JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal
Published by Faculty of Biotechnology and Food Sciences

GENETIC DIVERSITY ANALYSIS OF *TEUCRIUM POLIUM* POPULATIONS IN AYDIN/TÜRKİYE BASED ON RAPD-PCR

Emre Sevindik[1*], *Selin Gül Özdemir*[1], *Eda Nur Çırak*[1]

Address(es):
[1]Aydın Adnan Menderes University, Faculty of Agriculture, Department of Agricultural Biotechnology, South Campus, Cakmar, Aydin, Türkiye.

*Corresponding author: ph.d-emre@hotmail.com

https://doi.org/10.55251/jmbfs.9630

ARTICLE INFO

Received 16. 11. 2022
Revised 20. 1. 2023
Accepted 23. 1. 2023
Published 1. 4. 2023

Regular article

OPEN ACCESS

ABSTRACT

In this study, genetic diversity analysis of eight *Teucrium polium* L. populations distributed in Aydın province of Türkiye was conducted using ten RAPD markers. As a result of the study, a total of 45 bands were obtained, of which eight were monomorphic and 37 were polymorphic. The total polymorphism rate was determined as 82.22%. UPGMA dendrogram consists of two clades. The lowest similarity (-0.100) was observed between the Koçarlı and Hamitler village populations, while the highest coefficient (0.646) was observed with the Çakmar (road) and Hamitler village populations. As a result, RAPD marker technique can be a useful marker for revealing the genetic diversity of *Teucrium polium* populations. In addition, this results it will be a references for different marker and sequence-based studies in the future.

Keywords: *Teucrium polium*, genetic diversity, RAPD, Aydin

INTRODUCTION

The *Lamiaceae* family consists of 258 genera and more than 6000 species (**Valerio *et al.*, 2021**). *Lamiaceae* family, which includes medicinal plants rich in secondary metabolites, species with great economic value due to their potential as ornamental plants, culinary plants, and production of valuable bioactive compounds, contains a large number of species mostly seen in Asia and the Mediterranean (**Çelik *et al.*, 2021; Aebisher *et al.*, 2021; Gonçalves *et al.*, 2021**). The genus *Teucrium* L belongs to *Lamiaceae* family is mainly distributed in the Mediterranean region (**Sadeghi *et al.*, 2021**). *Teucrium polium* contains various components such as terpenoids, flavonoids and iridoids (**Bahramikia & Yazdanparast, 2012**). *T. polium* is a medicinal plant used in folk medicine for various purposes such as anti-inflammatory, antimicrobial, antitumour, anti-nociceptive, hypoglycemic, anti-hypertensive and anti-hyperlipedemic effects (**Gharaibeh *et al.*, 1988; Ardestani & Yazdanparast, 2007; Sevindik *et al.*, 2016; Elmasri *et al.*, 2016**). Biotechnology is known as a popular branch of science that relies on biological traits to obtain products with desired positive traits in all living organisms with genetic material (**Eren, 2021**). DNA markers have been developed to provide reliable results for genetic identification of varieties and genotypes (**Karakaya *et al.*, 2022**). The current molecular markers used in genetic variation identification are RFLP, PCR-RFLP, RAPD, ISSR, SSR, and they are preferred because they are simpler, cheaper, and faster (**Aydın & Özden, 2021**). Among molecular methods or markers, RAPD is very responsive to detecting variability among individuals of a species. The RAPD method is inexpensive and can work with limited sample sizes. Moreover, RAPD can amplify and target genomic regions with potential and diverse markers (**Vivodik *et al.*, 2022**). The RAPD-PCR technique in many plants has been used to examine genotype and population polymorphisms and genetic diversity of species (**Vierling *et al.*, 1992; Iqbal *et al.*, 1997; Sensoy *et al.*, 2007; Pinheiro *et al.*, 2012; Doğru Çokran *et al.*, 2019; Balážová *et al.*, 2021; Sekar *et al.*, 2021; Aydın & Özden, 2021; Qian & Mehri *et al.*, 2021; Bi *et al.*, 2021; Vivodik *et al.*, 2022; Yin et al., 2022; Al-Khayri *et al.*, 2022**; **etc.**). In this study, genetic relationships among some *Teucrium polium* populations distributed in Aydın province were determined using RAPD-PCR marker technique.

