

■ РЕФЕРАТИ СТАТЕЙ, ОПУБЛІКОВАНИХ В «CYTOLOGY AND GENETICS», № 4, 2023 р.

GENIC SSR DEVELOPMENT AND DIVERSITY ASSESSMENT OF PERSIAN HALOPHYtic GRASS, *AELUROpus LITTORALIS*

M. MEIDANSARY¹, N. NASIRI¹,
E. SHOKRI²*, H. ASKARI³

¹ Department of plant breeding and biotechnology,
Faculty of Crop Science, Sari Agricultural Sciences and Natural
Resources University (SANRU), Sari, Iran

¹ Center of Excellence in Electrochemistry, Faculty of Chemistry,
University of Tehran, Tehran, Iran

² Department of Nanotechnology, Agricultural Biotechnology
Research Institute of Iran (ABRII), Agricultural Research,
Education, and Extension Organization (AREEO), Karaj, Iran

³ Department of Biotechnology, Faculty of New Technologies
and Energy Engineering, Shahid Beheshti University (G.C), Tehran, IR Iran

E-mail: maryammeidansari@ymail.com, najmeh.nasiri@gmail.com,
e.shokri62@gmail.com, askarihossein@yahoo.com

Aeluropus littoralis is a valuable halophyte grass belonging to the same family of wheat and is used as forage. Although *A. littoralis* has the potential to become an important genetic resource for improving salt and drought tolerance in economically important crops, no SSR markers have been developed for it. The main goal was to rapidly develop a set of genic SSR markers for *A. littoralis*. Repeat analysis of non-redundant EST sequences of *Aeluropus* and transferability assessment of 110 SSR-rich loci from rice and wheat were used to identify EST-SSRs. Then selected EST-SSR loci and some physiological traits includings Na⁺, K⁺ and Ash content were utilized for marker characterization and assessment of genetic diversity among *A. littoralis* accessions collected from all around the country. The results showed that 6.7 % of EST records of *A. littoralis* comprising SSR motifs which was used for designing 18 primer pairs (ALES). In addition 48 SSR loci (GDES) from 110 of the gramineae were shown to be transferable to *A. littoralis* based on the PCR profiles. Finally, genotypic clustering based on EST-SSR markers divided the accessions into seven groups. The accessions were also categorized into six groups according

to the physiological traits. Our finding indicated that there are remarkable variations about 33 % in coding regions of Iranian *Aeluropus* accessions. The results of both genotypic and physiologic clustering were partially consistent and most groups corresponded to geographic regions.

Key words: EST-SSRs, EST Repeat analysis, Genetic variation, Transferability.

РОЗРОБКА ГЕННИХ SSR-МАРКЕРІВ ТА ОЦІНКА РІЗНОМАНІТТЯ ГАЛОФІТОВОЇ ПЕРСЬКОЇ ТРАВИ, *AELUROpus LITTORALIS*

Aeluropus littoralis – це цінна галофітова трава, яка належить до одного з пшеницею сімейства і використовується як корм для тварин. Хоча в *A. littoralis* є потенціал можливого генетичного ресурсу для покращення стійкості економічно важливих сільськогосподарських рослин до солей та посухи, для цієї рослини не було розроблено жодних SSR-маркерів. Головна мета полягала в швидкій розробці набору генних SSR-маркерів для *A. littoralis*. Для ідентифікації EST-SSR проводили повторний аналіз ненадлишкових послідовностей EST *Aeluropus* і оцінку переносимості 110 локусів рису та пшениці з багатьма SSR. Потім вибрані локуси EST-SSR та деякі фізіологічні ознаки, зокрема вміст Na⁺, K⁺ та попелу, використали для характеризування маркерів та оцінки генетичного різноманіття в ізолятах *A. littoralis*, зібраних по всій країні. Результати продемонстрували, що 6.7 % записів EST щодо *A. littoralis* містили мотиви SSR, які було використано для розробки 18 пар праймерів (ALES). Крім того, було показано можливість переносу 48 локусів SSR (GDES) із 110 від Gramineae до *A. littoralis* на основі профілів ПЛР. Зрештою генотипне кластерування на основі маркерів EST-SSR дозволило поділити ізоляти на сім груп. Також ізоляти було категоризовано в шість груп за фізіологічними ознаками. Наши результати продемонстрували значне різноманіття (блізько 33 %) кодуючих участків ізолятів іранських рослин *Aeluropus*. Результати генотипного та фізіологічного кластерування частково узгоджувалися між собою і більшість груп відповідала географічним регіонам.

