Bangladesh J. Bot. **54**(3): 537-545, 2025 (September) DOI: https://doi.org/10.3329/bjb.v54i3.84618

GENETIC DIVERSITY IN *RUMEX NERVOSUS* VAHL FROM SARAWAT MOUNTAINS OF SAUDI ARABIA

NORAH A ALYAHYA, SULAIMAN ALRUMMAN AND MAHMOUD MOUSTAFA*

Department of Biology, College of Science, King Khalid University, Abha, Saudi Arabia

Keywords: *Rumex nervosus*, Genetic diversity, Sarawat Mountains, RAPD-ISSR markers

Abstract

This study assessed the genetic diversity of *Rumex nervosus* in southern Saudi Arabia using RAPD, ISSR, and combined RAPD-ISSR markers. A total of 218 RAPD and 211 ISSR bands were produced, with polymorphism rates of 47.7% and 63.5%, respectively. The highest polymorphism was recorded in primer OPE-3 (67.5%) for RAPD and UBC 826 (83.8%) for ISSR. Dendrogram analysis revealed distinct clusters with varying linkage distances, reflecting genetic relationships shaped by geographic proximity and ecological adaptation. The highest genetic similarity was observed between Bahat Rabiea and Alssawda (84.9% RAPD), Bahat Rabiea and Alothrban (82.9% ISSR), and Tabab and Bahat Rabiea (100% RAPD-ISSR). Conversely, the lowest similarity (16.7% RAPD-ISSR) occurred between Abha and Tabab, and Abha and Bahat Rabiea, indicating strong genetic divergence. These findings confirm that geographic isolation and genetic factors influence population structure, providing valuable insights for conservation, genetic resource management, and evolutionary and ecological research in the region.

Introduction

The genus *Rumex*, belonging to the Polygonaceae family, encompasses approximately 250 species with a cosmopolitan distribution (Desta *et al.* 2016). *Rumex nervosus* is a perennial herbaceous species occurring across Yemen, Saudi Arabia, Ethiopia, Somalia, Kenya, and Tanzania. In traditional medicine, *R. nervosus* has been utilized for the treatment of inflammatory diseases, wounds, typhus, rabies, and dermatological disorders (Al-Sunafi 2016, Ibrahim *et al.* 2024). Additionally, its stem has demonstrated potential therapeutic applications for urease-related disorders (Khan *et al.* 2014), and its crushed leaves, combined with wheat flour, are employed in ethno-veterinary medicine to address cattle constipation (Alyemeni*et al.* 2010). Through GC-MS and HPLC various phytochemicals and vitamins were identified from this plant (Al Yahya *et al.* 2018). Molecular markers have proven indispensable for investigating genetic diversity, phylogenetics, and population structure across plant taxa. Techniques such as RAPD and ISSR offer robust tools for assessing genetic variation due to their efficiency, simplicity, and cost-effectiveness (Mir *et al.* 2021). RAPD (Random Amplified Polymorphic DNA), a PCR-based approach, amplifies random DNA sequences, facilitating the analysis of genetic variation across multiple loci without requiring prior sequence knowledge (Gogoi *et al.* 2020). ISSR (Inter-Simple Sequence Repeats) markers, similarly sequence-independent, are particularly valued for their high reproducibility and ability to uncover polymorphic patterns in genomes (Trieu *et al.* 2016). These methods have been widely applied to characterize genetic diversity and structure in plant species (Dasgupta *et al.* 2015, Tiwari *et al.* 2015,Ait Bella *et al.* 2021, Ahmed and Islam 2024).

In the present study, genetic variability among populations of *R. nervosus* collected from seven distinct geographic locations in the Aseer region of Saudi Arabia was analyzed using RAPD, ISSR, and hybrid RAPD-ISSR molecular markers. These tools facilitated an in-depth investigation of genetic diversity, providing insights into the population structure of this species and contributing to its genetic characterization.

*Author for correspondence: <mfmostfa@kku.edu.sa>.

