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RESEARCH ARTICLE

Integrative palynological and molecular characterization of *Pulicaria crispa* populations from the central region of Saudi Arabia

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Abstract

Pulicaria crispa, a xerophytic species of the family Asteraceae, demonstrates significant ecological adaptability across arid regions of Saudi Arabia. This study aimed to investigate the genetic and palynological diversity among four geographically distinct populations of *P. crispa* using a combined approach of morphological, palynological and molecular analyses. Pollen grains were extracted from mature anthers, acetalized and examined under light and scanning electron microscopes to measure key characteristics such as polar and equatorial diameter, aperture type and exine ornamentation. Additionally, genomic DNA was isolated from fresh leaf tissues and amplified using ISSR and ISJ molecular markers to assess genetic variation. A total of 134 ISSR bands and 112 ISJ bands were generated, with ISSR markers showing higher polymorphism (79.4 %) than ISJ (62.5 %). Morphological and palynological features displayed significant inter-population variability. Mantel tests revealed a moderate correlation ($r = 0.52$, $p < 0.05$) between molecular and morphological distances, indicating potential adaptive divergence. The King Abdulaziz Royal Reserve population exhibited the highest genetic and palynological diversity. These findings underscore the value of using integrated molecular and palynological tools to assess biodiversity in desert-adapted plant species, highlighting the evolutionary significance of environmental pressures shaping *P. crispa* populations. This work contributes to the ecological and conservation understanding of xerophytic flora in the Arabian Peninsula.

Keywords: genetic diversity; ISSR markers; palynology; *Pulicaria crispa*; Saudi Arabia

Introduction

P. crispa (Forssk.) Oliv. is an ecologically and medicinally important member of the Asteraceae family, native to arid and semi-arid regions of the Arabian Peninsula. Its wide distribution across the central region of Saudi Arabia, particularly in fragmented populations, has led to notable variations in morphological, physiological and chemical traits that reflect adaptation to diverse environmental conditions (1). This geographic isolation offers an excellent opportunity to investigate evolutionary processes and population divergence.

Moreover, *P. crispa* is particularly suitable for integrative studies because it exhibits substantial ecological plasticity and phenotypic variability in response to arid environments. This makes it an ideal model for investigating correlations between morphological traits and underlying genetic structure. Its medicinal significance, taxonomic complexity within the genus and wide ecological amplitude further enhance its relevance as a candidate for combined palynological and molecular approaches.

Palynology in plant taxonomy and systematics contributes to understanding species differentiation and evolutionary relationships. Due to their durable and specialized structures, pollen grains are often preserved in modern and fossil specimens. Their surface ornamentation is frequently species-specific, making them useful taxonomic indicators, particularly among closely related taxa. Their resistance to environmental degradation also supports their role in species identification when other morphological traits are ambiguous. Studies have shown that examining pollen morphology can help clarify species boundaries and inform interpretations of evolutionary history (2).

Pulicaria species, with their pollen aperture types and variation in exine structures, present a relevant case for palynological study. Differences in these traits among species within the genus can provide taxonomic information and support interpretations of evolutionary patterns. These pollen characteristics may also indicate adaptations to specific environmental conditions (3). Studying these features in *Pulicaria* species may help clarify taxonomic relationships and contribute to understanding the genus's evolutionary history.

Molecular approaches, including using inter-simple sequence repeats (ISSR) and intron splice junction (ISJ) markers, are commonly used to assess genetic diversity and population relationships. These markers are helpful because they can detect polymorphisms across the genome without requiring prior sequence information, which is beneficial in studies lacking genomic data. ISSR and ISJ markers are also known for their

reproducibility and capacity to capture genetic variation within and among populations (4). Research has shown that these markers can identify genetic differentiation among *Pulicaria* populations, contributing to the understanding of geographic distribution as well as evolutionary patterns (5).

Although both palynological and molecular data are useful for assessing plant diversity, few studies have integrated these methods to investigate the population structure of desert-adapted species. This study combines pollen trait analysis with molecular profiling to examine *P. crispa* populations. By comparing morphological and molecular data, this approach aims to support species identification and contribute to understanding desert-adapted plants' genetic and ecological characteristics.

Materials and Methods

Study area and plant material

Samples of *P. crispa* were collected from four ecologically distinct sites in the central region of Saudi Arabia, namely, King Abdulaziz Royal Reserve (formerly Tanahat, TN), Imam Abdulaziz Bin Mohammed Royal Reserve (formerly Khurai, KH), Nafud Al-Dahna (DH) and East Riyadh along Dammam Road (ER). Five healthy and mature individuals were sampled from each population during the peak flowering season. The GPS coordinates and environmental characteristics of each site were recorded (Table 1).

The collected specimens were compared with reference materials archived at the Botanical Herbarium of the Department of Botany and Microbiology, King Saud University, Riyadh, Saudi Arabia, to verify species identity. A voucher specimen was prepared and deposited in the same herbarium under accession number KSU-2464 (Fig. 1). Our laboratory collection retains a duplicate sample for further reference.

