Current Trends in Natural Sciences
Vol. 11, Issue 22, pp. 100-107, 2022
https://doi.org/10.47068/ctns.2022.v11i22.012
Current Trends in Natural Sciences (on-line)
ISSN: 2284-953X
ISSN-L: 2284-9521
Current Trends in Natural Sciences (CD-Rom)
ISSN: 2284-9521
ISSN-L: 2284-9521

DETERMINATION OF THE GENETIC SIMILARITIES/DIFFERENCES OF SOME ANNUAL GRASS VARIETIES

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Current Trends in Natural Sciences

Abstract
This study was conducted to determine the genetic similarities/differences of twenty-five annual grass varieties using the SRAP marker system. While the mean polymorphism rate was determined as 87%, the highest polymorphism was determined in the Me4 Em6, Me4 Em7, Me2 Em13, Me4 Em4, Me4 Em9, Me4 Em11, Me4 Em12, Me2 Em14 and Me3 Em2 primer combinations. The lowest polymorphism rate was obtained from Me3 Em3 (70%) primer combination. According to the dendrogram obtained from the molecular characterization of grass varieties using SRAP molecular markers, great variation was observed between the genotypes. The degree of similarity in the dendrogram varied between 0.54-0.90. According to the findings, it can be said that the SRAP marker system was an effective marker system in determining the genetic similarities and differences between different grass varieties. In addition, the variation obtained among the annual grass varieties used in the study can be used both in the grass application and in breeding programs.

Keywords: Annual grass, molecular characterization, SRAP

1. INTRODUCTION

Annual grass which can be used as fodder by grazing beef cattle, as hay by drying or used as silage for feeding and development of dairy cows, especially in milk production (Kusvuran, 2011; Taşsever, 2019) (Kusvuran and Tansi, 2011; Durst et al. 2013), is a highly nutritious feed source. In addition, the plant also plays an important role in soil improvement by leaving dense organic matter in the soil (Elçi 2005; Taşsever, 2019). With its high protein content, richness in minerals and water-soluble carbohydrates, it is one of the most important forage crops suitable for quality grass production (Kusvuran and Tansi, 2005). Studies have shown that Italian lawn grasses have a positive effect on milk yield and composition (Miller et al. 2001; Bernard, 2003) thanks to their high dry matter digestibility (71% to 78%) (Catanese et al. 2009; Amaral et al. 2011). It was also shown that it increased body weight (De Villiers et al. 2002; Zaman et al. 2002; Van Niekerk et al. 2008). Annual grass is one of the important forage crops in many parts of the world such as northwest Europe, South America, New Zealand, Australia and South Africa due to its high nutritional value and digestibility and is an important source of silage (Xu et al. 2007; Shao et al. 2002; Lopes et al. 2009; Ding et al. 2011). Annual grass is one of the valuable feeds for different animals such as cattle, sheep and rabbits (Ding et al. 2011). As with other species, there is a genetic

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variation in annual grasses, both commercially used varieties and natural populations. However, revealing genetic diversity can be used to distinguish varieties and can make an important contribution to breeding programs. In addition to the use of morphological features in the determination of genetic diversity, a significant advantage can be obtained by incorporating molecular methods.

The use of molecular markers in the characterization of genetic resources provides great convenience. Morphological features are widely used in the creation of germplasms. However, the effect of environment has started to be very limited due to their low heritability and polymorphism (Smith and Smith, 1992). There is no such restriction for DNA markers. DNA markers are very useful in distinguishing between close genotypes. Different types of molecular markers are widely used to determine genetic diversity in different products, but each technique has its own advantages (Kafkas et al., 2008; Pavlovic et al., 2012). Various marker systems are used in the characterization of genetic resources and one of them is the SRAP marker system. SRAP marker system is a molecular marker system based on PCR, which is applied for the first time in cabbage (Li and Quiros, 2001) that gives bands that are simple, reliable, sufficient and easily obtainable. It consists of forward and reverse primers of 17 and 18 bp lengths. The forward primers consist of a 13 or 14 bp main sequence followed by the CCGG sequence. It contains 3 selective (splitting) nucleotides at the 3' end. Reverse primers are similar to forward primers and contain the AATT sequence instead of the CCGG sequence. The AATT sequence also has three selective nucleotides added at the 3' end of the primer. SRAP markers provide more consistent results than RAPD markers and are cheaper and require less labor than AFLP markers (Li and Quiros, 2001).