Materials and Methods

Rumex nervosus specimens were obtained from seven distinct locations within the Aseer region of Saudi Arabia. The altitudes of these sites were recorded as follows: Rejal Almea (1512.53 m), Billahmir (2002.63 m), Abha (2234 m), Tabab (2400 m), Alothrban (2289 m), Alssawda (2751 m), and Bahat Rabiea (2563 m).

Genomic DNA was isolated from fresh young leaves of the collected plants using the DNeasy Plant Mini Kit (QIAGEN, USA). DNA concentration was quantified using a Thermo Scientific™ BioMate 3S UV-Visible spectrophotometer at 260 nm, and the purity was evaluated by the OD260/OD280 absorbance ratio. A ratio of 1.7 was considered indicative of high-quality DNA. DNA integrity was assessed using 1% agarose gel electrophoresis.

Amplified DNA fragments obtained from RAPD-PCR, ISSR-PCR, and combined RAPD-ISSR-PCR reactions were separated on 1.5% agarose gels using a 1 kb DNA ladder (100–10,000 bp). Visualization and documentation of DNA bands were performed with a gel documentation system (ProXima AQ-4) and a UV transilluminator operating at 365 nm.

Primers used in the study were divided into three categories: seven RAPD markers, seven ISSR markers, and seven combined RAPD-ISSR markers. The RAPD primers and their sequences were as follows: Oligo345 (5'-GCG TGA CCC G-3'), Oligo42 (5'-TTA ACC CGG C-3'), Oligo211 (5'-GAA GCG CGA T-3'), OPL-3 (5'-CCAGCAGCTT-3'), HB 15 (5'-GC GTC GTG GTG GC-3'), OPE-3 (5'-CCAGATGCAC-3'), and OPJ-1 (5'-CCCGGCATAA-3'). The ISSR primers and their sequences were as follows: Primer 3 (5'-TGGATGGATGGATGGA-3'), UBC823 (5'-TCTCTC TCT CTC TCT CC-3'), Primer 2 (5'-GA GAGAGAGAGAGAGAGAGAGA-3'), UBC826 (5'-ACA CAC ACA CAC ACA CC-3'), UBC842 (5'-GAG AGA GAG AGA GAG ACG-3'), ISSR06 (5'-GAG AGA GAG AGA GAG AC-3'), and Primer 5 (5'-GA GAGAGAGAGAGAGA CG-3').

The mixed RAPD-ISSR primers as follows: Primer A (Oligo211 + Primer 2), Primer B (Oligo211 + Primer 2 + OPJ-1 + UBC842), Primer C (Oligo211 + Primer 2 + OPJ-1 + UBC842 + HB 15 + Primer 5), Primer D (Oligo211 + Primer 2 + OPJ-1 + UBC842 + HB 15 + Primer 5 + OPL-3 + ISSR06), Primer E (Oligo211 + Primer 2 + OPJ-1 + UBC842 + HB 15 + Primer 5 + OPL-3 + ISSR06 + Oligo345 + UBC823), Primer F (Oligo211 + Primer 2 + OPJ-1 + UBC842 + HB 15 + Primer 5 + OPL-3 + ISSR06 + Oligo345 + UBC823 + OPE-3 + UBC826), and Primer G (Oligo211 + Primer 2 + OPJ-1 + UBC842 + HB 15 + Primer 5 + OPL-3 + ISSR06 + Oligo345 + UBC823 + OPE-3 + UBC826 + Oligo42 + Primer 3).

PCR reactions were conducted in 25 µl reaction volumes containing 12.5 µl of GoTaq Green Master Mix, 7 µl of primer, 5 µl of genomic DNA, and nuclease-free water to adjust the final volume. Amplification was carried out using a PTC 200 Peltier Thermal Cycler (MJ Research, USA). The PCR protocol included an initial denaturation step at 99°C for 5 min, followed by 49 cycles of denaturation at 92°C for 1 minute, annealing at 29°C for 1 min, and extension at 72°C for 2 min, with a final extension at 72°C for 7 min (Vendramin *et al.* 2014). Clustering was performed using Ward's method with Euclidean distance and, additionally, with the Community Analysis Package (CAP, version 6.0; Pisces Conservation Ltd., Lymington, UK), where similarity matrices and UPGMA dendrograms were generated to illustrate genetic relationships.

