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# ASSESSMENT OF GENETIC DIVERSITY IN PAKISTANI DATE PALM (*PHOENIX DACTYLIFERA* L.) CULTIVARS BY GENOTYPING AND SSR MARKERS

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**Abstract**

Date palm (*Phoenix dactylifera* L.) is a major fruit crop cultivated in arid and semi-arid regions and plays a vital role in the agricultural economy of Pakistan, understanding its genetic diversity is crucial for conservation, breeding, and sustainable crop improvement. As one of the top three fruit crops in the country, date palm is grown widely across Pakistan, except in the northern highlands. This study aimed to evaluate the genetic variation and population structure of 31 date palm cultivars, including both local and exotic varieties, collected from various regions of South Punjab, representing a key date palm-growing area with date plam genetic resources existing all over Pakistan. Sixteen SSR markers were initially screened, and ten were selected for genotyping. Principal component analysis (PCA) and cluster analysis revealed considerable genetic variation, with 14 alleles detected across six successfully amplified SSR primers. Among them, mPdCIR025 exhibited the highest polymorphic information content (PIC) value of 0.37, indicating its strong discriminatory power. Major allele frequencies ranged from 0.5484 (mPdCIR025) to 0.9193 (mPdCIR015), while gene diversity values ranged from 0.1436 to 0.4953. Cluster analysis grouped the cultivars into three main clusters, highlighting clear genetic distinctions between local and exotic genotypes. These findings offer valuable insights for breeding programs, conservation efforts, and the sustainable utilization of date palm genetic resources in Pakistan.

**Key words:** Genotyping; Microsatellite markers; Genetic structure; Population clustering; Polymorphism

## Introduction

Date palm (*Phoenix dactylifera* L.), a perennial monocotyledonous member of the Arecaceae family, is extensively cultivated in arid and semi-arid regions due to its remarkable adaptability and economic significance (Ahmed *et al.*, 2021) Its resilience in hot climates, tolerance to drought, and ability to access deep soil moisture through a robust root system make it particularly suited to harsh environments. These adaptive traits contribute to its longevity and sustained productivity, establishing it as a key crop for agricultural sustainability in countries like Pakistan.

Pakistan ranks among the top seven global producers and exporters of dates. The crop is cultivated across 90,000 hectares in all four provinces and yields approximately 600,000 metric tons annually, making it the nation's third most important fruit crop after citrus and mango (Rahman *et al.*, 2022). The presence of over 300 indigenous verities highlights the country's rich genetic diversity and potential for crop improvement.

Despite the crop's agronomic and economic importance, limited molecular data is available on the genetic diversity of Pakistani date palm cultivars. Gaining insight into this diversity is essential for germplasm conservation, varietal improvement, and the development of climate-resilient cultivars. Genetic variability supports the enhancement of important agronomic traits, including fruit quality, stress tolerance, and disease resistance (Metlo *et al*., 2021).

Microsatellites, also known as simple sequence repeats (SSRs), are widely used molecular markers for genetic diversity studies due to their high polymorphism, reproducibility, and co-dominant inheritance (Reddy *et al.*, 2022). Despite the availability of more advanced molecular techniques such as SNP arrays and genotyping-by-sequencing (GBS), SSR markers remain a valuable tool for assessing genetic variation, particularly in developing countries due to their cost-effectiveness and accessibility. However, SSRs target only known loci and thus offer lower resolution compared to next-generation sequencing (NGS) approaches.

In this study, SSR-based genotyping was applied to assess the genetic diversity and phylogenetic relationships among 31 local and exotic date palm cultivars collected from South Punjab, Pakistan. The findings aim to inform future breeding programs, conservation strategies, and the sustainable utilization of date palm genetic resources.

## Material and Methods

**Plant genetic resources:** This study was conducted on 31 cultivars of date palm (*Phoenix dactylifera* L.), comprising both male and female, native and exotic varieties. Leaf samples were collected from different geographical locations across South Punjab, Pakistan (Fig. 1; Table 1). All procedures were carried out in accordance with the applicable institutional biosafety policies and protocols.

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Fig. 1. Map Highlighting Key Date Palm Growing Regions in Pakistan. (Abdul Soad *et al.*, 2015).

**DNA isolation:** The total genomic DNA was isolated via the modified CTAB technique and subsequently treated with RNase. Using a BioSpec Nano spectrophotometer (Shimadzu, Japan) and 1% agarose gel, the genomic DNA was analyzed quantitatively and qualitatively. The extracted DNA was used for SSR genotyping (Aboul-Maaty & Oraby, 2019).

**Primer selection:** Primer selection was a critical step in this study to ensure accurate and reliable analysis of date palm genotypes. Initially, 100 reported SSR primers were shortlisted based on their relevance to date palm and prior reports of polymorphism. These primers were screened for amplification quality and polymorphic potential, leading to the selection of 16 SSR primers (Table 2) that demonstrated clear, reproducible, and polymorphic bands across the studied genotypes.

**Amplification by SSR markers:** An Applied Biosystems 96-well Veriti® thermal cycler was used to conduct polymerase chain reaction (PCR) to identify SSR polymorphisms. Using 5 primers and an annealing temperature of 54°C, 15 samples were chosen at random for SSR marker amplification. Genetic diversity among 31 date palm varieties was evaluated using 16 SSR markers.