There are many reports that SRAP markers have been used successfully in genetic diversity studies in many species in recent years. In this sense, initially, studies were carried out in recombinant breeding and diploid lines of *Brassica oleracea* L. and it was reported that 45% of the bands isolated from the gel matched with known genes in the gene bank after sequencing analysis. In addition, after sequencing analysis, 20% of the SRAP markers were determined to be co-dominant markers. As a result of the study, the glycosylate desaturation gene (BoGLS-ALK) found in Brassica was identified with the use of SRAP (Li and Quiros, 2001).

In this study, molecular characterization of 25 different annual grass varieties was carried out with the use of SRAP molecular marker system.

2. MATERIALS AND METHODS

In this study, 25 different annual grass varieties registered in Turkey (Vallivert, Master, Hellen, Venus, Excellent, BigBoss, Jivet, Cesco, Trinova, Tornado, First Step, Ration, Quickston, Jako, Elif, Braulio, Baqueno, Kar Tetra, Devis), Rambo, Teanna, Efe 82, Medoacus, Caramba, Koga) were used as plant material.

2.1. Molecular Characterization

Genetic similarities/differences between twenty-five grass genotypes were determined with the use of SRAP marker system.

2.2. DNA Isolation

The CTAB method reported by Doyle and Doyle (1990) was used for DNA isolation. DNA concentrations were determined with the aid of a semi-automatic micro plate reader (Power Wave HT, BIO-TEK Instruments, Inc., Winooski, VT, USA) and DNA concentrations of 10ng/µl were prepared for PCR studies. In this study, 208 SRAP primer combinations were tested and 12 primer combinations with a high total number of bands and scoreable bands were selected (Table 2.1).

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Table 2.1. Primer sequence of SRAP markers

Marker Combination	Forward	Reverse
Me4 Em5	TGA GTC CAA ACC GGA CC	GAC TGC GTA CGA ATT AAC
Me4 Em6	TGA GTC CAA ACC GGA CC	GAC TGC GTA CGA ATT GCA
Me4 Em7	TGA GTC CAA ACC GGA CC	GAC TGC GTA CGA ATT CAA
Me2 Em13	TGA GTC CAA ACC GGA GC	GAC TGC GTA CGA ATT CTG
Me3 Em3	TGA GTC CAA ACC GGA AT	GAC TGC GTA CGA ATT GAC
Me4 Em4	TGA GTC CAA ACC GGA CC	GAC TGC GTA CGA ATT TGA
Me4 Em9	TGA GTC CAA ACC GGA CC	GAC TGC GTA CGA ATT CAG
Me4 Em11	TGA GTC CAA ACC GGA CC	GAC TGC GTA CGA ATT CTA
Me4 Em12	TGA GTC CAA ACC GGA CC	GAC TGC GTA CGA ATT CTC
Me2 Em9	TGA GTC CAA ACC GGA GC	GAC TGC GTA CGA ATT CAG
Me2 Em14	TGA GTC CAA ACC GGA GC	GAC TGC GTA CGA ATT CTT
Me3 Em2	TGA GTC CAA ACC GGA AT	GAC TGC GTA CGA ATT TGC

2.3. SRAP Marker Analysis

PCR components and conditions were created by modifying the method used by Gülsen et al. (2005). PCR (Polymerase Chain Reaction) was performed using different combinations of SRAP primers for amplification of DNAs. PCR protocol; a total volume of 15 µl PCR mixture was prepared with 1.5 µl 10 X, 1.8 µl MgCl2, 1.2 µl dNTP, 0.5 µl reverse primer, 0.5 µl forward primer, 0.15 µl Taq Polymerase, 5.35 µl H2O, 4 µl DNA. PCR program in Eppendorf PCR device; 1) 5 minutes at 94°C 2) 1 minute at 94°C 3) 1 minute at 35°C 4) 2 minutes at 72°C 5) Repeat 4 times after the 2nd step 6) 94 1 minute at °C, 7) 1 minute at 50°C, 8) 2 minutes at 72°C, 9) 29 times after step 6, 10) 5 minutes at 72°C.

