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Full Length Article

Genetic Marker Identification and Functional Genomics in Date Palm (*Phoenix dactylifera* L.) for Economic Trait Improvement and Resilience Enhancement

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Abstract

Date palm (*Phoenix dactylifera* L.) is one of the most economically valuable fruit trees and its genetic markers are essential for future improvement in key economic traits along with stresses resistance. Through *in silico* PCR, traits including drought and salinity tolerance were linked to ten Date palm (*Phoenix dactylifera* L.) cultivars including Zaghloul, Hayani, Siwi, Amry and Sakkoty. *In silico* PCR and PCR validation techniques confirmed the three significant markers, PR-4, S.2, and NPR1-like, that exhibited cultivar-specific banding patterns linked to drought and salinity tolerance. Moreover, SCoT analysis revealed a 22% polymorphism among the ten cultivars studied, indicating a substantial level of genetic variability. Also, Genetic similarity indices ranged from 83% to 98% highlighting both the close relationships and the distinct genetic backgrounds among the studied cultivars, particularly between soft and dry types. Such markers and clustering patterns provide useful information for breeding programs to develop date palms that are more tolerant to stress while enhancing yield. This study underlines the importance of genomic data for breeding resilient and high-yield date palm cultivars, which can contribute to food and economic security in arid regions. These results provide genetic insight essential for improving date palm in the face of climate stress, which will ultimately support sustainable agriculture and development in an economically important growing region.

Keywords: *Phoenix dactylifera*; *In silico*; PCR; Drought; Salinity; Sustainable agriculture

Introduction

Date palm (*Phoenix dactylifera* L.) is a plant of significant economic, social, and historical importance. Recent advances in genomics have revealed the extensive genetic diversity of date palm, particularly within the wild populations of oases, emphasizing its adaptation to arid environments and its potential for sustainable agriculture. Understanding the genetic composition of date palm cultivars is essential for developing resilient plants that can withstand stress factors such as drought and salinity, thereby increasing agricultural productivity and enabling date palms to thrive in challenging conditions (Elmeer *et al*. 2011; Mathew *et al*. 2014; Saboori *et al*. 2021). In addition to their economic value as a high-priced commodity, date palms contain bioactive compounds with potential medicinal applications, offering new opportunities for pharmacological research. The genetic study of date palms not only enhances our understanding of their ancient history but also drives progress in both medicine and agriculture (Sattar *et al*. 2017; Rahman *et al*. 2022).

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By exploring the genetic landscapes that determine varietal variations and economic traits, research aims to optimize cultivation practices and expand the utility of date palms beyond traditional uses (Saboori *et al*. 2021). Integrating traditional agricultural significance with modern genetic insights is a key focus of current studies, aiming to substantially increase date palm production, enhance its resilience, and improve its nutritional quality. This not only boosts the economic vitality of regions where date palms are essential but also supports conservation efforts and breeding programs focused on developing superior cultivars adapted to diverse environments (Al-Najm *et al*. 2016; He *et al*. 2017). Molecular markers play a crucial role in unraveling the genetic complexity of plant species, facilitating genetic diversity assessments, genetic mapping, and the selection of plants with desired traits. Techniques such as microsatellites and SNPs have gained widespread acceptance in genetic studies, offering valuable insights into genetic diversity, trait selection, and breeding programs for crop improvement (Elshibli and Korpelainen 2009; Moussally *et al*. 2010; Maher *et al*. 2021). The use of molecular markers in plant breeding programs holds great promise for enhancing crop improvement and promoting food sustainability (Gros-Balthazard *et al*. 2021; Zhang 2024). *In silico* PCR, a computational tool for predicting PCR amplicons from sequenced genomes, has revolutionized molecular marker studies by enhancing primer selectivity and optimizing primer design for various genetic and genomic applications. This tool has been instrumental in improving the accuracy and efficiency of PCR-based assays, thereby advancing genetic research and molecular diagnostics (Amjad *et al*. 2022; Abdel-Baky *et al*. 2023). The continuous refinement and validation of *in silico* PCR tools is essential to ensure the accuracy and reproducibility of PCR-based assays across various research fields (Mokhtar *et al*. 2016). While existing molecular tools have significantly advanced the identification of individual date palm cultivars and the understanding of their genetic control, a research gap remains in developing molecular tools specifically tailored to date palms. There is a need to focus on creating genetic markers associated with key economic traits in date palms, using markers such as SSRs and microsatellites, which have proven effective in characterizing date palm genotypes (Elmeer *et al*. 2011; Rahman *et al*. 2022). Our research aims to address this gap by developing specific genetic markers linked to key economic traits of date palms. We conducted a comprehensive analysis of the date palm genome and perform laboratory experiments to validate our results, ultimately identifying genetic markers associated with important traits of date palm.