Two microliters of 25 ng of DNA template, 10µl of Bio-Basic Canada Red Dye Taq PCR master mix (Cat no. BS9297/BS9298), and 2µl of each 10 nM primer made up the 20µl volume used to conduct the PCR reactions. In the PCR protocol, the primers were heated to temperatures between 48 and 60°C for 90 seconds, the DNA was denatured at 94°C for 5 minutes, 35 cycles of denaturation at 94°C for 30 seconds, annealing at 72°C for 60 seconds, and finally, an extension step lasting 5 minutes at 72°C were performed. The images were obtained via the Alpha Innotech Gel Doc System with Alpha view software version 1.0.1.14. PCR amplification was visualized on a 2% agarose gel using Syber safe dye (Alpha Innotech Corporation) (Ahmed *et al.*, 2021).

**Genotyping by SSR markers:** Ten SSR primers (Table 3) were selected and confirmed via PCR amplification. Each SSR-PCR mixture contained a reaction buffer, 2 mmol/L $MgCl_2$, a dNTP mixture (40 µmol /L), reverse and forward primers (0.5 µmol /L each), 50 ng of genomic DNA, and DNA polymerase. Thermal cycling involved initial denaturation at 94°C for 5 minutes, followed by 36 cycles of denaturation at 94°C for 50 seconds, annealing at the optimum temperature for 1 minute, and extension at 72°C for 1 minute, with a final extension step at 72°C for 7 minutes.

The PCR findings were visualized via polyacrylamide gel electrophoresis (PAGE). The fluorescence dye-conjugated SSR primers enabled genotyping through capillary electrophoresis via the ABI system 3730 DNA Analyzer. The PCR products were introduced into capillaries with a polyacrylamide matrix for electrophoresis. Luminescent peaks in the electropherogram corresponded to amplified alleles (Saha *et al.*, 2017).

**Table 1. Geographic origin and collection sources of local and exotic date palm (*Phoenix dactylifera* L.) accessions.**

| Sr. No. | Name | Varieties | Sources |
|---|---|---|---|
| 1. | Takala, Karbalain, Halavi, Shukri | Indigenous female plants | "Gillani Farm, Bastimalook, District Multan, Central Punjab" |
| 2. | Toshabusra, Khudravi<br>Barhee , Amber, Khallas, Umm-ul-dahan, Fard white, Majdoul, Amber | Indigenous female plants<br>Imported female plants | AL-Qasim-Bagh Dera Ghazi Khan Punjab |
| 3. | Kehridora, Ghulistan, Shereen, Dhaki,<br>Ghanami | Indigenous female plants<br>Indigenous male plant | 18 Hazari date palm farm district jhang Punjab |
| 4. | Burhee<br>Khallas<br>Ganada, Ajwa, Nawader, Sultana, Sheeshe | Indigenous male plants<br>Exotic male plants<br>Exotic female plants | Al WathbaMarionnet L.L.C. Tissue Cultured Date Palms Abu Dhabi, United Arab Emirates. P. O. Box: 41522 |
| 5. | Karbalain, Angoor, Khudravi, Naboot, Ghush<br>Ajwa | Indigenous male plants<br>Imported male plant | Government Date Palm Research sub-station jhang, Punjab |

**Table 2. List of SSR markers selected for genetic diversity.**

| Sr. No. | Primers names | Forward sequence | Reverse sequence | Repeat motifs | Expected allele sizes | References |
|---|---|---|---|---|---|---|
| 1. | mPdCIR010 | ACCCCGGACGTGAGGTG | CGTCGATCTCCTCCTTTGTCTC | (GA)22 | 118-161 | (Ahmed & Al-Qaradawi, 2009) |
| 2. | mPdCIR015 | AGCTGGCTCCTCCCTTCTTA | GCTCGGTTGGACTTGTTCT | (GA)15 | 120-156 | (Ahmed & Al-Qaradawi, 2009) |
| 3. | mPdCIR016 | AGCGGGAAATGAAAAGGTAT | ATGAAAACGTGCCAAATGTC | (GA)14 | 130-138 | (Ahmed & Al-Qaradawi, 2009) |
| 4. | mPdCIR025 | GCACGAGAAGGCTTATAGT | CCCCTCATTAGGATTCTAC | (GA)22 | 199-231 | (Ahmed & Al-Qaradawi, 2009) |
| 5. | mPdCIR032 | CAAATCTTTGCCGTGAG | GGTGTGGAGTAATCATGTAGTAG | (GA)19 | 284-305 | (Ahmed & Al-Qaradawi, 2009) |
| 6. | mPdCIR035 | ACAAACGGCGATGGGATTAC | CCGCAGCTCACCTCTTCTAT | (GA)15 | 175-221 | (Ahmed & Al-Qaradawi, 2009) |
| 7. | mPdCIR044 | ATGCGGACTACACTATTCTAC | GGTGATTGACTTTCTTTGAG | (GA)19 | 281-332 | (Ahmed & Al-Qaradawi, 2009) |
| 8. | mPdCIR048 | CGAGACCTACCTTCAACAAA | CCACCAACCAAATCAAACAC | (GA)32 | 156-192 | (Ahmed & Al-Qaradawi, 2009) |
| 9. | mPdCIR050 | CTGCCATTTCTTCTGAC | CACCATGCACAAAAATG | (GA)21 | 154-208 | (Ahmed & Al-Qaradawi, 2009) |
| 10. | mPdCIR057 | AAGCAGCAGCCCTTCCGTAG | GTTCTCACTCGCCCAAAAATAC | (GA)20 | 251-278 | (Ahmed & Al-Qaradawi, 2009) |
| 11. | mPdCIR063 | CTTTTATGTGGTCTGAGAGA | TCTCTGATCTTGGGTTCTGT | (GA)17 | 121-156 | (Ahmed & Al-Qaradawi, 2009) |
| 12. | mPdCIR070 | CAAGACCCAAGGCTAAC | GGAGGTGGCTTTGTAGTAT | (GA)17 | 182-208 | (Ahmed & Al-Qaradawi, 2009) |
| 13. | mPdCIR078 | TGGATTTCCATTGTGAG | CCCGAAGAGACGCTATT | (GA)13 | 117-152 | (Ahmed & Al-Qaradawi, 2009) |
| 14. | mPdCIR085 | GAGAGAGGGTGGTGTTATT | TTCATCCAGAACCACAGTA | (GA)29 | 152-183 | (Ahmed &Al-Qaradawi, 2009) |
| 15. | mPdCIR090 | GCAGTCAGTCCCTCATA | TGCTTGTAGCCCTTCAG | (GA)26 | 142-175 | (Ahmed &Al-Qaradawi, 2009) |
| 16. | mPdCIR093 | CCATTTATCATTCCCTCTCTTG | CTTGGTAGCTGCGTTTCTTG | (GA)16 | 153-184 | (Ahmed &Al-Qaradawi, 2009) |

