Research Article

Genetic Diversity and Population Structure Reveal Signatures of Local Adaptation in *Melilotus indicus* (L.) All Populations from Arid Regions of Pakistan

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Received | September 24, 2026; **Accepted** | October 16, 2026; **Published** | November 14, 2026
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Citation | Sana, H., N. Ullah, S. Ali, M.A. Khan, A. Shehzad, M. Khan, N.U. Khan. 2026. Genetic diversity and population structure reveal signatures of local adaptation in *Melilotus indicus* (L.) all populations from arid regions of Pakistan. *Journal of Plant Health, Ecology and Evolution*, 2(1): 81-91.
DOI | https://dx.doi.org/10.17582/journal.jphee/2026/2.1.81.91
Keywords | *Melilotus indicus*, SSR markers, Genetic diversity, Population structure, Local adaptation, Arid ecosystems, Pakistan

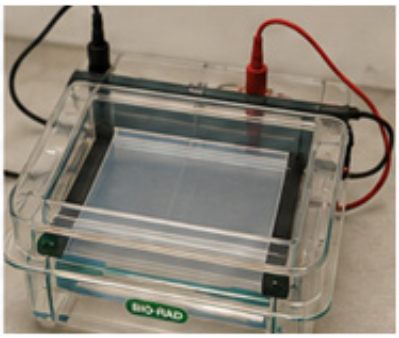
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Supplementary Table S1: *Summary of SSR primer performance: total primers tested, primers producing clear reproducible amplification, primers excluded, and total loci analysed.*

Description	Number
Total SSR primers tested	15
Primers producing clear and reproducible patterns	13
Primers with weak/inconsistent amplification (excluded)	2
Total loci analyzed	13

Supplementary Table S2: *PCR thermal cycling conditions used for amplification of SSR markers in Melilotus indicus (right panel), alongside the thermal cycler and gel electrophoresis apparatus used.*

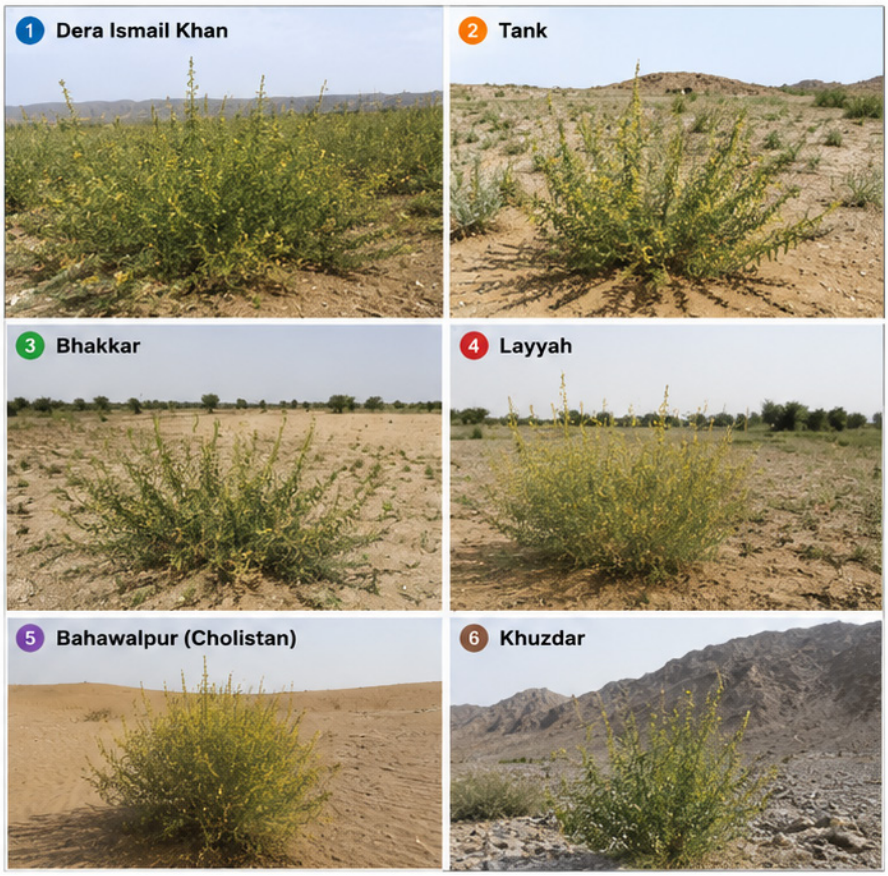




Step	Temperature	Time	Cycles
Initial denaturation	94 °C	4 min	1
Denaturation	94 °C	30 sec	30–35
Annealing	Ta (primer)	30 sec	
Extension	72 °C	45 sec	
Final extension	72 °C	7 min	1
Hold	4 °C	∞	1

Supplementary Table S3: *Environmental characteristics (altitude, rainfall, temperature, soil pH, electrical conductivity, organic matter) of representative sampling sites (S1–S8), corresponding to* **Figure 2**.