Table 1. Collection sites of *P. crispa* populations in the central region of Saudi Arabia

Species	Collection area	Coordinates
P. crispa (DH)	Nafud Al-Dahna	N25.4813250 E46.4524190
P. crispa (KH)	Imam Abdulaziz Bin Mohammed Royal Reserve	N25.4576490 E46.4503860
P. crispa (TN)	King Abdulaziz Royal Reserve	N26.111282 E46.482880
P. crispa (ER)	East Riyadh - Dammam Road	N24.859583 E46.840523

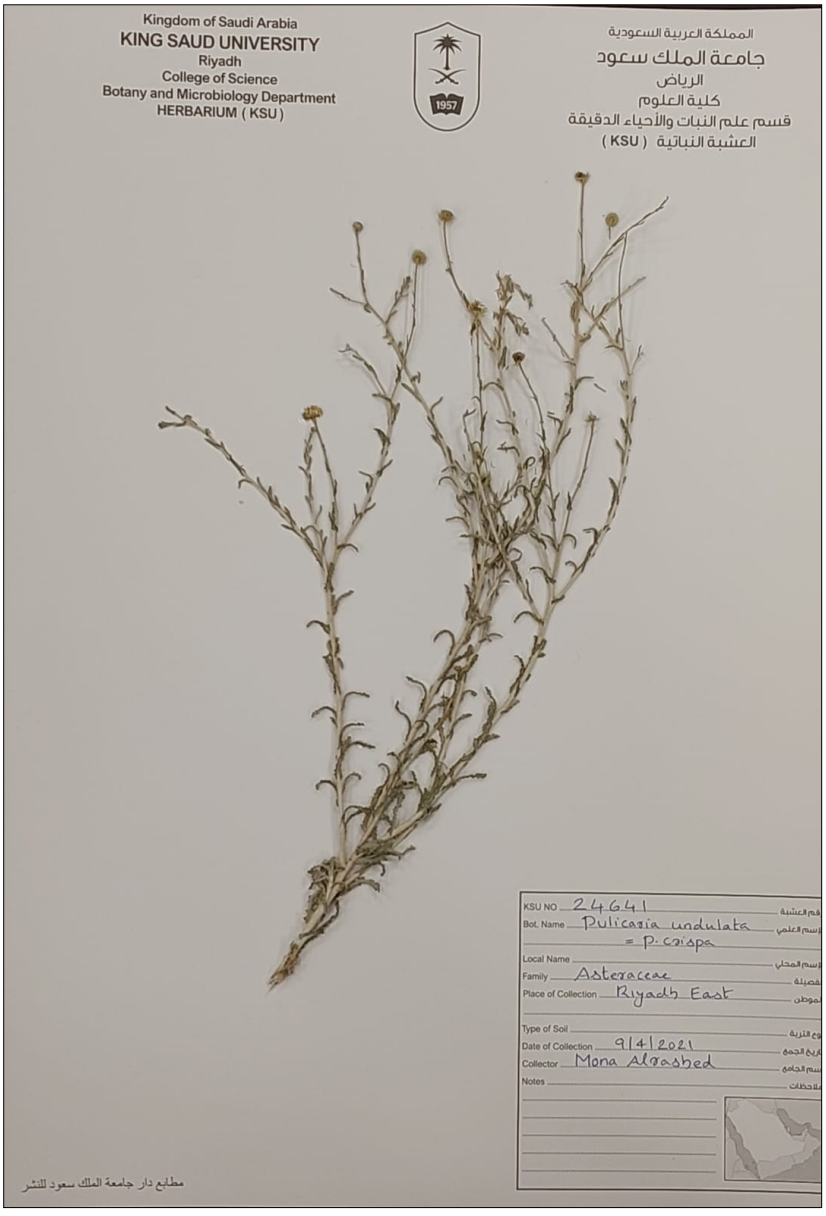

Fig. 1. The voucher specimen of *P. crispa* (KSU No. 24641) deposited in the Botanical Herbarium of the Department of Botany and Microbiology at King Saud University.

Pollen grain preparation and data analysis

Pollen grains were extracted from the mature anthers of *P. crispa* flowers and subjected to a dehydration process using an ethanol series, followed by acetolysis according to standard palynological protocols (6). The prepared grains were examined under light microscopy (LM) and scanning electron microscopy (SEM) to document their detailed morphological characteristics. Key pollen traits, including polar diameter, equatorial diameter, shape index, aperture type and exine ornamentation, were measured using Motic Images Plus 2.0 (MI PLUS) imaging software. Twenty pollen grains were counted for each sample and the values were averaged to obtain representative measurements.

Samples were collected from five individual plants within each of four distinct populations in the central region of Saudi Arabia. Individual-level averages were calculated for each population and subsequently used to determine the overall population means. Quantitative traits were treated as continuous data, while categorical attributes were transformed into binary form, with the presence of a given characteristic scored as "1" and its absence as "0". The compiled dataset was organized into a morphological data matrix and subjected to numerical analysis using XLSTAT software (version 2023). A similarity/dissimilarity matrix was generated and cluster analysis was performed to construct a dendrogram based on pollen morphology to illustrate the phenotypic relationships among the studied populations.

DNA extraction, PCR amplification and marker analysis

Genomic DNA was extracted from young leaf tissue of *P. crispa* using the CTAB protocol, with slight modifications (7). Specifically, during the DNA drying step, tubes were placed horizontally on sterile cheesecloth for 10-20 min to ensure complete evaporation of residual ethanol. DNA concentration and purity were assessed through spectrophotometry and gel electrophoresis. Twenty-three molecular markers were employed to analyse genetic variation among twenty *P. crispa* DNA samples. These included twelve intron splice junction (ISJ) markers and eleven inter-simple sequence repeat (ISSR) primers following the protocol and shows in in Table 2 (8).

Polymerase chain reaction (PCR) amplification was performed in a 20 µL reaction mixture containing 25 ng of genomic DNA, 15 pmol of each primer, 2.5 mM of dNTPs and 1X PCR buffer composed of 50 mM Tris-HCl (pH 9.0), 20 mM $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$ and 0.25 mg/mL bovine serum albumin (BSA). One unit of Taq DNA polymerase was added per reaction. Amplifications were conducted using a Bio-Rad C1000 thermal cycler.

The thermal cycling conditions were as follows: initial denaturation at 94°C for 5 min; 40 cycles of denaturation at 94°C for 30 sec, annealing at a primer-specific temperature ranging from 42°C to 65°C for 45 sec and extension at 72°C for 1 min; followed by a final extension at 72°C for 10 min. PCR products were resolved on 1.5 % agarose gels stained with ethidium bromide (0.5 µg/mL) and visualized under UV light using a Biometra gel documentation system (Germany). Banding patterns were scored manually as present (1) or absent (0) and the resulting binary matrix was used for subsequent genetic diversity and population structure analysis.

