The Journal of Animal & Plant Sciences, 30(4): 2020, Page: 931-943
ISSN (print): 1018-7081; ISSN (online): 2309-8694

GENETIC DIVERSITY AND MOLECULAR TAXONOMY STUDY OF GENUS *FESTUCA*

A. Alsaleh[1*], M. C. Doğrusoz[2], U. Basaran[2], A. Tamkoc[3] and M. A. Avci[3]

[1]Science and Technology Application and Research Center (BİLTEM), Yozgat Bozok University, Yozgat, Turkey.
[2]Department of Field Crops, Faculty of Agricultural, Yozgat Bozok University, Yozgat, Turkey.
[3]Department of Field Crops, Faculty of Agricultural, Selcuk University, Konya, Turkey.
Corresponding author E-mail: ahmad.alsaleh@bozok.edu.tr

ABSTRACT

The genus *Festuca* (Poaceae) occupies a wide range of lands in both hemispheres, with astounding significance endowed by the genetic diversity, intra/inter-species discriminations, and structure analysis of *Fescue* species based on a reliable molecular marker. While still in the infancy stage, information on genetic structure and relationships of *Festuca* species from Turkish gene pool have rarely, or never, been subjected to the most needed studies. Six species of genus *Festuca* were put in the limelight. A total of 598 loci were generated through molecular characterization of 68 accessions by using 19 inter-simple sequence repeats primers. Molecular variance revealed a variation within species and among species of 75% and 25, respectively. The *F. ovina* showed high values in relation to the number of different alleles, Shannon's information index, and percentage of polymorphic loci. The highest genetic variability, expected and unbiased expected heterozygosity values were detected for *F. arundinacea*. Nei genetic distance showed that the lowest value was found between *F. ovina* and *F. valesiaca* species, while the highest was identified between *F. heterophylla* and *F. pratensis* species. An obvious convergence has been detected through Principal Coordinate Analysis, neighbor-joining dendrogram and Structure output, with accessions divided into number-of- species-based groups. The study resulted in implications for genetic revision, which, in turn, may clear the misty vision that geneticists might have regarding fescue; and could be exploited in future genetic resources conservation and breeding programs.

Key words: *Festuca*, genetic diversity, genetic structure, ISSR, Molecular marker, phylogenetic.

https://doi.org/10.36899/JAPS.2020.4.0109 *Published online April 25, 2020*

INTRODUCTION

Festuca (*Poaceae*), one of the most complex genera of *Poaceae*, presents a cosmopolitan distribution worldwide with over 600 species of multiple ploidy levels ranging from diploid ($2n = 2x = 14$) up to dodecaploid ($2n = 12x = 84$) (Cheng *et al.,* 2016). This widespread genus is well adapted to diverse climatic conditions and soil types across the temperate zones and utilized as pasture turf grass and in erosion control (Gibson and Newman, 2001). In Turkey, *Festuca* is represented by 52 species (Guner, 2012). Tall fescue (*F. arundinacea* Schreb), is the most important C3 species of bunchgrass, commonly described with three major morphotypes (Continental, Mediterranean and rhizomatous) showing a significant variation in terms of agronomic, morphological and physiological traits (Hand *et al.,* 2010). Tall fescue varieties have been developed mainly for forage production, while turf-type varieties that are designated for recreational uses have been developed from several different species (*F. ovina L., F. arundinacea*, *F. rubra and F. pratensis*) (Demiroğlu *et al.,* 2010; Hand *et al.,* 2010; Ogle *et al.,* 2010; Özpinar *et al.,* 2014).

Festuca arundinacea shows high genetic diversity and tolerance to a broad range of habitats and environmental conditions (Cuyeu *et al.,* 2013). It has numerous cultivars, which makes it a highly important pasture grass. In addition, *F. arundinacea* is important in the Mediterranean region, and locally dominant species of arid zonal meadows (Gibson and Newman, 2001). Five such species (*F. filiformis* Pourret, *F. idahoensis* Elmer, *F. ovina* L. ssp. *hirtula* (Hackel ex Travis) M. Wilkinson, *F. trachyphylla* (Hackel) Krajina, and *F. valesiaca* Schleich. ex Gaudin) form the *Festuca ovina* aggregate that is often called the "*ovina* complex". Species within the *F. ovina* complex are cross compatible and, thus, hybrids between these and other *Festuca* species can occur frequently in nature (Ma, 2012). The species *F. ovina* may harbor novel variations for crop improvement, while the red fescue (*F. rubra*), characterized by a strong tillering capacity, and seems to be more suitable as a pasture plant (Özpinar *et al.,* 2014).

Taxonomically, fescue is one of the most complex genera in the *Poaceae* family with substantial morphological, phylogenic and anatomic variation. Genetic variability of individuals varies from one population to another (Balkan, 2018). Although the genetic variability has been earlier reported in tall fescue *(F. arundinacea* Schreb.) by Nelson *et al.* (1975) with the taxonomic classification of the genus described by many researchers, this matter has not been solved clearly yet

(Hand *et al.,* 2012). This necessitates a further investigation of the taxonomic relationships among the species of this genus using molecular markers. Characterization of germplasm belonging to different species of this genus must be in place as a first step, with the genetic structure and diversity at the inter/intra-genus level understood at the DNA level. DNA molecular analysis significantly contributes to characterization at the genetic and/or molecular level (Alsaleh *et al.,* 2016). Among the several molecular marker techniques developed over the past two decades, the technique of inter simple sequence repeats (ISSR) markers remains attractive for genetic research so far. It is quick, low cost and easy to handle dominant genetic marker system. This marker system was developed by Zietkiewicz *et al*. (1994) and used for studying the genetic diversity, phylogeny, gene tagging, genome mapping and evolutionary biology studies (Reddy *et al*., 2002). ISSR is a semi-arbitrary marker; amplified by polymerase chain reaction (PCR). It does not require genome sequence information and leads to multi-locus and highly polymorphous patterns (Nadeem *et al*., 2018). ISSR markers have been used for genetic diversity assessment and phylogenetic relationships in many different species such as fine-leaved fescue (Armoniene *et al.,* 2010; Kumar *et al*., 2014; Lamare and Rao, 2015; Stukonis *et al.,* 2015), *P. pratensis* (Szenejko *et al*., 2016) and in many different crops such as chickpea (Andeden *et al.,* 2013), lentil (Baloch *et al.,* 2015), soybean and peanut (Baloch *et al.,* 2010) alfalfa (Habibi *et al*., 2012), wheat (Abou-Deif *et al*., 2013), barley (Rahimi *et al*., 2014) and rice (Al-Turki *et al.,* 2015).

Information available on genetic resources and the related geographical locations can be used to gain insight into population divergence. Phenotypic divergence among fescue populations has been described earlier, with particular attention paid to some of the tall fescue populations; while genetic diversity has been the subject of multiple studies in different parts of the world (Armoniene *et al*., 2010; Ma, 2012; Stukonis *et al*., 2015; Cheng *et al*., 2016; Zhang *et al*., 2017; Akbari *et al*., 2018; Rahmati *et al*., 2018; Talukder *et al*., 2018). Although Turkey is considered as hotspot zone of origin and domestication of many species under the genus of *Poaceae* (Baloch *et al*., 2017), no information describing the genetic structure of the *Festuca* species from the Turkish gene pool has been found in literature so far. Therefore, the objective of this study was to bridge in the gap, as well as to evaluate the genetic diversity, determining species structure and understanding the phylogenetic and genetic relationships among six *Festuca* species.

MATERIALS AND METHODS

Plant material: A panel of 68 fescue (*Festuca* L.) accessions belonging to 6 different species including 37 accessions of *F. ovina*, 13 *F. arundinacea*, 12 *F. valesiaca*, two each of *F. heterophylla F. rubra*, and *F. pratensis*, collected from a diverse range of 17 different provinces and various ecological conditions in Turkey have been used as a plant material in this study. Full details of these accessions are presented in Table 1.