Materials and Methods

Plant materials

Ten cultivars of date palm (*Phoenix dactylifera* L.); Zaghloul-a (1), Zaghloul-b (2), Hayani-a (3), Hayani-b (4), Siwi-a (5), Siwi-b (6), Amry-a (7), Amry-b (8), Sackouttti-a (9), and Sackouttti-b (10), were kindly provided by the Central Laboratory for Date Palm Research and Development, Agricultural Research Center, Giza, Egypt. Date palm leaves were stored at -80°C prior to use.

DNA extraction

DNA extraction from 200 mg of leaf tissue of date palm samples was performed using the DNeasy Plant Mini Kit (QIAGEN, Germany, Cat. No./ID: 69104,). The DNA was then quantified and tested for the purity with the NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific, USA).

In silico analysis

The *in silico* PCR analysis was conducted using the NCBI date palm genome (Reference ID: GCF_000413155.1). The gene data were processed using Practical Extraction and Reporting Language (PERL) scripts, and the local sequence extraction software in EMBOSS was used to convert the GenBank format data to FASTA format. Gene sequences associated with specific biological processes, cellular components, and molecular functions in *Phoenix dactylifera* were identified using the 'Gene Ontology' tool with the FASTA file. The gene sequences associated with specific processes were extracted and used as input for the 'Primer3' software package. The parameters ensured a primer product size between 200 and 500 bp, with an optimal primer size of 20 bp. The resulting maximum amplicon length was ≤ 1500 bp. The *in silico* PCR analysis was conducted using PERL scripts with these conditions, where P represents the PCR primer pair. The local EMBOSS software's 'Overlap Layout Consensus' algorithm was used to report overlapping regions during primer genome coverage statistics, ensuring high congruence of overlaps. This algorithm is also employed for genome sequence assembly (Li *et al*. 2015). Net coverage was determined for each individual *in silico* PCR amplicon belonging to the marker type and for all amplicons combined. For phylogenomic analysis, the software package MEGA is known for its sophisticated methods and tools. PyElph is Python-based open-source software designed for analyzing genetic variation in DNA from different species or populations. It processes gel electrophoresis data patterns of genetic DNA markers (PyElph, available directly from Source Forge with a printed manual 2012; Pavel and Vasile 2012) and creates phylogenetic trees based on this analysis.

Primers design

NCBI Primer-BLAST database was used to design specific primers using the accession numbers of three markers specific to genes; the pathogenesis-related protein PR-4

Table 1: Primer sequences designed using NCBI Primer-BLAST database using the accession numbers of three markers specific to genes; the pathogenesis-related protein PR-4 (LOC103695821), the receptor-like protein kinase S.2 (LOC103697920), and the BTB/POZ domain and ankyrin repeat-containing protein NPR1-like (LOC113461809)

Locus code	Primer Sequences	
LOC103695821	F: 5′- GCCACAGCCCAATCCTAGTT-3′	R: 5′- TACAACGACTACAACCCGGC-3′
LOC103697920	F: 5′- AGATCATCCACCGCGATGTC-3′	R: 5′- GCTTCATTTCTGCGAGCGAG-3′
LOC113461809	F: 5′- GAGAACCCATGTCCAGCCAA-3′	R: 5′- GGTTTTGCTCGTGTGTCGAC-3′

Table 2: The list of SCoT primers name, sequence, total number of bands (TB), monomorphic bands (MB), polymorphic bands (PB), percentage of polymorphism (%P), Size range (bp), and the Polymorphism Information Content (PIC) as revealed by SCoT analysis

Name	Sequence (5'-3')	TB	MB	PB	%P	bp	PIC
SCoT-1	ACGACATGGCGACCACGC	18	14	4	22	160-1450	0.19
SCoT-2	ACCATGGCTACCACCGGC	12	9	3	25	150-1300	0.23
SCoT-3	ACGACATGGCGACCCACA	13	10	3	23	110-1400	0.21
SCoT-4	ACCATGGCTACCACCGCA	16	12	4	25	210-1500	0.26
SCoT-6	CAATGGCTACCACTACAG	17	12	5	29	180-1100	0.28
SCoT-10	ACAATGGCTACCACTACC	18	15	3	17	220-1250	0.20
SCoT-11	ACAATGGCTACCACCAGC	14	12	2	14	160-1000	0.18
SCoT-12	AAGCAATGGCTACCACCA	21	17	4	19	150-1750	0.21
SCoT-13	ACGACATGGCGACCAACG	18	13	5	28	100-1200	0.30
SCoT-14	AACCATGGCTACCACCAC	11	9	2	18	190-970	0.20
Total		158	123	35	-		-
Mean		15.8	12.3	3.5	22		0.23

(LOC103695821), the receptor-like protein kinase S.2 (LOC103697920) and the BTB/POZ domain and ankyrin repeat-containing protein NPR1-like (LOC113461809) as represented in Table 1.

