



Genetic Diversity *Robinia pseudoacacia* L. Growing in Drought Conditions using ISSR

P.A. Kuzmin¹, P.A. Krylov¹

10.18805/ag.DF-605

ABSTRACT

Background: The aim of this study was to evaluate the genetic polymorphism of two populations of introduced *R. pseudoacacia* growing under drought conditions using ISSR markers. The individuals of *R. pseudoacacia* from two populations grow in the Ergeninskaya Upland, where a low level of moisture supply is observed. The difference between the populations lies in the conditions of growth, with the first population having a less favorable hydrothermal regime.

Methods: ISSR markers were used to analyze the genetic structure of *R. pseudoacacia*. During this analysis, 6 out of 18 ISSR primers with a high information content were selected. Polymorphism and genetic diversity of ISSR loci in *R. pseudoacacia* were evaluated in two populations based on the obtained electropherograms using POPGENE v 1.31.

Result: The study revealed that individuals of *R. pseudoacacia* growing in less favorable conditions have the greatest polymorphism of ISSR loci. Among individuals of the first population of *R. pseudoacacia*, the highest proportion of polymorphism was observed when using ISSR primers UBC 808 and UBC 826. Comparison of the obtained results of genetic structure analysis and the vital state showed that individuals with higher polymorphism, particularly using UBC 808 and UBC 826, have a more pronounced resistance to soil moisture deficit. The obtained results can be further used for selection and developing new resistant forms of *R. pseudoacacia*.

Key words: DNA polymorphism, Genetic diversity, ISSR, *Robinia pseudoacacia* L.

INTRODUCTION

Currently, one of the tasks of population genetics is to identify the genetic diversity and features of intraspecific differentiation of woody species under arid conditions (Andablo-Reyes. *et al.*, 2023; Santos *et al.*, 2023; Firmat *et al.*, 2022; Pathirana *et al.*, 2022). Genetic diversity evaluation is carried out using various molecular markers (Sun *et al.*, 2023; Yan *et al.*, 2022; De Mori *et al.*, 2023), which allow to identify the features of intraspecific population structure and develop effective conservation measures based on them in the future (Zakhoshyi *et al.*, 2020). Moreover, based on the evaluation of genetic intraspecific diversity, individuals with economically valuable traits, including resistance to various types of stress, can be identified and used in selection. This, in turn, reduces the time required for selection activities.

Various non-specific markers, such as ISSR (Ramzan *et al.*, 2021; Abd-Dada *et al.*, 2023), RAPD (Kader *et al.*, 2022, Matsiakh *et al.*, 2023), SSR (Paliwal *et al.*, 2022; Zhao *et al.*, 2023, Nezami *et al.*, 2023; Hamm *et al.*, 2023) and others (Müller *et al.*, 2023; Vaishnav *et al.*, 2023), are used to evaluate genetic diversity of tree species, DNA barcoding (Panigrahy *et al.*, 2024) and GWAS (Banerjee *et al.*, 2023). This is due to the fact that most of woody species growing in arid areas do not have sequenced and annotated genomes (Belyaev *et al.*, 2023). A feature of marker-mediated analysis is its ability to be conducted at any stage of plant development (Novikova *et al.*, 2012). ISSR markers are used for a more informative determination of genetic diversity. ISSR markers are non-specific multi-locus markers that amplify DNA fragments of various lengths up to 5000 base pairs. These markers are located in the

¹Laboratory of Molecular Breeding and Laboratory of Genomic and Postgenomic Technologies, Federal State Budget Scientific Institution "Federal Scientific Centre of Agroecology, Complex Melioration and Protective Afforestation of the Russian Academy of Sciences" 400062, Volgograd, pr-t Universitetskij, 97, Russia.