About 3 µl of loading buffer (20 ml of glycerol (40%), 30 ml of sterile water, 0.05 g of bromophenol blue) was added to the PCR products obtained from the PCR studies and the resulting mixture was loaded on a 2.5% agarose gel and run under 115 V electric current for 3 hours. 1X TAE buffer was used in the preparation of the agarose gel and 25 µl (0.5 mg/ml) ethidium bromide solution was added into it. 100 bp DNA ladder was loaded as standard in each electrophoresis procedure. After the electrophoresis process, the gels were taken to the computer-connected gel imaging and the gel images were recorded on the computer under UV.

2.4. Data Analysis

All gel images were scored as present (1) or absent (0) and their files were created. These obtained data were analyzed in the computer package program of NTSYS (Numerical Taxonomy Multivariate Analysis System, NTSYS-pcversion 2.11, Exeter Software, Setauket, N.Y., USA, Rohlf, 2000). Similarity index values were calculated according to the Dice (1945) method and the dendrogram was created according to the UPGMA (Un-weighted Pair-Group Method with Arithmetic Average) method. In addition, principal components analysis (PCA) was performed showing the distances between genotypes on a two-dimensional graph.

3. RESULTS AND DISCUSSIONS

Average values for fresh herbage yields of annual grass cultivars harvested in different periods are In this study, genetic similarities/differences between twenty-eight grass genotypes were determined by using the SRAP marker system. The obtained findings are given in Table 3.1.

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A total of 61 bands were obtained from 12 SRAP primer combinations and 57 of these bands were determined as polymorphic. The highest number of bands was obtained from ME3 Em3 primer combination, while the lowest number of bands was obtained from Me2 Em13 and Me4 Em4 primer combinations. While the mean polymorphism rate was determined as 87%, the highest was determined in the primer combinations Me4 Em6, Me4 Em7, Me2 Em13, Me4 Em4, Me4 Em9, Me4 Em11, Me4 Em12, Me2 Em14 and Me3 Em2 SRAP. The lowest polymorphism rate was obtained from Me3 Em3 (70%) primer combination.

Table 3.1. Total number of bands, number of polymorphic bands and polymorphism ratios (%) obtained after amplification of ISSR primers

Marker Combination	Total number of bands	Number of polymorphic bands	Polymorphism ratio (%)
Me4 Em5	8	7	87.5
Me4 Em6	7	7	100.0
Me4 Em7	5	5	100.0
Me2 Em13	1	1	100.0
Me3 Em3	10	7	70.0
Me4 Em4	1	1	100.0
Me4 Em9	5	5	100.0
Me4 Em11	4	4	100.0
Me4 Em12	3	3	100.0
Me2 Em9	3	3	100.0
Me2 Em14	7	7	100.0
Me3 Em2	7	7	100.0
Total	61	57	93.4
Maximum	10	7	70.0
Minimum	1	7	700.0
Mean	5.46	4.75	87.0

According to the dendrogram obtained from the characterization of grass genotypes using SRAP molecular markers, great variation was observed between the genotypes. The degree of similarity in the dendrogram varied between 0.54-0.90. As a result, two main groups were formed and genotypes 1, 2, 3, 4 and 5 (Vallivert, Master, Hellen, Venus, Excellent) were placed into the first group, while the others formed the second group. The closest genotypes to each other were seen as genotypes 20 (RAMBO) and 25 (KOGA) (Figure 4.1).