PCR validation

Ten Start Codon Target (SCoT) primers were used to detect the polymorphism among the studied date palm cultivars (Table 2). The amplification reaction was carried out in a 25 μL reaction volume containing 12.5 μL DreamTaq Green PCR Master Mix (2X), 2 μL primer (10 pmol), 2.5 μL template DNA (10 ng/μL) and 8 μL dH_2O. The PCR technique was executed using the Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems). The amplification of SCoT markers was performed for 40 cycles, with an initial denaturation at 94°C for 45 sec, annealing at 50°C for 50 sec, elongation at 72°C for 1 min, followed by a final extension at 72°C for 5 min. The PCR amplification products were run on a 1.5% agarose gel supplemented with ethidium bromide (0.5 μg/mL) in 1X TBE buffer at 95 V. The PCR products were visualized under UV light and photographed using a gel documentation system (Bio-Rad 2000).

Data analysis

For SCoT analysis, only clear and unambiguous bands were visually scored as either present (1) or absent (0) for all samples. The final dataset included both polymorphic and monomorphic bands. A binary statistical matrix was then constructed. Dice's similarity coefficients were calculated between genotypes using the unweighted pair group method with arithmetic averages (UPGMA). This matrix was used to construct a phylogenetic tree (dendrogram) based on the Euclidean similarity index, using PAST software Version 1.91 (Hammer *et al.* 2001).

Results

Enrichment analysis

Biological processes (BP): Our analysis revealed that the 19 crucial pathways identified (Fig. 1) are notably enriched in BP. Importantly, the "developmental process" (GO: 0032502) was significantly enriched, further revealing that one of the major functions of date palm genes are those involved in growth and differentiation regulation. These developmental processes are crucial for the proper formation of tissues and organs at different stages of plant development such as when establishing seedlings, during fruiting and upon maturation. Cellular Process (GO: 0009987) occupies the largest proportion. These genes are potential candidates for improving yield and stress adaptation traits in date palm breeding programs. Particularly, genes included in "cellular process" (GO: 0009987) which involved in cellular activities to maintain basic functions of the cell such as cell division, growth and metabolism. Metabolic process (GO: 0008152) gene also occupies significant proportion. These genes control essential process in dates and also enable the stress tolerance in plants.

Molecular functions (MF): Regarding the molecular pathways (Fig. 2), there were eight distinct pathways highlighted in our analyses. The "Translation regulator

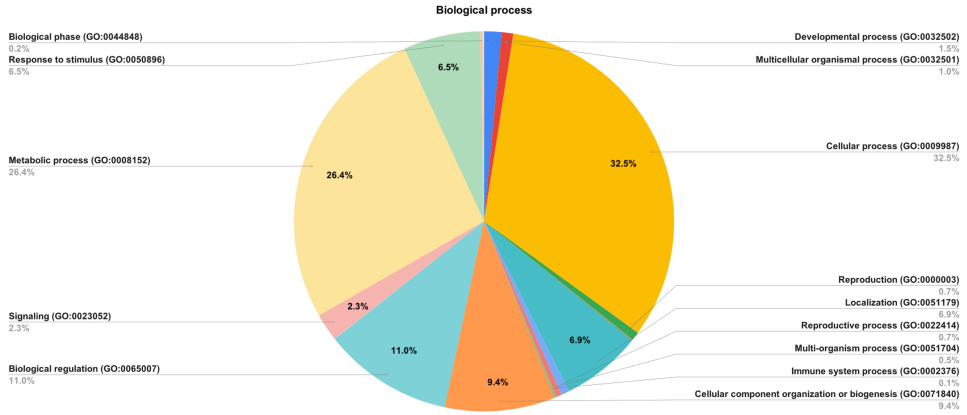

Fig. 1: A graphical representation of gene counts associated with various biological processes in date palm cultivars, highlighting genes involved in developmental and stress response pathways