The current study's schematic diagram integrates palynological and molecular analyses of *P. crispa* populations from four locations in the central region of Saudi Arabia (Fig. 2).

Table 2. Sequences of DNA primers used for ISSR and ISJ markers in *P. crispa* populations in the central region of Saudi Arabia

Type of Markers	Primer Name	Primer sequence	TM (°C)
ISJ	ISJ-1	CAGACCTGC	33
	ISJ-2	ACTTACCTGAGGCGCCAC	57
	ISJ-3	TGCAGGTCA	33
	ISJ-4	GTCGGCGGACAGGTAAGT	57
	3ISJ-5	CAGGGTCCCACCTGCA	54
	ISJ-6	ACTTACCTGAGCCAGCGA	57
	ISJ-7	TGCAGGTCAGGACCCT	54
	ISJ-8	GACCGCTTGCAGGTAAGT	57
	ISJ-9	AGGTGACCGACCTGCA	54
	ISJ-10	ACTTACCTGCATCCCCCT	57
	ISJ-11	TGCAGGTCAAACGTCG	54
	ISJ-12	GGACTGGAGCAGGTAAGT	57
ISSR	ISSR-P1	GTTGTTGTTGTTGTTGTTGTTC	56.6
	ISSR-P2	GTTGTTGTTGTTGTTGTTGTTA	54.7
	ISSR-P3	GTTGTTGTTGTTGTTGTTGTTT	54.7
	ISSR-P4	CACACACACACACACACACAG	61.3
	ISSR-P5	CACACACACACACACACACAC	61.3
	ISSR-P6	CACACACACACACACACACAA	59.4
	ISSR-P7	CACACACACACACACACACAT	59.4
	ISSR-P8	TGTGTGTGTGTGTGTGTGTGG	61.3
	ISSR-P9	TGTGTGTGTGTGTGTGTGTGA	59.4
	ISSR-P10	TGTGTGTGTGTGTGTGTGGTG	61.3
	ISSR-P11	GAGAGAGAGAGAGAGAC	52.4

Results

Palynological characteristics and analysis

Pollen grains of *P. crispa* from all four populations were spheroidal and tricolporate, with variations in size and surface ornamentation. Polar diameter ranged from 17.8 to 19.2 µm and equatorial diameter ranged from 17.5 to 18.6 µm. Pollen grain surfaces for all *P. crispa* samples displayed spines and a perforated sculpture observed using LM and SEM (Table 3). The diameter of the sculpture increased near the base of the grain. All samples were similar except for the tips of the spines. The sample displayed obtuse or tapered circular spines from one location. In contrast, the others exhibited convex conical spines (Fig. 3). A hierarchical cluster analysis was performed using XLSTAT software to explore population phenotypic relationships. The resulting dendrogram delineates two primary clusters based on pollen morphological traits (Fig. 4).

Cluster I

Comprised samples from (DH) and (ER) populations, indicating high morphological similarity between these ecotypes.

Cluster II

Consisted of samples from (KH) and (TN), suggesting shared characteristics distinct from Cluster I.

Genetic diversity through ISSR and ISJ markers

A total of 10 ISSR and 8 ISJ primers generated 132 and 94 scorable bands respectively. The ISSR profile and ISJ profile are shown in Fig. 5 & 6. The proportion of polymorphic bands was remarkably high-100 % for ISSR and 98.21 % for ISJ, indicating strong discriminatory power. Polymorphic information content (PIC) values ranged from 0.30 to 0.40, reflecting a high level of genetic variability among the samples. Based on Nei and Li's similarity coefficient, cluster analysis grouped the TN and KH populations into one cluster and the DH and ER populations into another, suggesting distinct genetic structuring among populations. A phylogenetic tree constructed from 20 *P. crispa* samples using ISJ