Corresponding Author: P.A. Krylov, Laboratory of Molecular Breeding and Laboratory of Genomic and Postgenomic Technologies, Federal State Budget Scientific Institution "Federal Scientific Centre of Agroecology, Complex Melioration and Protective Afforestation of the Russian Academy of Sciences" 400062, Volgograd, pr-t Universitetskij, 97, Russia. Email: krylov-p@vfanc.ru

How to cite this article: Kuzmin, P.A. and Krylov, P.A. (2024). Genetic Diversity *Robinia pseudoacacia* L. Growing in Drought Conditions using ISSR. Agricultural Science Digest. DOI: 10.18805/ag.DF-605.

Submitted: 08-03-2024 **Accepted:** 22-06-2024 **Online:** 10-07-2024

genome between microsatellites. ISSR markers are best suited for genotyping and are effective in the absence of specific markers (Luo *et al.*, 2018).

R. pseudoacacia is a promising introduced species that enhances species diversity of woody species in arid regions. This species has several advantages in terms of adaptation to dry conditions, good vital state and functionality compared to other indigenous and introduced species (Lazarev 2020). Given the justification of the chosen research topic, the aim of this study was to evaluate the genetic polymorphism of two populations of *R. pseudoacacia*, growing in the Ergeninskaya Upland under drought conditions using ISSR markers.

MATERIALS AND METHODS

Laboratory and field studies were conducted on the basis of the FSC of agroecology RAS in the laboratory of molecular breeding from October 2022-November 2023. The research objects were two populations of *R. pseudoacacia* with 30 individuals from each population, growing in the northern part of the Ergeninskaya Upland. The study was carried out based on cluster dendrological collections (cadastre №34:34:070010:21-Kirov Forestry, №34:34:000000:122-arboretum of the FSC of agroecology RAS). The two populations differ in terms of moisture supply, geological history and relief.

The first population (RPK) was localized in the Kirov Forestry on the upland, which contributed to the formation of specific local growing conditions characterized by a low level of moisture supply. The average height of trees was 6.5 m. The second population of *R. pseudoacacia* (RPP) grew in the arboretum of the FSC of agroecology RAS, in a low relief area. The average height of trees in this population was around 8 m.

The vital state of *R. pseudoacacia* was determined visually based on the degree of damage to the assimilation apparatus and tree crowns. According to the method of V.S. Nikolaevsky (1999), the following parameters were evaluated on a ten-point scale: the number of living branches in the tree crowns (P1); the degree of foliage in the crowns (P2); the number of living (without necroses) leaves in the crowns (P3); the average amount of living leaf area (P4). Then the total score (maximum 40 points) of the condition of *R. pseudoacacia* was determined and the distribution was carried out on a scale of categories: 39-40 points-good condition; 35-38-satisfactory; less than 35 points-weakened plants.

The genomic DNA was extracted from 50 mg leaf samples using the modified cetyltrimethylammonium bromide (CTAB) method with 0.2% β -mercaptoethanol (Shestibratov *et al.* 2021). Spectrostar-Nano (BMG Labtech, Germany) was used for measuring extracted DNA concentrations in the samples. The concentrations of DNA were set up to 50 ng- μ L⁻¹ in TE-buffer and DNA was stored at -20°C for the next amplification experiments. ISSR-PCR-based reaction was performed to detect the polymorphism of *R. pseudoacacia* genotypes using the synthesized primers presented in Table 1 (CJSC Evrogen, Russia).

The ISSR-PCR-based amplification was conducted using kit qPCRmix-HS (cat: #PK145L) (CJSC Evrogen, Russia). The ISSR-PCR-based amplification was performed using kit qPCRmix-HS (cat: #PK145L, CJSC Evrogen, Russia) according to the manufacturer's instructions. The Applied Biosystems QuantStudio 5 (Thermo Fisher Scientific, USA) was used to amplify genomic DNA with ISSR markers. The conditions of ISSR-PCR amplifications included initial denaturation at 95°C for 10 min followed by 40 cycles at 95°C for 30 sec, annealing temperature for the various primers at 55°C for 30 sec (extension) and final extension at 72°C for 4 min.