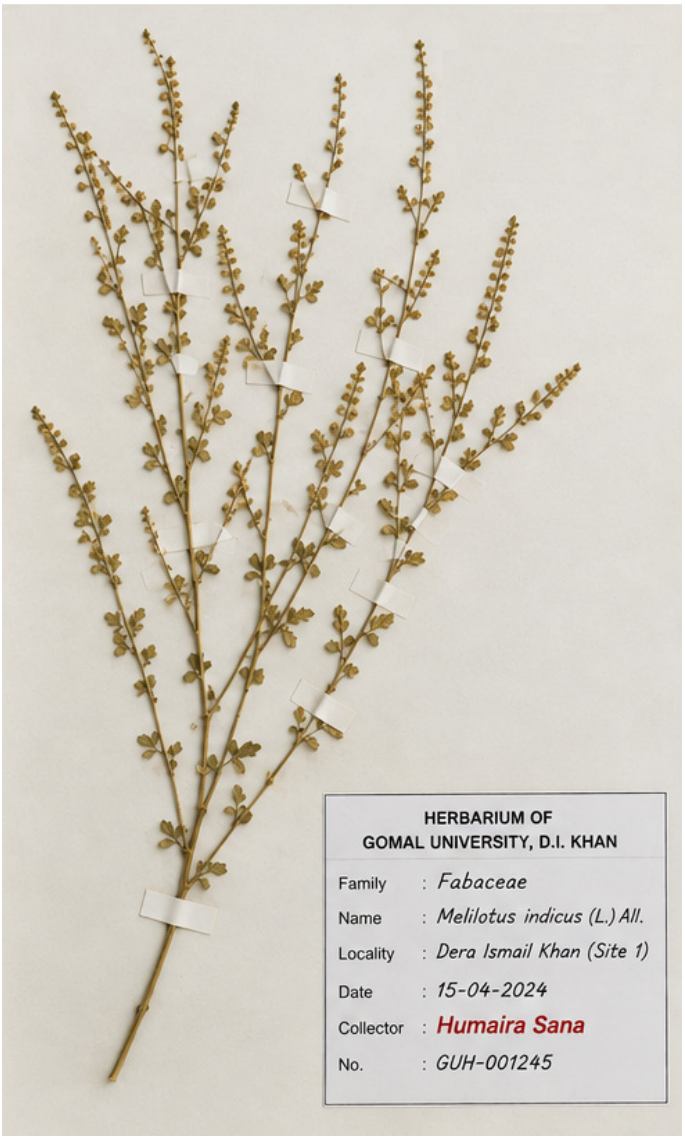
Site	Location	Altitude (m a.s.l.)	Annual rainfall (mm)	Mean annual temperature (°C)	Soil pH	Electrical conductivity (dS m^{-1})	Organic matter (%)
S1	Dera Ismail Khan	165	315	31.6	8.5	0.48	1.08
S2	Taunsa Sharif	132	263	30.8	8.2	0.81	0.74
S3	Tank	610	142	25.2	7.6	1.76	0.39
S4	Bhakkar	138	198	29.4	7.8	1.23	0.63
S5	Paharpur	210	128	26.1	7.3	2.12	0.32
S6	D.G. Khan	145	204	30.0	7.9	1.58	0.88
S7	Kallur Kot	298	92	24.8	7.4	2.61	0.34
S8	Jatoi	176	167	27.3	8.1	0.93	0.57