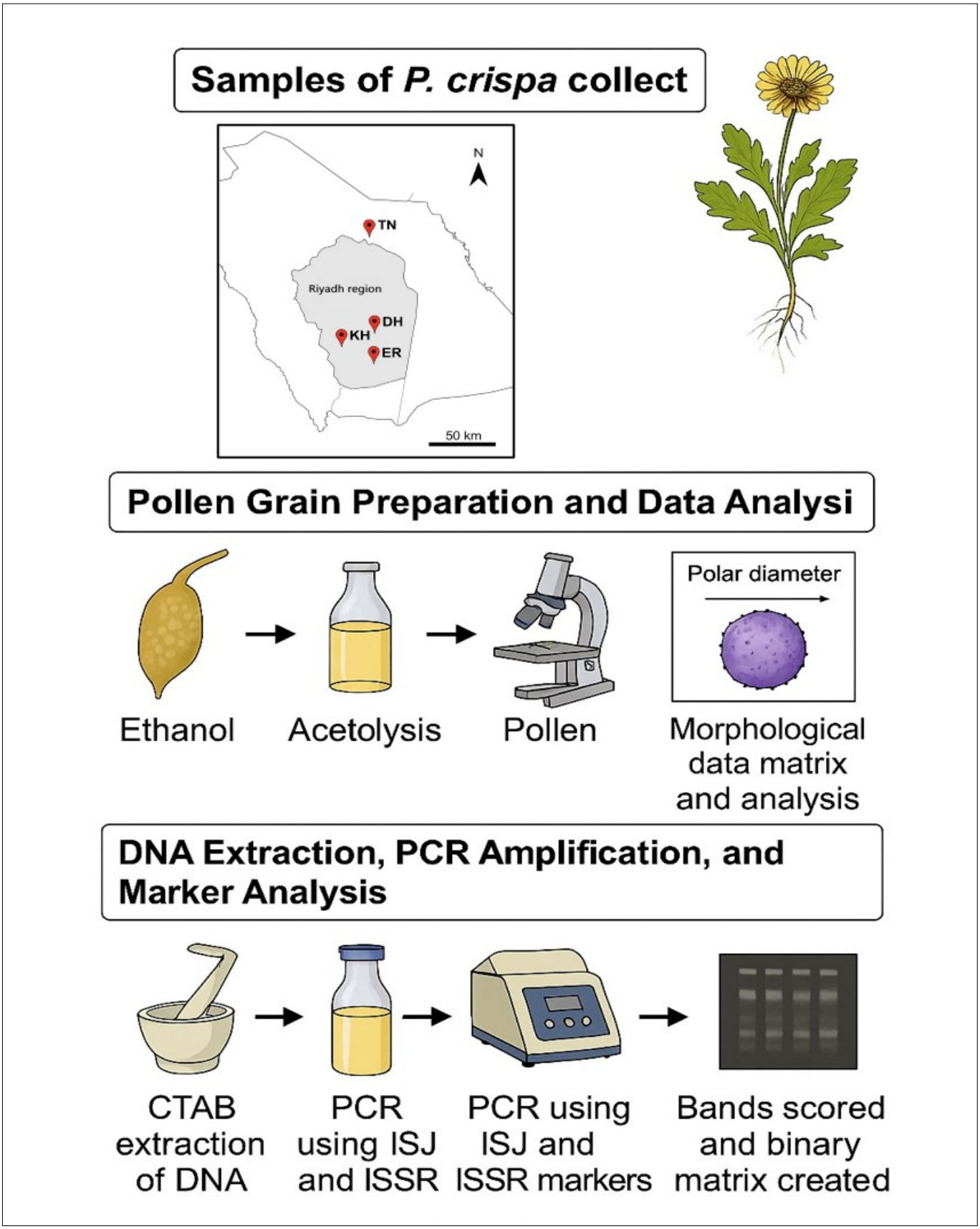

Fig. 2. Schematic diagram of the current study, integrating palynological and molecular analyses of *P. crispa* populations in the central region of Saudi Arabia.

Table 3. Pollen morphological traits of *P. crispa* populations in the central region of Saudi Arabia based on palynological traits

Location	Polar Axis (P) (µm)	Equatorial Diameter (E) (µm)	P/E Ratio (%)	Pollen Shape	Exine Ornamentation	Pollen Volume (µm³)
DH	19.117	19.117	100.00	Spheroidal	Spiny	3,635.233
KH	16.176	17.640	91.66	Spheroidal	Spiny	2,203.820
TN	20.290	19.117	106.13	Spheroidal	Spiny	4,354.037
ER	18.230	18.230	100.00	Spheroidal	Spiny	3,157.955

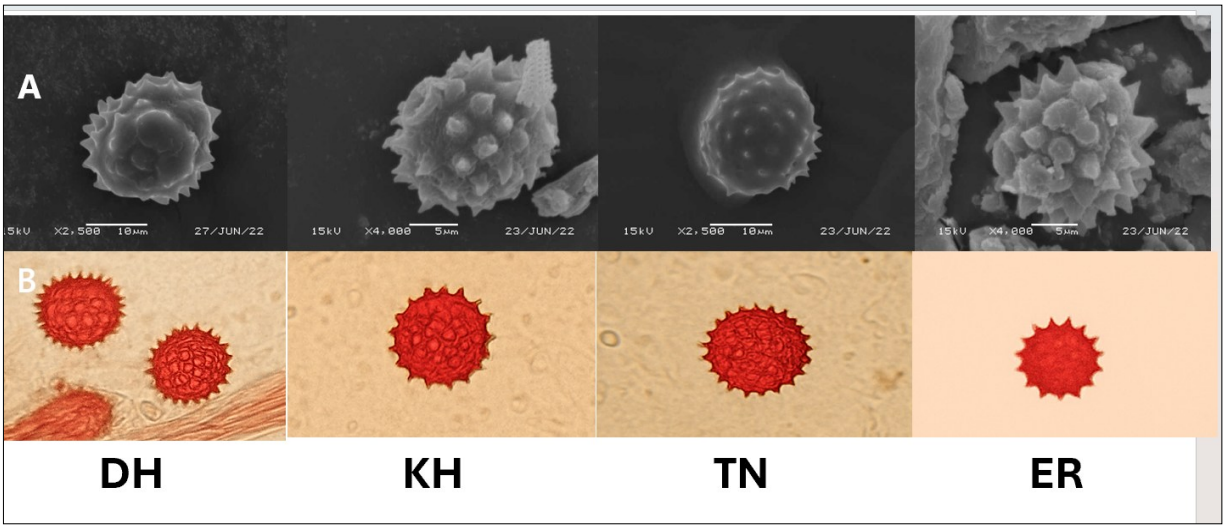

Fig. 3. Pollen grain shapes: (A) scanning electron micrograph; (B) light micrograph of *P. crispa* populations in the central region of Saudi Arabia.