Results and Discussion

The results indicate that the markers varied in their effectiveness in detecting genetic differentiation among populations. For RAPD, seven primers produced 218 stable bands, including 104 polymorphic bands (47.71%), 9 monomorphic bands (4.13%), and 105 unique bands (48.17%). The highest polymorphism rate (67.5%) was observed in primer OPE-3, while

the lowest (28.5%) in Oligo 345 (Fig. 1. Panels A and C). The dendrogram generated using Ward's method with Euclidean distance grouped the locations into two primary clusters at a linkage distance of 76.8 (Fig. 1. Panel B). The first cluster comprised genotypes from Rejal Almae, Billahmir, and Abha, which were further divided into two subclusters, with Abha separated from Rejal Almae and Billahmir. The second cluster included the Tabab genotype as an outgroup, distinct from those of Bahat Rabiea, Alssawda, and Alothrban. This cluster also split into two subclusters, with Alothrban clearly separated from Bahat Rabiea and Alssawda.

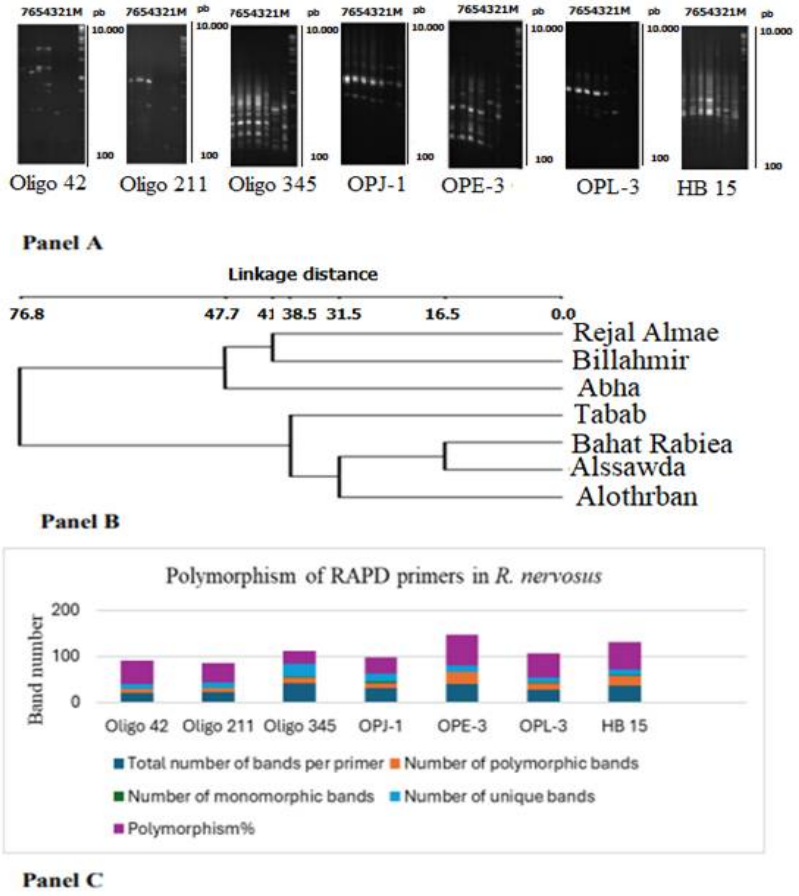

Fig. 1. Panel A: RAPD profiles of *Rumexnervosus* populations (1–7: Rejal Almae, Billahmir, Abha, Tabab, Bahat Rabiea, Alssawda, Alothrban; M: 1 kb ladder). Panel B: Dendrogram of genetic relationships. Panel C: Summary of total bands/primer, polymorphic, monomorphic, unique bands, and % polymorphism.