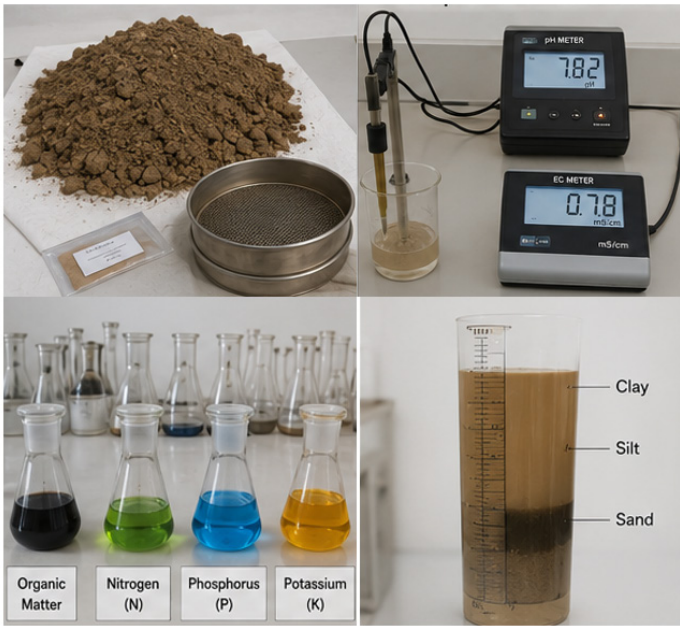
Supplementary Figure S1: *Representative field photographs of Melilotus indicus populations collected from Dera Ismail Khan, Tank, Bhakkar, Layyah, Bahawalpur (Cholistan), and Khuzdar.*



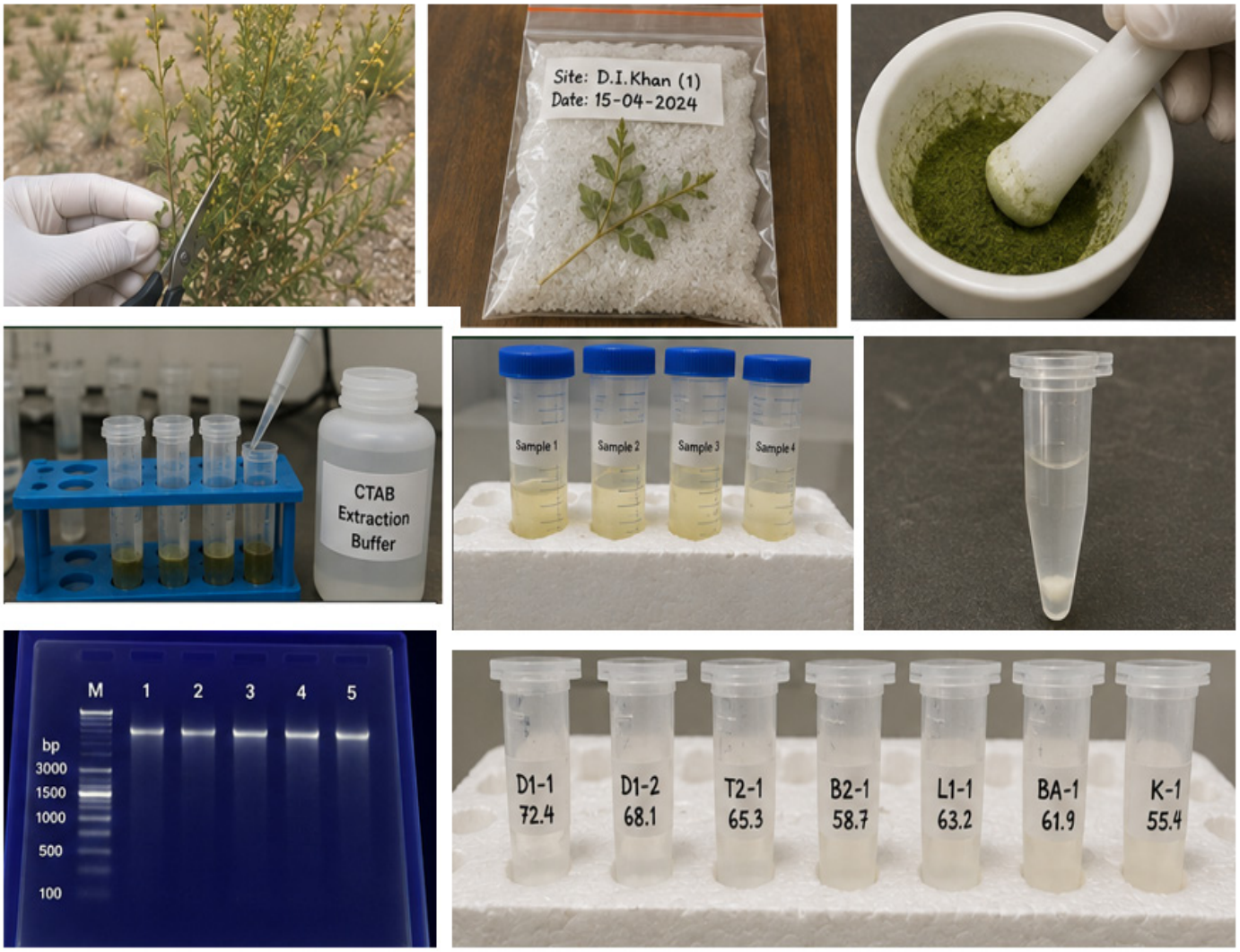
Supplementary Figure S2: *Field collection and preparation of Melilotus indicus herbarium specimens, showing plant pressing, drying, and mounting.*