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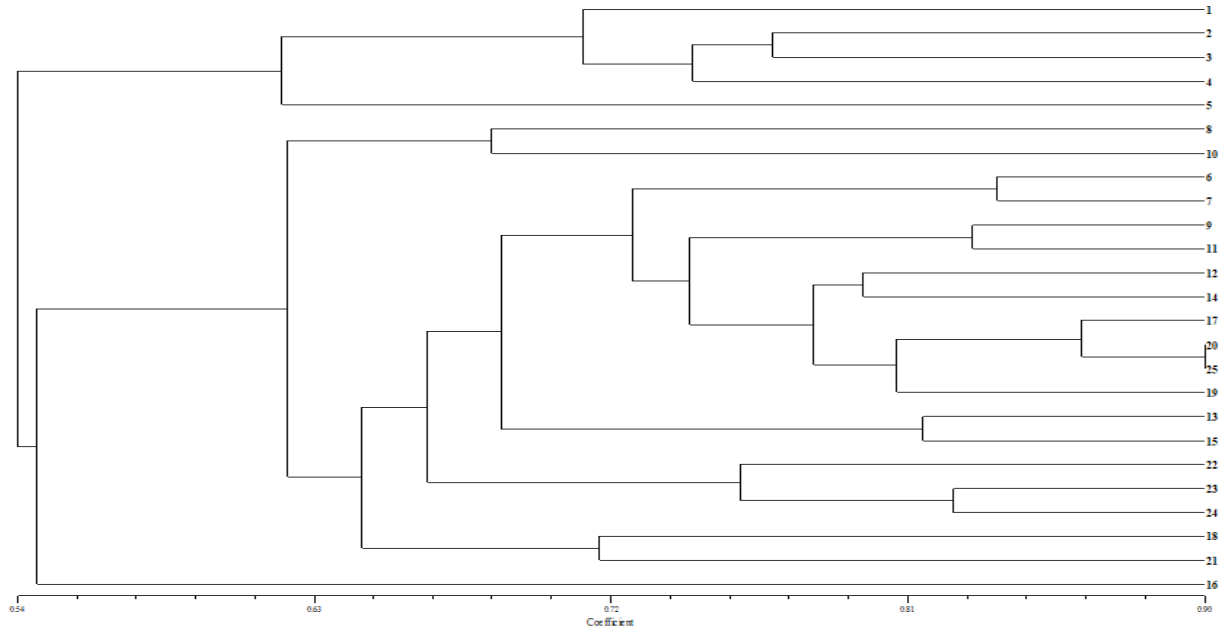

Figure 4.1. Dendrogram for characterization of annual grass varieties with the use of SRAP molecular markers

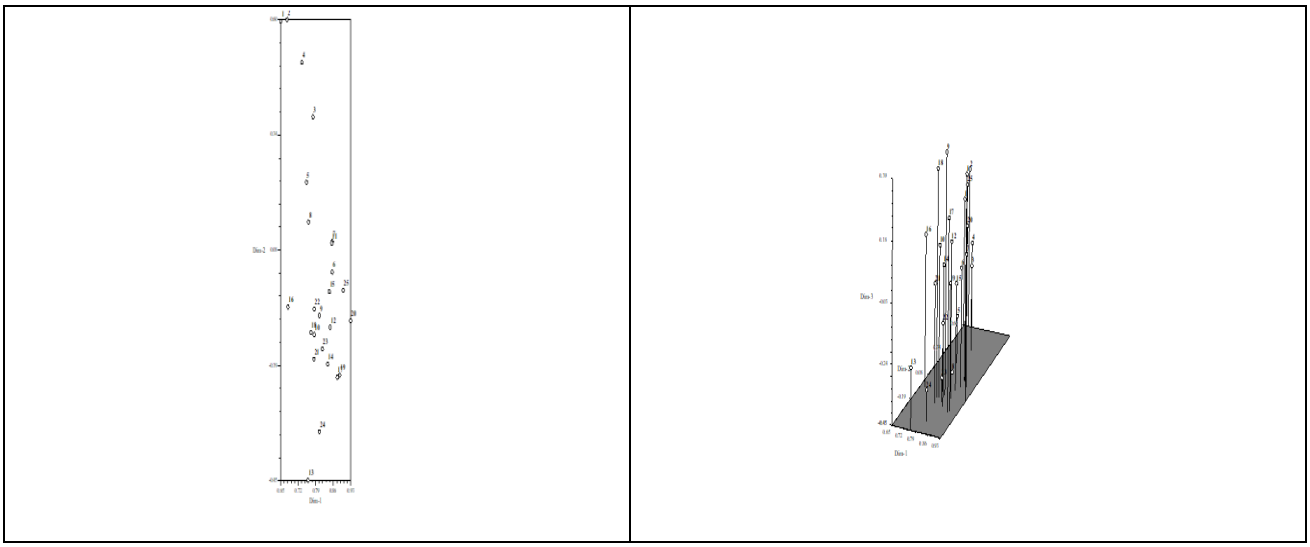

Figure 4.2. Principle component analysis (PCA) for characterization of annual grass varieties with the use of SRAP molecular markers

Li et al. (2011) determined the similarities and molecular variation between 95 wild Bermuda grasses collected from 11 provinces in China and 1 commercial variety, 'Tift3', using the ISSR technique. According to the findings obtained, they reported that the genetic similarity coefficient with 29 ISSR primers varied between 0.51 and 0.97. On the other hand, Wang et al. (2010) in a study conducted to determine the similarity and molecular variation among 24 Bermuda grass varieties developed in the USA, Australia and China, in which 90 SRAP primer combinations were used, reported the genetic similarity coefficient between the genotypes as between 0.57 and 0.97.