RAPD marker analysis revealed considerable variation in genetic similarity among the studied populations, based on binary presence/absence data and simple matching coefficients. These values offer critical insights into the genetic architecture, historical gene flow, and evolutionary dynamics across populations. The highest genetic similarity was recorded between Bahat Rabiea and Alssawda (84.9%), followed by Alssawda-Alothrban (78.4%) and Bahat Rabiea-Tabab (76.6%) (Table 1). These elevated values suggest strong genetic affinities, likely reflecting recent gene flow, shared ancestry, or convergence under similar environmental pressures. Notably, the

close relationship between Alssawda and Alothrban points to substantial genetic exchange or a common evolutionary history. In contrast, the lowest similarity values were observed between Billahmir-Tabab (50%) and Rejal Almae-Tabab (50.9%), indicating marked genetic divergence. These differences may be attributed to geographic isolation, genetic drift, or localized adaptation leading to restricted gene exchange. Moderate similarity levels were observed in several population pairs, including Rejal Almae-Billahmir (62.4%) and Abha-Tabab (64.2%), suggesting partial genetic connectivity or shared lineage. Rejal Almae exhibited intermediate similarity with Abha (60.1%), Bahat Rabiea (56%), Alssawda (56.4%), and Alothrban (57.8%), indicating historical gene flow or similar ecological influences. Similarly, Billahmir showed moderate affinities with Abha (55.5%), Bahat Rabiea (55.1%), Alssawda (52.8%), and Alothrban (52.3%). Abha consistently demonstrated mid-range similarity with most populations, Bahat Rabiea (58.3%), Alssawda (58.7%), and Alothrban (59.2%), suggesting it may function as a genetic intermediary within the region, potentially linking divergent gene pools (Table 1). These results demonstrate the efficiency of RAPD markers in detecting genetic variability, which is consistent with the findings of Fernandez *et al*. (2002).

Table 1. Genetic similarity among seven populations of*Rumex nervosus* based on RAPD, ISSR and RAPD-ISSR markers.

Population pair	RAPD (%)	ISSR (%)	Mixed RAPD-ISSR (%)
Same population	100	100	100
Rejal Almae - Billahmir	62.4	57.8	91.7
Rejal Almae - Abha	60.1	54	66.7
Rejal Almae - Tabab	50.9	40.3	50
Rejal Almae - Bahat Rabiea	56	37	50
Rejal Almae - Alssawda	56.4	41.2	41.7
Rejal Almae - Alothrban	57.8	36	41.7
Billahmir - Abha	55.5	64	41.7
Billahmir - Tabab	50	46.5	41.7
Billahmir - Bahat Rabiea	55.1	42.2	41.7
Billahmir - Alssawda	52.8	45.5	33.3
Billahmir - Alothrban	52.3	41.2	33.3
Abha - Tabab	64.2	69.2	16.7
Abha - Bahat Rabiea	58.3	54.5	16.7
Abha - Alssawda	58.7	55	41.7
Abha - Alothrban	59.2	56.4	41.7
Tabab - Bahat Rabiea	76.6	78.7	100
Tabab - Alssawda	70.6	71.6	75
Tabab - Alothrban	60.1	73.9	58.3
Bahat Rabiea - Alssawda	84.9	82.5	75
Bahat Rabiea - Alothrban	70.6	82.9	58.3
Alssawda - Alothrban	78.4	80.6	83.3

Using ISSR primers, a total of 211 bands were recorded (Fig. 2. Panels A and C). Among these, 134 bands (63.51%) were polymorphic, 9 bands (4.27%) were monomorphic, and 68 bands

(32.23%) were unique. Primer UBC 826 exhibited the highest polymorphism rate (83.8%), while primer UBC 842 showed no polymorphism. The dendrogram constructed from ISSR data using Ward's method with Euclidean distance grouped the genotypes into two major clusters at a linkage distance of 112 (Fig. 2. Panel B). The first cluster comprised genotypes from Rejal Almae, Billahmir, and Abha, further divided into two subclusters: one containing Rejal Almae, and the other including Billahmir and Abha. The second cluster grouped the genotype from Tabab as an outgroup, distinct from the genotypes of Bahat Rabiea, Alssawda, and Alothrban. Within this group, Alssawda was clearly separated from Bahat Rabiea and Alothrban.