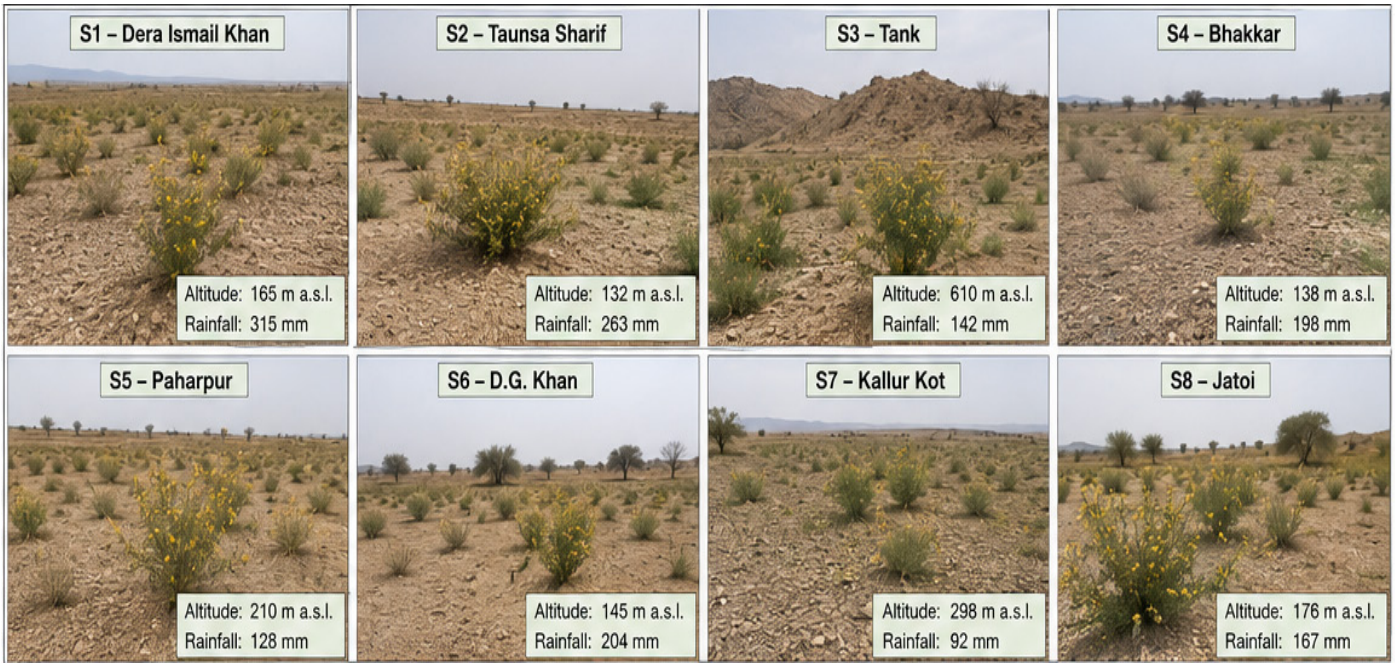
Supplementary Figure S3: *Authenticated herbarium voucher specimen of Melilotus indicus deposited in the Herbarium, Institute of Biological Sciences, Gomal University, Dera Ismail Khan, Pakistan.*



