MOLECULAR CHARACTERIZATION OF SOME LETTUCE SAMPLES (LACTUCA SATIVA) GROWN IN TURKEY USING SIMPLE SEQUENCE REPEAT (SSR)

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Received on August 20, 2021
Presented by A. Atanassov, Member of BAS, on October 28, 2021

Abstract

This study aims to determine the differences between genotypes of some Lactuca sativa (lettuce) samples commonly grown in Turkey. In the study, Lactuca sativa genotypes were obtained from the samples of three different companies and their molecular analyses were carried out. Fourteen SSR primers were used for the analysis of genetic differences between Lactuca sativa genotypes. As a result of the analysis with SSR markers, the differences according to the UPGMA method were considered statistically significant at 95% sensitivity and shown in the distance matrix. As a result of this study, 23 genotypes were divided into two main groups and the genetic difference between these groups was determined as 12%. Among the Lactuca sativa genotypes, Yedikule and Eskule genotypes were genetically determined to be more distant than expected. The SSR findings gathered as a result of this study will contribute to the selection of appropriate SSR markers for future scientific studies and thus minimize the loss of time and effort for the studies.

Key words: Lactuca sativa, SSR, genetic relationship, molecular markers, lettuce

Introduction. The genus Lactuca L. is a member of the family Compositae (Asteraceae), subfamily Cichorioideae, tribe Cichorieae, subclade Lactucinae [1]. The genus is distributed in temperate and warm regions of the northern
hemisphere: Europe, Asia, Indonesia, North and Central America, Africa. The Mediterranean region and the Middle East are considered the centres of *Lactuca* biodiversity, and are thought to be the probable centre of domestication of cultivated lettuce (*Lactuca sativa*) [3–5]. *Lactuca sativa* is considered the most important vegetable in the group of leafy vegetables. It is almost exclusively used as a fresh vegetable in salads, but some forms are also cooked. Lettuce is produced commercially in many countries worldwide and is also widely grown as a vegetable in home gardens [6]. It is a widely consumed vegetable throughout the world and is produced in Turkey every month of the year. It is a cool climate plant and needs humid weather conditions. With its short vegetation period, it can be cultivated in all regions of the country [7]. There are several features that make lettuce suitable for genetic studies. It has a relatively short life cycle, it is fully self-fertile with a high rate of natural self-pollination, it is possible to carry out a large number of crosses on one plant, and individual plants require only a moderate amount of space [8].

Since the 1980s, molecular markers became frequently used in plant genetic studies and breeding programmes, thus shifting the orientation from phenotype-based genetics to genotype-based approaches. Microsatellites or simple sequence repeats (SSRs) are short, tandemly repeated motifs of DNA ubiquitous in all analyzed eukaryotic genomes [10–12]. Most of the molecular studies on lettuce have focused on the analysis of genetic variation among effective lettuce genotypes [13]. Due to the limited gene information of lettuce, ISSR (Inter Simple Sequence Repeat) and SSR (Simple Sequence Repeat) markers are generally the most suitable techniques for the analysis of lettuce diversity. SSR markers have become one of the most preferred marker systems due to their typically codominant, reproducible, cross-species transferable and extremely polymorphic qualities. Although molecular tools are less in lettuce than many types of greens, significant amounts of SSR markers have been developed in recent years [14]. The most common method for mapping genes in lettuce is genetic linkage mapping that involves generating populations derived from a single cross. Recently, the association mapping technique became a useful tool for detecting markers linked to the genes underlying the variation of a trait among cultivars. However, the main drawback of association mapping is the possibility of false-positive results due to an unrecognized population structure [15]. The best approach to avoid spurious associations in lettuce association studies is to assess the relatedness of accessions with molecular markers and to include this information into the statistical model [14]. Particularly useful for the study of population structure and demographic history of domesticated species are SSR markers because of their high level of allelic diversity [16].

This study aims to determine the differences between genotypes of some *Lactuca sativa* samples commonly grown in Turkey. In the study, 23 *Lactuca sativa* genotypes were obtained from the samples of three different companies and their molecular analyses were carried out using 14 SSR primers. DNA isolations were
carried out from young leaves of *Lactuca sativa* species grown in the greenhouse. After performing electrophoresis by performing PCR reaction, DNA band images were recorded. The phylogenetic tree was created using the data obtained from GeneTools, UPGMA and MEGA7 Programs resulting in the degree of relationship between genotypes.

