41° 06. 426'N	26° 20. 542'E
16			EDI-S17	41° 03. 192'N	26° 21. 810'E
17		Merkez	EDI-S18	41° 30. 844'N	26° 36. 642'E
18			EDI-S19	41° 29. 712'N	26° 37. 067'E
19	Samsun	Alaçam	SAM–S1	41° 37. 400'N	35° 43. 456'E
20			SAM–S2	41° 38. 824'N	35° 49. 332'E
21		Bafra	SAM–S3	41° 42. 043'N	35° 55. 014'E
22			SAM–S4	41° 43. 412'N	35° 57. 281'E
23		Çarşamba	SAM–S5	41° 16. 568'N	36° 44. 104'E
24			SAM–S6	41° 12. 494'N	36° 36. 012'E
25		Ondokuz Mayıs	SAM–S7	41° 32. 075'N	36° 03. 828'E
26		Terme	SAM–S8	41° 13. 500'N	36° 58. 096'E
27			SAM-S9	41° 11. 305'N	36° 59. 033'E
28		Yakakent	SAM–S10	41° 37. 656'N	35° 33. 829'E
29	Kırklareli	Babaeski	KIR-S1	41° 20. 940'N	27° 07. 340'E
30			KIR-S2	41° 21. 425'N	27° 04. 110'E
31		Pehlivanköy	KIR-S3	41° 22. 044'N	26° 52. 956'E
32	Kastamonu	Hanönü	KAS-S1	41° 37. 248'N	34° 28. 703'E
33			KAS-S2	40° 56. 368'N	33° 52. 502'E
34		Tosya	KAS-S3	41° 02. 654'N	34° 11. 373'E
35			KAS-S4	41° 03. 730'N	34° 12. 300'E
36	Tekirdağ	Hayrabolu	TEK-S1	41° 03. 275'N	27° 03. 625'E
37			TEK-S2	41° 03. 229'N	27° 03. 672'E
38		Malkara	TEK-S3	40° 56. 830'N	27° 01. 020'E
39	Bursa	Merkez	BUR-S1	40° 10. 356'N	28° 11. 256'E
40			BUR-S2	40° 11. 873'N	28° 11. 337'E
41			BUR-S3	40° 11. 758'N	28° 11. 300'E
42	Sinop	Saraydüzü	SIN-S1	41° 23. 532'N	34° 56. 981'E
43		Boyabat	SIN-S2	41° 37. 290'N	34° 36. 730'E
44			SIN-S3	41° 32. 955'N	34° 42. 959'E
45		Durağan	SIN-S4	41° 26. 722'N	34° 54. 735'E
46			SIN-S5	41° 25. 954'N	34° 56. 650'E
47	Balıkesir	Gönen	BAL-S1	40° 07. 161'N	27° 43. 387'E
48			BAL-S2	40° 07. 056'N	27° 42. 101'E
49		Manyas	BAL-S3	40° 04. 680'N	28° 02. 410'E
50			BAL-S4	40° 04. 987'N	28° 02. 578'E
51			BAL-S5	40° 04. 993'N	28° 02. 581'E
52			BAL-S6	40° 06. 140 'N	28° 08. 241'E
53	Çorum	Kargı	ÇOR-S1	41° 06. 098 'N	34° 24. 910'E
54			ÇOR-S2	41° 04. 986'N	34° 26. 134'E
55			ÇOR-S3	41° 07. 123'N	34° 25. 272'E
56		Osmancık	ÇOR-S4	40° 58. 821'N	34° 55. 776'E
57			ÇOR-S5	40° 57. 726'N	34° 50. 011'E
58			ÇOR-S6	40° 56. 319'N	34° 51. 357'E
59		Bayat	ÇOR-S7	40° 31. 376'N	34° 20. 545'E
60		Dodurga	ÇOR-S8	40° 49. 609'N	34° 51. 519'E
61		İskilip	ÇOR-S9	40° 36. 055'N	34° 28. 523'E
62		Laçin	ÇOR-S10	40° 49. 602'N	34° 51. 529'E

2.2. Morphological Studies

Seeds of *S. mucronata* from each population were sown in plastic pots (diameter 20 cm; height 25 cm) and cultivated in a screen house. The pots were filled with rice soil, and the experiments were set up with five replicates according to a randomized block trial design. Fertilizer used in the regions and sufficient water were provided to the pots. In the cultivation process, the biological stages of the plants from seed to seed were monitored through daily observations, and morphological parameter data were recorded. The examined parameters included plant height (cm), first cotyledon time (day), second cotyledon time (day), leaf number (per plant), rays number (per spike), spike length (cm), spikelet lenght (cm), rays lenght (cm per spike), inflorescence number (per bract), total

inflorescence number (per plant), fresh weight (g) and dry biomass (g) (Tayyar et al., 2003; Więcław et al., 2021). After harvesting, the plants were dried at 70°C for 3 days for dry biomass measurement.