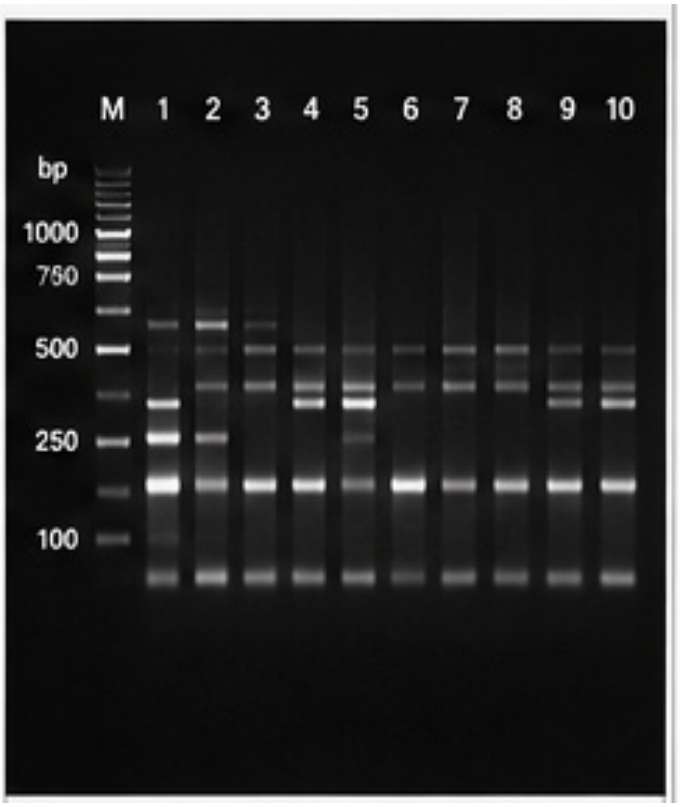
Supplementary Figure S4: *Soil sampling and physicochemical analysis procedures (pH, electrical conductivity, organic matter, nitrogen, phosphorus, potassium, and texture) for the sampled M. indicus sites.*



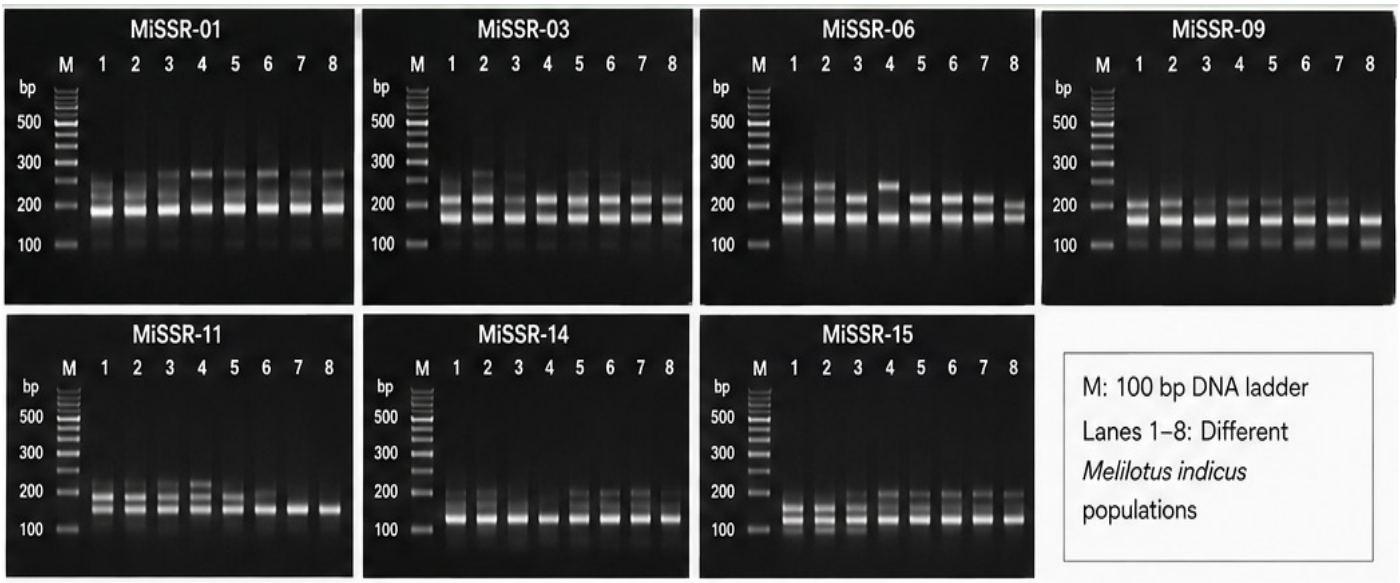
Supplementary Figure S5: *CTAB-based genomic DNA extraction workflow for Melilotus indicus leaf tissue, from field collection through DNA quality assessment.*



Supplementary Figure S6: *Representative field habitats of eight sampled sites, with altitude and annual rainfall indicated.*



Supplementary Figure S7: *Representative agarose gel electrophoresis profile of SSR-PCR amplified DNA fragments from ten Melilotus indicus samples (M = 100 bp ladder; lanes 1–10 = individual samples).*



Supplementary Figure S8: *Representative SSR amplification profiles at seven loci (MiSSR-01, -03, -06, -09, -11, -14, -15) across eight Melilotus indicus populations.*