**Table 3. List of SSR primers used for genotyping.**

| Sr. No. | Primer code | Forward sequence | Reverse sequence | Temp. |
|---|---|---|---|---|
| 1. | mPdCIR010 | ACCCCGGACGTGAGGTG | CGTCGATCTCCTCCTTTGTCTC | 54°C |
| 2. | mPdCIR015 | AGCTGGCTCCTCCCTTCTTA | GCTCGGTTGGACTTGTTCT | 56°C |
| 3. | mPdCIR016 | AGCGGGAAATGAAAAGGTAT | ATGAAAACGTGCCAAATGTC | 58°C |
| 4. | mPdCIR025 | GCACGAGAAGGCTTATAGT | CCCCTCATTAGGATTCTAC | 58°C |
| 5. | mPdCIR035 | ACAAACGGCGATGGGATTAC | CCGCAGCTCACCTCTTCTAT | 54°C |
| 6. | mPdCIR070 | CAAGACCCAAGGCTAAC | GGAGGTGGCTTTGTAGTAT | 56°C |
| 7. | mPdCIR078 | TGGATTTCCATTGTGAG | CCCGAAGAGACGCTATT | 56°C |
| 8. | mPdCIR085 | GAGAGAGGGTGGTGTTATT | TTCATCCAGAACCACAGTA | 58°C |
| 9. | mPdCIR090 | GCAGTCAGTCCCTCATA | TGCTTGTAGCCCTTCAG | 59°C |
| 10. | mPdCIR093 | CCATTTATCATTCCCTCTCTTG | CTTGGTAGCTGCGTTTCTTG | 58°C |

**Data scoring and analysis:** Scorable fragments were defined as any visible and clearly scorable fragments amplified by the SSR primers. Researchers evaluated the germplasm amplification profiles and assigned a presence/absence score to bands of DNA fragments. A number of shared amplification products were used to determine genetic similarity via primer DNA fingerprinting data. The genetic relationships among the germplasms were estimated via cluster dendrogram that was built through vegan package in R language version 4.2.3. The efficacy of a molecular marker technique relies on a specific set of parameters. To evaluate this, we calculated the polymorphic information content (PIC) using Nei's and Botstein methods. Principal component analysis was performed in Python using libraries (numpy, pandas, matplotlib, sklearn).

## Results

To better understand the genetic profiles of selected date palm (*Phoenix dactylifera* L.) cultivars, 16 SSR primers were initially screened for amplification. Of these, 6 primers successfully produced distinct banding patterns across all 31 genotypes. The 6 SSR primers collectively produced a total of 14 alleles, with amplified fragment sizes across all primers ranging from 100 to 280 bp. Specifically, the fragment size ranges (Fig. 2) for these primers were as follows: mPdCIR015 (120-160 bp), mPdCIR016 (180-200 bp), mPdCIR025 (180-190 bp), mPdCIR035 (170-190 bp) mPdCIR085 (160-210 bp), and mPdCIR090 (130-180 bp).

The effectiveness of each primer in detecting polymorphism was evaluated by calculating the polymorphism information content (PIC), major allele frequency, and gene diversity (Table 4). PIC values ranged from 0.13 to 0.37, with the highest value observed for the primer mPdCIR025 (0.37), followed by mPdCIR085 and mPdCIR090, each with a PIC value of 0.33. Based on these results, the primers mPdCIR025, mPdCIR085, and mPdCIR090 were identified as the most informative for distinguishing date palm genotypes. In contrast, the lowest PIC value (0.13) was recorded for mPdCIR015. Overall, the 6 SSR primers used in this study yielded an average PIC value of 0.28, indicating an intermediate polymorphism. Genetic diversity was highest for the mPdCIR025 primer (49%) and lowest for mPdCIR015.

**Table 4. SSR polymorphisms.**

| Primer | Major Allele frequency | Sample size | Gene diversity | PIC Value |
|---|---|---|---|---|
| mPdCIR015 | 0.9193 | 31 | 0.1436 | 0.13 |
| mPdCIR016 | 0.7419 | 31 | 0.3080 | 0.24 |
| mPdCIR025 | 0.5484 | 31 | 0.4953 | 0.37 |
| mPdCIR035 | 0.7258 | 31 | 0.3975 | 0.32 |
| mPdCIR085 | 0.6774 | 31 | 0.4287 | 0.33 |
| mPdCIR090 | 0.6935 | 31 | 0.4204 | 0.33 |

**Cluster analysis based on SSR amplification:** The 31 date palm types were categorized into three chief clusters, with further subdivisions based on genetic similarity using the Jacquard similarity coefficient (Fig. 3). Cluster 1 exhibited 2 subclusters, each further separated into groups. Subcluster 1.1 comprised 2 groups, with Shereen in group 1.1/1 and Ghulistan in group 1.1/2. Subcluster 1.2 consisted of 3 groups, with Khudravi and Kehridora in one group and Takala and Toshabusra in separate groups. Cluster 2 was divided into 2 subclusters, with subcluster 2.1 containing 2 groups showing notable genetic similarity. Subcluster 2.2 displayed diversity, comprising 2 varieties, Angoor male and Ajwa. Cluster 3 exhibited distant genetic relationships between subclusters, with subcluster 3.1 being divided into 2 groups and subcluster 3.2 containing 4 varieties. Overall, maximum genetic distinctness was observed within subclusters of Cluster 1 and Cluster 3, where red-colored genotypes represent exotic varieties, and green-colored represent local cultivars.