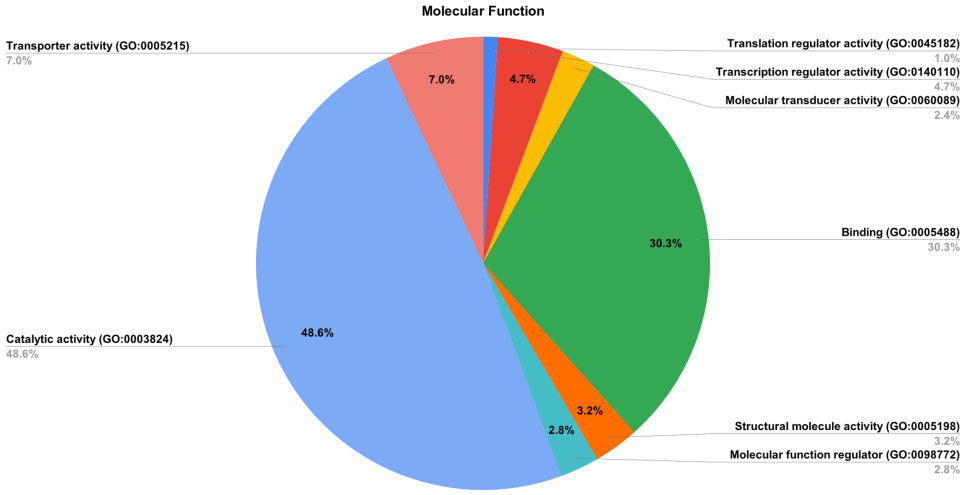

Fig. 2: A graphical representation of the molecular function of genes identified in date palm cultivars, emphasizing the roles in transporter and structural molecule activities

activity" (GO: 0045182) was not only one of the three most highly enriched categories but also is a key pathway regulating macromolecule biosynthesis. Other significant annotations were associated with the "molecular transducer activity" (GO: 0060089), indicating an extensive signaling network in date palms for sensing and responding to environmental cues.

Cellular Components (CC)

Out of the 15 cellular components pathways that were identified by GO enrichment analysis (Fig. 3), we found significant enrichment for plasmodesma (GO: 0009506). Plasmodesmata – specialized channels which transport nutrients, hormones and various signaling molecules which are essential for keeping the structure and functionality of the plant in check should any stresses inadvertently attack it. Additionally, the membrane part (GO: 0044425) category was also enriched.

PCR validation

To validate our results, PCR confirmation reaction (Fig. 4) was done using those three mentioned above primers. The first primer, LCO-1 yielded two different bands one at 400 bp and another at 360 bp for the two cultivars Zaghloul and Hayani respectively. The most important of these findings is that this assures the specificity of the design with regard to these two cultivars which is essential for breeding and genetic studies among date palms. The second primer, LOC-2 (LOC103697920), yielded a pattern of three fragments. A fragment of about 750 bp identifying the four samples of Zaghloul and Hayani cultivars indicate a specific genetic marker associated with these cultivars. This larger fragment may be useful in breeding programs as a marker for selection due to its likely association with these cultivars. All ten samples also possessed the second fragment (380 bp), Indicating that this particular fragment may belong to conserved regions in various cultivars.

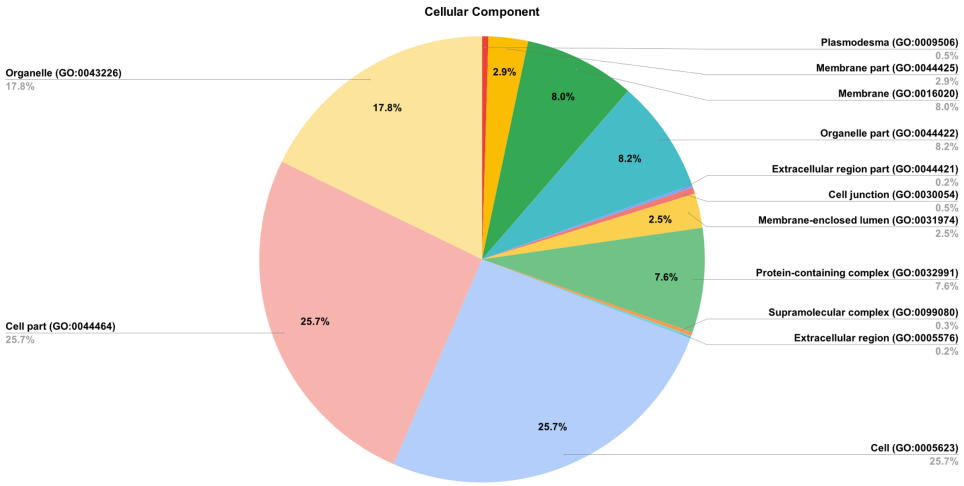

Fig. 3: A graphical representation of gene counts related to cellular component functions, demonstrating the involvement of genes in cellular structure maintenance and stress adaptation