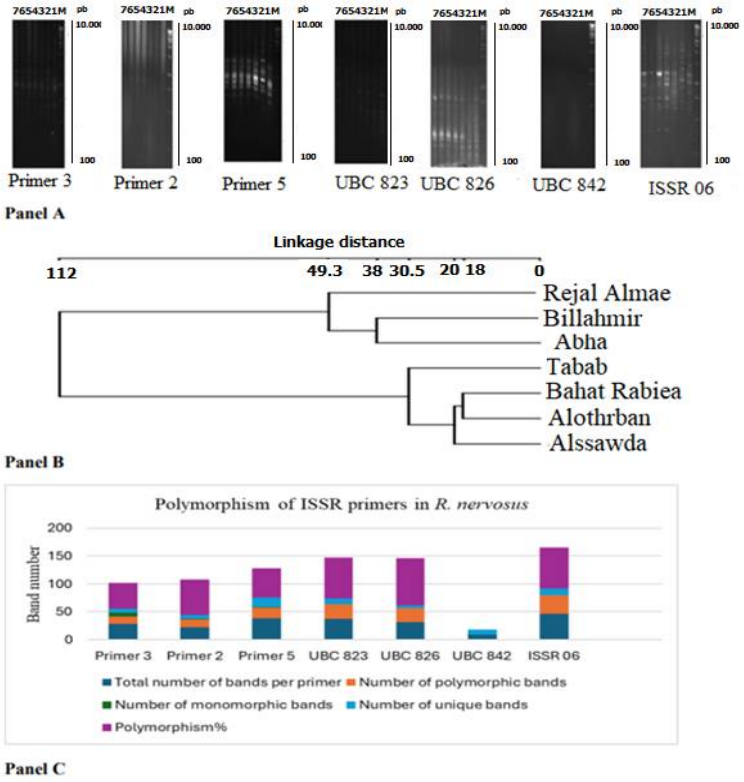

Fig. 2. Panel A: ISSR profiles of *Rumexnervosus* populations (1-7: Rejal Almae, Billahmir, Abha, Tabab, Bahat Rabiea, Alssawda, Alothrban; M: 1 kb ladder). Panel B: Dendrogram of genetic relationships. Panel C: Summary of total bands/primer, polymorphic, monomorphic, unique bands, and % polymorphism.

ISSR marker analysis revealed notable variation in genetic similarity among *R. nervosus* populations, reflecting differences in genetic structure and evolutionary relationships. The highest similarity values were observed between Bahat Rabiea and Alothrban (82.9%), Bahat Rabiea and Alssawda (82.5%), and Alssawda and Alothrban (80.6%), indicating strong genetic connectivity likely due to shared ancestry, gene flow, or similar environmental pressures. Tabab also showed relatively high similarity with Alssawda (71.6%) and Alothrban (73.9%), suggesting its inclusion within this genetically cohesive group. In contrast, Rejal Almae showed the lowest similarity values, particularly with Alothrban (36%) and Bahat Rabiea (37%), suggesting significant genetic divergence potentially driven by isolation or local adaptation. Moderate similarities between Rejal Almae and Billahmir (57.8%) and Abha (54%) indicate limited connectivity. Abha displayed

intermediate similarity with several populations and may serve as a genetic link between more distinct groups. Overall, the data reveal a structured genetic pattern in *R. nervosus*, with close relationships among Bahat Rabiea, Alssawda, Alothrban, and Tabab, and marked differentiation of Rejal Almae (Table 1). These findings highlight the roles of gene flow, geography, and adaptation in shaping population structure.

In the case of mixed primers (Fig. 3. Panels A and C), only primers (C) and (G) produced reproducible bands, while the other primers failed to generate any bands. Together, these two primers produced a total of 12 reproducible bands, of these, 8 (66.67%) were polymorphic, and 4 (33.33%) were unique. Neither primer generated any monomorphic bands. Primers (A), (B), (D), (E), and (F) did not produce any detectable bands, which may be attributed to the high number ofprimers in a single PCR reaction. This may due to competition for binding sites, increased primer-dimer formation, and nonspecific amplification. Additionally, the inherent complexity of the plant genome may further contribute to the lack of amplification. This issue requires further investigation to identify the precise cause and optimize the reaction conditions.