DNA extraction: Total genomic DNA was isolated from young leaves according to QIAGEN kit (DNeasy Plant Mini kit, Valencia, California). The extracted DNA was qualitatively and quantitatively evaluated, and measured by 0.8 % Agarose gel electrophoresis. Before using DNA for PCR analysis, the concentrated DNA was diluted to a 10 ng/µl.

ISSR-PCR: To detect their polymorphism level, a total of 50 ISSR primers were first screened on four DNA of randomly selected accessions of different species. The primers that showed poor or no patterns were discarded and those showing clear bands with higher allelic frequencies were selected. 19 polymorphic ISSR primers were selected and screened for the whole set of accessions. Information about the ISSR primers used in this study is briefly described in Table 2.

PCR amplifications were carried out according to the protocol of Zietkiewicz *et al*. (1994). PCR amplification of ISSRs was performed in a 10µl PCR mix of approximately 50 ng genomic DNA, 0.4 mM dNTPs, 0.8 pmol ISSR primers, 0.2 U of *Taq* DNA polymerase and 1X PCR buffer (10 mM Tris HCl pH 8.3, 50 mM KCl, 3 mM $MgCl_2$). The Eppendorf Mastercycler (Germany) was programmed for an initial denaturation step of 94°C for 2 min, followed by 40 cycles at 94°C for 1 min, at the specific annealing temperature of each primer (as described in Table 2), 72°C for 2 min and a final extension at 72°C for 10 min, ahold temperature of 4°C at the end was applied.

According to their molecular weight, the ISSR-PCR products were separated by Fisher scientific electrophoresis chamber (UK) on 2.5% Agarose gels submerged in 1X TBE buffer and accompanied with a standard size marker 100 bp DNA Ladder (Jena Bioscience GmbH, Germany) for molecular size estimations. They were finally stained with ethidium bromide solution (0.5 µg/ml final concentration) for 20 min. PCR fragments were visualized on UV trans-illuminator using the Gel Documentation System (VILBER, Quantum, France).

Data analysis: The amplified 598 ISSR loci were scored and checked twice using Cross Checker 2.91 as described by Buntjer (1999). ISSR markers were scored as binary data (1/0), indicating the presence (1) or absence (0) of a

marker in the genomic representation of each sample; data are constructed in a binary matrix. For each primer, polymorphism information content value (PIC) has been calculated by MS Excel using PIC = $1 - [f^2 + (1 - f)^2]$ formula, where (f) is the marker frequency in the data set (Chesnokov and Artemyeva, 2015). Principal Coordinate Analysis (PCoA), Analysis of Molecular Variance (AMOVA) and other diversity analysis were performed using computer software program GenAlEx 6.1 (Peakall and Smouse, 2001). A Cluster analysis of all accessions has been made using the DARWin 6.0.13 software (Perrier *et al.,* 2003) as per the neighbor-joining method is attributed to Sokal and Michener (1958).

The population structure was performed with the STRUCTURE v.2.3.4 software (Pritchard *et al.,* 2000) with a model-based clustering method applied. The structure analysis was carried out with K = 10, 20000 burning length and 50,000 reps over 10 iterations, then the results were run in the Structure Harvester version 0.6.94 software (Earl and Von Holdt, 2012) to estimate the number of subpopulations (K). Delta K (ΔK) is based on the rate of change in the log probability of data between successive K values. This was used to determine the number of clusters (K) in the population (Evanno *et al*., 2005).

RESULTS AND DISCUSSION

Molecular variance and population diversity analysis: For genetic diversity and genetic evaluation, the dominant data would be robust for studying population structuring and genetic differentiation among populations, where the molecular markers such as ISSR are more effective because they present a lot of details on genetic difference at the DNA level. Therefore, extensive information about the genetic diversity of genus *Festuca* collected from many eco-geographical regions is expected to have a remarkable impact on the preservation and usage of genus *Festuca*. A total of 68 accessions belonging to 6 species of *Festuca* were screened with nineteen polymorphic ISSR primers, with a total of 598 polymorphic bands produced. The fragment size varied from 400 to 4000 bp. The highest amplified fragment number (46) was produced by IS6 primer, while the lowest bands (16) were displayed by IS16, with an average of 31.5 fragments per primer. The informativeness of the ISSR markers was estimated by the PIC values ranged from 0.07 (UBC844) to 0.21 (IS6) with an average of 0.15 (Table 2), which is lower than the average PIC value of 0.33 reported by Rahmati *et al.,* (2018) using ISSR markers, lower than the value of 0.21 identified by Zhang *et al*., (2017) using Amplified Fragment Length Polymorphism (AFLP) and lower than the PIC values of 0.27 and 0.22 obtained using genomic SSR and EST-SSR (Tehrani *et al.,* 2009).

To detect species differentiation using molecular markers, all data were analyzed for the frequency and the overall distribution of alleles among and within the suggested groups. Comparison of AMOVA in all species clearly demonstrated that groups within a given comparison have genetically a higher degree of differentiation than when contrasted among groups. This was reflected by the higher proportion of total variance for the 'within' (75%) and for the 'among' (25%) analyses. These results is consistent with the analysis reported by Zhang *et al*. (2017) (77.8%) and (20.7%), respectively. Other studies also reveal a high proportion of total variance for the within populations, for example, Kumar *et al.* (2014) reported 92.2% variance within populations and 7.8% variance among *J. adhatoda* populations. This could be interpreted that genus *Festuca* is a polyploidy complex, or that out-crossing species would favor the maintenance of high levels of intra-accession genetic diversity (Zhang *et al.,* 2017).

The diversity parameters were also investigated for the whole species, *F. ovina* showed the highest values in three of the diversity parameters such as No. of different alleles (Na), Shannon's information index (I), and Percentage of Polymorphic Loci (PPL), with values of 1.492, 0.157, and 74.6%, respectively. The highest values (1.130, 0.090 and 0.094) of No. of effective alleles (Ne), expected heterozygosity (He), and unbiased expected heterozygosity (uHe) were detected in *F. arundinacea,* respectively (Table 3). On the other hand, *F. valesiaca* showed a significant number of effective alleles detected by the primer IS12. While for *F. arundinacea* the highest value was detected by the primer IS6. Other primers IS1, IS9, UBC808, UBC812, and UBC864 showed the same value of (Ne), but for *F. rubra*, and *F. ovina*, the highest value was detected by UBC741, with similar results reported by Fasih *et al*. (2013). Thus, these primers will be advantageous and helpful to easily distinguish the fescue species.

Actually, the understanding of the genetic relationships among fescue species would improve the understanding of evolutionary relationships among *Festuca* species (Ma, 2012). With *F. ovina* accessions exceeding 54% of the total studied number, because its importance where high tolerance to stress, abundance, and perseveration in multiple environments (Zhang *et al*., 2017), each species has been grouped into portions of variant proportions. This factor may have a positive effect on the high diversity parameter values (Na, I, and PPL) noticed for *F. ovina*, while it may has negatively affected other species which contained a fewer number of accessions. For example, the number of *F. arundinacea* accessions (13 accessions) in our study was slightly higher than that of *F. valesiaca* accessions (12 accessions); differences have been observed between these two species, where *F. arundinacea* showed higher values in all genetic parameters. Similarly, a small

number of accessions may arguably lose large amounts of genetic variation, where, for example, by comparing *F. heterophylla, F. rubra* and *F. pratensis*, three species of the same accessions number, *F. pratensis* showed higher values of the whole diversity parameters compared to *F. heterophylla and F. rubra.* While; comparing these species of 'two accessions' with species of bigger accessions numbers low genetic diversity values obviously have been obtained. Overgrazing and human over exploitation may trigger a rising trend in irregular distribution and isolation of this species, with low genetic diversity revealed (Table 3).