**SSR genotyping and genetic diversity analysis in date palm:** Genetic mapping of 31 date palm (*Phoenix dactylifera* L.) varieties was conducted using 10 highly polymorphic SSR markers. Genomic DNA was analyzed through the microsatellite genotyping service of CD Genomics (USA), where PCR amplification with fluorescently labeled primers was followed by capillary electrophoresis on ABI genetic analyzers.

Allele sizing and genotype scoring were performed using Applied Biosystems GeneMapper® 5 software, which provided allele sizes, peak heights, and area values for precise genotyping. The SSR profiles revealed both homozygous and heterozygous patterns among the varieties. For instance, the 'Takala' variety showed two distinct peaks at 121.34 bp and 133.21 bp for marker mPdCIR010, indicating heterozygosity at this locus (Fig. 4). These clear and reproducible polymorphisms highlight the utility of SSR markers in assessing genetic diversity and distinguishing genotypes. Data, including complete Excel sheets and graphical allele profiles for all ten markers, are provided in the supplementary data.

**Cluster analysis based on genotyping of SSR marker:** Cluster Analysis based on Genotyping divided the varieties into 2 main clusters (Fig. 5). In Cluster 1, 2 subclusters were identified. Subcluster 1.1 comprised 3 varieties, with Burhee and Majdoul paired closely together, exhibiting maximum similarity (index value close to zero), whereas Sultana presented moderate similarity to this pair. Subcluster 1.2 included 4 date palm varieties, with Ganada grouping closely with Sheeshe, and Naboot Male clustering with Ghush Male. This pairing suggests high similarity within each pair and notable differentiation between them, supported by an index value of 44. Cluster 2 was also subdivided into 2 subclusters. Subcluster 2.1 consisted solely of the Burhee Male variety, which showed the greatest divergence from the varieties in Subcluster 2.2. The latter was further separated into 2 main groups, with Group 2.2/1 containing 6 distinct varieties, where Tosha busra and Kehri dora exhibited the greatest similarity, closely followed by Takala. Karbalain and Ghanami formed another pair closely related to Umm-ul-dahan. Group 2.2/2 included 11 varieties, with Angoor males and Ajwa males showing maximum similarity, whereas Karbalain and Ghulistan exhibited distinctness. Shereen was closely related to Khallas, Halavi to Fard White, and Amber to Nawader. Additionally, Amber and Ajwa showed maximum similarity, which was distinct from that of Khallas males.