2.3. Genetic Studies

For DNA extraction, seeds of the 62 populations of *S. mucronata* were germinated in Petri dishes and then transferred to pots, where they were grown until the 4-6 leaf stage under controlled conditions at 30°C with a 12/12-hour lighting period in a greenhouse. Genomic DNAs from leaf samples were extracted using the DNeasy DNA extraction kit (Qiagen, Qiagen GmbH, Hilden, Germany) according to the kit protocol. The isolated DNA concentration and relative purity were checked using a Nanodrop ND-1000 (Thermo Scientific) and adjusted to 25 µL-1 (Danquah et al., 2002; Ruiz-Santaella et al., 2006; Kaya Altop and Mennan, 2011).

In the ISSR-PCR Analysis, 11 primers were tested, and 8 of them were successfully used for *S. mucronata* samples (Table 2). The primers were synthesized by Genox company (Ankara, Türkiye). The PCR reaction was prepared with a total volume of 25 µL, including 50 ng genomic DNA, 0.2 mM oligonucleotide primer, 1.5 mM $MgCl_2$, 0.4 mM dNTP, 0.2 units Taq DNA Polymerase, 1X PCR buffer, and 11 µL sdH_2O, and placed in a PCR device (Rotor-Gene Q 5plex HRM, Qiagen). The reaction conditions were set as follows: initial denaturation at 95°C for 1 min, followed by 45 cycles of denaturation at 95°C for 1 min, annealing at 72°C for 2 min, and extension at 72°C for 5 min.

After PCR, the resulting DNA fragments were subjected to agarose gel electrophoresis (Biorad), using a 2% agarose gel. A 1 Kb DNA marker (New England Biolabs) was used as a reference, and photographs of the DNA bands on the gel were taken using a gel imaging device. The evaluation of bands relied on whether the bands were visible or not on the gel after electrophoresis. In this study, optimal PCR conditions were established by repeating amplifications several times, and conditions that provided stable band profiles for each primer were selected. Monomorphic and polymorphic bands in the gels were identified to obtain statistical analysis.

2.4. Statistical Analyses

Different methods were used for the statistical analysis of morphological and molecular findings. The data obtained from morphological studies were subjected to hierarchical cluster analysis using IBM SPSS Statistics 20 (for Windows) statistical package program. Duncan's multiple comparison test was applied to the parameter values, and a dendrogram created using hierarchical clustering method. Euclidean distances were calculated across landraces and a distance matrix was produced. Moreover, a principal component analysis (PCA) plot was constructed from the combined morphological parameters (Kaya Altop and Mennan, 2011; Moore, 2014).

For molecular studies, band sizes were entered as present (1) or absent (0) and values were calculated using observed and expected heterozygosities, using the NTSYS package program. Band matrices were thus created for use in subsequent stages. In the final step, dendrograms of the varieties were drawn using the SAHN (Sequential, Agglomerative, Hierarchical, and Nested Clustering) clustering subprogram and the UPGMA algorithm based on similarity matrices according to Jaccard (Jaccard, 1908; Nei, 1972; Nei and Li, 1979; Mujeeb et al., 2017). The genetic similarity matrix is based on Euclidean distances. The Principal Component Analysis (PCA) is relatively objective and provides a reasonable indication of relationships, it was used to confirm the similarity of the grouping obtained with the UPGMA dendrogram (Więcław et al., 2021).