Genetic distance and neighbor joining tree: The genetic divergence between species was measured as genetic distance. The pairwise population matrix of the Nei genetic distance showed that the lowest genetic distance was found between *F. ovina* and *F. valesiaca* species (0.012), while the highest genetic distance was observed between *F. heterophylla* and *F. pratensis* species (0.060) (Table 4). Genetic distance was calculated also between whole accessions by applying DARWin software, showed that the minimum value (0.083) was noticed between the accessions of No: 54 and 55 from *F. valesiaca* specie collected from the same place (Kayseri-Tuzla Golu), while the maximum distance value was noticed between two accessions collected from same location (Adana-Pozantı) but from different species, accession with accession number of 32 belonging to *F. ovina* and the accession with accession number of 65 belonging to *F. rubra* (Figure 1). Genetic distance average within the whole collection is 0.126 (0.083 - 0.9166). These values are close to their counterparts of 0.1094 (0.0802 - 0.1508) reported by Zhang *et al*. (2017), and lower than the value of 0.369 identified by Szenejko *et al*. (2016) for *P. pratensis* L only. The average of genetic distance identified among *F. ovina* accessions (0.815) showed higher value than those of *F. arundinacea* and *F. valesiaca* accessions, (0.708) and (0.585), respectively (data not shown).

Correlation coefficients between the genetic diversity parameters and an average of altitude values of collection areas of the accessions were calculated and have been negative generally with zero significance, where high altitudes were correlated obviously with low genetic diversity parameters and confirmed, where some of biotic and/or abiotic factors could be the reasons behind, and this result is consistent with Zhang *et al.* (2017) (Table 5).

In addition, to check and confirm the genetic variations for each species, the genetic distance based neighbor joining dendrogram was constructed, with results showing clear diversity among accessions from each species. Accessions were splatted into the three main clusters: A, B and C. Cluster (A) is contained two sub-clusters; A-1 consists mainly of *F. ovina* and A-2 composed of *F. valesiaca*. Cluster B was divided also into two sub-clusters, *F. arundinacea* was present in B-1 and *F. pratensis* in B-2, but convergence among two different species *F. heterophylla* and *F. rubra* noted at C-1 cluster, confirming the clear convergence and overlapped noted among these species (Figure 1). The species *F. ovina* and *F. valesiaca* have all been gathered within cluster A, while *F. arundinacea* and *F. pratensis* within cluster B, *F. heterophylla* and *F. rubra* have been gathered within cluster C, indicating their association. These results were compatible with the calculated genetic distance values for these species, where the Nei genetic distance values were the lowest for these species 0.012, 0.029, and 0.020, respectively (Table 4). Ma (2012) considers *F. valesiaca* and *F. ovina* as one species in the *F. ovina* complex. Despite of the discrete genetic entities, much research carried out for decades has revealed an affinity between many fescues species. Borrill *et al*. (1977) suggests that *F. pratensis* is one progenitor of *F. arundinacea.* In addition, Malik & Thomas (1967), Xu and Sleper (1994), Rouf Mian *et al*. (2005), Hand *et al*. (2010), Angelov & Ivanova (2012), Ma (2012) and Cheng *et al*. (2016) all confirmed their close relationship, with *F. arundinacea* as the most relevant species, which is evolution-wise close to other *F. pratensis* species. On the other hand, Bulifiska-Radomska and Lester (1986) confirm the association between *F. rubra* and *F. heterophylla*.

Despite the fact that grouping does not show a full relationship with geographical origin, it partially confirms with their geographical source, whereas this relationship confirms in many sub-clusters (Figure 1). The reason behind this could be associated with many factors, which are the gene flow, out-crossing and ploidy level difference. The analyses, therefore, relatively indicates that accessions weakly categorized on the basis of geographical origin, and that geographical convergence factor may be the reason behind the prominent similarity that indicates a close relationship among them, with a common progenitor probably existing.

Principal Coordinate Analysis: In order to identify the relationships within and among species, Principal Coordinate Analysis was carried out using the dominant genotypic distance and the covariance-standardized methodology to elucidate the patterns of species structure. The first two principal coordinates were drawn in a two-dimensional space graph to show the clustering of different accessions. The percentage of accessions variations explained by the first 3 axes were15.69%, 5.52% and 5.38% of the variance, respectively with a percentage of cumulative 15.69%, 21.21%, and 26.59%, respectively. While higher of percentage found of species variations explained by the first 3 axes were 81.27%, 10.55% and 5.37% of the variance, respectively with a

percentage of cumulative 81.27%, 91.82%, and 97.19%, respectively. The accessions were divided simply into many species-specific groups; *F. ovina* accessions were gathered at "A" group; *F. arundinacea* accessions were located at "B", the third "C" for *F. valesiaca*, "D" group represents *F. heterophylla* accessions, "E" group for *F. rubra* and "F" for *F. pratensis* accessions (Figure 2). Furthermore, the PCoA results are consistent with the neighbor-joining clusters and with the genetic distance, as well. Similarly to AMOVA analysis in PCoA, a high diversity has been clearly identified within species, although the accessions have been collected from a varying range of different provinces and ecological conditions in Turkey, the PCoA diagram also showed that their proliferation was very close or indicates they are gathered according to each species. Whereas an obvious convergence among some species has been noticeable also particularly for *F. heterophylla* and *F. rubra* accessions (Figure 2). The relationships among the six species were also visualized by PCoA and demonstrated in Figure 3. Likewise, a consistency between PCoA result and that of Neighbor-Joining tree, as well as a clear genetic differentiation also have been clearly noticed, confirming the clear convergence and indicating high probability that has a progenitor common with those species.

Defining population structure: Numerous biometrical models have been developed for population stratification (Zhu *et al*., 2008). A favorite method to detect population structure has been introduced by Pritchard *et al.* (2000), in which, molecular marker information has been used to assign group membership probabilities to the genotypes utilizing a Bayesian framework. Genetic distance and genetic relationships among the *Festuca* panel are therefore, graphically investigated via PCoA, and by neighbor-joining cluster analyses. These are used as complementary approaches to confirm the results obtained using STRUCTURE. Different accessions belonging to various species have been assigned to the related groups as per the membership of coefficient (Q matrices). Accessions having a membership of coefficient (Q matrices) greater than 0.5 are assigned to related group. The optimal number of populations (*K*) was derived by Δ*K* values (Evanno *et al*., 2005), the highest value of ΔK was observed at K = 3, is shown 64.7% of the accessions have a complex genetic structure (Figure 4). Minute details of the second highest value of ΔK is shown at K = 6, which corresponded to the number of sub-populations, where the accessions were classified into eight groups, the accessions were lined up and obviously gathered based on related species, comprise a "simple" or "simple and admixture" pattern genetic structure, while the other groups are of "complex" or "complex and admixture" pattern of a genetic structure (Figure 4). For example, group IV was simple pattern of a genetic structure consisted of *F. arundinacea*, while group I was simple pattern, but has been indicating admixture also between *F. valesiaca, F. heterophylla* and *F. rubra* carrying a large dark red segment for membership coefficient, leads to confirm their relationship and suggests that genetic structure composition of this group is substantially affected by the alleles introduced from *F. valesiaca*. However, 70.6% of the accessions have a complex genetic structure, where groups II complex structure pattern restricted to *F. valesiaca*, while groups III, VI, VII and VIII consisted of *F. ovina* are complex also with no admixture noticed. The other group, V in addition to complex structure pattern, showed admixture of various alleles with two different species *F. arundinacea and F. pratensis*, introduced from species with an ancestral crossing parent in their lineages (Figure 5). At K = 6 the estimated Ln Prob = 8988.9, with a standard deviation of 390.1, mean value of Ln likelihood = -7359.1, variance of Ln likelihood = 3259.6 and mean value of α = 0.0374. High value was noticed for average of six population differentiation Fst 51.7% (12.8% - 97.1%) with SE of 0.1196. The average genetic variability of species determined in this study (51.7%) was higher than FsT value of (20.71%) reported by Zhang *et al*. (2017) and by Fjellheim *et al*. (2004) for both the Norwegian meadow fescue populations and Nordic cultivars. High variability value determined in our study indicating high diversity, and has been probably affected by using different molecular markers, DNA strategies, or habitat fragmentation, where random genetic drift over time has managed to both enter genetic differentiation, and cause a loss of heterozygosity within the population. However, it is necessary to keep in mind that structure and all other Bayesian methods are model-based, with strong priors and hypotheses, and all limitations and restrictions of these approaches considered in terms of ensuring a correct analysis of the results.