MATERIALS AND METHODS

Plant Materials and Genomic DNA Isolation

Eight populations of *Teucrium polium* populations were collected from different districts of Aydın province. gDNA was isolated using a commercial kit (GeneMark catalog no: DP022). Extracted genomic DNA samples were stored at -20 ºC.

PCR and agarose electrophoresis

The RAPD primers selected for PCR amplifications are given in Table 1 below.

Table 1 RAPD primer sequences, Tm, RAPD- PCR amplification conditions and amplification results

Primers	DNA sequences	Tm ºC	PCR Amplification (35 Cycles except final extension step)	Amplification
OPA-15	5'-TTCCGAACCC-3'	32	94 °C /2 min	+
OPA-20	5'-GTTGCGATCC-3'	32		+
OPA-02	5'- TGCCGAGCTG-3'	34	94 °C /1 min	+
OPA-13	5'- CAGCACCCAC-3'	34		+
OPA-16	5'-AGCCAGCGAA-3'	32	32-34 °C /1 min	-
OPA-18	5'-AGGTGACCGT-3'	32		+
OPA-05	5'-AGGGGTCTTG-3'	32	72 °C /1 min	+
OPJ-10	5'-AAGCCCGAGG-3'	32		-
OPA-03	5'-AGTCAGCCAC-3'	32	72°C/10min(final extension: 1 cycle)	-
OPA-07	5'-GAAACGGGTG-3'	32		-

PCR applications were performed using a Thermocycler Gradient device. Ready mix was used as an alternative route for the PCR reaction. Amplification was performed with 0.5 µL genomic DNA, 1 µL primers, 10 µL master mix and 8.5 µL ddH_2O in a PCR tube. The PCR programs used were generated separately for each primer in accordance with the TM values of the primers used in the studies based on referenced articles. 1.5% agarose gel electrophoresis was applied to separate the

bands formed as a result of PCR. To do this, 1.5 g agarose was weighed and dissolved in 100 mL 1 X TBE (Tris-Boric EDTA) buffer by boiling in a microwave oven. The mixture was cooled, 1.25 µL EtBr was added and casted. Then 5 µL of PCR product and 1 µL of loading dye (6X DNA loading dye) were added, stained and loaded into the wells with a micropipette. To determine the size of the PCR products, 5 µL of DNA size marker (1 kb DNA ladder) was loaded into an empty well and the samples were run at 100 volts for 90 min. The gel was then taken to the gel imaging device, the bands were observed under UV light and the data were recorded by taking photographs with the help of the gel imaging device's computer program and camera.

RAPD-PCR analysis

After RAPD-PCR analyses, DNA bands were assigned a value of "1" if DNA was present and "0" if DNA was absent, and the polymorphism rate was determined by scoring the DNA bands. The genetic diversity of the *Teucrium polium* samples used in the study was analyzed using MVSP 3.22 program (**Kovach, 200**7).

RESULTS AND DISCUSSION

As a result of RAPD-PCR analysis, 10 RAPD primers were used, six of them yielded positive results and 4 of them displayed negative results. In scoring the bands, a total of 45 bands were obtained, of which 37 were polymorphic and eight were monomorphic. Polymorphism rate was 82.22%. The genetic distance matrix between the UPGMA tree and Principal component analysis (PCA) of *Teucrium polium* populations were generated using the MVSP 3.22 analysis program. The UPGMA tree consisted of two clusters. Cluster 1 consisted of Çakmar (road) and Hamitler village populations, while cluster 2 consisted of two subgroups. Subgroup A consisted of only Didim population, while subgroup B consisted of Aydın-Centre, Danişment village, Çine (road), Koçarlı and Çakmar (mountain) populations (Figure 1).