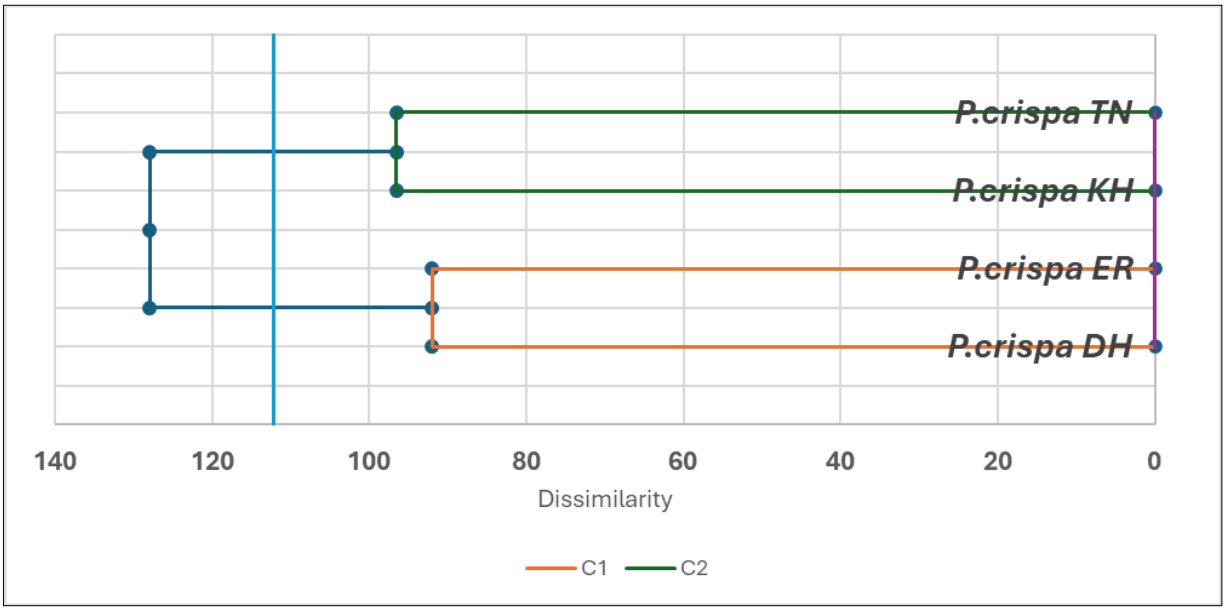

Fig. 4. Dendrogram of *P. crispa* populations in the central region of Saudi Arabia.

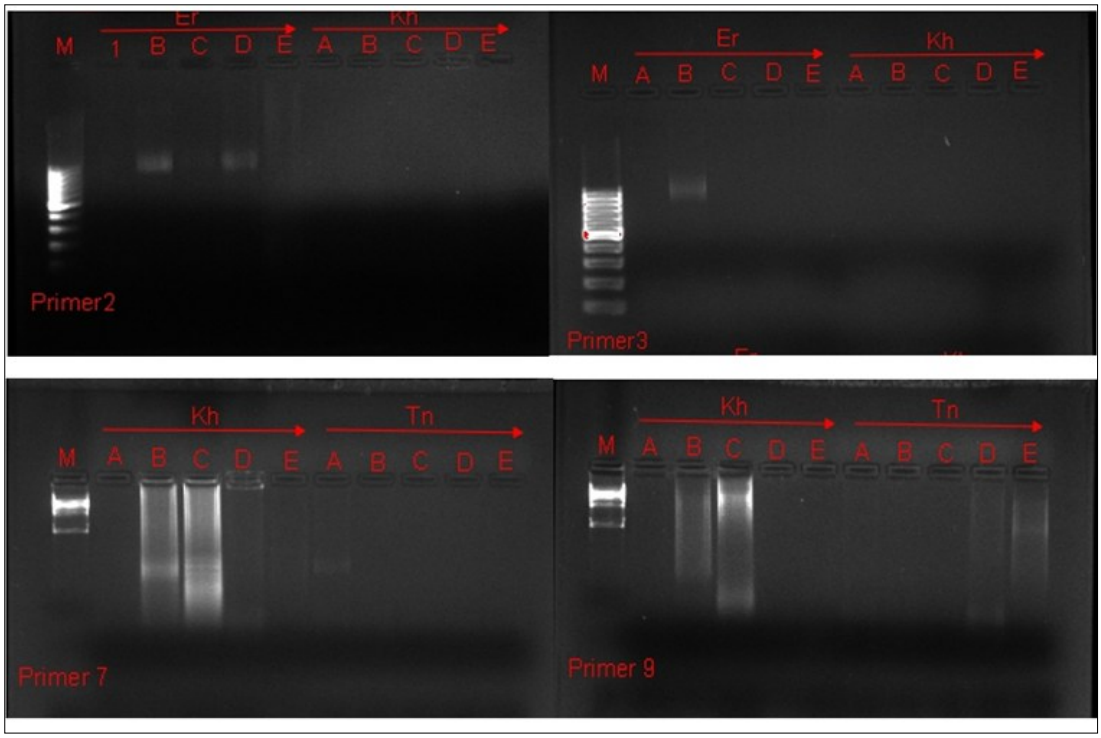

Fig. 5. ISSR profiles of *P. crispa* populations from four locations in the central region of Saudi Arabia: East Riyadh (ER), Khurai (KH), Tanahat (TN) and Dahna (DH), generated using different primers.