Table 2. Information about the primers used in ISSR application

Locus	Repeat motif	Primer sequence (5′–3′)	Ta (°C)
SM2	$(GA)_{14}$	GTCTCACGAGAGAGAGAGAGA	54.5
		GCTTGTTCGGAGTAGGTGTG	
SM4	$(GA)_{15}$	TACTGCAGAGAGAGAGAGAG	53
		GCGAAAGTAGAGGAGATAA	
SM5	$(CT)_{15}$	GGGGCGCCTCTCTCTCTCTC	54.5
		AGGCTCCAACAATCCAGTAA	
SM6	$(AG)_{15}$	CGGCTTGCCTTTGGTTTCAT	57
		GGGGGGGCTCTCTCTCTCTC	
SM7	$(CT)_{15}$	TTGACAGCTCTCTCTCTCT	50
		GAATCTTTGAGCGTTTAGT	
SM11	$(CT)_{10}$	TAATGGATGGAGCAGAGACAG	54.5
		CGCAGTGGAGTCGGAGA	
SM12	$(AC)_6(AT)_4\ (GC)_3$	ATTTTTCTTTCTCCACACACTCT	54.5
		CGCTCGCTCGTCGCTAAA	

Ta= annealing temperature, GenBank Accession numbers= EU121661- EU121669 (Zhou et al., 2009).

3. Results

3.1. Morphological Studies

It has been observed that plants from 62 *S. mucronata* populations collected from different geographic locations exhibit an average height of 80.48 cm, with the tallest plant belonging to the Edirne-Keşan (EDI-S13) population (126.03 cm) and the shortest plant belonging to the Samsun-Terme (SAM-S8) population (64.45 cm). The population Edirne-Ipsala (EDI-S5) takes the longest time, up to 14 days, to develop its first cotyledon leaf, while the population Samsun-Alaçam (SAM-S1) has the earliest development times for both the first and second cotyledon leaves, with 7.10 and 6.98 days, respectively.

Even within the same province, the number of leaves varies significantly, with the EDI-S16 population having the least number of leaves per plant (17.86) and the EDI-S4 population having the highest number of leaves per plant (44.06). The second cotyledon leaf in the EDI-S4 population emerges approximately 19 days later than in other populations, making it the latest to develop. The BAL-S1 biotype from Balıkesir-Gönen location achieved the highest values in terms of rays number (2.61 per spike), fresh weight (31.99 g), and dry weight (8.69 g) parameters, while the EDI-S17 biotype from Edirne-Meriç location reached the highest values in spikelet and rays height (32.31 mm and 16.45 mm) parameters. Regarding the parameters of inflorescence number, total inflorescence number, and spike height, the highest values were obtained from the EDI-S19 (21.08 per bract) population in Edirne and the SAM-S5 (175.92 per bract) and SAM-S10 (84.51 mm) populations in Samsun, which are the two most important provinces for rice farming in Türkiye. EDI-S12 from Edirne-Keşan showed the lowest values in terms of total inflorescence number and dry weight parameters, while SAM-S3 from Samsun-Bafra locations had the lowest values for spikelet height, rays height, and inflorescence number (data not shown).

The Table 3 illustrates the PC components obtained from the principal component analysis (PCA) results based on the morphological characteristics of *S. mucronata* populations. Three PC components, representing a total variation of 80.74%, were obtained among the total 12 features examined. The first PC component, which accounts for 54.44% of the total variation among the morphological characteristics, is primarily influenced by the feature dry weight (0.95 g), contributing the most to its explanation, while plant height (0.19 cm) has the least impact. The second PC component, representing 14.80% of the variation, is positively related to total inflorescence number (0.69 per bract). Both PC2 and PC3, which account for 11.50% of the total variation, are significantly influenced by plant height and spike length. It was observed that total inflorescence number, plant height and spike length have limited associations with other parameters (see Figure 1).

When the entire set of morphological parameter data from the populations was subjected to hierarchical clustering analysis, the dendrogram in Figure 2 was generated based on similarity levels. According to this analysis, two main groups have formed at a taxonomic distance of 25%. The first group consists of two subgroups, with the KAS-S2 population standing out as distinctive within this division. The second main group is represented solely by the SAM-S8 population. High similarity regions among the other populations were not significantly affected by geographical differences. No geographical isolations were observed.

3.2. Genetic Studies

In the genetic analysis, 17 primers were tested, of which 8 consistently produced amplified ISSR fragments. These ISSR primers generated a total of 32 alleles, with an average allele count of 4. The highest number of alleles was observed in loci SM2 and SM7. The ISSR primers produced band profiles with lengths ranging from 76 bp (SM7) to 258 bp (SM1). When examining the findings obtained from the 8 primers used, it was observed that HO (observed heterozygosity) values ranged from 0.192 to 0.970, with the highest value obtained from the SM12-SM1 primers. On the other hand, HE (expected heterozygosity) values ranged from 0.136 to 0.566, primarily derived from the SM12-SM7 primers.