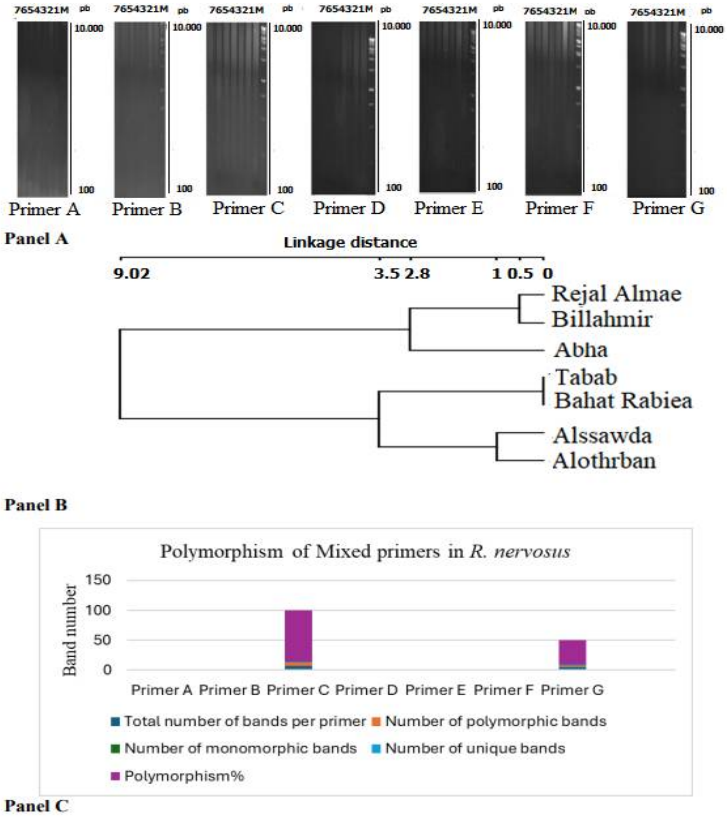

Fig. 3. Panel A: Mixed RAPD-ISSR profiles of *Rumexnervosus* populations (1–7: Rejal Almae, Billahmir, Abha, Tabab, Bahat Rabiea, Alssawda, Alothrban; M: 1 kb ladder). Panel B: Dendrogram of genetic relationships. Panel C: Summary of total bands/primer, polymorphic, monomorphic, unique bands, and % polymorphism.

The first cluster contained genotypes from Rejal Almae, Billahmir, and Abha, which split into two subclusters: one separated Abha from Rejal Almae and Billahmir. The second cluster comprised genotypes from Tabab and Bahat Rabiea, which showed 100% similarity. Genotypes

from Alssawda and Alothrban formed a separate clade within the same cluster.The combined RAPD–ISSR analysis revealed the highest genetic similarity between Tabab and Bahat Rabiea (100%), followed by Alssawda and Alothrban (83.3%), and then both Tabab–Alssawda and Bahat Rabiea–Alssawda (75%). These results suggest strong genetic cohesion, likely attributable to shared ancestry or ongoing gene flow. In contrast, Abha exhibited very low similarity with Tabab and Bahat Rabiea (16.7%), reflecting significant genetic divergence, likely due to geographic or ecological isolation. Rejal Almae showed high similarity with Billahmir (91.7%) and moderate similarity with Abha (66.7%), suggesting partial connectivity. However, its similarity with Alssawda and Alothrban (41.7%) was lower, indicating genetic differentiation.

Billahmir also showed limited similarity with most populations, particularly with Alssawda and Alothrban (33.3%), reinforcing its relatively distinct genetic position.Overall, the combined marker data suggest a clear genetic cluster comprising Tabab, Bahat Rabiea, Alssawda, and Alothrban, while Abha, Rejal Almae, and Billahmir appear more genetically isolated to varying extents (Table 1).