On the other hand, *F. ovina* and *F. valesiaca* accessions investigated in this study are spread in many different groups, probably due to the five different ploidy levels reported by Šmarda *et al.* (2005), the six subspecies existed according to Sheidai and Bagheri-Shabestarei (2007), or to some other reasons. Generally, the examined species have shown that the groups are in good agreement in terms of the result of the PCoA and neighbor-joining analysis.

Table 1. The list of accessions, Species, Collection place, Latitude, Longitude and Altitude.

No	Species	Collection place	Latitude	Longitude	Altitude
1	*F.ovina*	Konya Kent Ormani	37°53.351N	032°12.581E	1530m
2	*F.ovina*	Konya Kiziloren	37°51.571N	032°06.033E	1433m
3	*F.ovina*	Korkuteli	36°31.892N	029°59.445E	1043m
4	*F.ovina*	Kayseri	38°36.098N	035°30.595E	1884m
5	*F.ovina*	Konya Kiziloren	37°51.571N	032°06.033E	1433m
6	*F.ovina*	Antalya-Alanya	36°51.589N	032°31.228E	1734m
7	*F.ovina*	Antalya-Korkuteli	36°58.406N	030°09.097E	1224m
8	*F.ovina*	Antalya-Korkuteli	36°53.255N	030°02.478E	1188m
9	*F.ovina*	Antalya-Alanya	36°51.589N	032°31.228E	1734m
10	*F.ovina*	Seydisehir-Tınaztepe	37°16.097N	031°54.997E	1642m
11	*F.ovina*	Konya Kiziloren	37°51.571N	032°06.033E	1433m
12	*F.ovina*	Konya Kiziloren	37°51.571N	032°06.033E	1433m
13	*F.ovina*	Kizilcahamam	40°40.534N	032°19.500E	1251m
14	*F.ovina*	Karaman	36°42.073N	032°25.534E	1696m
15	*F.ovina*	Karabuk-Kastamonu	41°21.323N	033°39.731E	1177m
16	*F.ovina*	Corum	40°33.000N	034°57.000E	801m
17	*F.ovina*	Corum	40°33.000N	034°57.000E	801m
18	*F.ovina*	Corum	40°33.000N	034°57.000E	801m
19	*F.ovina*	Adana Kadirli-Karatepe	37°16.025N	036°13.276E	305m
20	*F.ovina*	Adana Kadirli-Karatepe	37°16.025N	036°13.276E	305m
21	*F.ovina*	Adana Kadirli-Karatepe	37°16.025N	036°13.276E	305m
22	*F.ovina*	Adana-Pozantı-Akcatekir	37°23.820N	034°50.962E	905m
23	*F.ovina*	Adana-Pozantı-Akcatekir	37°23.820N	034°50.962E	905m
24	*F.ovina*	Adana-Pozantı	37°21.648N	034°48.800E	1242m
25	*F.ovina*	Adana-Pozantı	37°21.648N	034°48.800E	1242m
26	*F.ovina*	Adana-Pozantı	37°21.648N	034°48.800E	1242m
27	*F.ovina*	Adana-Pozantı	37°21.648N	034°48.800E	1242m
28	*F.ovina*	Adana-Pozantı	37°21.648N	034°48.800E	1242m
29	*F.ovina*	Adana-Pozantı	37°21.648N	034°48.800E	1242m
30	*F.ovina*	Adana-Pozantı	37°21.648N	034°48.800E	1242m
31	*F.ovina*	Adana-Pozantı	37°21.648N	034°48.800E	1242m
32	*F.ovina*	Adana-Pozantı	37°21.648N	034°48.800E	1242m
33	*F.ovina*	Adana-Pozantı	37°21.648N	034°48.800E	1242m
34	*F.ovina*	Ankara-Elmadag	40°37.014N	032°30.504E	1387m
35	*F.ovina*	Ankara-Elmadag	40°37.014N	032°30.504E	1387m
36	*F.ovina*	Ankara-Elmadag	40°37.014N	032°30.504E	1387m
37	*F.ovina*	Adana Kadirli-Karatepe	37°16.025N	036°13.276E	305m
38	*F.arundinacea*	Cankiri	40°36.000N	033°36.600E	750m
39	*F.arundinacea*	Bolu Kartepe	40°39.674N	030°08.019E	1520m
40	*F.arundinacea*	Konya Kent Ormani	37°53.351N	032°12.581E	530m
41	*F.arundinacea*	Bursa	40°08.120N	029°01.498E	956m
42	*F.arundinacea*	Kastamonu	41°06.732N	033°44.978E	1355m
43	*F.arundinacea*	Yozgat	39°51.607N	034°55.494E	1285m
44	*F.arundinacea*	Kizilcahamam	40°40.534N	032°19.500E	1251m
45	*F.arundinacea*	Antalya-Akseki	37°07.290N	031°49.118E	1281m
46	*F.arundinacea*	Antalya-Akseki	37°07.290N	031°49.118E	1281m
47	*F.arundinacea*	Ankara-Kizilcahamam	40°40.534N	032°19.500E	1251m
48	*F.arundinacea*	Konya-Kampus	38°36.577N	031°07.646E	984m
49	*F.arundinacea*	Konya-Kampus	38°36.577N	031°07.646E	984m
50	*F.arundinacea*	Konya-Kampus	38°36.577N	031°07.646E	984m
51	*F.valesiaca*	Ankara-Kizilcahamam	40°37.014N	032°30.504E	1387m
52	*F.valesiaca*	Kayseri	38°36.098N	035°30.595E	1884m
53	*F.valesiaca*	Kayseri/Tuzla Golu	38°57.658N	035°45.614E	1324m
54	*F.valesiaca*	Kayseri/Tuzla Golu	38°57.658N	035°45.614E	1324m
55	*F.valesiaca*	Kayseri/Tuzla Golu	38°57.658N	035°45.614E	1324m
56	*F.valesiaca*	Kayseri	38°36.098N	035°30.595E	1884m

57	*F.valesiaca*	Konya-Antalya	37°08.884N	031°53.313E	1541m
58	*F.valesiaca*	Konya-Antalya	37°08.884N	031°53.313E	1541m
59	*F.valesiaca*	Konya-Basarakavak	37°53.510N	032°18.579E	1268m
60	*F.valesiaca*	Konya-Aksehir Beysehir	37°08.411N	031°47.805E	1204m
61	*F.valesiaca*	Adana-Pozantı	37°21.648N	034°48.800E	1242m
62	*F.valesiaca*	Ankara	40°37.014N	032°30.504E	1387m
63	*F.heterophylla*	Kizilcahamam	40°40.534N	032°19.500E	1251m
64	*F.heterophylla*	Kizilcahamam-Gerede	40°37.014N	032°30.504E	1387m
65	*F.rubra*	Adana Pozantı	37°21.648N	034°48.800E	1242m
66	*F.rubra*	Mersin-Tarsus	37°11.375N	034°48.534E	699m
67	*F.pratensis*	Yozgat	39°51.607N	034°55.494E	1285m
68	*F.pratensis*	Konya-Cevizli	37°12.169N	031°46.334E	1111m

Table 2. ISSR primers used for screening of polymorphism, their Sequences, Annealing temperatures, Fragment sizes and numbers as well as their Polymorphic Index contents.