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Huang et al. (2010) used the SRAP technique to investigate the relationship between 57 *C. dactylon* genotypes collected from 17 countries from 5 continents. As a result of the cluster analysis, it was determined that the Chinese genotypes were genetically different from the others. The genetic similarity coefficient of 57 genotypes varied between 0.53 and 0.97. Relatively greater variation was detected in the large number of *C. dactylon* collected from all geographic regions, particularly in genotypes from China and Australia.

Wang et al. (2010) investigated the effectiveness of SSR markers in commercially used and vegetatively propagated cultivars. Thirty-two clones of bermuda grass genotypes consisting of 29 commercial cultivars and 3 Oklahoma State University experimental lines were evaluated with 11 microsatellite markers in the study. The genetic similarity coefficient among 55 genotypes was between 0.52 and 0.95, which revealed that there was a high level of variation in the *Cynodon* genotypes.

Huang et al. (2013) used the SRAP markers and 15 primer combinations to amplify a specific *C. radiatus* genome sequence to evaluate the genetic relationship between 33 *C. radiatus* genotypes collected from different regions of China. It was determined that the genetic similarity coefficient among 33 genotypes used in the study varied between 0.53 and 0.95.

Wang et al. (2013) used ISSR and SSR markers to evaluate genetic diversity among 33 *C. dactylon* genotypes and 22 cultivars from four different countries to provide information on how to increase the use of bermuda grass gene resources. It was determined that SSR primers were 97.7% and ISSR primers were 86.9% polymorphic. Genetic similarity coefficient, gene diversity and Shannon index were respectively found to be 0.58 – 0.97, 0.27 and 0.41 for for ISSR and 0.52 – 0.97, 0.29 and 0.43 for SSR. According to the UPGMA analysis, 55 genotypes were clustered, and the cluster results produced by ISSR data 20 were found to be close to the SSR data results. Analysis based on combined ISSR and SSR data revealed that the gene sources tested were more related to geographic distribution.

Huang et al. (2012) conducted a study with 50 ISSR primers to genetic relationships among 29 *C. radiatus* genotypes collected from different parts of China and 14 primers were selected which were capable of producing multiple band samples to amplify the specific *C. radiatus* genome sequence. It was determined that the genetic similarity coefficient among 29 genotypes varied between 0.45 and 0.90. This study showed that the ISSR technique was a powerful technique for the identification and molecular classification of *C. radiatus* genotypes and their genetic relationships.

Huang et al. (2013) conducted a study to determine the genetic variation and similarities among 29 *C. arcuatus* genotypes collected from different regions of China using ISSR and SRAP marker techniques. The polymorphism rates of ISSR and SRAP markers were 98.84% and 94.61%, respectively; genetic similarity coefficient ratios were 0.45-0.90 for ISSR markers, 0.48-0.94 for SRAP markers and 0.48-0.90 for ISSR+SRAP markers. Findings from this study confirmed a rich diversity of *C. arcautus* in Hainan with the use of ISSR and SRAP markers, which provide a basis for molecular breeding and generation of gene resources of *C. arcuatus*.

Kang et al (2008) evaluated the genetic diversity of Korean bermuda grass at the morphological, cytological and molecular levels. Morphological parameters, nuclear DNA content and ploidy level were investigated in 43 bermuda grass ecotypes. AFLP technique was used to determine genetic diversity and it was determined that genetic similarity ratios ranged between 0.42-0.94 and ecotypes were divided into 6 different groups according to UPGMA and basic coordinate analysis.

In a study conducted by Jewell et al (2012), large-scale collection studies of *Cynodon* genotypes across Australia aimed to optimize the use of the core collection in breeding and research. The

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genetic diversity of the collected 690 *Cynodon* genotypes was determined using 16 EST-SSR markers and the average number of alleles obtained was 7.44 per marker. It was determined that the basic collection consisted of 13% of the gene sources and reflected 96% of the allele diversity.

4. CONCLUSIONS

The studies mentioned above show that there was a wide variation among grass genotypes. It also shows that there may be differences in the determination of genetic diversity between the marker systems used in the studies. In this study, a wide variation was obtained among the annual grass varieties in the characterization made with the primer combinations of the SRAP marker system and the variation obtained was complying with the findings of the previous studies. This variation obtained can be used in grass improvement studies.

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