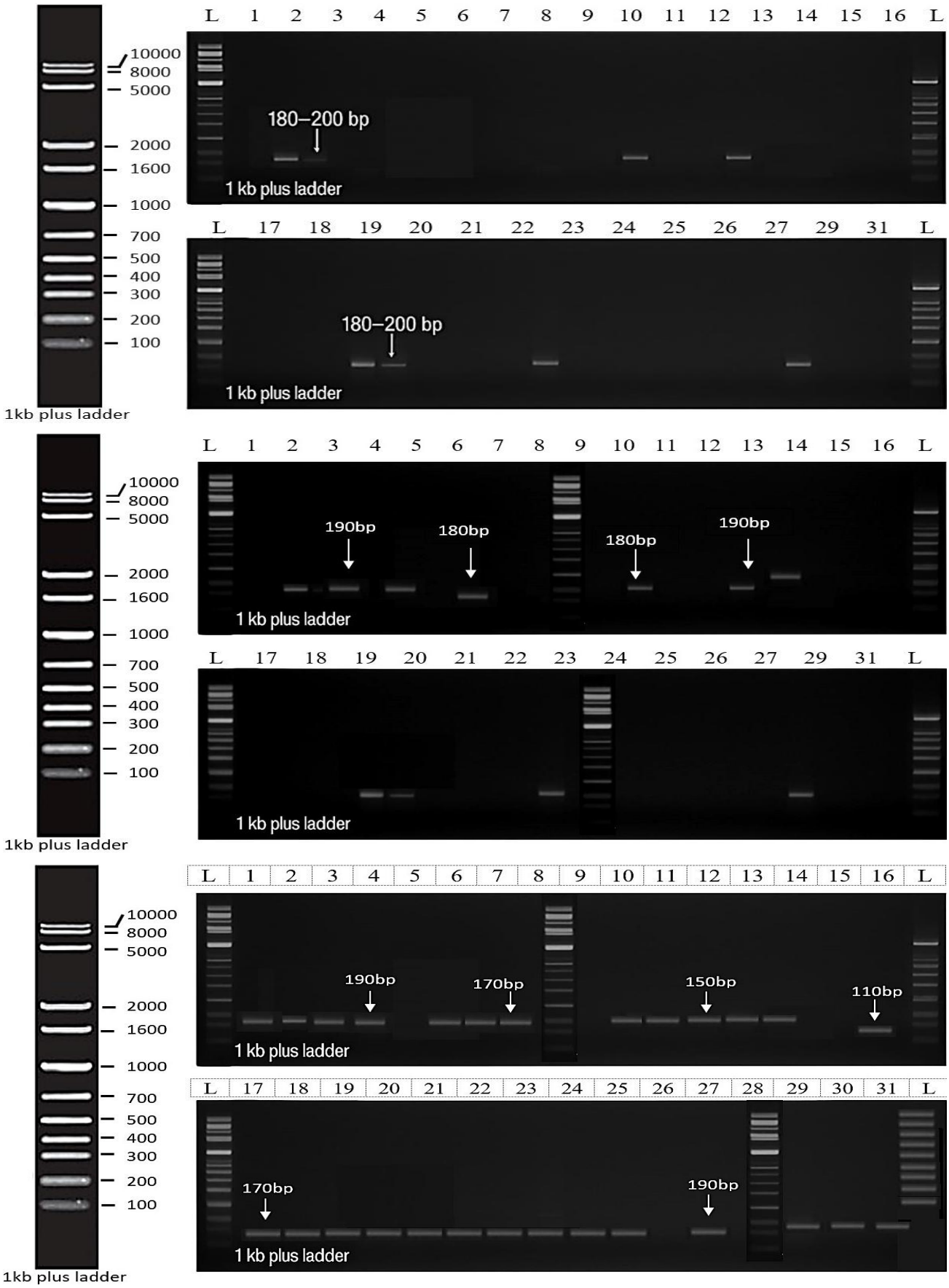


Fig. 2. PCR amplification profiles of three SSR primers in 31 date palm (*Phoenix dactylifera* L.) cultivars.
(A) mPdCIR016, (B) mPdCIR025, (C) mPdCIR035. PCR products were resolved on 2% agarose gel stained with ethidium bromide and visualized under UV light.
(Distinct banding patterns confirmed successful amplification and polymorphism, with each primer showing varying resolution and ability to separate alleles)

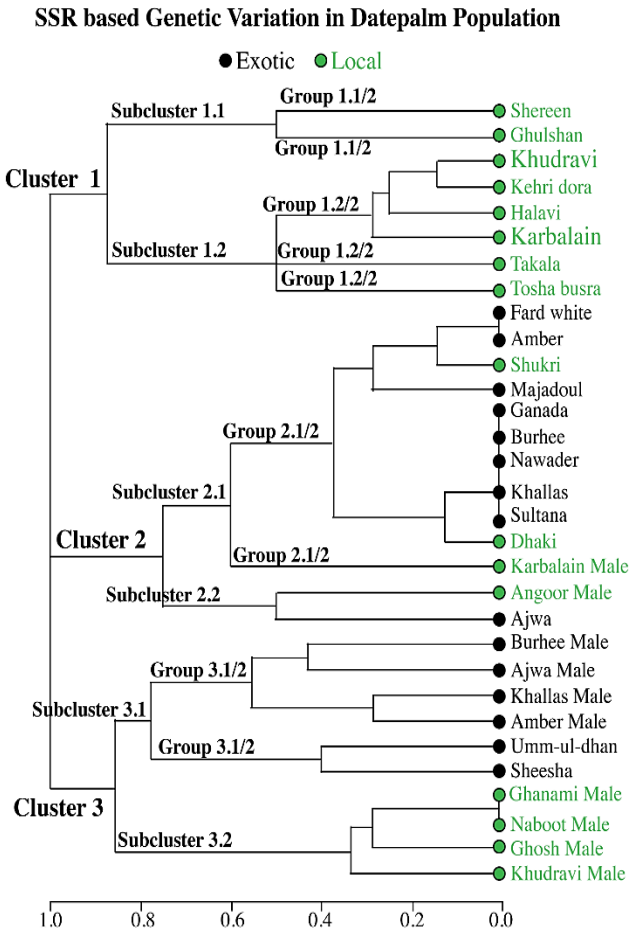


Fig. 3. Cluster dendrogram of 31 date palm (*Phoenix dactylifera* L.) cultivars based on binary data from SSR marker profiles.

**Principal component analysis:** In the PCA scatter plot, each point represents a genotype analyzed using SSR markers (Fig. 6). Principal Component 1 (PC1) (or Dim1) separates the genotypes along the x-axis and explains 22.72% of the total variation in the dataset. This component reflects major genetic differences among the cultivars, possibly due to their geographic origin or shared allelic composition. Principal Component 2 (PC2) (or Dim2), aligned on the y-axis, accounts for 17.44% of the variation and reveals additional diversity, which may be linked to sex-specific alleles or cultivar-specific markers. Combined, PC1 and PC2 explain 40.17% of the total variation.

Including PC3 increases the cumulative variability to 56.56%, and PC4 brings it to 67.58%. Variability explained beyond PC5 (75.95%) is comparatively minor. Therefore, the first three to four components are sufficient for capturing the main genetic structure of the dataset (Table 5).

The spatial arrangement of genotypes in the PCA plot illustrated meaningful clustering patterns.

Genotypes such as "Ajwa," "Amber," and "Karbalain Male" grouped closely together, indicating high genetic similarity. In contrast, genotypes like "Ghulistan" and "Shereen" appeared more isolated from other groups, suggesting distinct genetic features. Furthermore, "Tosha Busra" and "Ghulistan" are positioned far apart, implying the presence of unique alleles or divergence in lineage. Male genotypes such as "Khudravi Male" and "Burhee Male" showed tendencies to group separately from female cultivars, which may point to potential sex-linked genetic variation.