Primer name	Sequences 5'- 3'	Tm	Fragments size (bp)	Fragments Number	PIC
G07	$(GAA)_5CG$	47	400 – 3500	31	0.16
G11	$(AC)_8CG$	47	410 – 3200	35	0.15
IS1	$(GA)_8C$	52	1000 – 3200	23	0.16
IS5	$(AG)_8C$	52	575 – 3000	28	0.19
IS6	$DBD(AC)_7A$	49 - 56	420 – 3500	46	0.21
IS9	$(GA)_8YC$	54 - 56	540 – 4000	31	0.18
IS10	$(AC)_8YA$	51 - 54	575 – 3500	25	0.11
IS12	$(AG)_8YT$	51 - 54	550 – 3200	38	0.12
IS13	$(GACA)_4$	48	580 – 3500	29	0.15
IS16	$(AC)_8C$	52	680 – 3100	16	0.16
UBC807	$(AG)_8T$	50	580 – 3000	32	0.18
UBC808	$(AG)_8C$	52	550 – 3000	35	0.15
UBC812	$(GA)_8A$	50	540 – 3000	29	0.14
UBC826	$(AC)_8C$	52	550 – 2800	36	0.16
UBC841	$(GA)_8YC$	54 - 56	560 – 4000	45	0.19
UBC842	$(GA)_8YG$	54 - 56	520 – 3500	30	0.12
UBC844	$(CT)_8RC$	54 - 56	730 – 3300	24	0.07
UBC847	$(CA)_8RC$	54 - 56	580 – 3200	38	0.14
UBC864	$(ATG)_6$	47	595 – 2000	27	0.13
Min			**400**	**16**	**0.07**
Max			**4000**	**46**	**0.21**
Average				**31.5**	**0.15**
Total				**598**	

Tm: Annealing Temperature, **PIC**: polymorphism information content

Table 3. Genetic parameters, Mean and standard Error over Loci for each population, Grand Mean and SE over Loci of studies species.

Pop		Na	Ne	I	He	uHe	PPL%
F. ovina	Mean	1.492	1.102	0.157	0.082	0.084	74.6
	SE	0.036	0.005	0.006	0.004	0.004	
F. arundinacea	Mean	0.866	1.130	0.151	0.090	0.094	43.3
	SE	0.041	0.009	0.008	0.005	0.006	
F. valesiaca	Mean	0.314	1.082	0.075	0.049	0.052	15.7
	SE	0.030	0.009	0.008	0.005	0.005	
F. heterophylla	Mean	0.080	1.018	0.015	0.010	0.014	2.5
	SE	0.014	0.005	0.004	0.003	0.004	
F. rubra	Mean	0.119	1.035	0.030	0.021	0.028	5.0
	SE	0.019	0.006	0.005	0.004	0.005	
F. pratensis	Mean	0.134	1.040	0.034	0.024	0.031	5.7

	SE	0.020	0.007	0.006	0.004	0.005	
Grand Mean and SE over Loci and Pops							
Total	Mean	0.501	1.068	0.077	0.046	0.050	24.5
	SE	0.014	0.003	0.003	0.002	0.002	11.8

Na = No. of different alleles, Ne = No. of effective Alleles, I = Shannon's information index, He = Expected heterozygosity, uHe = Unbiased expected heterozygosity, PPL= Percentage of Polymorphic Loci, and SE= standard error.

Table 4. Pairwise Species Matrix of Nei Genetic Distance.

	F. ovina	*F. arundinacea*	*F. valesiaca*	*F. heterophylla*	*F. rubra*	*F. pratensis*
F. ovina	0.000					
F. arundinacea	0.015	0.000				
F. valesiaca	0.012	0.023	0.000			
F. heterophylla	0.040	0.048	0.046	0.000		
F. rubra	0.030	0.038	0.036	0.020	0.000	
F. pratensis	0.028	0.029	0.033	0.060	0.050	0.000

Table 5. Correlation coefficient between values of genetic diversity parameters and average of altitude values of the accession collection areas.

	Na	Ne	I	He	uHe	PPL
Ne	0.787**					
I	0.934**	0.955**				
He	0.881**	0.984**	0.992**			
uHe	0.871**	0.985**	0.988**	0.998**		
PPL	0.999**	0.795**	0.938**	0.887**	0.877**	
Altitude	-0.119	-0.030	-0.096	-0.085	-0.123	-0.115

Ne = No. of effective alleles, I = Shannon's information index, He = expected heterozygosity, uHe = unbiased expected heterozygosity, PPL= Percentage of Polymorphic Loci.
*. Correlation is significant at the P≤0.05 level, **. Correlation is significant at the P≤0.01 level.

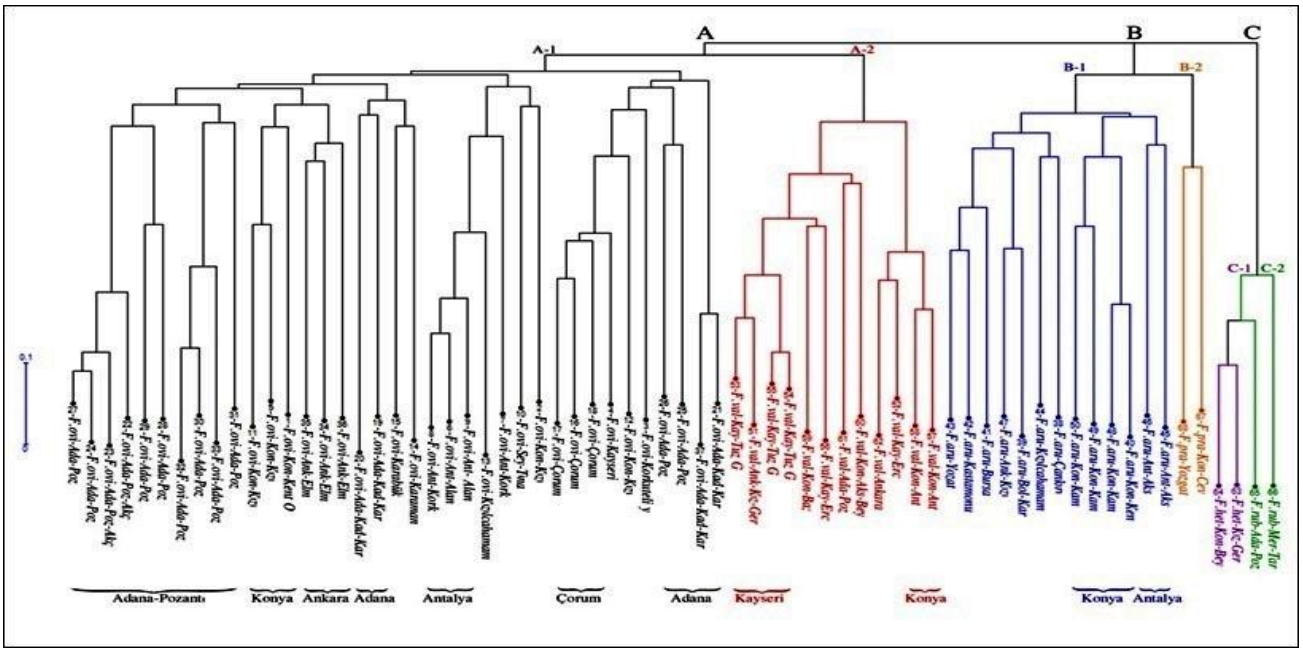

Figure 1. Neighbor-Joining tree showing relationships between 68 accessions revealed by overall genetic markers. The colors correspond to the species; black branches and names for *F. ovina,* blue for *F. arundinacea*, red for *F. valesiaca*, purple for *F. heterophylla*, green for *F. rubra*, and dark yellow for *F. pratensis*, the corresponding accessions numbers indicated at the bottom of the plot.