Table 3. Correlation matrix of morphological parameters

Parameter	PH	FCT	SCT	LN	RN	SH	STH	RH	IN	TIN	FW	DW
PH	1.00											
FCT	0.09	1.00										
SCT	0.07	0.92**	1.00									
LN	0.12*	0.41**	0.45**	1.00								
RN	0.01	0.84**	0.84**	0.41**	1.00							
SH	0.50**	0.12*	0.13*	0.17**	0.07	1.00						
STH	0.23**	0.67**	0.69**	0.34**	0.67**	0.48**	1.00					
RH	0.16**	0.79**	0.80**	0.44**	0.89**	0.35**	0.81**	1.00				
IN	0.10	0.67**	0.68**	0.37**	0.74**	0.23**	0.60**	0.74**	1.00			
TIN	0.18**	0.10	0.13*	0.50**	0.02	0.20**	0.04	0.14*	0.34**	1.00		
FW	0.19**	0.44**	0.48**	0.69**	0.51**	0.31**	0.42**	0.58**	0.51**	0.57**	1.00	
DW	0.08	0.78**	0.79**	0.59**	0.94**	0.18**	0.66**	0.90**	0.75**	0.25**	0.73**	1.00

PH= plant height (cm), FACT= first cotyledon time (day), SCT= second cotyledon time (day), LN= leaf number (per plant), RN= rays number (per spike), SH= spike length (mm), STH= spikelet height (mm), RH= rays height (mm/per spike), IN= inflorescence number (per bract), TIN= total inflorescence number (per plant), FW= fresh weight(g), DW= dry weight (g)., *P≤0.05, **P≤0.01.

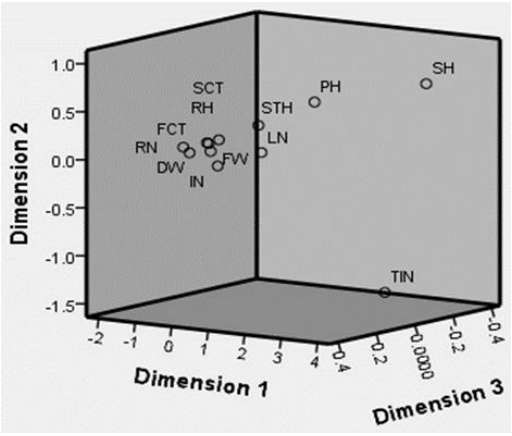

Figure 1. Morphological parameter relationship graph created according to the Euclidean distance model.