These populations may share some genetic traits while also displaying divergence due to local adaptations or genetic drift. These results aligned with the findings of Alsenidi *et al.* (2018), who demonstrated that various biomarkers, including RAPD, ISSR, and the combined RAPD-ISSR approach, effectively distinguished between the genotypes of *Nicotiana glauca* plants in the Abha region, Saudi Arabia.

The study revealed clear genetic differentiation among *R. nervosus* populations, especially with RAPD and ISSR markers, and indicated that geographical and ecological variation, particularly differences in altitude among the studied sites (ranging from 1512.53 m in Rejal Almea to 2751 m in Alssawda) and their geographical location, may play an important role in shaping the observed genetic divergence. The findings of Nagaoka and Ogihara (1997) demonstrate that environmental and geographical isolation can drive genetic differentiation in plant populations. The genetic relationships observed in this study are consistent with those reported in other species, such as *Trigonella* (Sundaram and Purwar 2011) and *Chrysanthemum* (Wolff and Peters-van Rijn 1993), where RAPD and ISSR markers effectively distinguished populations based on genetic variation. The genetic differentiation observed in this study can be attributed to both geographical distances between populations and ecological factors such as climate and altitude (Moustafa *et al.* 2016,Hassan *et al.* 2021, Al-Qarni *et al.* 2024). Similar trends have been reported in other species, where populations from higher altitudes showed greater genetic variation due to harsher environmental conditions (Hu *et al.* 2010). The genetic variability observed among *R. nervosus* populations reflects the complexity of evolutionary processes acting at the species level. The RAPD and ISSR markers in this study provided valuable insights into the genetic variation within *R. nervosus.* The results revealed varying degrees of genetic similarity, with some populations exhibiting substantial divergence, while genetic connectivity may be influenced by geographic proximity.

In conclusion, altitudinal variation, geographic separation, and ecological factors collectively shape the genetic structure of *R. nervosus.* RAPD and ISSR markers proved to be effective tools for assessing genetic relationships, and the findings underscore the importance of incorporating these factors into conservation strategies to preserve the species' genetic diversity.

Acknowledgments

The authors gratefully acknowledge the support of King Khalid University (KKU), KSA, for this work.

References

Al-Qarni M, Alrumman S and Moustafa M 2024. Anticandidal activity and chemicals of *Commiphoragileadensis* (L.) C. Chr. (Burseraceae) growing in the southwestern region of Saudi Arabia. Bangladesh J. Bot. **53**: 1-8.

Al-Sunafi SMY 2016. Pharmacognostical study of *Rumex nervosus* Vahl. family (Polygonaceae) growing in Yemen. Ph.D. Thesis, Cairo University, Cairo, Egypt.

Al Yahya NA, Alrumman SA and Moustafa MF 2018. Phytochemicals and antimicrobial activities of *Rumex nervosus* natural populations grown in Sarawat Mountains, Kingdom of Saudi Arabia. Arab. J. Sci. Eng. **43**: 3465-3476.

Alyemeni MN, Sher H and Wijaya L 2010. Some observations on Saudi medicinal plants of veterinary importance. J. Med. Plants Res. **4**: 2298-2304.

Ait Bella Y, Bouda S, Khachtib Y and Haddioui A 2021. Genetic variability of cultivated plum (*Prunus domestica* L. and *Prunus salicina* Lindl.) in Morocco assessed by ISSR markers. Aust. J. Crop Sci. **15**: 948-954.

Alsenidi MD, Moustafa MF and Alrumman SA 2018. Assessing genetic diversity of invasive plant *Nicotiana glauca* in the Abha region, Saudi Arabia by RAPD, ISSR, and mixed biomarkers. Int. J. Agric. Biol. **20**: 1471-1478.

Dasgupta N, Nandy P, Sengupta C and Das S 2015. RAPD and ISSR marker-mediated genetic polymorphism of two mangroves, *Bruguieragymnorrhiza* and *Heritiera fomes*, from Indian Sundarbans in relation to their sustainability. Physiol. Mol. Biol. Plants **21**: 375-384.