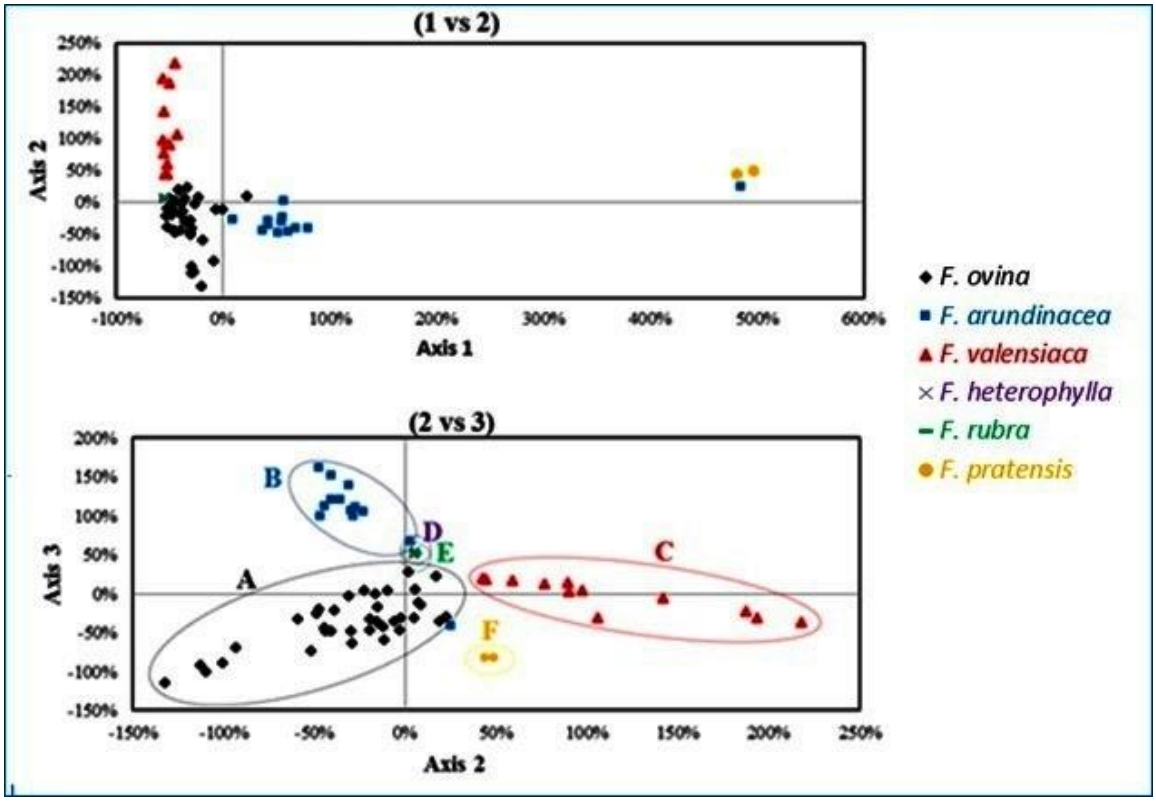

Figure 2. Relationship between accessions visualized by PCoA using ISSR markers.

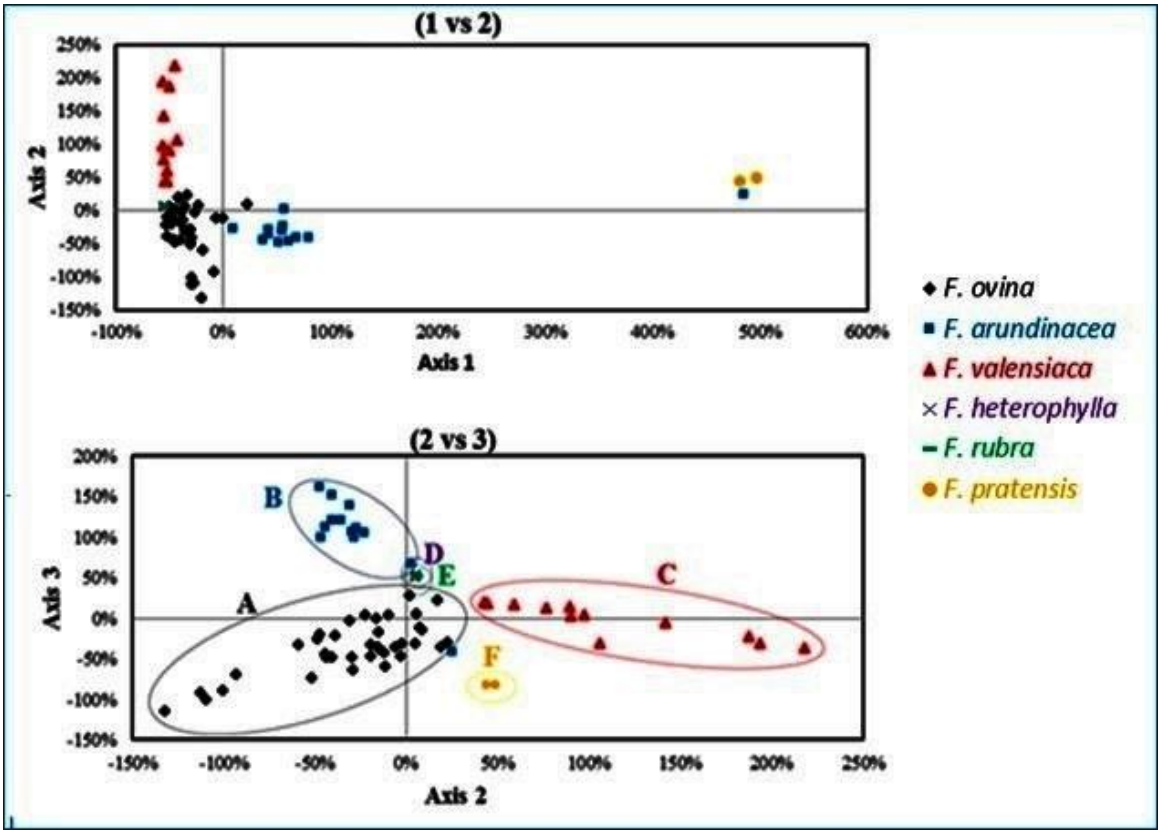

Figure 3. The relationships among six species based on the binary genetic distance visualized by PCoA.

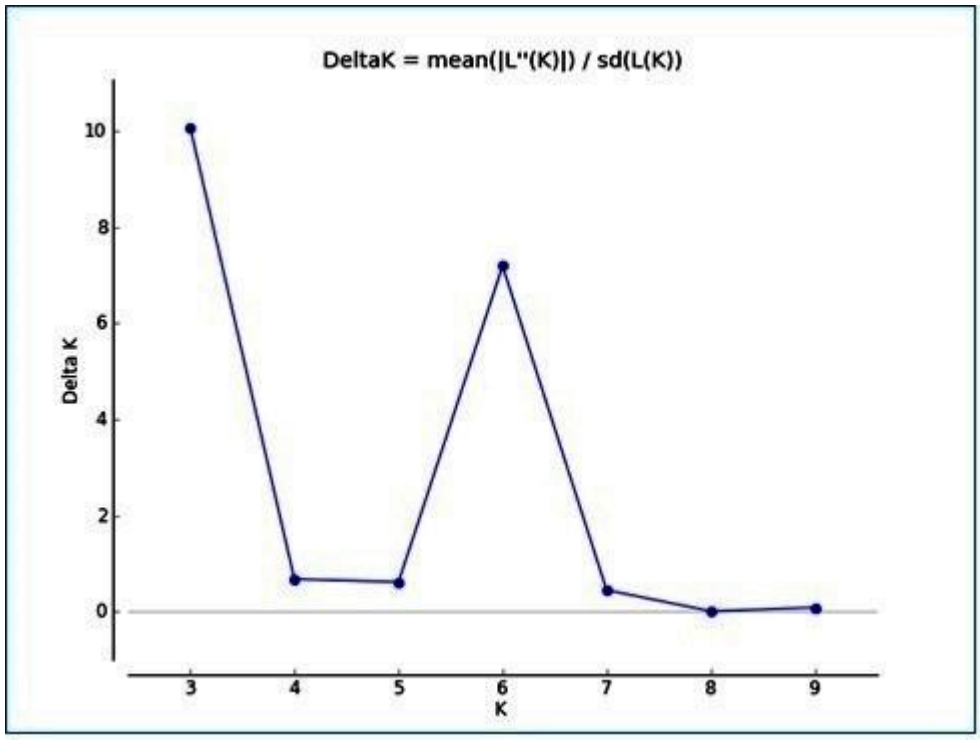

Figure 4. Inference of the optimal K value obtained from the STRUCTURE analysis of a dataset containing 68 *Festuca* accessions.