**Materials and methods.** **Plant materials and DNA extraction.** In this study, a total of 23 *Lactuca sativa* genotypes obtained from DIKMEN, Syngenta, and Enza Zaden Seedling and Agriculture Company were used. DNA isolations were carried out from young leaves of *Lactuca sativa* species grown in the greenhouse. Number, name and genotype of the samples are as follows: 1. Sürpriz *Lactuca sativa* var. *crispa*; 2. Funfix *Lactuca sativa* var. *crispa*; 3. Fun-time *Lactuca sativa* var. *crispa*; 4. Festival *Lactuca sativa* var. *crispa*; 5. Armoni *Lactuca sativa* var. *crispa*; 6. Rüzgâr *Lactuca sativa* var. *crispa*; 7. Yedikule *Lactuca sativa* var. *longifolia*; 8. Eskule *Lactuca sativa* var. *longifolia*; 9. Maritima *Lactuca sativa* var. *crispa*; 10. M45B *Lactuca sativa* var. *crispa*; 11. M46B *Lactuca sativa* var. *crispa*; 12. M47 *Lactuca sativa* var. *crispa*; 13. M63 *Lactuca sativa* var. *crispa*; 14. M155 *Lactuca sativa* var. *crispa*; 15. M162 *Lactuca sativa* var. *crispa*; 16. M182 *Lactuca sativa* var. *crispa*; 17. M187 *Lactuca sativa* var. *crispa*; 18. M194 *Lactuca sativa* var. *crispa*; 19. M199 *Lactuca sativa* var. *crispa*; 20. M241 *Lactuca sativa* var. *crispa*; 21. M242 *Lactuca sativa* var. *crispa*; 22. M259 *Lactuca sativa* var. *crispa*; 23. M274 *Lactuca sativa* var. *crispa*. Samples No 2,3 were obtained from Syngenta, No 3 were obtained from Enza Zaden and the rest of the samples were obtained from Dikmen, Seedling and Agriculture Companies. Genomic DNA isolation from lettuce samples was performed using a commercial kit (Plant Genomic DNA Miniprep System, Viogene) according to Hong’s protocol \[13\]. Biospec-nano Life Science Spectrophotometer was used to determine the amount of DNA and the degree of purity. Samples with purity ranges of 1.8–2.0 nm were prepared for PCR reactions.

**PCR and electrophoresis.** Fourteen different primer sets used for each *Lactuca sativa* genotype are given in Table 1. For the amplification of 23 genotypes, 2 µl template DNA, 1 µl forward and reverse primers, 9.5 µl ddH₂O, 1.5 µl 10X Buffer, 1 µl 2.5 mM dNtp, 0.17 µl Taq Polymerase PCR components were used. All primer combinations consisted of 4 min of initial denaturation at 94°C, 35 PCR cycles of 30 s at 94°C, 30 s at 55–58°C, 45 s at 72°C and the last 6 min at 72°C extension reaction conditions in Thermo Thermal Cycler (Tc-5000) device. Three percent agarose gel was prepared for screening the PCR products. Six µL of PCR product was mixed with loading dye and loaded into the wells formed in the gel. Thermo Scientific Gene Ruler 100 bp Plus DNA Ladder was used to determine the size of PCR products. PCR products were run at 120 V 300 amperes for 150 min. Subsequently, the gel was examined with a transilluminator (Biolab Uvitec) with 312 nm wavelength UV.
**Statistical analysis.** QUANTUM ST4 program was used for PCR imaging of Lactuca sativa DNA samples. The tape images were recorded and loaded into the GeneTools automatic image analysis program to convert the obtained data into numerical values. On this program, the faulty band images were cleaned and graded by transferring to the spreadsheet as 0 (none) or 1 (var) according to the polymorphism status by looking at the data generated. The obtained genetic distance values were grouped with the UPGMA (Unweighted Pair-Group Method with Arithmetic Average) method and uploaded to the MEGA7 (Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets) program to convert the generated code into a dendrogram.