The separation of genotypes along PC1 and PC2 offered insights into the genetic relationships, diversity, and potential lineage of *Phoenix dactylifera* L. varieties in Pakistan. This analysis also supports the utility of SSR markers in differentiating both local and exotic varieties, identifying potential parental lines, and informing breeding programs focused on genetic improvement and conservation.

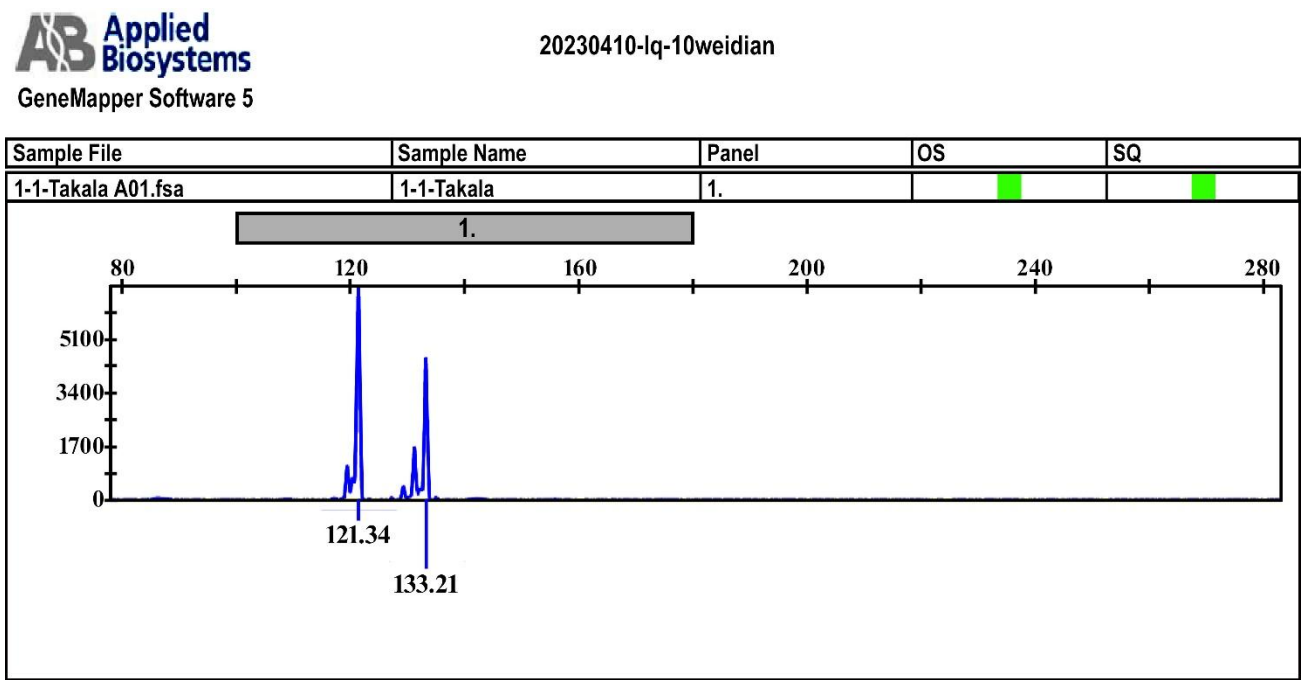


Fig. 4. Genetic mapping analysis using Applied Bioscience Gene Mapper 5 software with the date palm variety 'TAKALA'. Graph illustrating the distribution of the mPdCIR010 marker across the genome.