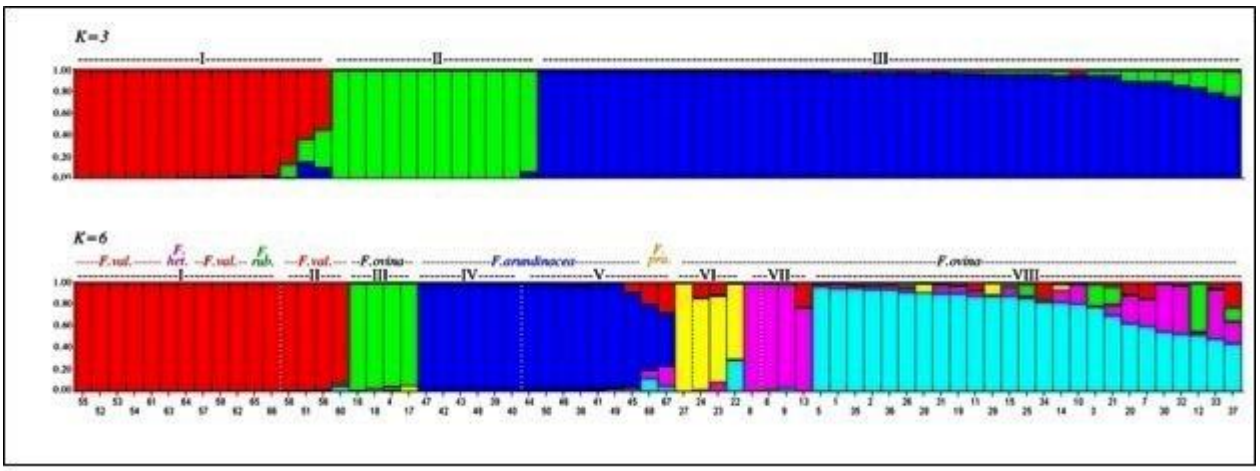

Figure 5. The survey of the genetic associations among 68 accessions using STRUCTURE. The models of K=3 and K=6 showed the ΔK value at the highest log-likelihood value. A vertical axis indicates a membership coefficient in each optimum number of K.

Conclusion: The study demonstrated genetic and taxonomic relationship among 6 species of genus *Festuca* using ISSR markers. A different statistical algorithm was used. An obvious convergence has been detected through PCoA, NJ tree, and Structure output, with accessions divided into number-of-species-based groups. *F. ovina* and *F. valesiaca* species showed the highest genetic relationship, while the highest genetic distance was identified between *F. heterophylla* and *F. pratensis* species. More deep studies are indispensable using next-generation sequencing based molecular markers covering the whole genome in order to get a clearer picture and revising the taxonomy of this complex genus.

Acknowledgements: The authors are grateful to Prof. Dr. Ruştu Hatipoğlu, University Cukurova, Adana, Turkey, for his contribution and valuable scientific inputs.

The authors express their thanks also to Yozgat Bozok University, Scientific Research Projects Unit, for financial support (Project No. 6603c-ZF/17-128).

REFERENCES

Abou-Deif, M.H., M.A. Rashed, M.A.A. Sallam, E.A.H. Mostafa, and W.A. Ramadan (2013). Characterization of Twenty Wheat Varieties by ISSR Markers. Middle East J. Sci. Res. 15 (2): 168-175.

Akbari, M., H. Salehi, and A. Niazi (2018). Evaluation of Diversity Based on Morphological Variabilities and ISSR Molecular Markers in Iranian *Cynodon dactylon* (L.) Pers. Accessions to Select and Introduce Cold‐Tolerant Genotypes. Molecular Biotechnology. 60:259–270.

Alsaleh, A., F.S. Baloch, M. Nachit and, H. Ozkan (2016). Phenotypic and genotypic intra-diversity among Anatolian durum wheat "Kunduru" landraces, Biochem. Syst. Ecol. 65: 9–16.

Al-Turki, T.A., and M.A. Basahi (2015). Assessment of ISSR based molecular genetic diversity of Hassawi rice in Saudi Arabia. Saudi J. Biological Sciences. 22: 591–599.

Andeden, E.E., F.S. Baloch, B. Kilian, and H. Ozkan (2013). iPBS-retrotransposons based genetic diversity and relationship among wild annual Cicer species. J. Plant Biochem. Biotechnol. 22: 453-466.

Angelov, G. B., and T.A. Ivanova (2012). Isoenzyme variation and genetic affinities among four species of the genus *Festuca* L. (Poaceae) Biodiv. Res. Conserv. 28: 3-8.

Armoniene, R., V. Stukonis, V. Paplauskiene, and G. Brazauskas (2010). The Genetic Diversity of Fine-Leaved Fescue (*Festuca* L.) Species in Lithuania. C. Huyghe (ed), Sustainable Use of Genetic Diversity in Forage and Turf Breeding, Springer Science +Business Media B. V. 2010. P: 41-45.

Balkan, A. (2018). Genetic variability, heritability and genetic advance for yield and quality traits in M2-4 generations of bread wheat (*Triticum Aestivum* L.) genotypes. Turk J Field Crops. 23(2): 173-179.

Baloch, F.S., A. Alsaleh, M.Q. Shahid, V. Ciftci, L.E. Saenz de Miera, M. Aasim, M. A. Nadeem, H. Aktas, H. Ozkan, and R. Hatipoğlu (2017). A Whole Genome DArTseq and SNP Analysis for Genetic Diversity Assessment in Durum Wheat from Central Fertile Crescent. PLoS One. 12 (1).

Baloch, F.S., M. Derya, E.E. Andeden, A. Alsaleh, G. Comertpay, B. Kilian, and H. Ozkan (2015). Inter-primer binding site retrotransposon and inter-simple sequence repeat diversity among wild Lens species. Biochem. Syst. Ecol. 58: 162-168.

Baloch, F.S., C. Kurt, H. Arıoglu, and H. Ozkan (2010). Assaying of diversity among soybean (*Glycin max* L. Merr.) and peanut (*Arachis hypogaea* L.) genotypes at DNA level. Turk. J. Agric. For. 34: 285-301.

Borrill, M., M. Kirby, and W.G. Morgan (1977). Studies in *Festuca* 11. Interrelationships of some putative diploid ancestors of the polyploid broad-leaved fescues. New Phytol. 78:661-674.

Bulifiska-Radomska, Z., and R. N. Lester (1986). Species Relationships in *Festuca* (Sect. *Ovinae*, Poaceae) Pi. Syst. Evol. 154: 175- 182.

Buntjer, J.B. (1999). Cross Checker Fingerprint analysis software v2.9, Wageningen University and Research Centre, The Netherlands.

Cheng, Y., K. Zhou, M.W. Humphreys, J.A. Harper, X. Ma, X. Zhang, H. Yan, and L. Huang (2016). Phylogenetic Relationships in the *Festuca*-Lolium Complex (Loliinae; Poaceae): New Insights from Chloroplast Sequences. Front. Ecol. Evol. 4: 89.

Chesnokov, Yu. V., and A.M. Artemyeva (2015) Evaluation of the Measure of Polymorphism Information of Genetic Diversity, Agricultural Biology, V. 50 (5): 571-578.

Cuyeu, R., B. Rosso, E. Pagano, G. Soto, R. Fox, and N.D. Ayub (2013). Genetic diversity in a world germplasm collection of tall fescue. Genet. Mol. Biol. 36 (2): 237-242.