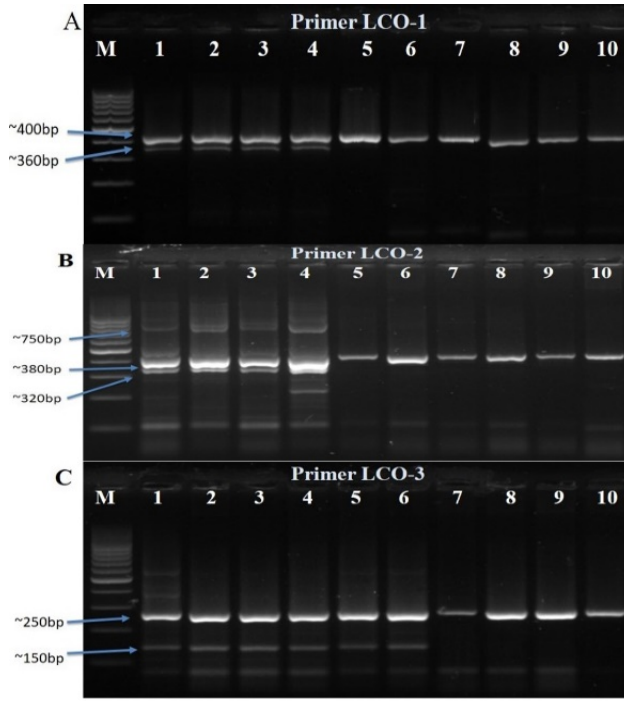

Fig. 4: PCR banding patterns of the ten date palm samples (representing five cultivars). M: Thermo Scientific GeneRuler 100 bp DNA Ladder. A: PCR amplification with primer LCO-1. B: PCR amplification with primer LCO-2. C: PCR amplification with primer LCO-3

Because of this conservation, it may be important for key biological processes and could therefore be useful to further functionality. The third fragment was around 320 bp and was only observed in the same four samples as the first fragment, suggesting a cultivar specific marker. In addition, the third primer, LOC-3 (LOC113461809) produced two different fragments. The first one (ca 250 bp) was detected in all ten samples, whereas the second one (ca 150 bp) was found in six of these samples belonging to the Zaghloul, Hayani and Siwi cultivars. Despite their limited numbers, these results show that the specific primers designed can amplify different markers that could act as cultivar-specific genetic marker amplifiers. All the samples have a 250 bp fragment, which represents a conserved material (gene). The specific fragment of 150 bp in some cultivars namely Zaghloul, Hayani and Siwi can be used as a cultivar-specific marker.

Polymorphism as revealed by SCoT markers

Ten SCoT primers showing clear amplification profiles (Table 2) were used to analyze the genetic polymorphism among date palm cultivars (Fig. 5). A total of 153 bands were obtained, of which 35 were polymorphic, giving an average percentage of polymorphism equal to 22%. The total number of bands per primer varied from 11 (SCoT-14) to 21 (SCoT-12), with an average of 15.3 bands per primer among the different cultivars.

Moreover, Polymorphic bands ranged from 2 (SCoT-11 and SCoT-14) to 5 (SCoT-06; SCoT-13), with an average of 3.5 polymorphic bands per primer. This difference in polymorphic bands indicated that SCoT markers are more specific and sensitive for evaluating genetic diversity among the studied date palm cultivars.

Polymorphism Information Content (PIC) values ranged from 0.18 in primer SCoT-11 to a high of 0.30 for primer SCoT-13 indicating the efficiency of these markers used in providing information about genetic variations within populations studied. The similarity matrix between the studied date palm cultivars (Table 3) was used to construct a dendrogram using UPGMA cluster analysis (Fig. 6). The obtained dendrogram was divided into two main clusters. The first one included the dry cultivars "Sakkoty-a" and "Sakkoty-b." The second was further divided into two subclusters; the first included the semi-dry cultivars "Siwi-a" and "Siwi-b," while the second one

Table 3: Matrix of Dice similarity coefficients representing the genetic similarity across the ten date palm cultivars based on SCoT markers (Zaghloul-a (Z-a), Zaghloul-b (Z-b), Hayani-a (H-a), Hayani-b (H-b), Siwi-a (S-a), Siwi-b (S-b), Amry-a (Am-a), Amry-b (Am-b), Sackouttti-a (Sk-a), and Sackouttti-b (Sk-b))