The 2% agarose gel electrophoresis with TAE buffer was used to separate the results of the ISSR-PCR reactions (Al-Khayri *et al.*, 2022). The size of DNA bands on the gel was determined using a 100+bp DNA ladder (cat: #NL002, CJSC Evrogen, Russia). Ethidium bromide (Helicon, Russia) was used to stain the agarose gel, which was then visualized using a UV illuminator (VilberLourmat, France). The frequencies of polymorphisms and the number of bands produced by each primer were calculated individually.

Data were generated based on the triplicate amplification of each studied primer with consistent bands. The ISSR-based PCR loci were scored as present (1) or absent (0), each of which was treated as independent. Data were compiled in a binary (0/1) matrix using MS Excel. The binary generated data were used to calculate primer binding characteristics using POPGENE Version 1.31 (Louati *et al.*, 2019). The following parameters of genetic variability were used to estimate the population: n_a = Absolute number of alleles, N_e = Effective number of alleles Kimura and Crow (1964), h = Nei's (1973) gene diversity, I = Shannon's Information index Lewontin (1972). Nei's Original Measures of Genetic Identity and Genetic distance See Nei (1972) was also calculated between 2 populations *R. pseudoacacia*.

Quantitative data were processed using Statistica 10.0 (StatSoft, Inc. USA) and presented as median (Me) and upper and lower quartiles (Q1-Q3). Statistical significance between samples was assessed using the Mann-Whitney nonparametric test with a confidence level of $p < 0.05$.

RESULTS AND DISCUSSION

Assessment of the vital condition of trees

The evaluation of the vital state of the studied populations of *R. pseudoacacia* was conducted according to the method of Nikolaevsky, which allows determining the condition of aboveground shoots, leaf blades and the plant photosynthetic apparatus (Fig 1). The analysis of the vital state showed that individuals from the RPP population scored 39.5 points, corresponding to a good vital state. Individuals from the RPK population had a satisfactory vital state. The high scores of vital state in the RPP population were due to a greater number of living branches in the tree crowns, better foliage in the crowns, a larger number of living leaves and a larger leaf area (Fig 1A, 1B).

A decrease in the vital state of plants in the RPK population was noted due to a significant decrease in foliage coverage by 4-6 points ($p < 0.05$, $n = 30$) and the number of living leaves in the tree crown by 5.5-6 points (Fig 1C). The parameter of the amount of living leaf area also showed a significant decrease for plants from the RPK population by 1.2-1.5 points, compared to this parameter for plants from the RPP population ($p < 0.05$, $n = 30$) (Fig 1C). This indicates the presence of a morpho-physiological adaptation strategy elements for plants growing in arid conditions. The total viability score of RPP

population individuals of *R. pseudoacacia* was 39.5, which is 3.5 points higher than in the RPK population ($p < 0.05$, $n = 30$) (Fig 1D).

Table 1: Codes and sequences of ISSR.

Code	Sequence '5-3'	Primer annealing temperature (T_m , °C)
UBC808	AGA GAG AGA GAG AGA GC	50.6
UBC818	CACACACACACACACAG	53.0
UBC823	TCT CTC TCT CTC TCT CC	50.7
UBC826	ACA CAC ACA CAC ACA CC	54.7
UBC857	ACA CAC ACA CAC ACA CYG	54.0
UBC860	TGT GTG TGT GTG TGT GRA	51.5

ISSR-screening

To identify the intraspecific genetic diversity of *R. pseudoacacia*, 18 ISSR primers were tested in both populations. As a result of the testing, 6 ISSR primers were selected for their high efficiency in amplifying a larger number of genomic DNA fragments (Table 2).