Desta KT, Lee WS, Lee SJ, Kim YH, Kim GS, Lee SJ, Kim ST, Abd El-Aty AM, Warda M, Shin HC, Shim JH and Shin SC 2016. Antioxidant activities and liquid chromatography with electrospray ionization tandem mass spectrometry characterization and quantification of the polyphenolic contents of *Rumex nervosus* Vahl leaves and stems. J. Sep. Sci. **39**: 1433-1441.

Fernandez M, Figueiras A and Benito C 2002. The use of ISSR and RAPD markers for detecting DNA polymorphism, genotype identification, and genetic diversity among barley cultivars with known origin. Theor. Appl. Genet. **104**: 845-851.

Gogoi B, Wann SB and Saikia SP 2020. Comparative assessment of ISSR, RAPD, and SCoT markers for genetic diversity in *Clerodendrum* species of North East India. Mol. Biol. Rep. **47**: 7365-7377.

Hassan A, Alrumman S and Moustafa M 2021. Effect of elevation on biological activity of *Juniperus procera*Hochst. ex Endlicher populations grown in Al Soda Mountains, Saudi Arabia. Pak. J. Bot. **53**: 1371-1377.

Hu Y, Wang L, Xie X, Yang J, Li Y and Zhang H 2010. Genetic diversity of wild populations of *Rheum tanguticum* endemic to China as revealed by ISSR analysis. Biochem. Syst. Ecol. **38**: 264-274.

Ibrahim BM, Awad HM, Shaffie NM, Youness ER and Mahmoud AH 2024. *Rumex nervosus* nanoparticles versus aqueous extract: Introduction of novel immunomodulatory anti-inflammatory nano-preparation for management of paw oedema and gastritis in rats. Bull. Natl. Res. Cent. **48**: 2-16.

Khan R, Quradha MM, Saif AQ, Ali J, Rauf A and Khan A 2014. Comparative urease enzyme inhibition profile of leaves and stems of *Rumex nervosus* Vahl. Nat. Prod. Res. **28**: 2355-2357.

Mir MA, Mansoor S, Sugapriya M, Alyemeni MN, Wijaya L and Ahmad P 2021. Deciphering genetic diversity analysis of saffron (*Crocus sativus* L.) using RAPD and ISSR markers. Saudi J. Biol. Sci. **28**: 1308-1317.

Moustafa MF, Hesham AE, Quraishi MS and Alrumman SA 2016. Variations in genetic and chemical constituents of *Ziziphus spina-christi* L. populations grown at various altitudinal zonation up to 2227 m height. J. Genet. Eng. Biotechnol. **14**: 349-362.

Nagaoka T and Ogihara Y 1997. Applicability of inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. Theor. Appl. Genet. **94**: 597-602.

Sundaram S and Purwar S 2011. Assessment of genetic diversity among fenugreek (*Trigonella foenum-graecum* L.) using RAPD molecular markers. J. Med. Plants Res. **5**: 1543-1548.

Tiwari V, Mahar KS, Singh N, Meena B, Nair KN, Datt B and Rana TS 2015. Genetic variability and population structure of *Bergenia ciliata* (Saxifragaceae) in the Western Himalaya inferred from DAMD and ISSR markers. Biochem. Syst. Ecol. **60**: 165-170.

Trieu LN, Mien NT, Tien TV, Ket NV and Duy NV 2016. Genetic diversity of *Panax stipuleanatus* Tsai in North Vietnam detected by inter-simple sequence repeat (ISSR) markers. Biotechnol. Biotechnol. Equip. **30**: 506-511.

Vendramin E, Pea G, Dondini L, Pacheco I, Dettori MT, Gazza L, Scalabrin S, Strozzi F, Tartarini S, Bassi D, Verde I and Rossini L2014. A unique mutation in a MYB gene cosegregates with the nectarine phenotype in peach. PLOS ONE **9**: e90574.

Wolff K and Peters-van Rijn J 1993. Rapid detection of genetic variability in chrysanthemum (*Dendranthema grandiflora*Tzvelev) using random primers. Heredity **71**: 335-341.

(Manuscript received on 10 January, 2025; revised on 24 July, 2025)