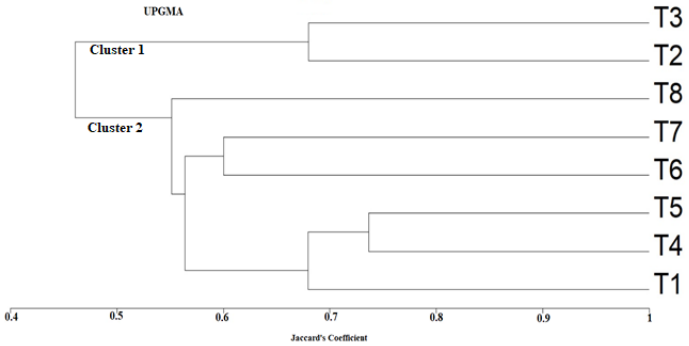

Figure 1 The UPGMA tree generated using RAPD data (T1: Çakmar (mountain), T2: Çakmar (road), T3: Hamitler village, T4: Aydın-Centre, T5: Danişment village, T6: Çine (road), T7: Koçarlı, T8: Didim populations)

Using the MVSP 3.22 program, the PCA image in Figure 2 was obtained. As a result of the analysis, three groups were obtained. The UPGMA dendrogram and PCA analysis were compatible with each other.

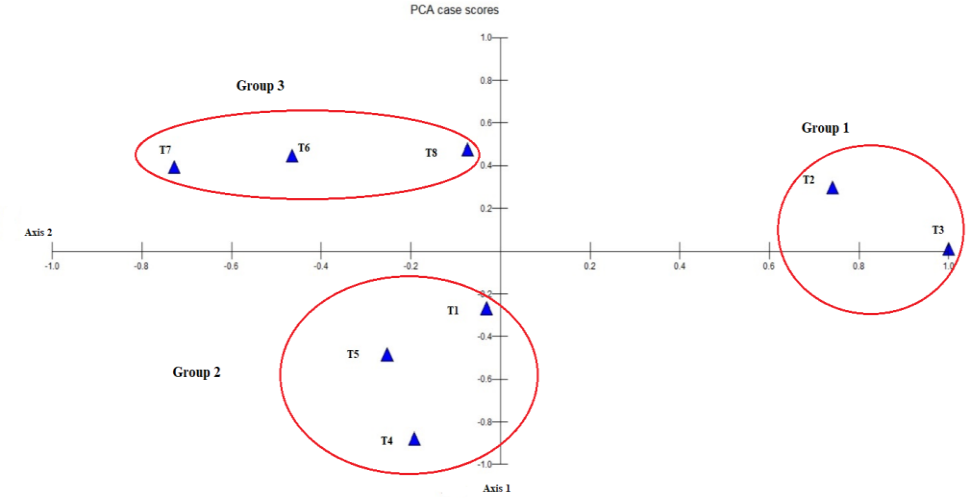

Figure 2 Principal component analyses of *Teucrium polium* populations using MVSP 3.22 software (T1: Çakmar (mountain), T2: Çakmar (road), T3: Hamitler village, T4: Aydın-Centre, T5: Danişment village, T6: Çine (road), T7: Koçarlı, T8: Didim populations)

According to RAPD molecular data, the lowest similarity (-0.100) was observed between the Koçarlı and Hamitler village populations, while the highest coefficient (0.646) was observed with the Çakmar (road) and Hamitler village populations (Table 2).

Table 2 Similarity matrix between the *Teucrium polium* populations

Populations	T1	T2	T3	T4	T5	T6	T7	T8
Çakmar (mountain)	1							
Çakmar (road)	0.409	1						
Hamitler	0.348	0.646	1					
Aydın-Centre	0.416	0.202	0.263	1				
Danişment	0.339	0.273	0.311	0.449	1			
Çine (road)	0.346	0.245	0.070	0.226	0.399	1		
Koçarlı	0.316	0.159	-0.100	0.167	0.365	0.467	1	
Didim	0.440	0.334	0.249	0.226	0.303	0.377	0.378	1