### Table 1
Primer sets used in the study

<table>
<thead>
<tr>
<th>Primer</th>
<th>Primer sequences (5′−3′)</th>
<th>T (Annealing) S (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KSL-092</td>
<td>F: GGTCTCTTTCTTCGCCCCCTG&lt;br&gt;R: TCGGGTTCTGAAAGTACCAT</td>
<td>58°C</td>
</tr>
<tr>
<td>KSL-097</td>
<td>F: CCAGAAAAAGGGATCGACCA&lt;br&gt;R: TCAGAGACACTGCAAGGGA</td>
<td>58°C</td>
</tr>
<tr>
<td>KSL-119</td>
<td>F: TTCTCTGACCTGCTGACC&lt;br&gt;R: CGATGTCACAGCCACATCT</td>
<td>57.5°C</td>
</tr>
<tr>
<td>KSL-123</td>
<td>F: ATGTAACCTCTGCGGCGCT&lt;br&gt;R: GCCCTCACCATGTTCTGCCC</td>
<td>57.5°C</td>
</tr>
<tr>
<td>KSL-137</td>
<td>F: TTCTCTAGGCTCCTACAAGAAGG&lt;br&gt;R: TCATCACCATCAGTCATCCC</td>
<td>58°C</td>
</tr>
<tr>
<td>KSL-173</td>
<td>F: ATATGCACGGCTCACTCCCA&lt;br&gt;R: CCAATTTTCTCTCTTGCA</td>
<td>58°C</td>
</tr>
<tr>
<td>KSL-271</td>
<td>F: GCAAGGGCAAGAFFGGGCT&lt;br&gt;R: GCGATATGCGCGCATAACA</td>
<td>57°C</td>
</tr>
<tr>
<td>KSL-322</td>
<td>F: CTTCCAAGGAAAATATGGA&lt;br&gt;R: TCTCCAACAAAACACCCCACC</td>
<td>57°C</td>
</tr>
<tr>
<td>KSL-7</td>
<td>F: TGTCCTAATTCGAGCCTTATCT&lt;br&gt;R: ATGTGCCAAGGAAAGACA</td>
<td>57°C</td>
</tr>
<tr>
<td>SML-002</td>
<td>F: GTGATTGCATGCCCAATG&lt;br&gt;R: TTAGTAGCCCGCATGTTT</td>
<td>55.7°C</td>
</tr>
<tr>
<td>SML-022</td>
<td>F: GGGCCTCAAATCTCTCTCTG&lt;br&gt;R: TGTCCTCCCTCTTCTGGAA</td>
<td>55.7°C</td>
</tr>
<tr>
<td>SML-028</td>
<td>F: TGGATCCAGGCTCTCAGGAAAT&lt;br&gt;R: CACGACCATTGTTGATTGTCG</td>
<td>58°C</td>
</tr>
<tr>
<td>SML-039</td>
<td>F: ATACACCCCTGGCTCATTG&lt;br&gt;R: TCGTATCTTGGCCTCTCCCAT</td>
<td>57°C</td>
</tr>
<tr>
<td>SML-057</td>
<td>F: TCCCATGAGAGAGACACTCA&lt;br&gt;R: CCCAAAGGGAAATGCAACC</td>
<td>57°C</td>
</tr>
</tbody>
</table>
Results. In this study, 23 *Lactuca sativa* genotypes were obtained from the samples of three different companies and their molecular analyses were carried out using 14 SSR primers. PCR products were run on the gel and subsequently, the gel was examined with a transilluminator. The gel images of 23 *Lactuca sativa* genotypes used in the study are presented in Fig. 1. PCR imaging of *Lactuca sativa* genotypes used in the study was provided in the QUANTUM ST4 program. The data obtained after the gel imaging process was loaded into the GeneTools automatic image analysis program to determine the locations of the bands and convert them into numerical data. Genetic distance values were classified by UPGMA (Unweighted Pair-Group Method with Arithmetic Average) clustering method in order to see the genetic differentiations of *Lactuca sativa* genotypes on a