During the analysis, ISSR primer UBC808 showed differences in locus polymorphism between the two *R. pseudoacacia* populations. The other 5 primers, UBC818, UBC823, UBC826, UBC857, UBC860, had similar polymorphic loci ranging from 20% to 45.5%. DNA marking of the first and second population using selected ISSR primers allowed for the identification of 33 amplified genomic DNA fragments, 14 of which were polymorphic. The number of amplified fragments varied from 3 to 11

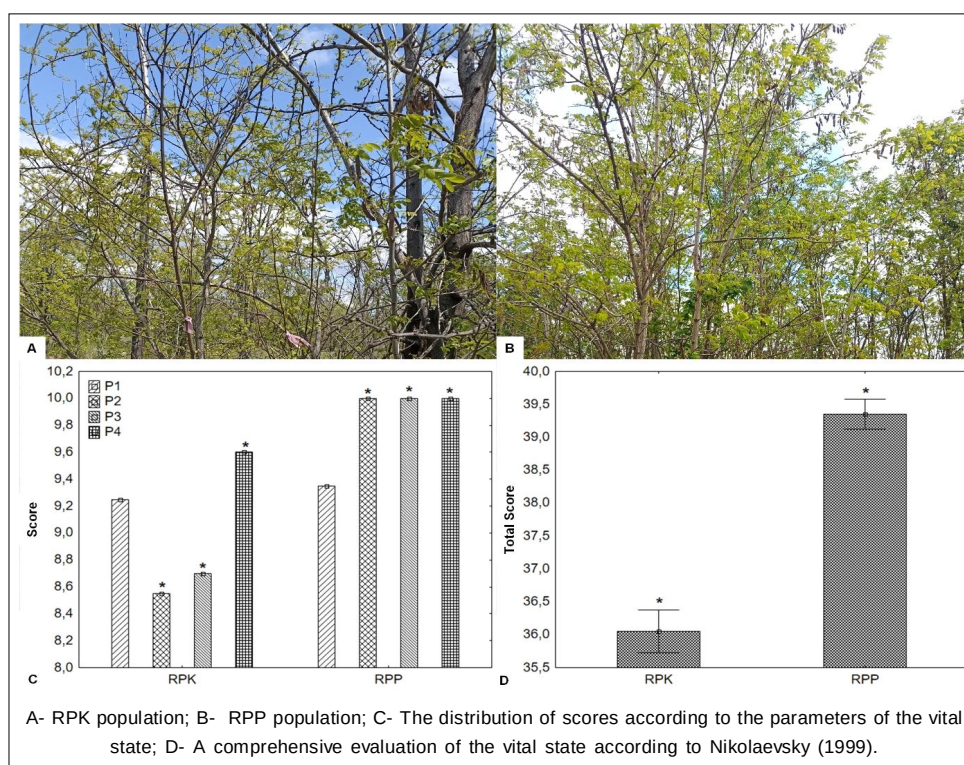


Fig 1: The appearance of individuals and the analysis of the vital state of *R. pseudoacacia*.

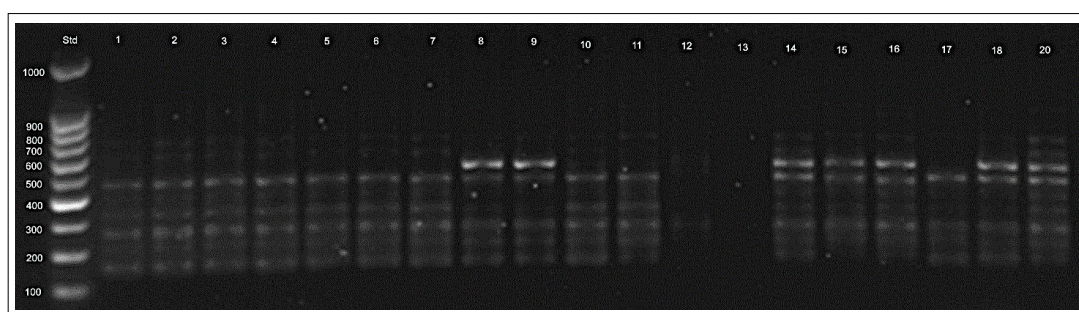


Fig 2: Example of an electrophoregram of amplification products of two populations of *R. pseudoacacia* with ISSR primer UBC826, Std marker of molecular weight (100-1000 bp), 2-10 – RPK, 11-18 – RPP.

Table 2: Evaluation of PCR amplification product of ISSR markers in populations *R. pseudoacacia*.