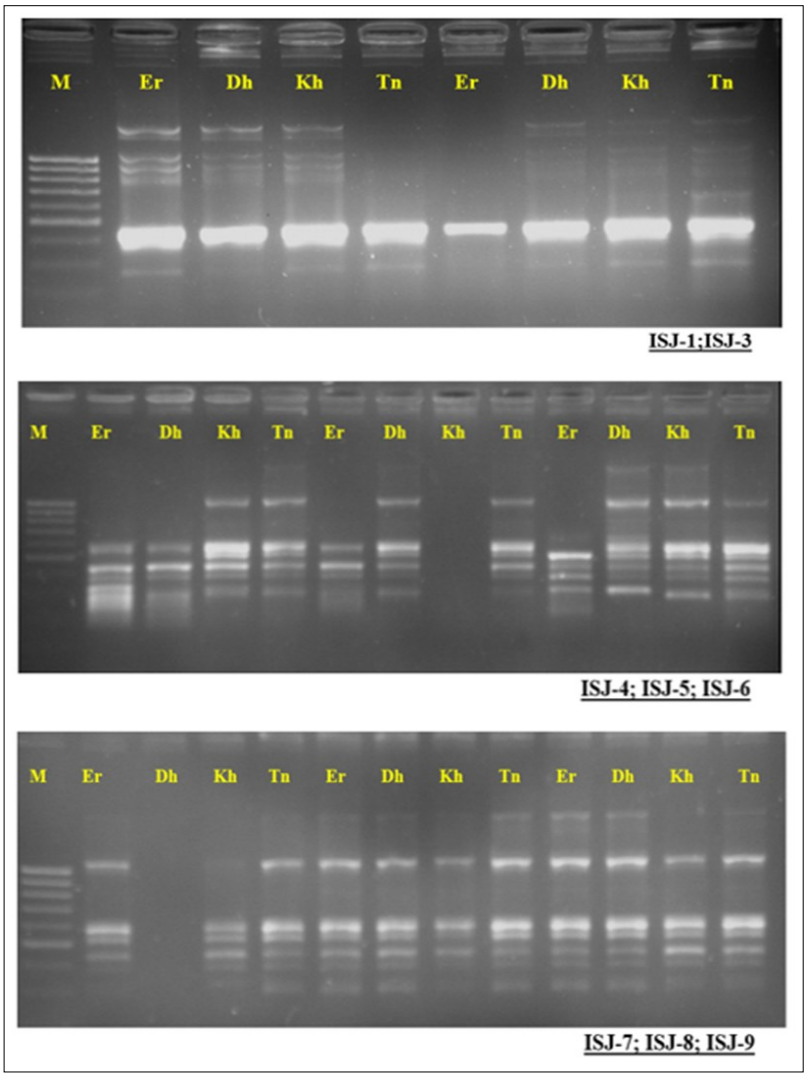

Fig. 6. ISJ profiles of *P. crispa* populations from four locations in the central region of Saudi Arabia: East Riyadh (ER), Khurai (KH), Tanahat (TN) and Dahna (DH), generated using different primers.

and ISSR marker data revealed three main clusters and four individual branches, indicating complex genetic relationships (Table 4, Fig. 7).

- Cluster I grouped all ER samples with DHA-DHC from DH, while DhD formed a separate branch.
- Cluster II included DHE and KH-A-C, with KH-D on an independent branch.
- Cluster III grouped KH-E with TN-A and TN-B, whereas TN-C, TN-D and TN-E each formed individual branches, highlighting significant intra-population diversity. Overall, the broad range of genetic distances (0-1) further supports the presence of substantial genetic diversity both within and among *P. crispa* populations from central Saudi Arabia.

Correlation between pollen traits and genetic structure

The dendrograms derived from palynological and molecular data revealed congruent clustering patterns among *P. crispa* populations. Notably, populations exhibiting reticulate pollen ornamentation (TN and KH) demonstrated higher genetic similarity, whereas those with microreticulate pollen patterns (DH and ER) were genetically distinct. These findings suggest a potential correlation (PC) between morphological adaptation and underlying genetic divergence. This relationship is further supported by the principal component analysis (PCA) biplot, which visualizes genetic distance derived from ISSR and ISJ markers across the studied populations (Fig. 8). The first two principal components captured the majority of variance and revealed apparent clustering by geographic origin. Samples from the exact location consistently grouped, reinforcing the patterns observed in cluster analysis and Mantel tests. Collectively, these results validate the association between pollen traits and genetic structuring, reflecting adaptive divergence shaped by environmental pressures.

Discussion

Integrating palynological and molecular data in this study provides a broader framework for examining intraspecific variation among *P. crispa* populations in central Saudi Arabia. Combining morphological and genetic analyses contributes to understanding diversity patterns within and between species populations.

Pollen morphology, a key component of palynological studies, exhibited consistent qualitative traits across all populations, including a spheroidal shape, tricolporate apertures and spiny exine ornamentation. These features are generally stable at the species level and are consistently observed across *P. crispa* populations (2). However, quantitative differences in pollen size and sculptural dimensions were identified, which are often associated with ecological and environmental variation. These differences served as useful indicators for distinguishing among populations and align with findings from other arid-zone plant studies, where environmental factors have been linked to morphological

Table 4. The genetic distance matrix among *P. crispa* populations in the central region of Saudi Arabia is based on combined ISSR and ISJ marker data