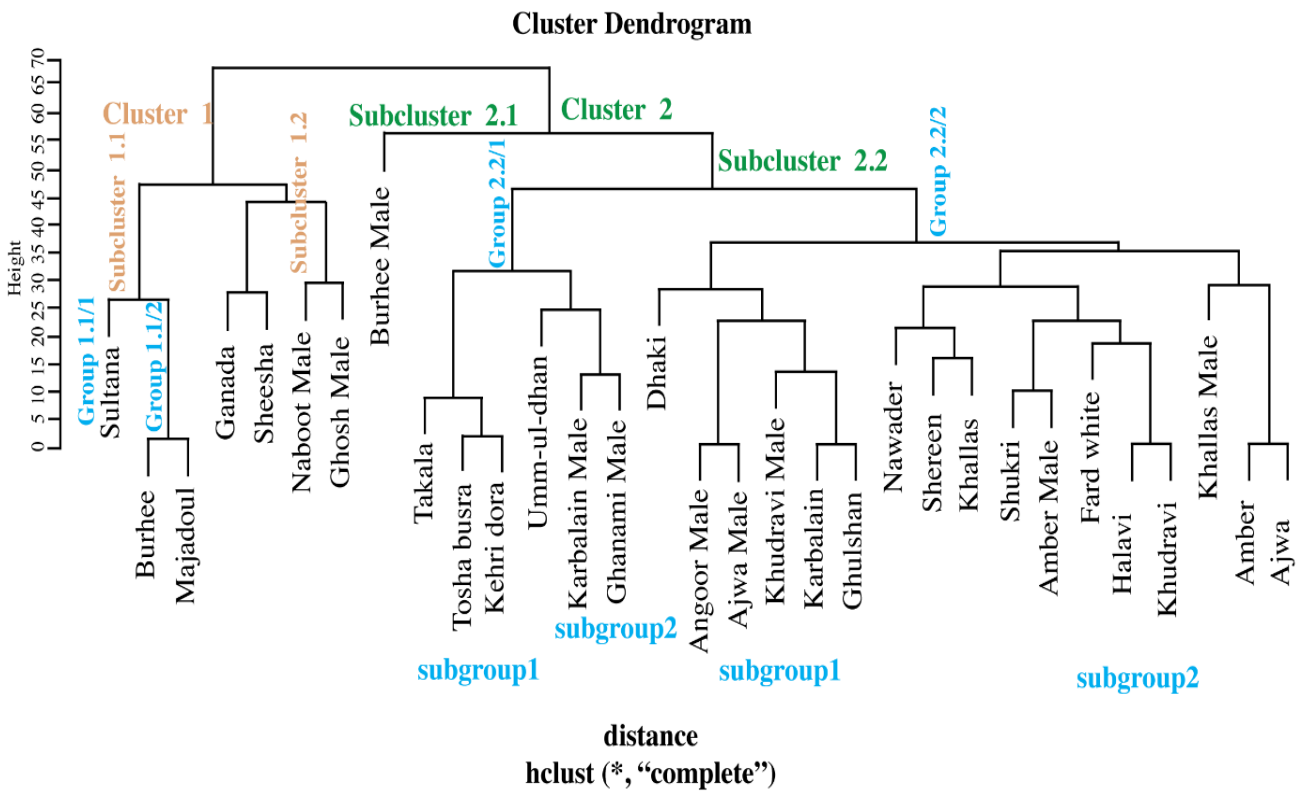


Fig. 5. Cluster dendrogram of 31 date palm (Phoenix dactylifera L.) cultivars based on genotyping data from ten SSR markers.

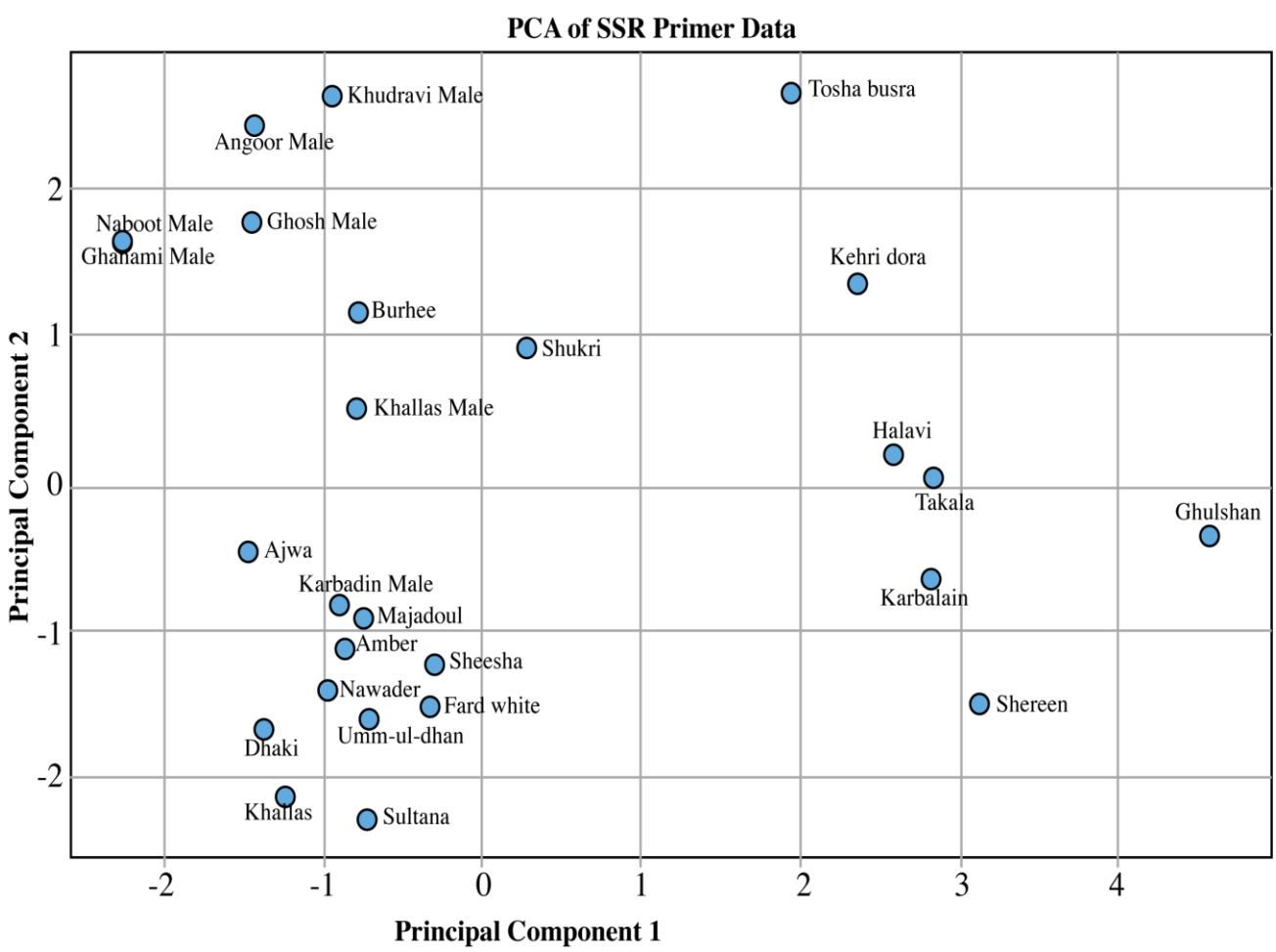


Fig. 6. Principal Component Analysis (PCA) showing the spatial distribution of date palm (*Phoenix dactylifera* L.) varieties based on genetic similarity.