Primer code	Populations	Size range (bp)	Total loci	Polymorphic loci	% polymorphism
UBC808	RPK	125-850	7	3	42.9
	RPP			2	28.6
UBC818	RPK	700-1250	5	1	20.0
	RPP				
UBC823	RPK	700-950	3	1	33.3
	RPP				
UBC826	RPK	200-950	11	5	45.5
	RPP				
UBC857	RPK	500-950	4	1	25.0
	RPP				
UBC860	RPK	500-950	3	1	33.3
	RPP				

Table 3: The frequency of ISSR markers of the two studied populations of *R. pseudoacacia*.

Primer code	Size range (bp)	Frequency	
		RPK	RPP
UBC808	125	0.444	0.556
	200	0.444	0.556
	400	0.556	0.556
	500	0.333	0.444
	600	0.444	0.333
	700	0.667	0.556
	850	0.222	0.111
UBC818	700	0.111	0.333
	800	1.0	1.0
	900	0.778	0.667
	1000	0.444	0.444
	1250	0.889	0.444
UBC823	700	1.0	0.889
	900	0.778	0.289
	950	0.667	0.222
UBC826	200	0.222	0.444
	300	0.778	0.667
	400	1.0	1.0
	500	1.0	1.0
	550	0.222	0.557
	600	1.0	1.0
	650	0.556	0.556
	700	1.0	1.0
	800	1.0	1.0
	900	1.0	1.0
UBC857	950	1.0	1.0
	500	0.556	0.778
	700	0.556	1.0
	800	0.667	0.778
UBC860	950	0.778	1.0
	500	0.667	0.889
	800	0.556	1.0
	950	0.667	0.778

depending on the primer. The size of the fragments ranged from 125 to 1250 bp. The highest number of identified fragments was observed with primer UBC826. The remaining 12 primers did not amplify with the samples of isolated genomic DNA and were therefore excluded from further research. For a more detailed evaluation of the effectiveness of the used ISSR primers, the frequency of occurrence of each locus was evaluated in two populations of *R. pseudoacacia*, obtained through electrophoresis of amplicons (Table 3). Each analyzed locus of ISSR marker formed a specific spectrum of amplification products in each individual of *R. pseudoacacia* in the populations (Fig 2).

Based on the obtained amplicon spectra shown in Fig 1, binary matrices were compiled for further mathematical processing in POPGENE to evaluate the genetic diversity in two populations of *R. pseudoacacia* growing in different territories. The results of the evaluation of genetic variability parameters are presented in Table 4.

In population RPK, the average absolute number of alleles and effective number of alleles per locus were higher by 4% compared to population RPP, indicating greater individuality in genetic structure. We also observed a 12% decrease in the N_e parameter compared to N_a in both populations. The results of genetic distance evaluation according to Nei showed that the two populations have high genetic similarity. The Shannon index showed low values for both populations, indicating low inter-population diversity, while the proportion of polymorphic loci was higher in the RPK population at 72%.

Thus, 6 of the 18 primers tested are suitable for the analysis of *R. pseudoacacia* DNA polymorphism, since they can be used to identify a large number of loci and evaluate their polymorphism (Guo *et al.*, 2006). Only 30% of the primers showed effective performance. This may indicate high intraspecific polymorphism in the genetic structure of *R. pseudoacacia*. Comparative analysis of multi-locus spectra of amplified products of the studied markers in populations revealed high genetic similarity among individuals. However, plants growing in more arid conditions

Table 4: Indicators of genetic diversity in two populations of *R. Pseudoacacia*.

Populations	Na	Ne	h	I	% Polymorphic loci
RPK	1.73±0.45	1.53±0.39	0.30±0.20	0.43±0.29	72.73
RPP	1.67±0.48	1.48±0.40	0.27±0.21	0.39±0.30	66.67

N_a - Absolute number of alleles per locus; N_e - Effective number of alleles per locus; h - Nei's genetic distance; I - Shannon index; Standard deviations are given in parentheses for all the above parameters.

showed a higher degree of polymorphism, potentially enhancing their survival (Shaban *et al.*, 2022). At the same time, there is evidence that less adapted genotypes are eliminated under the pressure of stress factors (Mollashahi *et al.*, 2023). The growing conditions for the first populations of *R. pseudoacacia*, growing at a higher geographical point on the Ergeninskaya Upland, were more arid and therefore the vital state was worse than that of the second population.