Ключові слова: EST-SSR, повторний аналіз EST, генетична варіація, переносимість.

© ІНСТИТУТ КЛІТИННОЇ БІОЛОГІЇ ТА ГЕНЕТИЧНОЇ
ІНЖЕНЕРІЇ НАН УКРАЇНИ, 2023

REFERENCES

- Ahmadi J, Fotokian MH (2011) Identification and mapping of quantitative trait loci associated with salinity tolerance in rice (*Oryza sativa*) using SSR markers. *Iran J Biotech* 9(1):21–30.

Arbeiter AB, Hladnik M, Jaklje J, Bandelj D (2017) Identification and validation of novel EST-SSR markers in olives. *Sci Agri* 74(3):215–225. <https://doi.org/10.1590/1678-992x-2016-0111>.

Ashraf J, Malik W, Iqbal MZ, Ali KA, Qayyum A, Noor E, Abid MA, Naseer CH, Ahmad MQ (2016) Comparative analysis of genetic diversity among bt cotton genotypes using est-ssr, issr and morphological markers. *J Agr Sci Tech* 18(2):517–531.

Barhoumi Z, Djebali W, Abdelly C, Charbi W, Smaoui A (2008) Ultrastructure of *Aeluropus littoralis* leaf salt glands under NaCl stress.. *Protoplasma* 233(3–4):195–202. <https://doi.org/10.1007/s00709-008-0003-x>.

Chai L, Biswas MK, Yi H, Guo W, Deng X (2013) Transferability, polymorphism and effectiveness for genetic mapping of the Pummelo (*Citrus grandis Osbeck*) EST-SSR markers. *Sci Horti-Amsterdam* 155:85–91. <https://doi.org/10.1016/j.scienta.2013.02.024>.

Cortese LM, Honig J, Miller C, Bonos SA (2010) Genetic diversity of twelve switchgrass populations using molecular and morphological markers. *Bioenerg Res* 3(3):262–271. <https://doi.org/10.1007/s12155-010-9078-2>.

Dellaporta SL, Wood J, Hicks JB (1983) A plant DNA minipreparation: version II. *Plant Mol Biol Rep* 1(4):19–21 <https://doi.org/10.1007/BF02712670>.

Ebrahimi S, Seyed TB, Sharif NB (2010) Microsatellite isolation and characterization in pomegranate (*Punica granatum* L.). *Iran J Biotech* 8(3):156–163.

EST-SSR markers. *Genome* 60(3):193–200. <https://doi.org/10.1139/gen-2016-0116>.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131(2):479–491.

García-Gymez B, Razi M, Salazar JA, Prudencio AS, Ruiz D, Dondini L, Martínez-Gymez P (2018) Comparative Analysis of SSR Markers Developed in Exon, Intron, and Intergenic Regions and Distributed in Regions Controlling Fruit Quality Traits in *Prunus* Species: Genetic Diversity and Association Studies. *Plant Mol Biol Rep* 36(1):23–35. <https://doi.org/10.1007/s11105-017-1058-7>.

Jiang Y, Li H, Zhang J, Xiang J, Cheng R, Liu G (2018) Whole Genomic EST-SSR Development Based on High-Throughput Transcript Sequencing in Proso Millet (*Panicum miliaceum*). *Int J Agric Biol* 20(3):617–620. <https://doi.org/10.1795/IJAB/15.0531>.