Pesaraklu *et al.* (2013) determined the genetic diversity of six *Teucrium polium* populations collected from different locations in Iran utilizing RAPD markers. In their study, 61 bands were obtained from 8 RAPD primers and they determined the polymorphism rate as 89.71%. In their study, genetic similarity between genotypes was determined between 0.33 and 0.58. **Boulila *et al.* (2010)** evaluated the genetic diversity of seven diploid and polyploid *Teucrium polium* populations in five bioclimatic areas in Tunisia utilizing RAPD markers. They obtained 141 bands in RAPD-PCR results and found 129 of them to be polymorphic. **Baghizadeh *et al.* (2017)** determined the genetic diversity of 15 *Teucrium polium* genotypes collected from Kerman province of Iran by RAPD analysis. In the study, 182 bands were obtained from 15 RAPD primers and 169 (93%) of them were polymorphic. They also determined the genetic similarity of the genotypes to be between 0.37 and 0.72. **Norouzi Ghare Tapeh *et al.* (2018)** determined the genetic diversity of 77 *Teucrium polium* populations collected from different regions of Iran using ISSR marker technique. They obtained 198 bands from 18 ISSR markers, 184 of which were polymorphic and the polymorphism rate was 92.9%. Also, Polymorphic Information Content (PIC), Shannon's Information index (I), and Number of effective alleles (Ne) have been determined as 0.39, 0.526 and 1.6, respectively. **Esfandyari *et al.* (2018)** revealed the genetic diversity of 17 *Teucrium polium* ecotypes collected from Kermanshah (Iran) with 15 ISSR primers. In their study, 82 bands were obtained from 12 ISSR primers and 80 of them were polymorphic. The average number of bands for each primer was 6.83. **Mohajer-Tabrizi *et al.* (2020)** extracted genetic diversity parameters of 16 *T. polium* populations in Albozr mountain range of Iran using ISSR technique. As a result of the study, the means of Polymorphism (%P), Nei's genetic diversity (H) and Shannon's Information Index (I) were found to be 33.24%, 0.118 and 0.179, respectively, while AMOVA analysis revealed a large genetic variation (77%) within the population and a relatively high genetic differentiation (Gst: 0.311) and gene flow (Nm: 1.107). **Farshadfar *et al.* (2020)** used the SCoT marker to determine genetic variation among 17 *Teucrium polium* genotypes collected from Kermanshah province, Iran. In their study, they obtained 48 polymorphic bands in all SCoT primers. They calculated the average PIC=0.361, MI=1.525, EMR=4.197, polymorphism percentage 98.485% and RP=2.837 indices for all primers. In the studies conducted in previous years, the polymorphism rate was high as indicated in our study.

CONCLUSION

The present study suggests novel applications for our country in many aspects. RAPD analysis revealed a high rate of polymorphism and genetic diversity in *Teucrium polium* populations. As a result of the study, 45 bands were obtained from 10 RAPD primers, and the polymorphism rate was 82.22%. In this respect, determining the molecular characteristics of *Teucrium polium* populations growing in the region will contribute to both science and future economic studies. In addition, phylogenetic relationships and genetic distances between populations will be revealed and taxonomic problems will be solved. It will also be a reference for future phylogenetic studies.

Acknowledgments This research was supported by the TUBITAK 2019/2209-A (Project no: 1919B011901957)

REFERENCES

Aebisher, D., Cichonski, J., Szpyrka, E., Masjonis, S., & Chrzanowski, G. (2021). Essential oils of seven Lamiaceae plants and their antioxidant capacity. *Molecules*, 26, 3793. https://doi.org/10.3390/molecules26133793

Al-Khayri, J.M., Mahdy, E.M.B., Taha, H.S.A., Eldomiaty, A.S., Abd-Elfattah, M.A., Abdel Latef, A.A.H., Rezk, A.A., Shehata, W.F., Almaghasla, M.I., & Shalaby, T.A. et al. (2022). Genetic and morphological diversity assessment of five kalanchoe genotypes by SCoT, ISSR and RAPD-PCR markers. *Plants, 11*, 1722. https://doi.org/10.3390/plants11131722

Ardestani, A., & Yazdanparast, R. (2007). Inhibitory effects of ethyl acetate extract of *Teucrium polium* on in vitro protein glycoxidation. *Food and Chemical Toxicology, 45*(12), 2402-2411. https://doi.org/10.1016/j.fct.2007.06.020

Aydın, A., & Özden, E. (2021). Determination of genetic variation in some teff genotypes using RAPD markers *Journal of the Institute of Science and Technology, 11*, 3496-3506. https://doi.org/10.21597/jist.1029010

Bahramikia, S., & Yazdanparast, R. (2012). Phytochemistry and medicinal properties of *Teucrium polium* L.(Lamiaceae). *Phytotherapy Research, 26*(11), 1581-1593. https://doi.org/10.1002/ptr.4617

Baghizadeh, A., Moghaddari, M., & Khaniki, G. B. (2017). Investigation of genetic and phytochemical diversity of *Teucrium polium* L. germplasm in Kaerman province by RAPD molecular marker and GC/MS method. *Iranian Journal of Medicinal and Aromatic Plants, 33*(4).