The features of the relief of the Ergeninskaya Upland have influenced the formation of specific habitat conditions (Melikhova, 2022), which in turn have contributed to the formation of populations of various tree and shrub species with different degrees of genetic polymorphism. The research confirms the theory that plants with higher genetic polymorphism are more resilient and capable of surviving in arid conditions. Therefore, it is promising to use more genetically polymorphic individuals in populations for selection of tree and shrub species, especially *R. pseudoacacia*, to solve the problems of agroforestry and protective afforestation in areas with arid climates.

CONCLUSION

As a result of the research on the genetic structure of *R. pseudoacacia* using ISSR markers, it was found that individuals with a higher proportion of polymorphic loci UBC808 and UBC826 may have high resistance to water deficit. The vital state of *R. pseudoacacia* individuals is satisfactory, while populations in less arid conditions show lower polymorphism of loci and good vital state. The obtained data can be used in the future for selecting more drought-resistant *R. pseudoacacia* individuals for agroforestry and protective afforestation in arid territories.

ACKNOWLEDGEMENT

The work was carried out within the framework of the State task "Search for breeding valuable genetic material for the creation of new genotypes of tree and shrub species by molecular breeding methods" (No. FNFE-2022-0009).

Conflict of interest

All authors declare that they have no conflicts of interest.

REFERENCES

- Abd-Dada, H., Bouda, S., Khachtib, Y., Bella, Y.A., Haddioui, A. (2023). Use of ISSR markers to assess the genetic diversity of an endemic plant of Morocco (*Euphorbia resinifera* O. Berg). *Journal, Genetic Engineering and Biotechnology*. 21(1): 91. doi: 10.1186/s43141-023-00543-4.
- Al-Khayri, J.M., Mahdy, E.M.B., Taha, H.S.A., Eldomiaty, A.S., Abd-Elfattah, M.A., Abdel Latef, A. A.H. *et al.* (2022). Genetic and morphological diversity assessment of five kalanchoe genotypes by SCoT, ISSR and RAPD-PCR markers. *Plants* (Basel, Switzerland). 11(13): 1722. doi: 10.3390/plants11131722.
- Andablo-Reyes, A.D.C., Moreno-Calles, A.I., Cancio-Coyac, B.A., Gutiérrez-Coatecatl, E., Rivero-Romero, A.D., Hernández-Cendejas, G., Casas A. (2023). Agri-silvicultures of mexican arid America. *Journal of Ethnobiology and Ethnomedicine*. 19(1): 39. doi: 10.1186/s13002-023-00612-5.
- Banerjee, R., Bharti, Begum, S., Das, P., Ahmad, T. (2023). Issues and challenges of imputation techniques in genome wide association studies (GWAS): A review. *Bhartiya Krishi Anusandhan Patrika*. 38(3): 193-202. doi: 10.18805/BKAP597.
- Belyaev, A.I., Krylov, P.A., Pugacheva, A.M., Derevshikova, L.V. (2023). Analysis of the presence of the genomes of wood and shrubs plants used in agro-forest-melioration in the southern regions of Russia. *Proceedings of Lower Volga Agro-University Complex: Science and Higher Education*. 2(70): 30-42. doi: 10.32786/2071-9485-2023-02-03.
- De Mori, G., Cipriani, G. (2023). Marker-assisted selection in breeding for fruit trait improvement: A review. *International Journal of Molecular Sciences*. 24(10): 8984. doi: 10.3390/ijms24108984.
- Firmat, C., Litrico, I. (2022). Linking quantitative genetics with community-level performance: Are there operational models for plant breeding? *Frontiers in Plant Science*. 13: 733996. doi: 10.3389/fpls.2022.733996.
- Guo, W.L., Li, Y., Gong, L., Li, F., Dong, Y., Liu, B. (2006). Efficient micropropagation of robinia ambigua and detection of genomic variation by ISSR markers. *Plant Cell Tissue and Organ Culture*. 84(3): 343-351. doi: 10.1007/s11240-005-9043-5.
- Hamm, T.P., Nowicki, M., Boggess, S.L., Ranney, T.G., Trigiano, R.N. (2023). A set of SSR markers to characterize genetic diversity in all *Viburnum* species. *Scientific Reports*. 13(1): 5343. doi: 10.1038/s41598-023-31878-0.
- Kader, A., Sinha, S.N., Ghosh, P. (2022). Clonal fidelity investigation of micropropagated hardened plants of jackfruit tree (*Artocarpus heterophyllus* L.) with RAPD markers. *Journal Genetic Engineering and Biotechnology*. 20(1): 145. doi: 10.1186/s43141-022-00426-0.
- Kimura, M., Crow, J.F. (1964). The number of alleles that can be maintained in a finite population. *Genetics*. 49: 725-738.
- Lazarev, S.E. (2020). Adaptation mechanisms and life strategies of species of the Robinia L. genus under the conditions of introduction. *World Ecology Journal*. 10(1): 48-67. doi: 10.25726/worldjournals.pro/WEJ.2020.1.3.