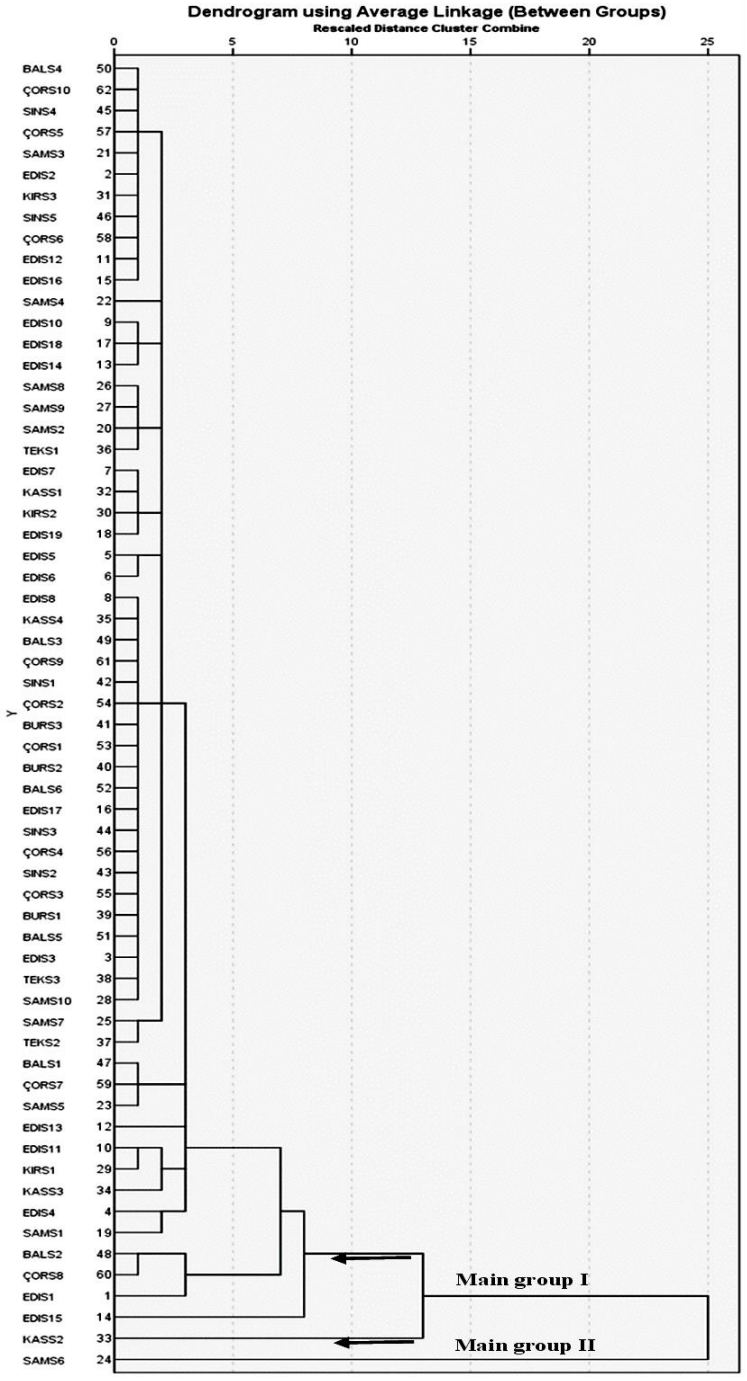

Figure 2. Phylogenetic dendrogram based on morphological characteristics.

The average HO value was determined as 0.501, and the average HE values were found to be 0.432 (Table 3).

The molecular data obtained from the ISSR analysis for *S. mucronata* populations were subjected to Principal Component Analysis (PCA) (Table 4 and 5). The PCA results indicated 9 PC axes explaining 87.41% of the variations and their corresponding factor groups. It was observed that the principal coordinates (PCA1-PCA9) were determined, and their ratios were closely related to each other. The first principal coordinate accounted for 39.85% of the variation, with significant contributions from the BUR-S3 and EDI-S9 populations. The second coordinate, explaining 24.78% of the variation, showed both positive and negative effects, with the BAL-S6 population contributing the most. Other coordinates (PCA3-PCA9) were influenced by various populations (data not shown).

In the hierarchical cluster analysis, where the presence or absence of band indices generated by polymorphic primers was considered, a dendrogram was created using the Average Linkage method. It was observed that populations analyzed at a genetic distance of 0.25 could be divided into two main groups (Figure 3). The populations were grouped into two main clusters based on the populations studied.

Table 4. Factor groups and PCoA axes of morphological parameters created by PCA (principal component analysis)

	PCA axes		
	1	2	3
Eigenvalues	6.53	1.78	1.38
Variance (%)	54.44	14.80	11.50
Cumulative (%)	54.44	69.24	80.74
		Factor groups	
Parametreler	PCA 1	PCA 2	PCA 3
PH	0.19	0.58	0.55
FCT	0.86	-0.29	0.04
SCT	0.88	-0.26	0.01
LN	0.61	0.39	-0.43
RN	0.90	-0.35	-0.02
SH	0.33	0.57	0.60
STH	0.79	-0.05	0.40
RH	0.93	-0.12	0.16
IN	0.82	-0.04	-0.03
TIN	0.31	0.69	-0.47
FW	0.72	0.43	-0.32
DW	0.95	-0.09	-0.14

PH= plant height (cm), FACT= first cotyledon time (day), SCT= second cotyledon time (day), LN= leaf number (per plant), RN= rays number (per spike), SH= spike length (mm), STH= spikelet height (mm), RH= rays height (mm/per spike), IN= inflorescence number (per bract), TIN= total inflorescence number (per plant), FW= fresh weight(g), DW= dry weight (g).

Table 5. Amplification results of Inter Simple Sequence Repeats (ISSR) analysis

Locus	Amplification			
	Size range (bp)	NA	*HO*	*HE*
SM1	254–258	4	0.970	0.566
SM2	113–151	5	0.642	0.493
SM4	242–311	3	0.723	0.495
SM5	135–150	4	0.483	0.593
SM6	149–183	4	0.346	0.269
SM7	76–97	5	0.323	0.594
SM11	232–322	4	0.330	0.310
SM12	112–143	3	0.192	0.136
Mean		4	0.501	0.432

NA= number of alleles, HO= observed heterozygosity, HE= expected heterozygosity.