	ErA	ErB	ErC	ErD	ErE	DhA	DhB	DhC	DhD	DhE	KhA	KhB	KhC	KhD	KhE	TnA	TnB	TnC	TnD	TnE
ErA	1.0	0.7	0.9	0.7	0.8	0.6	0.6	0.5	0.0	0.6	0.1	0.5	0.6	0.0	0.1	0.3	0.1	0.0	0.0	0.6
ErB	0.7	1.0	0.8	0.8	0.9	0.7	0.7	0.5	0.0	0.7	0.1	0.5	0.6	0.0	0.1	0.3	0.1	0.0	0.0	0.4
ErC	0.9	0.8	1.0	0.8	0.9	0.7	0.5	0.6	0.0	0.5	0.1	0.6	0.8	0.0	0.1	0.4	0.1	0.0	0.0	0.5
ErD	0.7	0.8	0.8	1.0	0.9	0.7	0.5	0.6	0.0	0.5	0.1	0.4	0.6	0.0	0.1	0.4	0.1	0.0	0.0	0.3
ErE	0.8	0.9	0.9	0.9	1.0	0.6	0.6	0.6	0.0	0.6	0.1	0.6	0.7	0.0	0.1	0.3	0.1	0.0	0.0	0.4
DhA	0.6	0.7	0.7	0.7	0.6	1.0	0.4	0.5	0.0	0.4	0.1	0.3	0.4	0.0	0.1	0.4	0.1	0.0	0.0	0.4
DhB	0.6	0.7	0.5	0.5	0.6	0.4	1.0	0.3	0.0	0.8	0.1	0.3	0.6	0.0	0.1	0.1	0.1	0.0	0.0	0.2
DhC	0.5	0.5	0.6	0.6	0.6	0.5	0.3	1.0	0.0	0.3	0.2	0.4	0.6	0.0	0.2	0.6	0.2	0.0	0.0	0.1
DhD	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DhE	0.6	0.7	0.5	0.5	0.6	0.4	0.8	0.3	0.0	1.0	0.1	0.3	0.4	0.0	0.1	0.3	0.1	0.0	0.0	0.4
KhA	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.0	0.1	1.0	0.2	0.2	0.0	1.0	0.3	1.0	0.0	0.0	0.0
KhB	0.5	0.5	0.6	0.4	0.6	0.3	0.3	0.4	0.0	0.3	0.2	1.0	0.6	0.0	0.2	0.3	0.2	0.0	0.0	0.3
KhC	0.6	0.6	0.8	0.6	0.7	0.4	0.6	0.6	0.0	0.4	0.2	0.6	1.0	0.0	0.2	0.3	0.2	0.0	0.0	0.3
KhD	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
KhE	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.0	0.1	1.0	0.2	0.2	0.0	1.0	0.3	1.0	0.0	0.0	0.0
TnA	0.3	0.3	0.4	0.4	0.3	0.4	0.1	0.6	0.0	0.3	0.3	0.3	0.3	0.0	0.3	1.0	0.3	0.0	0.0	0.2
TnB	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.0	0.1	1.0	0.2	0.2	0.0	1.0	0.3	1.0	0.0	0.0	0.0
TnC	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
TnD	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
TnE	0.6	0.4	0.5	0.3	0.4	0.4	0.2	0.1	0.0	0.4	0.0	0.3	0.3	0.0	0.0	0.2	0.0	0.0	0.0	1.0

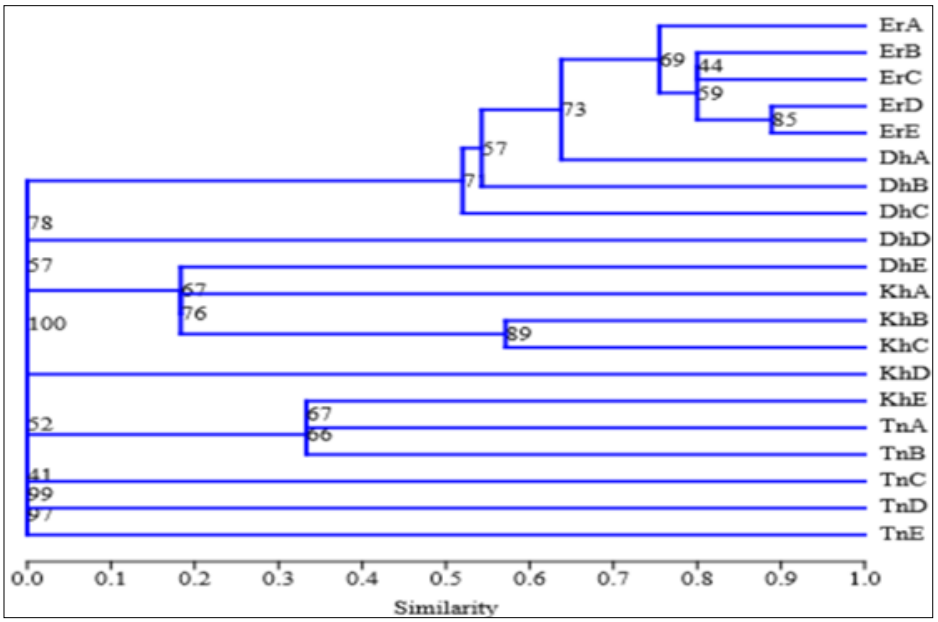

Fig. 7. Phylogenetic tree illustrating genetic relationships among 20 *P. crispa* samples in the central region of Saudi Arabian populations, based on Nei and Li's similarity analysis using combined data from 8 ISJ and 10 ISSR primers.

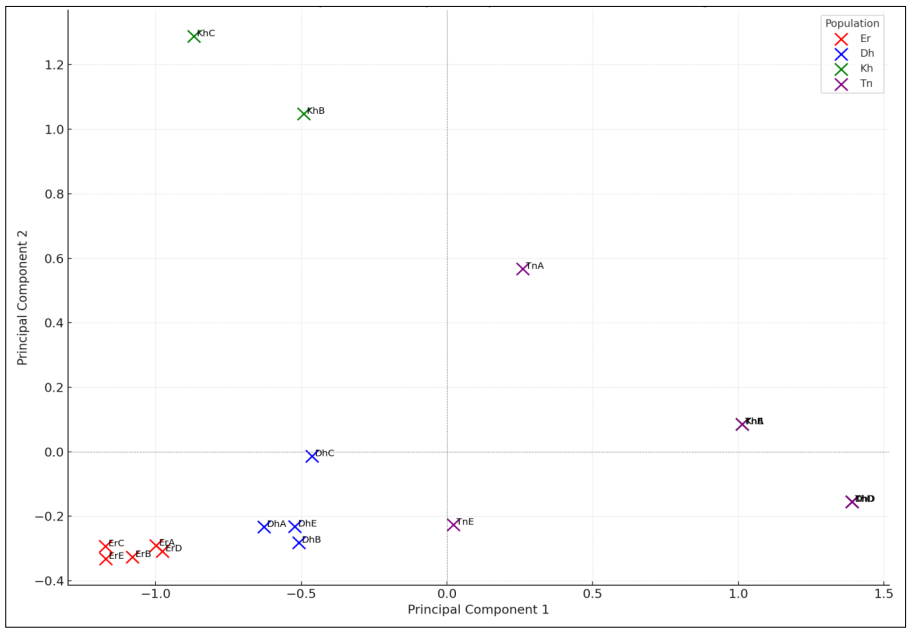

Fig. 8. Principal component analysis (PCA) biplot based on ISSR and ISJ marker data across 20 *P. crispa* samples. Samples cluster according to geographic origin, indicating population genetic structuring.

divergence (9, 10). The morphological distinctions observed in this study suggest that pollen traits may have potential as diagnostic tools for identifying population-level variation in *P. crispa* (1, 9).