- Louati, M., Ucarli, C., Arikan, B., Ghada, B., Hannachi, A.S., Turgut-Kara, N. (2019). Genetic, morphological and biochemical diversity of argan tree (*Argania spinosa* L.) (Sapotaceae) in tunisia. *Plants* (Basel, Switzerland). 8(9): 319. doi: 10.3390/plants8090319.
- Luo, C., Chen, D., Cheng, X., Liu, H., Li, Y., Huang, C. (2018). SSR analysis of genetic relationship and classification in chrysanthemum germplasm collection. *Horticultural Plant Journal*. 4(2): 73-82. doi: 10.1016/j.hpj.2018.01.003.
- Matsiakh, I., Menkis, A. (2023). An overview of *Phytophthora* species on woody plants in sweden and other nordic countries. *Microorganisms*. 11(5): 1309. doi: 10.3390/microorganisms11051309.
- Melikhova, A.V. (2022). Spatial analysis of protective forest plantations in the northern part of the Ergeninskaya upland. *Scientific Agronomy Journal*. 3(118): 43-48. doi: 10.34736/FNC.2022.118.3.006.43-48.
- Mollashahi, H., Urbaniak, J., Szymura, T.H., Szymura, M. (2023). Genetic structure of *Trifolium pratense* populations in a cityscape. *Peer J*. 11: e15927. doi: 10.7717/peerj.15927.
- Müller, M., Kües, U., Budde, K.B., Gailing, O. (2023). Applying molecular and genetic methods to trees and their fungal communities. *Applied Microbiology and Biotechnology*. 107(9): 2783-2830. doi: 10.1007/s00253-023-12480-w.
- Nei, M. (1972). Genetic distance between populations. *American Naturalist*. 106: 283-292.
- Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proc Natl Acad Sci USA*. 70: 73321-73323.
- Nezami, E., Gallego, P.P. (2023). History, phylogeny, biodiversity and new computer-based tools for efficient micropropagation and conservation of pistachio (*Pistacia* spp.) germplasm. *Plants* (Basel, Switzerland). 12(2): 323. doi: 10.3390/plants12020323.
- Nikolaevsky, V.S. (1999). Ecological assessment of environmental pollution and the state of terrestrial ecosystems by phytoindication methods. *MGUL*: 193 p.
- Novikova, A.A., Sheikina, O.V., Novikov, P.S., Doronina, G.U. (2012). Estimation of the ISSR-markers application for systematization and genetic certification of Genus *Rhododendron*. *Scientific Journal of KubSAU*. 82: 79-89.
- Paliwal, R., Singh, R., Choudhury, D.R., Tiwari, G., Kumar, A., Bhat, K.C., Singh, R. (2022). Molecular characterization of *tinospora cordifolia* (Willd.) Miers using novel g-SSR markers and their comparison with EST-SSR and SCoT markers for genetic diversity study. *Genes*. 13(11): 2042. doi: 10.3390/genes13112042.
- Panigrahy, A.R., More, P.M., Prashant, S., Nair, S.S., Chitnis, K.S. (2024). Biochemical analysis and DNA barcoding of millet *Echinochloa frumentacea*. *Bhartiya Krishi Anusandhan Patrika*. 38(4): 397-402. doi: 10.18805/BKAP677.