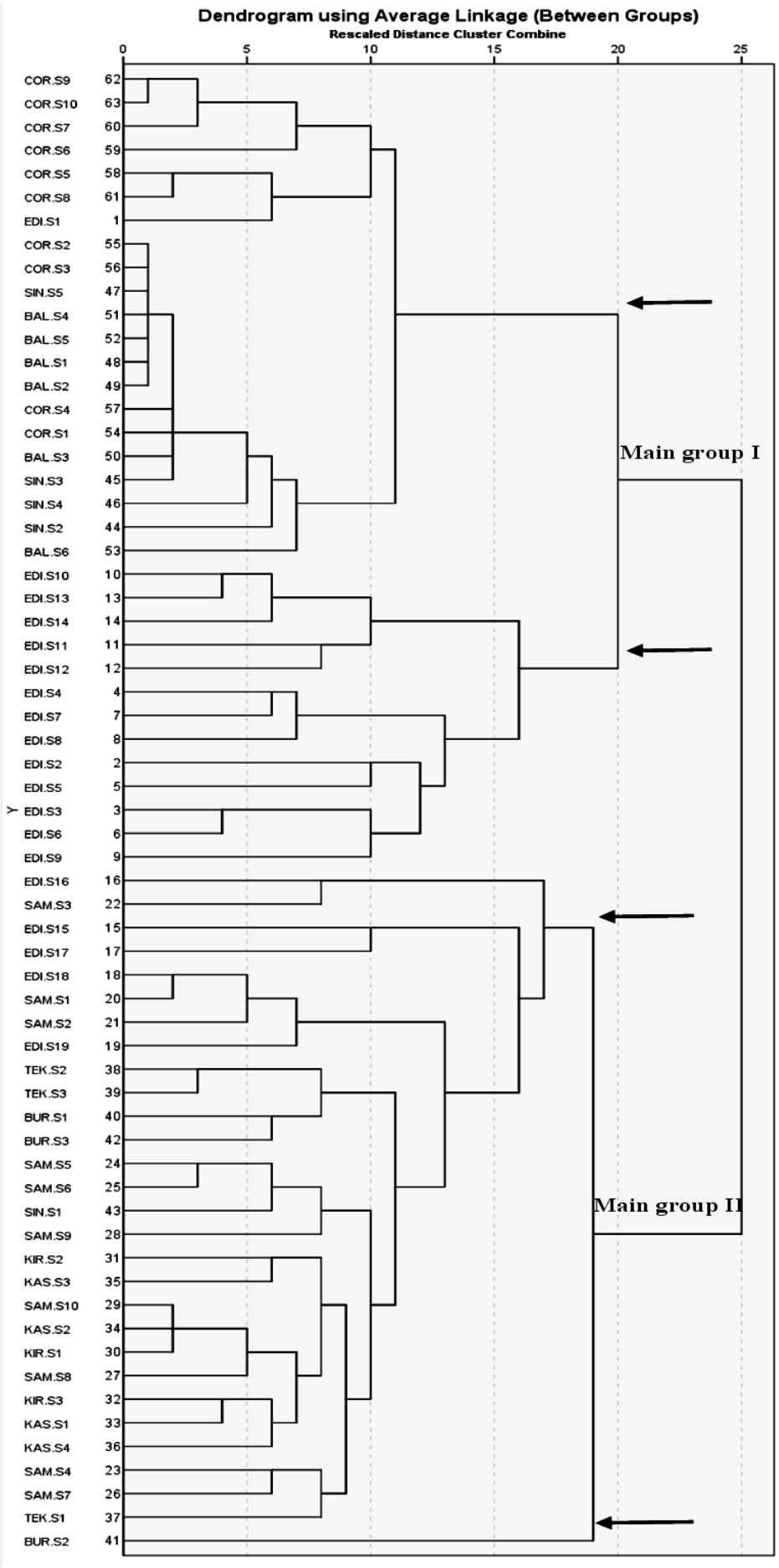

Figure 3. Phylogenetic dendrogram based on genetic characteristics.