Balážová, Ž., Vivodík, M., & Gálová, Z. (2021). Assessment of RAPD polymorphism in ricin genotypes. *Journal of Microbiology, Biotechnology and Food Sciences, 2021*, 386-388. https://doi.org/10.15414/jmbfs.2016.5.4.386-388

Bi, D., Chen, D., Khayatnezhad, M., Hashjin, Z. S., Li, Z., & Ma, Y. (2021). Molecular identification and genetic diversity in *Hypericum* L.: A high value medicinal plant using RAPD markers markers. *Genetika, 53*(1), 393-405. https://doi.org/10.2298/GENSR2101393B

Boulila, A., Béjaoui, A., Messaoud, C., & Boussaid, M. (2010). Genetic diversity and population structure of *Teucrium polium* (Lamiaceae) in Tunisia. *Biochemical Genetics, 48*(1), 57-70. https://doi.org/10.1007/s10528-009-9295-6

Çelik, G., Kılıç, G., Kanbolat, Ş., Özlem Şener, S., Karaköse, M., Yaylı, N., & Karaoğlu, Ş. A. (2021). Biological activity, and volatile and phenolic compounds from five Lamiaceae species. *Flavour and Fragrance Journal, 36*(2), 223-232. https://doi.org/10.1002/ffj.3636

Doğru Çokran, B., Karadeniz, T., & İkten, H. (2019). Analysis of genetic diversity among 'misket' apple clones using AFLP, SSR and RAPD markers. *Erwerbs-Obstbau, 61*, 293–302.https://doi.org/10.1007/s10341-019-00430-8

Elmasri, W. A., Hegazy, M. E. F., Mechref, Y., & Paré, P. W. (2016). Structure-antioxidant and anti-tumor activity of *Teucrium polium* phytochemicals. *Phytochemistry Letters, 15*, 81-87. https://doi.org/10.1016/j.phytol.2015.11.007

Eren, B. (2021). Gen transfer teknolojisi ve yağ asidi kompozisyonlarına katkısı-CRSPR/Cas teknolojisi. *Avrupa Bilim ve Teknoloji Dergisi, (22)*, 300-305. https://doi.org/10.31590/ejosat.853850

Farshadfar, M. , Shirvani, H., Amjadian, M., & Noorian, A.M. (2020). Genetic variation study of *Teucrium polium* genotypes in the west of Iran using SCoT molecular markers. *Cellular and Molecular Researches (Iranian Journal of Biology), 33*(3), 392-404.

Gharaibeh, M. N., Elayan, H. H., & Salhab, A. S. (1988). Hypoglycemic effects of *Teucrium polium*. *Journal of Ethnopharmacology, 24*(1), 93-99. https://doi.org/10.1016/0378-8741(88)90139-0

Gonçalves, S., Mansinhos, I., Rodríguez-Solana, R., Pereira-Caro, G., Moreno-Rojas, J.M., & Romano, A. (2021). Impact of metallic nanoparticles on ın vitro culture, phenolic profile and biological activity of two mediterranean Lamiaceae species: *Lavandula viridis* L'Hér and *Thymus lotocephalus* G. López and R. Morales. *Molecules, 26*, 6427. https://doi.org/10.3390/molecules26216427

Esfandyari, S., Farshadfar, M., Safari, H., & Shirvani, H. (2017). Genetic variability of *Teucrium polium* ecotypes using ISSR molecular markers. *Iranian Journal of Rangelands and Forests Plant Breeding and Genetic Research, 25*(1).

Iqbal, M. J., Aziz, N., Saeed, N. A., Zafar, Y., & Malik, K. A. (1997). Genetic diversity evaluation of some elite cotton varieties by RAPD analysis. *Theoretical and Applied Genetics, 94*(1), 139-144. https://doi.org/10.1007/s001220050392

Karakaya, O., Yaman, M., Balta, F., Yilmaz, M., & Balta, M. F. (2022). Assessment of genetic diversity revealed by morphological traits and ISSR markers in hazelnut germplasm (*Corylus avellana* L.) from Eastern Black Sea Region, Turkey. *Genetic Resources and Crop Evolution*, 1-13. https://doi.org/10.1007/s10722-022-01444-0

Kovach WL (2007). MVSP-A MultiVariate Statistical Package for Windows, ver. 3.1. Kovach Computing Services, Pentraeth, Wales, U.K