Variable	Z-a	Z-b	H-a	H-b	S-a	S-b	Am-a	Am-b	Sk-a	Sk-b
Z-a	1.00									
Z-b	0.98	1.00								
H-a	0.94	0.95	1.00							
H-b	0.94	0.96	0.98	1.00						
S-a	0.90	0.92	0.93	0.92	1.00					
S-b	0.90	0.92	0.91	0.93	0.95	1.00				
Am-a	0.92	0.93	0.94	0.94	0.93	0.90	1.00			
Am-b	0.93	0.92	0.92	0.92	0.93	0.91	0.95	1.00		
Sk-a	0.85	0.85	0.83	0.85	0.85	0.86	0.83	0.87	1.00	
Sk-b	0.87	0.87	0.85	0.88	0.87	0.87	0.88	0.91	0.94	1.00

included the soft cultivars "Zaghoul-a" and "Zaghoul-b," "Haiany-a" and "Haiany-b," as well as the semi-dry cultivars "Amry-a" and "Amry-b."

However, the clear separation of the two dry cultivars "Sakkoty-a" and "Sakkoty-b" into a separate group might indicate a close relationship and similar adaptation to arid climate. The second cluster containing semi-dry "Siwi-a" and "Siwi-b"and soft cultivars "Zaghoul" and "Haiany" suggests a more diverse genetic background among those cultivars.

The dendrogram established in this study shows a clear distinction among the various groups used of date palm cultivars, indicating the great potential for taxonomic information at genetic level. All the studied date palm cultivars were found highly similar genetically with estimated genetic similarities ranging from 83–98%. The maximum similarity of (98%) was noticed for Zaghoul-a & Zaghoul-b and Haiany-a & Haiany-b respectively. On the other side, the lowest genetic similarity (83%) was observed between "Haiany-a" (soft cultivar) and "Sakkoty-a" (hard cultivar). Such notable divergence indicates that these herbaceous cultivars have experienced uniqueness in their genetic backgrounds over time, possibly resulting from differences in ecological niches they occupied and cultivation practices.

Discussion

The date palm (*Phoenix dactylifera* L.) is a keystone species of economic and ecological importance in arid and semi-arid regions (Pandey *et al*. 2024). The species, famous for tolerating high environmental conditions, is vital to sustainable agriculture in adverse climates. The present study investigates the genetic foundation for economically and ecologically important traits in date palms. It also provides molecular markers to facilitate a marker-assisted breeding strategy for increased stress resistance and productivity (Osnato *et al*. 2022; Redmond *et al.* 2023).

Drought and salinity are environmental stresses that greatly affect the phenotypic response capabilities of date palms. Notably, flowering time and other critical developmental processes in plants are controlled by complex genetic networks that respond to environmental signals, elucidated by genes such as TFL1 (Osnato *et al*. 2022). Such genes not only assist reproductive success but also maximize vegetative growth through efficient resource utilization under stress conditions. Examples of transcriptional regulators, which have been isolated and studied extensively, most commonly *via* single-plant omics, suggest the complex interactions linking environmental signal transduction and developmental pathways but also provide a glimpse of how to deploy the bet-hedging potential of improved growth rates under conditions where they are otherwise neither optimal nor expeditious (Redmond *et al.* 2023). Interestingly, interactions at the phytohormonal level further emphasize the genetic regulation of stress adaptation. For instance, cytokinins have a key impact on cell division, differentiation, and organogenesis, connecting developmental plasticity with environmental signals (Přerostová *et al*. 2022). The crosstalk between auxin-cytokinin signaling has been established for adaptive developmental responses for optimal resource mobilization during stress events (Min *et al*. 2022). Those hormonal interactions are even more important for date palms, as their capacity to respond to drought and salinity stress guarantees their survival and productive performance in extreme conditions (Gupta *et al*. 2020; Mishra and DiGennaro 2020).

The resilience of date palms in maintaining homeostasis under environmental stress is recognized at the cell level. At the level of cellular processes critical for stress adaptation, sugar signaling, and metabolic reprogramming are basic. For example, it has been reported that heat and drought-responsive genes that regulate sugar metabolism activate stress-induced pathways to improve cellular tolerance (Safronov *et al*. 2017). The signaling mechanisms reported reflect the evolutionary adaptations of the date palm and those genetic potentials can be used for the improvement of stress resistance (Du *et al*. 2019). They also stated that changes in metabolism, especially in systems of controlling reactive oxygen can be important for protecting cells from damage and enhancing recovery from damaging conditions. In drought or salinity stress, improved anti-oxidative capacity preserves leaf integrity from damage and recovers plant physiology. This metabolic reprogramming catalyzes a crucial basis for breeding stress-tolerant cultivars, as unlocking the genetic signatures associated with these pathways is a key for optimization.