Cui HR (2006) Analysis of SSR information in EST resource of tea plants (*Camellia sinensis*). *Bull Sci Technol* 4:471–476.

Jo WS, Kim HY, Kim KM (2017) Development and characterization of polymorphic EST based SSR markers in barley (*Hordeum vulgare*). *3 Biotech* 7(4):265. <https://doi.org/10.1007/s13205-017-0899-y>.

Kantety RV, LaRota M, Matthews DE, Sorrells ME (2002) Data mining for simple sequence repeats in expressed sequence tags from barley, maize, rice, sorghum and wheat. *Plant Mol Biol Rep* 48(5):501–510. <https://doi.org/10.1023/A:1014875206165>.

Khodashenas M (2008) *Aeluropus peterganicus* (Poaceae), a new species from Iran. *Iran J Bot* 14(1):13–15.

Khodashenas M (2009) Two new records and a new combination of the genus *Aeluropus* Trin (Poaceae) for the flora of Iran. *Iran J Bot* 15(1):61–62.

Liu C, Fan B, Cao Z, Su Q, Wang YA, Zhang Z, Wu J Tian J (2016) A deep sequencing analysis of transcriptomes and the development of EST-SSR markers in mungbean (*Vigna radiata*). *J Genet* 95(3):527–35. <https://doi.org/10.1007/s12041-016-0663-9>.

Ma JQ, Ma CL, Yao MZ, Jin JQ, Wang ZL, Wang XC, Chen L (2012) Microsatellite markers from tea plant expressed sequence tags (ESTs) and their applicability for cross-species/genera amplification and genetic mapping. *Sci Hortic Amsterdam* 134(1):167–175. <https://doi.org/10.1016/j.scienta.2011.10.029>.

Mohammadzadeh F, Monirifar H, Saba J, Valizadeh M, Haghghi AR, Zanjani BM, Barghi M, Tarhriz (2011) Genetic variation among Iranian alfalfa (*Medicago sativa* L.) populations based on RAPD markers. *Bangl J Plant Taxon* 18(2):93–104. <https://doi.org/10.3329/bjpt.v18i2.9296>.

Nasiri N, Shokri E, Nematzadeh GA (2012) *Aeluropus littoralis* NaCl-induced vacuolar H⁺-ATPase Subunit c: Molecular cloning and expression analysis. *Russ J Genet* 48(12):1199–1206. <https://doi.org/10.1134/S1022795412080054>.

Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. *P Natl Acad Sci USA* 76(10):5269–5273.

Parthiban S, Govindaraj P, Senthilkumar S (2018) Comparison of relative efficiency of genomic SSR and EST-SSR markers in estimating genetic diversity in sugarcane. *3 Biotech* 8(3):144–150. <https://doi.org/10.1007/s13205-018-1172-8>.

Rahemi A, Fatahi R, Ebadi A, Taghavi T, Hassani D, Gradziel T, Folta K, Chaparro J (2012) Genetic diversity of some wild almonds and related *Prunus* species revealed by SSR and EST-SSR molecular markers. *Plant Syst Evol* 298(1):173–192. <https://doi.org/10.1007/s00606-011-0536-x>.

- Rohlf FJ (1993) NTSYS-pc – Numeric taxonomy and multivariate analysis system.

Salimi H, Bahar M, Mirlohi A, Talebi M (2016) Assessment of the genetic diversity among potato cultivars from different geographical areas using the genomic and EST microsatellites. *Iran J Biotech* 14(4) 270. <https://doi.org/10.15171/ijb.1280>.

Shamasbi FV, Nasiri N, Shokri E (2018) Genetic Diversity of Persian Ecotypes of Indian Walnut (*Aeluropus littoralis* (Gouan) Pari.) by AFLP and ISSR Markers. *Cytol Genet* 52(3) 222–230. <https://doi.org/10.3103/S0095545271803012X>.