Mohajer-Tabrizi, F., Asri, Y., & Mehregan, I. (2020). Population genetic structure and diversity of *Teucrium polium* in Iran using ISSR markers. *Rostaniha, 21*(2), 248-263. https://doi.org/10.22092/BOTANY.2020.351672.1215

Norouzi Ghare Tapeh, R., Bernousi, I., Fayaz Moghadam, A., & Abdollahi Mandoulakani, B. (2018). Genetic diversity and structure of Iranian *Teucrium* (*Teucrium polium* L.) populations assessed by ISSR markers. *Journal of Agricultural Science and Technology, 20*(2), 333-345.

Qian, X., & Mehri, S. (2021). Detecting DNA polymorphism and genetic diversity in a wide pistachio germplasm by RAPD markers. *Genetika, 53*(2), 783-798. https://doi.org/10.2298/GENSR2102783Q

Pesaraklu, A., Mianabadi, M., Najjar, M. B. B., Sattarian, A., & Baghizadeh, A. (2013). Genetic diversity of different populations of Iranian *Teucrium polium* L. using RAPD markers. *Iranian Journal of Rangelands and Forests Plant Breeding and Genetic Research, 21*(1).

Pinheiro, L. R., Rabbani, A. R. C., da Silva, A. V. C., da Silva Lédo, A., Pereira, K. L. G., & Diniz, L. E. C. (2012). Genetic diversity and population structure in the Brazilian *Cattleya labiata* (Orchidaceae) using RAPD and ISSR markers. *Plant Systematics and Evolution, 298*(10), 1815-1825. https://doi.org/10.1007/s00606-012-0682-9

Sadeghi, Z., Yang, J. L., Venditti, A., & Moridi Farimani, M. (2021). A review of the phytochemistry, ethnopharmacology and biological activities of *Teucrium* genus (Germander). *Natural Product Research*, 1-18. https://doi.org/10.1080/14786419.2021.2022669

Sensoy, S., Büyükalaca, S., & Abak, K. (2007). Evaluation of genetic diversity in Turkish melons (*Cucumis melo* L.) based on phenotypic characters and RAPD markers. *Genetic Resources and Crop Evolution, 54*(6), 1351-1365. https://doi.org/10.1007/s10722-006-9120-6

Sekar, S., Jayachandran, S., & Kodukkur, P. V. (2021). Identıfıcatıon of *Gymnema* specıes by random amplıfied polymorphıc DNA technıque and chloroplast *trn*K gene. *Journal of Microbiology, Biotechnology and Food Sciences*, 266-270. https://doi.org/10.15414/jmbfs.2014-15.4.3.266-270

Sevindik, E., Abacı, Z. T., Yamaner, C., & Ayvaz, M. (2016). Determination of the chemical composition and antimicrobial activity of the essential oils of *Teucrium polium* and *Achillea millefolium* grown under North Anatolian ecological conditions. *Biotechnology & Biotechnological Equipment, 30*(2), 375-380. https://doi.org/10.1080/13102818.2015.1131626

Valerio, F., Mezzapesa, G. N., Ghannouchi, A., Mondelli, D., Logrieco, A. F., & Perrino, E. V. (2021). Characterization and antimicrobial properties of essential oils from four wild taxa of Lamiaceae family growing in Apulia. *Agronomy, 11*(7), 1431. https://doi.org/10.3390/agronomy11071431

Vierling, R. A., & Nguyen, H. T. (1992). Use of RAPD markers to determine the genetic diversity of diploid, wheat genotypes. *Theoretical and Applied Genetics, 84*(7), 835-838. https://doi.org/10.1007/BF00227393

Vivodik, M., Balazova, Z., Chnapek, M., Hromadova, Z., Mikolasova, L., & Galova, Z. (2022). Molecular characterızatıon and genetıc dıversıty studıe of soybean (*Glycıne Max* L.) cultıvars usıng RAPD markers. *Journal of Microbiology, Biotechnology and Food Sciences*, e9219-e9219. https://doi.org/10.55251/jmbfs.9219

Yin, J. (2022). Evaluation of genetic variability *Rindera* using RAPD markers. *Genetika, 54*(1), 173-186. https://doi.org/10.2298/GENSR2201173Y