Cluster analysis of the palynological traits further revealed that the populations could be divided into two main groups-DH/ER and TN/KH, suggesting an ecological gradient that may influence the morphological traits of these populations. This separation provides strong evidence of environmental adaptation and potential ecological factors, such as soil type, microclimate and elevation, playing a role in the morphological differentiation of these populations. Recent studies in arid environments have confirmed that ecological gradients often correlate with significant intraspecific variation in plant traits (11, 12). The strong alignment of this clustering with results obtained from molecular markers such as ISSR and ISJ highlights the complementary nature of these two approaches, reinforcing the idea that both morphological and molecular data provide complementary insights into the evolutionary history of a species (13, 14).

Molecular markers such as ISSR and ISJ help assess genetic variability, particularly in species that lack sufficient sequence data for other molecular techniques (15, 16). In *P. crispa*, genetic analysis showed relatively high levels of diversity, with ISSR and ISJ markers detecting polymorphic bands of 100 % and 98.21 %, respectively. These results suggest notable genetic variation within the studied populations, possibly associated with environmental variability in central Saudi Arabia. The polymorphism information content (PIC) values, ranging from 0.30 to 0.40, indicate that the markers have moderate discriminatory ability. This level of diversity aligns with findings from other desert-adapted species, where environmental stress is thought to contribute to genetic variation (17, 18). The observed genetic differentiation among populations may reflect influences such as geographical separation, local environmental conditions and potential adaptation processes (5).

Phylogenetic analysis provided additional insight into the genetic structure of the populations, identifying three main genetic clusters and four individual branches among the 20 genotypes examined. This pattern suggests a more complex population structure, potentially influenced by gene flow, genetic drift and selection processes. Genetic distance values ranging from 0 to 1 indicate notable divergence among populations, consistent with inter- and intra-population variation (19). Additionally, the alignment between pollen ornamentation and genetic clustering among *P. crispa* populations suggests that shared ecological pressures may influence both traits.

Reticulate pollen types corresponded to genetically similar populations, while microreticulate types were genetically distinct. This suggests that palynological traits may reflect phylogenetic relationships and ecological adaptation, supporting the notion that environmental factors drive divergence. Similar patterns have been reported in other desert plant species, where geographically isolated populations often exhibit substantial genetic differentiation (20). These findings underscore the importance of investigating evolutionary dynamics in *P. crispa*, particularly in relation to environmental factors that may influence genetic and morphological variation (9, 17).

The alignment between pollen morphology and molecular data suggests that populations may exhibit adaptive responses to their specific ecological contexts. Ecological and geographical separation among populations appears to be associated with distinct genetic patterns, which may reflect localized adaptation and ecological divergence. These findings indicate that *P. crispa* populations in the central region of Saudi Arabia could be influenced by a combination of evolutionary factors. Integrating morphological and molecular data offers a helpful approach for distinguishing species and examining processes contributing to variation in arid environments. Recent studies on desert plants have demonstrated that combining these methods can enhance the understanding of how species respond to environmental pressures (21, 22).

The broader implications of these findings are relevant for both plant systematics and conservation biology. They underscore the importance of integrating multiple data sources to assess genetic and morphological diversity within plant populations, which is crucial for effective conservation management in challenging environments such as deserts. Identifying genetically distinct populations indicates that conservation efforts should be tailored to protect these populations, which may possess unique adaptive traits and ecological functions (23). Conservation strategies that account for genetic and morphological differences between populations may be necessary to maintain the genetic integrity of *P. crispa* in the face of climate change and habitat degradation, ensuring the persistence of these populations in their respective ecological niches (24). Additionally, this study highlights the potential of combining palynological and molecular approaches to provide a more comprehensive understanding of plant biodiversity, supporting the development of more effective conservation policies and restoration practices in arid and semi-arid regions (25, 26).

Although our study revealed a moderate correlation between morphological and genetic diversity among *P. crispa* populations, the lack of direct environmental variable analysis limits the interpretative scope of these findings. Future research should consider integrating ecological parameters such as soil composition, moisture content and local microclimatic conditions. These factors can act as selective pressures that shape both genetic structure and phenotypic traits. By incorporating such data, it would be better to disentangle the environmental contributions from inherent genetic divergence, offering a more holistic understanding of adaptive evolution in desert-adapted plant species like *P. crispa*.

Conclusion

The present study confirms that *P. crispa* populations in central Saudi Arabia exhibit substantial morphological and genetic variability. Pollen morphological traits, including size and exine ornamentation, varied across populations and strongly correlated with genetic data derived from ISSR and ISJ markers. The congruence between palynological and molecular findings underscores the importance of using integrative methods in plant systematics. This approach enhances the resolution of population structure analysis and provides a robust framework for future studies on biodiversity conservation, especially in arid ecosystems. *P. crispa* can be a model species for investigating adaptation and differentiation in desert environments. Further research incorporating environmental variables and gene expression data is recommended to deepen our understanding of evolutionary dynamics in xerophytic taxa.

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Authors' contributions

MMA performed the palynological and molecular experiments, including DNA extraction, ISSR and ISJ marker amplification and pollen grain preparation, also contributed to the morphological measurements and drafted significant portions of the manuscript. MSA supervised the entire research process, coordinated the laboratory work, provided guidance on the methodology, assisted in data interpretation and contributed to the manuscript's critical revision and final preparation. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors do not have any conflicts of interest to declare.

Ethical issues: None

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