

Genetic diversity assessment of *Lotus corniculatus* L. using SCoT and DAMD molecular markers

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Abstract

Lotus corniculatus L., commonly known as bird's-foot trefoil (BFT), is a perennial forage legume of high agronomic value due to its ability to fix atmospheric nitrogen and its tolerance to diverse pedoclimatic conditions. In this study, 14 genotypes of *L. corniculatus* (LV-01 to LV-14), developed and maintained at the Agricultural Research and Development Station Lovrin (SCDA Lovrin, Romania), were evaluated. Genetic diversity was assessed using two types of molecular markers, namely DAMD (Directed Amplification of Minisatellite DNA) and SCoT (Start Codon Targeted) markers. The results revealed a high level of genetic variation among the analyzed genotypes. According to the results, SCoT primers amplified DNA fragments, resulting in a higher number of total bands (10.2) and polymorphic bands (8.4) compared to DAMD markers, which generated 8.2 total bands and 4 polymorphic bands. Among the two types of markers used, SCoT1 primer proved to be the most efficient, generating 20 bands, of which 19 were polymorphic, indicating a high discriminatory power for genetic diversity ($R_p = 11.71$). On the other hand, the best-performing DAMD markers was the DAMD01 primer, which produced a lower number of bands (16), with only 9 being polymorphic. However, SCoT and DAMD markers provide a more comprehensive genomic overview when combined. SCoT and DAMD markers target distinct regions of the genome, implying that a reliable assessment of genetic diversity requires the use of markers that amplify different genomic regions.

Keywords: *data reproducibility, genetic variation, genotype clustering, marker efficiency, phylogenetic relationships, sustainable breeding*

References:

1. Ammar MH, Alghamdi SS, Migdadi HM, Khan MA, El-Harty EH, Al-Faifi SA (2015). Assessment of genetic diversity among faba bean genotypes using agro-morphological and molecular markers. Saudi Journal of Biological Sciences 22(3):340-350. <https://doi.org/10.1016/j.sjbs.2015.02.005>
2. Bidyananda N, Jamir I, Nowakowska K, Varte V, Vendrame WA, Devi RS, Nongdam P (2024). Plant genetic diversity studies: Insights from DNA marker analyses. International Journal of Plant Biology 15(3):607-640. <https://doi.org/10.3390/ijpb15030046>
3. Bostan C, Horablaga NM, Boldea M, Onișan E, Istrate-Schiller C, Rechitean D, ... Samfira I (2025). Genetic variation and trait correlations in Lotus corniculatus L. as a basis for sustainable forage breeding. Sustainability 17(15):7007. <https://doi.org/10.3390/su17157007>
4. Cannon S (2008). Legume comparative genomics. In: Stacey G (Eds). Genetics and genomics of soybean. Plant genetics and genomics: Crops and models, vol 2. Springer, New York, NY pp 35-54. https://doi.org/10.1007/978-0-387-72299-3_3
5. Collard BCY, Mackill DJ (2008). Marker-assisted selection: An approach for precision plant breeding in the twenty-first century. Philosophical Transactions of the Royal Society B 363(1491):557-572. <https://doi.org/10.1098/rstb.2007.2170>
6. de Lourdes Corral-Bermudez M, Sánchez-Ortiz E, Álvarez-Bernal D, Gutiérrez-Montenegro MO, Cassio-Madrado E (2019). Scenarios of availability of water due to over-exploitation of the aquifer in the basin of Laguna de Santiaguillo, Durango, Mexico. PeerJ 7:e6814. <https://doi.org/10.7717/peerj.6814>
7. Dwivedi SL, Sahrawat KL, Upadhyaya HD, Mengoni A, Galardini M, Bazzicalupo M, ... Ortiz R (2015). Advances in host plant and rhizobium genomics to enhance symbiotic nitrogen fixation in grain legumes. Advances in Agronomy 129:1-116. <https://doi.org/10.1016/bs.agron.2014.09.001>
8. Fariaszewska A, Aper J, Van Huylenbroeck J, De Swaef T, Baert J, Pecio Ł (2020). Physiological and biochemical responses of forage grass varieties to mild drought stress under field conditions. International Journal of Plant Production 14(2):335-353. <https://doi.org/10.1007/s42106-020-00088-3>
9. Pederson GA (2000). Temperate forage legumes. J. Frame, J. F. L. Charlton, A. S. Laidlaw. The Quarterly Review of Biology 2000 75(1):60-60. <https://doi.org/10.1086/393300>
10. Gao R, Li Y, Shan X, Wang Y, Yang S, Ma S, ... Cronk Q (2025). A single nucleotide polymorphism affects protein translation and leads to post-anthesis color change