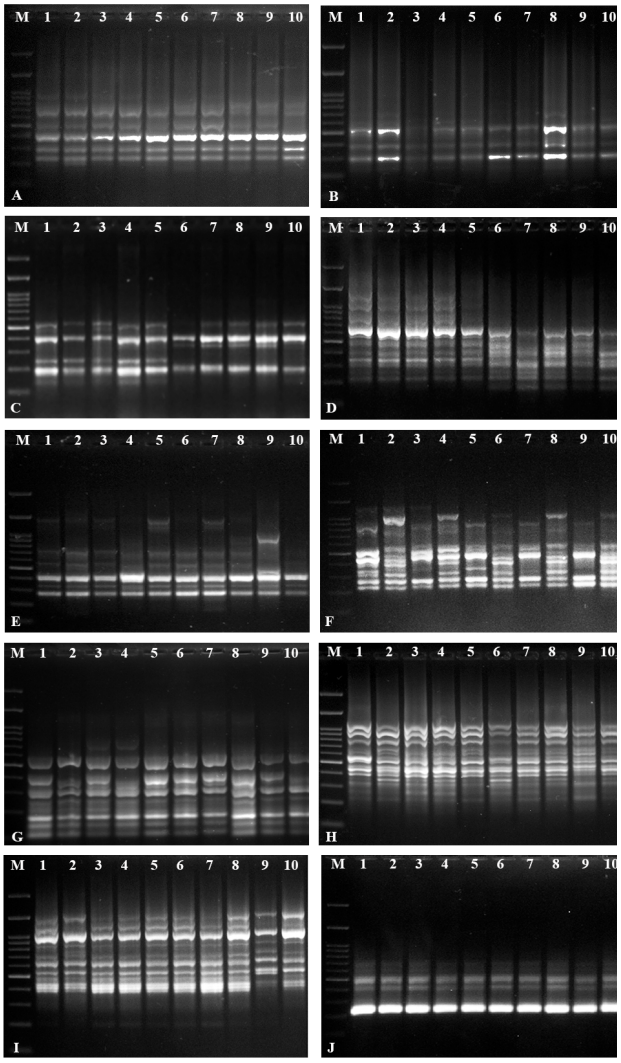

Fig. 5: Agarose gel electrophoresis of the ten SCoT markers; (**A**) SCoT 1, (**B**) SCoT 2, (**C**) SCoT 3, (**D**) SCoT 4, (**E**) SCoT 6, (**F**) SCoT 10, (**G**) SCoT 11, (**H**) SCoT 12, (**I**) SCoT 13 and (**J**) SCoT 14 used to assess the polymorphism among the ten date palm cultivars studied. Lanes 1–10 represent the ten cultivars: Zaghloul-a (1), Zaghloul-b (2), Hayani-a (3), Hayani-b (4), Siwi-a (5), Siwi-b (6), Amry-a (7), Amry-b (8), Sackouttti-a (9), and Sackouttti-b (10). M represents FastGene 100 bp DNA Ladder (NIPPON Genetics EUROPE). The amplified PCR products were resolved on a 1.5% agarose gel stained with ethidium bromide, and visualized under UV