Fig. 1. The gel images of 23 *Lactuca sativa* genotypes amplified by 14 primer sets: A) SML-028, B) SML-002, C) SML-022, D) SML-039, E) SML-057, F) KSL-092, G) KSL-097, H) KSL-119, I) KSL-123, J) KSL-137, K) KSL-173, L) KSL-7, M) KSL-322, N) KSL-271
Fig. 2. UPGMA dendrogram obtained 23 *Lactuca sativa* genotypes using 14 SSR visual graph. The MEGA7 (Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets) program was used to convert the code created with UPGMA to a dendrogram. The UPGMA dendrogram is presented in Fig. 2 and the distance matrix is shown in Fig. 3. *Lactuca sativa* genotypes were gathered under two main groups as a result of the examination of the dendrogram indicating the degree of relationships between genotypes according to the UPGMA method. Group 1 consists of Sürpriz, M274, Maritima, M47, M182, M187, M45B, M46B, M63, M162, M194, M199, M241, M242, M155 genotypes. Group 2 consists of Funfix, Funtime, Festival, Wind, Armoni, M259, Yedikule and Eskule genotypes.

**Discussion.** The nutritional problem that occurs with the rapid growth of the world population, the various problems that arise with global warming and climate change reveal the importance of plant genetic resources. The food needs
Plant gene sources and gene pools are of critical importance in terms of maintaining genetic diversity in plants for generations. Plant genetic resources should be evaluated with correct and appropriate techniques to gain agricultural and economic value and to be used in obtaining various products and characteristics. Since the 1990s, molecular studies have gained momentum as well as classical breeding methods. Molecular and biotechnological breeding methods make it possible to obtain new and efficient products suitable for the desired purpose. *Lactuca sativa* is an important vegetable that is produced and consumed all over the world today. A large number of populations have been formed as a result of the selections and studies made by the producers over the years in the places where they are grown in Turkey. Identification of these populations, which have significant genetic potential in terms of breeding, increases their genetic diversity and provides more productive and quality species. Complex and costly breeding systems lengthen the lettuce breeding period and complicate breeding studies. For this reason, molecular and biotechnological methods have become a necessity in accelerating the breeding process. This is a good property of the molecular methods used to determine the originality of new varieties, they have great importance in terms of providing defined and known material for subsequent breeding studies. Microsatellites (SSR markers) are very important in determining the diversity and relationships in plant species.

This study was conducted to reveal the relationships and genetic diversity between genotypes in *Lactuca sativa* to facilitate the work in breeding programmes and to ensure variety purity control. In this study, molecular analyses of 23 *Lactuca sativa* species, which are grown in Turkey and have commercial importance, were carried out using leaf samples collected after cultivation. After DNA isolation of leaf samples, PCR analyses were successfully performed using DNA samples with 14 SSR markers according to appropriate protocols. The gel images obtained from the experiments were analyzed in the Syngene GeneTools Program and the phylogenetic tree was obtained in the MEGA7 program according to the UPGMA algorithm.
As a result of the analysis, the differences were considered statistically significant at 95% sensitivity and are observed in the distance matrix. According to the study, 23 genotypes were divided into 2 main groups. Sürpriz, M274, Maritima, M47, M182, M187, M45B, M46B, M63, M162, M194, M199, M241, M242, M155 were found in the first group. It has been revealed that the second group includes Funfix, Funtime, Festival, Rüzgär, Armoni, M259, Yedikule and Eskule. Among the varieties, as expected, Yedikule and Eskule varieties were genetically more distant.

**Conclusion.** There are limited molecular characterization studies on *Lactuca sativa*. This study was carried out in order to increase the genetic diversity information of lettuce, which is one of the most consumed vegetables in the world, to obtain more productive species by crossbreeding with distant relatives. By the possible hybridizations to be done in the future, more productive, high temperature tolerant and disease resistant species can be obtained. For future scientific studies, researchers will be able to choose more suitable SSR markers in line with their research by the data at the genotypes and SSR markers used in this study. In this way, the loss of money, time and effort to be spent on studies will be minimized. This study contributes new aspects for the new molecular studies, it will serve as profit new *Lactuca sativa* genotypes that provide higher adaptation to the region and climate.

**REFERENCES**


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