According to the UPGMA analysis, the phylogenetic tree revealed a high level of genetic similarity among populations, particularly between COR-S9 (Çorum/İskilip), COR-S10 (Çorum/Laçin), COR-S2 (Çorum/Kargı), COR-S3 (Çorum/Kargı), SIN-S5 (Sinop/Duragan), BAL-S1 (Balıkesir-Gönen), BAL-S2 (Balıkesir-Gönen), BAL-S4 (Balıkesir-Manyas), and BAL-S5 (Balıkesir-Manyas) populations. The most distant genetic similarity was observed between BUR-S2 (Bursa/Merkez) and other population groups. The Jaccard genetic similarity coefficient ranged from 0.138 to 1.000, with an average genetic similarity of 54%. The results from the similarity index matrix were consistent with the dendrogram, highlighting the genetic distances among populations.

Notably, the population BAL-S6 exhibited low similarity, or in other words, genetic distance, with SAM-S3 (0.138) and EDI-S16 (0.171) populations. Furthermore, populations from Balıkesir province (BAL-S1-S2-S4-S5), Çorum province (ÇOR-S2-3), and Sinop province (SIN-S5) showed 100% genetic similarity. The same level of similarity was also observed between the populations' COR-S9 and COR-S10 (data not shown).

4. Discussion

The genetic and morphological diversity of *Scirpus mucronata* has been notably limited in the existing literature. In a study focusing on the morphological characteristics of *S. mucronata* populations, the obtained PC axes and corresponding factor groups were statistically evaluated. The findings, when assessing the PCA analysis of *Scirpus* species' morphological characterization based on morphological features, are supported by De Greef and Triest (1999)'s study. In their work, it was observed that the 2 PC axes explained 44% and 13% of the total variation, respectively. This highlights the relevance and applicability of PCA analysis in defining the morphological traits of *Scirpus* species.

In the molecular data obtained for *S. mucronata* populations, a total of 5 principal coordinates have been identified, with the proportions of genetic variation explained ranging between 2.18% and 42.7%. These results bear similarity to the component and variation findings reported by Danquah et al. (2002). The observed parallels suggest consistency in the genetic structure and variability patterns within *S. mucronata* populations, as indicated by both studies.

When compared to morphological studies, molecular investigations have yielded more detailed data. In the ISSR studies, eight different primers were employed. The utilization of numerous primers is known to be effective in genetic mapping (Vellend and Geber, 2005).

However, Millan et al. (1996) reported the use of 10 primers in roses, Abad et al. (1998) utilized 27 primers for *Cyperus esculentus* L., while *Ixora* varieties were studied with six primers. Schontz and Rether (1999) applied four primers in Italian ryegrass, Tasrif et al. (2004) and Juraimi et al. (2005) used four primers in *E. crus-galli* var. *crus-galli*, Tayyar et al. (2003) employed 16 primers for 35 populations, Merotto et al., (2010) utilized 75 SRAP markers for *Cyperus* spp., and Danquah et al., (2002) suggested that six AFLP primers and five microsatellite primers might be sufficient for detecting polymorphism in *E. crus-galli*.

Ren et al. (2005) suggested that if the variation among varieties is high, a limited number of primers may be sufficient. In the ISSR study conducted to assess the genetic diversity of the alien weed species *Xanthium italicum*, eight different primers were utilized across 10 distinct populations. The results indicated that the genetic variation among populations is likely primarily attributed to genetic drift and anthropogenic activities (Tang and Ma, 2020). In Türkiye, molecular studies on genotypes of *Polygonum cognatum* Meissn. have been carried out using 14 SSR and 23 RAPD primers (Önen et al., 2010). Additionally, successful applications of techniques were reported in wild poppy species belonging to the *Oxytona* section, using 12 SSR and 22 RAPD primers (Parmaksız et al., 2009).

All eight primers used in the study resulted in the formation of polymorphic bands. According to the available information, there is no existing study on the determination of genetic diversity in *S. mucronata* using ISSR markers.

While the genetic similarity rate varied between 1% and 98%, these findings are consistent with the results obtained by Samuelsson et al. (1997) through the examination of variation among *Vicia pisiformis* populations using morphological analyses and the RAPD marker system. Similarities were also observed with the results of Tayyar et al. (2003), who worked on *Cyperus* species. Furthermore, Budak et al. (2004) expressed confidence in the usability of a 57% genetic average obtained from their research using the RAPD method for result evaluation. The primers used in the study exhibited a high level of polymorphism, providing positive outcomes for the accurate assessment of the results.