- Pathirana, R., Carimi, F. (2022). Management and utilization of plant genetic resources for a sustainable agriculture. *Plants* (Basel, Switzerland). 11(15): 2038. doi: 10.3390/plants11152038.
- Ramzan, F., Kim, H.T., Younis, A., Ramzan, Y., Lim, K.B. (2021). Genetic assessment of the effects of self-fertilization in a *Lilium* L. hybrids using molecular cytogenetic methods (Fish and ISSR). *Saudi Journal of Biological Sciences*. 28(3): 1770-1778. doi: 10.1016/j.sjbs.2020.12.019.
- Santos, A.S., Cazetta, E., Faria, D., Lima, T.M., Lopes, M.T.G., Carvalho, C.D.S., Alves-Pereira, A., Morante-Filho, J.C., Gaiotto, F.A. (2023). Tropical forest loss and geographic location drive the functional genomic diversity of an endangered palm tree. *Evolutionary Applications*. 16(7): 1257-1273. doi: 10.1111/eva.13525.
- Shaban, A.S., Arab, S.A., Basuoni, M.M., Abozahra, M.S., Abdelkawy, A.M. (2022). SCoT, ISSR and SDS-PAGE investigation of genetic diversity I and drought conditions. *International Journal of Agronomy*. 1-14.
- Shestibratov, K.A., Mescherova, E.N., Krutovsky, K.V., Baranov, O.Y., Kiryanov, P.S., Panteleev, S.V., Mozharovskaya, L.V., Padutov, V.E. (2021) Structure and phylogeny of the curly birch chloroplast genome. *Frontiers in Genetics*. 12(FEB): 625764. doi: 10.3389/fgene.2021.625764.
- Sun, J., Wang, Y., Qiao, P., Zhang, L., Li, E., Dong, W., Zhao, Y., Huang, L. (2023). *Pueraria montana* population structure and genetic diversity based on chloroplast genome data. *Plants* (Basel, Switzerland). 12(12): 2231. doi: 10.3390/plants12122231.
- Vaishnav, K., Tiwari, V., Durgapal, A., Meena, B., Rana, T.S. (2023). Estimation of genetic diversity and population genetic structure in *Gymnema sylvestre* (Retz.) R. Br. ex Schult. populations using DAMD and ISSR markers. *Journal Genetic Engineering and Biotechnology*. 21(1): 42. doi: 10.1186/s43141-023-00497-7.
- Yan, H., Qi, H., Li, Y., Wu, Y., Wang, Y., Chen, J., Yu, J. (2022). Assessment of the genetic relationship and population structure in oil-tea camellia species using simple sequence repeat (SSR) markers. *Genes*. 13(11): 2162. doi: 10.3390/genes13112162.
- Zakhoshy, I.G., Shadrin, D.M., Pylina, Y.I., Chadin, I.F., Golovko, T.K. (2020). Genetic differentiation of two phenotypes of *Plantago media* L. In South Timan. *Ecological Genetics*. 18(2): 139-148. doi: 10.17816/ecogen15605.
- Zhao, Z., Zhang, H., Wang, P., Yang, Y., Sun, H., Li, J., Chen, X., Li, J., Ji, N., Feng, H., Zhao, S. (2023). Development of SSR molecular markers and genetic diversity analysis of clematis *acerifolia* from taihang mountains. *PloS One*. 18(5): e0285754. doi: 10.1371/journal.pone.0285754.