- variation in closely related Lotus species. The Plant Journal 121(1):e17188. <https://doi.org/10.1111/tpj.17188>
11. Heath DD, Lwama GK, Devlin RH (1993). PCR primed with VNTR core sequences yields species-specific patterns and hypervariable probes. Nucleic Acids Research 21(24):5782-5785. <https://doi.org/10.1093/nar/21.24.5782>
 12. Hughes AR, Inouye BD, Johnson MT, Underwood N, Vellend M (2008). Ecological consequences of genetic diversity. Ecology Letters 11(6):609-623. <https://doi.org/10.1111/j.1461-0248.2008.01179.x>
 13. Işık Mİ, Türkoğlu A, Demirel F, Aydın A, Eren B, Koç A, Armağan M, Haliloğlu K, Yaman M, Bocianowski J (2025). Genetic diversity and genetic structure of alfalfa (Medicago sativa L.) genotypes as revealed by Start Codon Targeted (SCoT) markers. Genetic Resources and Crop Evolution 72(8):10543-10558. <https://doi.org/10.1007/s10722-025-02587-6>
 14. Lee S, Yun J-M, Lee JY, Hong G, Kim J-S, Kim D, Han J-G (2021). The remediation characteristics of heavy metals on applying recycled food waste ash and electrokinetic remediation techniques. Applied Sciences 11(16):7437. <https://doi.org/10.3390/app11167437>
 15. Pakseresht F, Talebi R, Karami E (2013). Comparative assessment of ISSR, DAMD and SCoT markers for evaluation of genetic diversity in chickpea (Cicer arietinum L.) genotypes collected from north-west of Iran. Physiology and Molecular Biology of Plants 19(3):435-445. <https://doi.org/10.1007/S12298-013-0181-7>
 16. Petolescu C, Sarac I, Popescu S, Tenche-Constantinescu AM, Petrescu I, Camen D, ... Onisan E (2024). Assessment of genetic diversity in alfalfa using DNA polymorphism analysis and statistical tools. Plants 13(20):2853. <https://doi.org/10.3390/plants13202853>
 17. Promega (2025). Wizard genomic DNA purification kit technical manual TM050. Retrieved January 10 from <https://ita.promega.com/-/media/files/resources/protocols/technical-manuals/0/wizard-genomic-dna-purification-kit-protocol.pdf>
 18. Rai MK (2023). Start codon targeted (SCoT) polymorphism marker in plant genome analysis: Current status and prospects. Planta 257(2):34. <https://doi.org/10.1007/s00425-023-04067-6>
 19. Saleh B (2019). Molecular characterization using directed amplification of minisatellite-region DNA (DAMD) marker in Ficus sycomorus L. (Moraceae). The Open Agriculture Journal 13:74-81. <https://doi.org/10.2174/1874331501913010074>
 20. Samuil C, Stavarache M, Sirbu C, Vintu V (2018). Influence of sustainable fertilization on yield and quality food of Mountain Grassland. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 46(2):410-417. <https://doi.org/10.15835/nbha46210660>
 21. Schulman AH (2007). Molecular markers to assess genetic diversity. Euphytica 158(3):313-321. <https://doi.org/10.1007/s10681-006-9282-5>

22. Smýkal P, Coyne CJ, Ambrose MJ, Maxted N, Schaefer H, Blair MW, ... Varshney RK (2014). Legume crops phylogeny and genetic diversity for science and breeding. *Critical Reviews in Plant Sciences* 34(1-3):43-104. <https://doi.org/10.1080/07352689.2014.897904>
23. Sujkowska-Rybkowska M, Banasiewicz J, Rekosz-Burlaga H, Stępkowski T (2020). Anthyllis vulneraria and Lotus corniculatus on calamine heaps form nodules with Bradyrhizobium liaoningense-related strains harboring novel in Europe symbiotic nifD haplotypes. *Applied Soil Ecology* 151:103539. <https://doi.org/10.1016/j.apsoil.2020.103539>
24. Tekin F, Altın İ, Aydın F, Alkan M, Orel DC, Ardiç M, ... Özer G (2024). Unveiling genetic diversity among bacterial isolates using SCoT markers. *Current Microbiology* 81:409. <https://doi.org/10.1007/s00284-024-03938-9>
25. Tenche-Constantinescu AM, Lalescu DV, Popescu S, Sarac I, Petrescu I, Petolescu C, ... Onisan E (2024a). Exploring the genetic landscape of Tilia spp. with molecular and statistical tools. *Horticulturae* 10(6):596. <https://doi.org/10.3390/horticulturae10060596>
26. Tenche-Constantinescu AM, Lalescu DV, Popescu S, Sarac I, Petolescu C, Camen D, ... Onisan E (2024b). Juglans regia as urban trees: Genetic diversity and walnut kernel quality assessment. *Horticulturae* 10(10):1027. <https://doi.org/10.3390/horticulturae10101027>
27. Vântu V, Samuil C, Rotar I, Moisuc A, Razec I (2011). Influence of the management on the phytocoenotic biodiversity of some Romanian representative grassland types. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 39(1):119-125. <https://doi.org/10.15835/nbha3915867>
28. Vörösmarty CJ, McIntyre PB, Gessner MO, Dudgeon D, Prusevich A, Green P, ... Davies P (2010). Global threats to human water security and river biodiversity. *Nature* 467(7315):555-561. <https://doi.org/10.1038/nature09440>
29. Wang B, Lin Z, Li X, Zhao Y, Zhao B, Wu G, ... Wang H (2020). Genome-wide selection and genetic improvement during modern maize breeding. *Nature Genetics* 52(6):565-571. <https://doi.org/10.1038/s41588-020-0616-3>
30. Yang W, Guo Z, Huang C, Duan L, Chen G, Jiang N, ... Xiong L (2014). Combining high-throughput phenotyping and genome-wide association studies to reveal natural genetic variation in rice. *Nature Communications* 5:5087. <https://doi.org/10.1038/ncomms6087>