Singh R K, Jena S N, Khan S, Yadav S, Banarjee N, Raghuvanshi S, Bhardwaj V, Dattamajumder SK, Kapur R, Solomon S, Swapna M (2013) Development, cross-species/genera transferability of novel EST-SSR markers and their utility in revealing population structure and genetic diversity in sugarcane. *Gene* 524(2):309–29. <https://doi.org/10.1016/j.gene.2013.03.125>.

Ukoskit K, Posudsavang G, Pongsiripat N, Chatwachirawong P, Klomsa-ard P, Poomipant P, Tragoonrung S (2019) Detection and validation of EST-SSR markers associated with sugar-related traits in sugarcane using linkage and association mapping. *Genomics* 111(1):1–9. <https://doi.org/10.1016/j.ygeno.2018.03.019>.

Wang IJ, Glor RE, Losos JB (2013) Quantifying the roles of ecology and geography in spatial genetic divergence. *Ecol Lett* 16(2):175–182. <https://doi.org/10.1111/ele.12025>.

Wang J, Chen Z, Jin S, Hu Z, Huang Y, Diao Y (2017) Development and characterization of simple sequence repeat (SSR) markers based on a full-length cDNA library of Napier Grass (*Pennisetum purpureum* Schum). *Gene Genom* 39(12):1297–1305. <https://doi.org/10.1007/s13258-017-0536-5>.

Wang ML, Dzievit M, Chen Z, Morris JB, Norris JE, Barkley NA, Tonnis B and Pederson GA, Yu J (2016) Genetic diversity and population structure of castor (*Ricinus communis* L.) germplasm within the US collection assessed with

Watson L, Dallwitz MJ (1992) The grass genera of the world. Wallingford: CAB International, 1038 pp. <https://doi.org/10.1017/S0021859600076668>.

Wu BD, Fan R, Hu LS, Wu HS, Hao CY (2016) Genetic diversity in the germplasm of black pepper determined by EST-SSR markers. *Genet Mol Res* 15(1):8099. <https://doi.org/10.4238/gmr.15018099>.

Yang ZJ, Peng ZS, Yang H (2016) Identification of novel and useful EST-SSR markers from de novo transcriptome sequence of wheat (*Triticum aestivum* L.). *Genet Mol Res* 15(1):15017509. <https://doi.org/10.4238/gmr.15017509>.

Younesi-Melerdi E, Nematzadeh G, Shokri E (2014) Codon bias patterns in photosynthetic genes of halophytic grass *Aeluropus littoralis*, *J Plant Mol Breed* 2(1):12–20. <https://doi.org/10.22058/JPMB.2014.8425>.

Yu JK, Dake TM, Singh S, Benschoter D, Li W, Gill B, Sorrells ME (2004) Development and mapping of EST-derived simple sequence repeat markers for hexaploid wheat. *Genome* 47(5):805–818. <https://doi.org/10.1139/g04-057>.

Yu JK, LaRota M, Kantety RV, Sorrells ME (2004) EST derived SSR markers for comparative mapping in wheat and rice. *Genome* 271(6):742–751. <https://doi.org/10.1007/s00438-004-1027-3>.

Zhou Q, Luo D, Ma L, Xie W, Wang Y, Wang Y, Liu Z (2016) Development and cross-species transferability of EST-SSR markers in Siberian wildrye (*Elymus sibiricus* L.) using Illumina sequencing. *Sci Rep* 6: 20549. <https://doi.org/10.1038/srep20549>.

Zhu Y, Hao Y, Wang K, Wu C, Wang W, Qi J, Zhou J (2009) Analysis of SSRs information and development of SSR markers from walnut ESTs. *Int J Fruit Sci* 26(3):394–398.

Zouari N, Saad RB, Legavre T, Azaza J, Sabau X, Jaoua M, Masmoudi K, Hassairi A (2007) Identification and sequencing of ESTs from the halophyte grass *Aeluropus littoralis*. *Gene* 404(1–2):61–69 <https://doi.org/10.1016/j.gene.2007.08.021>.

Received June 19, 2021

Received October 25, 2021

Accepted July 18, 2023