Demiroğlu, G., H. Geren, B. Kir, and R. Avcıoğlu (2010). Performances of some cool season turfgrass cultivars in Mediterranean environment: II. *Festuca arundinacea* Schreb., *Festuca ovina* L., *Festuca rubra* spp. *rubra* L., *Festuca rubra* spp. trichophylla Gaud and *Festuca rubra* spp. commutata Gaud. Turk J Field Crops. 15 (2): 180-187.

Earl, D.A., and B.M. Von Holdt (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources. 4 (2) 359-361.

Evanno, G., S. Regnaut, and J. Goudet (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14: 2611-2620.

Fasih, Z., M. Farshadfar, and H. Safari (2013). Genetic diversity evaluation of within and between populations for *Festuca arundinacea* by ISSR markers. Intl J Agri Crop Sci. 5 (13): 1468-1472.

Fjellheim, S., Z. Grieg, and O.A. Rognli (2004). Aflp-marker analyses of genetic structure in nordic meadow fescue (*Festuca pratensis* huds.).

Tracing the origin of norwegian cultivars and local populations. Dev. Plant Breed. 11: 303–308.

Gibson, D.J., and J.A. Newman (2001). *Festuca arundinacea* Schreber (*F.elatior* L.ssp. *arundinacea* (Schreber) Hackel). J. Ecol. 89: 304-324.

Guner, A. (2012). Turkey List of plants, vascular plants. ANG vakfı. S 74-79.

Habibi, B., M. Farshadfar, and H. Safari (2012). Evaluation of Genetic Diversity in 18 Genotypes of Alfalfa (*Medicago* Sativa) Using of Molecular ISSR Markers. Intl J Agri Crop Sci. 4-21:1573-1578.

Hand, M.L., N.O.I. Cogan, and J.W. Forster (2012). Molecular characterisation and interpretation of genetic diversity within globally distributed germplasm collections of tall fescue (*Festuca arundinacea* Schreb.) and meadow fescue (*F. pratensis* Huds.). Theor Appl Genet. 124: 1127.

Hand, M.L., N.O.I. Cogan, A.V. Stewart, and J.W. Forster (2010). Evolutionary history of tall fescue morphotypes inferred from molecular phylogenetics of the *Lolium-Festuca* species complex. BMC Evol Biol. 10: 303.

Kumar, A., P. Mishra, S. C. Singh, and V. Sundaresan (2014). Efficiency of ISSR and RAPD markers in genetic divergence analysis and conservation management of *Justicia adhatoda* L., a medicinal plant. Plant Syst Evol. 300:1409–1420.

Lamare, A., and S.R. Rao (2015). Efficacy of RAPD, ISSR and DAMD markers in assessment of genetic variability and population structure of wild Musa acuminata colla. Physiol Mol Biol Plants. 21 (3):349–358.

Ma, Y. (2012). Genetic Characterization of Fine-leaved *Festuca valesiaca* Germplasm and Evaluation of Their Relationship to the *F. ovina* complex. M.Sc. thesis, 1352. Plant Science. Utah State University. USA.

Malik, C. P., and P.T. Thomas (1967). Cytological Relationships and Genome Structure of Some *Festuca* Species, Caryologia. 20: (1) 1-39.

Nadeem, M.A., A.A. Nawaz, Q. M. Shahid, Y. Doğan, G. Comertpay, M. Yıldız, R. Hatipoğlu, F. Ahmad, A. Alsaleh, N. Labhane, H. Ozkan, G. Chung, and F. S. Baloch (2018). DNA molecular markers in plant breeding: current status and recent advancements in genomic selection and genome editing. Biotechnol Biotec EQ. 32: (2) 261-285.

Nelson, C.J., K.H. Asay, and G. L. Horst (1975). Relationship of leaf photosynthesis to forage yield of tall fescue. Crop Sci. 15:476-478.

Ogle, D., M. Stannard, P. Scheinost, and S. L. John (2010). Plant guide for sheep fescue (*Festuca ovina* L.). USDA Natural Resources Conservation Service, Idaho and Washington Plant Materials Program.

Özpinar, H., C.O. Sabanci, A.A. Acar, S. Aksu, and İ. F. Niksarli (2014). Evaluation of *Fescue* (*Festuca arundinacea* Schreb. and *Festuca rubra* L.) populations grown under Aegean region conditions, Anadolu, J. of Aari. 24 (2): 32 – 40.

Peakall, R., and P.E. Smouse (2001). GenAlEx V5: Genetic Analysis in Excel. Population genetic software for teaching and research. Australian National University, Canberra, Australia.

Perrier, X., A. Flori, and F. Bonnot (2003). Methods for data analysis. In: Hamon P, Seguin M, Perrier X, Glazmann JC (eds) Genetic diversity of cultivated tropical plants. Science Publishers, Inc and Cirad, Montpellier. 31–63.

Pritchard, J.K., M. Stephens, and P. Donnelly (2000). Inference of population structure using multilocus genotype data. Genetics. 155: 945-959.

Rahimi, M., I.M. Hervan, M. Valizadeh, F.D. Kajori, and F. Ebrahimpour (2014). Genetic Diversity among Wild and cultivated barley by ISSR Marker. Bull. Env. Pharmacol. Life Sci. 3 (10) 57-62.

Rahmati, H., M. Farshadfar, and H. Shirvani (2018). Study of Genetic Diversity of *Festuca Arundinacea* Based on ISSR Molecular Markers**.** J. Crop Breed. Vol. 9, No 24**.**

Reddy, M.P., N. Sarla, and E.A. Siddiq (2002). Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding, Euphytica 128: 9–17.

Rouf Mian, M.A. M.C., Saha, A. Hopkins, and Z.Y. Wang (2005). Use of tall fescue EST-SSR markers in phylogenetic analysis of cool-season forage grasses. Genome 48, 637–647.

Sheidai, M., and E. Bagheri-Shabestarei (2007). Cytotaxonomy of some *Festuca* species and populations in Iran. Acta Bot Croat. 66 (2):143-151.

Šmarda, P., J. Müller, J. Vrána, and K. Kočí1(2005). Ploidy level variability of some Central European fescues (*Festuca* subg. *Festuca*, Poaceae). Biologia Bratislava. 60 (1): 25-36.

Sokal R.R., and C.D. Michener (1958). A statistical method for evaluating systematic relationships. Univ. Kans. Sci. Bull. 28:1409–1438.

Stukonis, V., R. Armonienė, N. Lemežienė, V. Kemešytė and G. Statkevičiūtė (2015). Identification of Fine-Leaved Species of Genus *Festuca* By Molecular Methods. Pakistan J. Bot. 47 (3): 1137-1142.

Szenejko, M., P. Smietana, and E. Stepien (2016). Genetic diversity of *Poa pratensis* L. depending on geographical origin and compared with genetic markers. PeerJ 4:e2489.

Talukder, S.K., P. Azhaguvel, K. Chekhovskiy, and M.C. Saha (2018). Molecular discrimination of tall fescue morphotypes in association with *Festuca* relatives. PLoS ONE. 13(1): e0191343.

Tehrani, M.S., M. Mardi, J. Sahebi, C. P. Pilar, and A. Dıaz-Perez (2009). Genetic diversity and structure among Iranian tall fescue populations based on genomic-SSR and EST-SSR marker analysis. Plant Syst Evol. 282:57–70.

Xu, W.W., and D.A. Sleper (1994). Phylogeny of tall fescue and related species using RFLPs Theor Appl Genet. 88:685-690.

Zhang, C., J. Zhang, Y. Fan, M. Sun, W. Wu, W. Zhao, X. Yang, L. Huang, Y. Peng, X. Ma, and X. Zhang (2017). Genetic Structure and Eco-Geographical Differentiation of Wild Sheep Fescue (*Festuca ovina* L.) in Xinjiang, Northwest China. Molecules. 22, 1316.

Zhu, C., M. Gore, E.S. Buckler, and J. Yu (2008). Status and Prospects of Association Mapping in Plants. The Plant Genome J. 1:5–20.

Zietkiewicz, E., A. Rafalski, and D. Labuda (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomic. 20 (2):176-83.