In the molecular dendrogram, geographical isolations are more clearly visible compared to the morphological dendrogram. Although populations are grouped together morphologically, the wide geographic distribution can be attributed to the soil cultivation and transportation through harvest machinery in the rice fields where the samples were collected. Ecotypes showing high phenotypic similarity may not necessarily exhibit genetic similarity (Vellend and Geber, 2005) due to the potential existence of different gene pools. The assessment of genetic and morphological similarities can be accurately interpreted by examining phylogenetic data to evaluate the changing rates of gene flow over time and genes associated with variation that enables expression. Similar views have been expressed by Bromham et al. (2002). Genetic diversity is inversely proportional to the rate of gene flow, meaning that the higher the genetic diversity among populations, the lower the gene flow, or vice versa

(Merotto et al. 2010). While some studies have linked gene flow between populations to factors such as distance, cultivation systems, pollination characteristics, plant species, environmental conditions, and vectors (Levin and Kerster 1974), there are also studies suggesting that genetic relationships between populations are not necessarily associated with geographic distance (Merotto et al., 2009). In the study, the interpretation of UPGMA dendrogram information revealed that genetic relationships are not always associated with geographic distance, but clear geographic isolation exists among populations. When the data from these situations are evaluated in the triangle of genetic relationship, geographic location, and herbicide activity, it is found that these three concepts cannot be directly correlated. However, this indicates that resistant populations are rapidly spreading. In various previous studies, it has been suggested that herbicide resistance can spread through gene flow between populations with high genetic similarity (Rutledge et al., 2000; Stankiewicz et al., 2001; Tsuji et al., 2003; Merotto et al., 2010). Considering the high rate of self-pollination in *S. mucronata*, it is emphasized that gene flow may occur more through seed dispersal than pollen dispersal (Baker et al., 2007). As mentioned by Roy et al. (2000), uncertified seeds being transported and used from one region to another, coupled with the rapid adaptation of this invasive weed species to the region where it is planted, can lead to the formation of morphologically similar groups despite genetic differences. Cross-pollination, strong clonal growth, sexual reproduction, and human-mediated spread factors are effective in the formation of variation, a viewpoint supported by researchers (Tayyar et al., 2003; Ren et al., 2005).

In the conducted morphological and molecular analyses, differences were identified among the examined populations in terms of certain quantitative characters. While populations of *S. mucronata* from different regions exhibited significant morphological similarities, higher genetic diversity was observed. This indicates a high potential for gene flow. In conclusion, the joint evaluation of morphological and molecular studies revealed that variation among populations is primarily characterized by low morphological but high genetic diversity. This is attributed to adaptation to geographical areas, the transportation of seeds between regions by humans and tools, and the development of resistance by the weed, especially against herbicides commonly used in weed control methods. In this context, it is emphasized that the authorization, marketing, and supervision services in the seed sector should be conducted in accordance with standards to prevent the spread of *S. mucronata* seeds over both short and long distances. Continuous monitoring of adaptation processes and the integration of preventive measures into weed control strategies are crucial.

5. Conclusion

The results of this study emphasize the importance of considering both morphological and genetic characteristics when assessing the diversity and relationships among *S. mucronata* populations. The variations observed in plant height, leaf development, leaf numbers, and inflorescence-related parameters reflect the adaptability and response of these populations to their specific environments.

The genetic analysis, based on ISSR markers, provided valuable insights into the genetic diversity and relationships among the populations. The high level of genetic diversity observed in *S. mucronata* populations suggests their potential for adaptation and evolution in response to changing environmental conditions.

The findings from this study can serve as a basis for further research into the conservation and management of *S. mucronata* populations, especially in regions like Edirne and Samsun, where rice farming is of great importance. Understanding the genetic diversity and relationships among populations is crucial for the development of effective conservation strategies.

In conclusion, the combined evaluation of morphological and molecular studies indicates that there is low morphological but high genetic variation among populations. This is primarily attributed to adaptation to geographical areas, transportation of seeds between regions by humans and tools, and the development of resistance by the weed to herbicides commonly used in weed control strategies. In this context, it is crucial to implement seed sector authorization, marketing, and monitoring services in accordance with standards to prevent the spread of *S. mucronata* seeds both short and long distances. Continuous monitoring of adaptation processes and the integration of preventive measures with weed control strategies are essential considerations.

Author Contributions

The percentage of the author contributions is presented below. The author reviewed and approved the final version of the manuscript.

	E.K.A.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

Acknowledgments

This research was supported by Ondokuz Mayıs University with the project number PYO.ZRT.1902.15.003.

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