**Table 5. Description of eigenvalues, variability, and cumulative variability of principal components for female varieties.**

| PC | Eigenvalue | Variability % | Cumulative % |
|---|---|---|---|
| PC1 | 3.287318 | 22.723399 | 22.723399 |
| PC2 | 2.523643 | 17.444535 | 40.167935 |
| PC3 | 2.370882 | 16.388582 | 56.556517 |
| PC4 | 1.594291 | 11.020443 | 67.57696 |
| PC5 | 1.212005 | 8.377918 | 75.954878 |
| PC6 | 1.054466 | 7.288939 | 83.243817 |
| PC7 | 0.839595 | 5.803654 | 89.047471 |
| PC8 | 0.446442 | 3.086004 | 92.133475 |
| PC9 | 0.375421 | 2.595077 | 94.728552 |
| PC10 | 0.293264 | 2.027167 | 96.755719 |
| PC11 | 0.194703 | 1.345871 | 98.10159 |
| PC12 | 0.115125 | 0.795792 | 98.897382 |
| PC13 | 0.084797 | 0.586157 | 99.483539 |
| PC14 | 0.074715 | 0.516461 | 100 |

## Discussion

The assessment of genetic diversity among 31 date palm cultivars using SSR markers revealed a moderate polymorphism. A total of 14 alleles were detected across 6 amplified primers, with an average PIC of 0.28. The highest, observed for mPdCIR025, was 0.37 suggesting its potential effectiveness in distinguishing between genotypes. These findings were consistent with earlier studies that reported moderate to high levels of diversity using SSR markers in date palm (Ahmed *et al.*, 2021; Saha *et al.*, 2017).

The dendrogram constructed from SSR marker data grouped the cultivars into 3 main clusters, indicating clear genetic differentiation between local and exotic varieties. This clustering pattern supports previous findings by (Al-Dous *et al.*, 2011) and (Khouane *et al.*, 2020), who also reported genetic separation based on origin and sex. Interestingly, the PCA results further confirmed this structure, with PC1 and PC2 explaining a significant proportion of total genetic variation. However, the principal components lacked clear interpretation in the original results section; thus, we highlight here that PC1 mainly captured variation due to geographic origin, while PC2 reflected genetic differences between male and female genotypes.

Compared to more advanced genotyping methods such as SNP arrays or GBS, SSR markers provide a lower-resolution but still informative approach for assessing genetic diversity. While SSRs offer cost-efficiency and ease of use, they are labor-intensive, cover fewer loci, and may miss subtle genomic variations detectable by genome-wide approaches. For example, studies such as (Al-Najm *et al.*, 2017) using SNP data have demonstrated finer-scale diversity and clearer population structures. Despite these limitations, the use of SSR markers in this study successfully revealed meaningful insights into the genetic structure of Pakistani date palms.

In support of SSR utility, (Taheri *et al.*, 2018) demonstrated the effective development of novel SSR markers from next-generation sequencing data, emphasizing their continued relevance in plant genomics despite the rise of high-throughput technologies. Similarly, (Yousaf *et al.*, 2015) validated microsatellite markers in medicinally important Epimedium species, demonstrating their utility across diverse plant species. These studies reinforce the credibility of SSR-based approaches in genetic diversity assessments, particularly where resources for NGS are limited.

This research highlights the importance of local cultivars, which exhibited greater genetic variability compared to exotic ones. This diversity is valuable for future breeding efforts aimed at improving traits such as drought tolerance, fruit quality, and pest resistance. Moreover, the identification of highly informative markers like mPdCIR025 and mPdCIR085 provides a useful resource for marker-assisted selection in breeding programs.

**Limitations:** While SSR markers offered important insights into the genetic diversity of date palm cultivars, several limitations should be acknowledged. SSR analysis is labor-intensive and time-consuming, and it targets a limited number of known loci, reducing its genome-wide representativeness. Unlike modern high-throughput techniques such as SNP arrays and genotyping-by-sequencing, SSRs have lower resolution and may overlook minor allelic variations. Additionally, SSR-based clustering may not fully reflect true genetic distances at the whole-genome level. Future studies incorporating next-generation sequencing technologies would enable more comprehensive assessments of genetic variation and provide higher resolution for mapping, population structure analysis, and marker-assisted breeding in date palm.

## Conclusion

In conclusion, assessing genetic diversity among date palms at the DNA level is crucial for breeding and conservation purposes. This study sheds light on the significant genetic diversity present among 31 local and exotic date palm varieties from South Punjab, Pakistan, using SSR markers. The moderate levels of polymorphism observed, along with the identification of informative primers, highlight the utility of SSRs for genetic studies in date palm. The distinct genetic groupings between local and exotic varieties emphasize the broader genetic base of local varieties highlighting their importance for breeding programs. The identification of SSR markers with high PIC values provides reliable tools for marker-assisted selection in date palm breeding programs. These findings contribute to our understanding of date palm germplasm and can guide efforts to improve fruit quality, stress tolerance, and disease resistance through breeding programs and conservation initiatives. Future studies incorporating high-throughput sequencing and broader sampling will enhance our understanding of date palm genetics and support sustainable conservation and breeding strategies.

**Conflict of interests:** The authors confirm that they do not have any identifiable conflicting financial interests or personal ties that could have potentially influenced the findings presented in this paper.

**Authors contribution:** Beenish Sadique designed and conducted the research, performed experiments, and wrote the manuscript. Aleena Sumrin supervised the research, provided overall guidance, assisted in data analysis, figure preparation, and manuscript revision. Shaukat Ali contributed to study design, sample collection, primer selection, and DNA extraction. Ana Caicedo conducted genotyping in her lab and contributed to data interpretation. All authors reviewed and approved the final manuscript.

**Data availability:** Supplementary material associated with this article, including all available data, is provided in the online version.

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