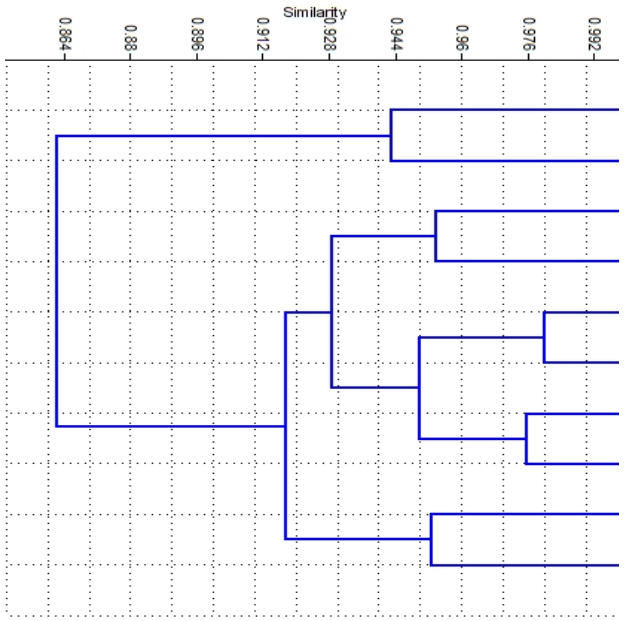

Fig. 6: Dendrogram of the ten date palm cultivars based on Dice similarity coefficients constructed from SCoT marker analysis. The cultivars were clustered according to their genetic similarity, with higher similarity values reflected by shorter branch lengths. Cultivars include Zaghloul-a (Z-a), Zaghloul-b (Z-b), Hayani-a (H-a), Hayani-b (H-b), Siwi-a (S-a), Siwi-b (S-b), Amry-a (Am-a), Amry-b (Am-b), Sackouttti-a (Sk-a) and Sackouttti-b (Sk-b)

Functions such as transcription regulation, translation regulation, and signal transduction are important for plants to adapt to stress. Translation regulators enable flexible protein synthesis in response to environmental stimuli, promoting fast cellular adaptation necessary for cell survival under extremes (Peng *et al*. 2018). Fine-tuning of gene/protein expression is a key adaptation for the stress of extended drought and salinity faced by date palms. Other examples include the molecular transducers such as G proteins that mediate the transduction of extracellular signals into intracellular responses, allowing plants to dynamically adapt to their fluctuating environments (Cheah *et al*. 2015; Kim *et al*. 2017). These molecular mechanisms are critical for stress tolerance and serve as potential platforms for genetic improvement programs (Sheikh *et al*. 2016; Ibrahimi *et al*. 2024).

The stress response pathways of date palm involve plasmodesmata, specialized pathways for intercellular transport (Naseeruddin *et al*. 2018). These structures ensure the controlled movement of nutrients, hormones, and signaling molecules among and between cells, regardless of whether the communication is redundant or specific to the stress conditions (Gopika *et al.* 2024). This suggests that the quick deposition of callose at plasmodesmata during stress events limits nutrient loss, providing a beneficial effect and protect ability for the plant (Yang *et al*. 2021). This mechanism is especially relevant at least for date palms, in which resource to assimilate is probably the most important key to survive in dry areas. Additionally, plasmodesmata also mediate the transport of phytohormones including auxins and cytokinins that modulate growth and stress tolerance (Ho *et al*. 2020). The relationship between plasmodesmata and hormonal signaling portrays a comprehensive mechanism that date palms use to adapt to stress and suggests targets of interventions to ameliorate stress tolerance (Hunziker *et al*. 2020; Khalofah *et al*. 2021).

SCoT markers analysis showed a 22% polymorphism, confirming the genetic diversity among the date palm cultivars studied. This is similar to findings in several studies that reported successful genetic variation indicated by SCoT markers for different plant species (Sari *et al*. 2018; Azim and Khashaba 2021). Such diversity is important for breeding programs to select traits related to stress tolerance, yield enhancement, and disease resistance (Heikrujam *et al*. 2015; Balážová *et al*. 2017; Sharma *et al*. 2019). Moreover, Dehghanian and Sheidai (2022) reported high genetic differences between date palm cultivars in Iran and suggested that the Iranian date palm gene pool is simply a complex international gene pool that arose from changes in human germplasm flows. Similarly, Khierallah *et al*. (2011) reported that substantial genetic diversity exists amongst Iraqi date palm varieties and most of this inter-varietal polymorphism was associated with high out-crossing mechanisms of this species. In summary, these studies emphasize the importance of molecular markers, such as SCoT, in genetic diversity identification which is useful for the conservation and breeding of date palm cultivars.

In addition, genetic diversity among Tunisian date palm ecotypes measured using SCoT markers confirm results of Hamza *et al*. (2011), who found that centuries of local and/or intensive selection in date palm oases have led to a clear genetic variability. This finding is in agreement with the general observation made by various workers on date palms in other parts of the world by molecular markers that have helped portray a genetic relationship and diversity level. For example, Saboori *et al*. (2021) highlighted the utility of morphological and molecular markers for increasing the resolution in the identification of closely related date palm cultivars. Such multidimensional genetic analysis not only endorsed our findings but also reaffirmed the potential of SCoT markers in the pursuit and conservation of genetic variation available for exploitation in the date palm gene pool.

Nevertheless, the clustering patterns can be attributed to the dissimilar genetic backgrounds of non-related cultivars and different adaptation mechanisms to non-specific environmental conditions that these cultivars of the same species may occupy. Conserved genetic fragments, for example, the common 250 base pair marker associated with genes functionally important for growth, development, and stress. Such markers serve as a consistent and effective means of identifying and choosing desirable traits in cultivars. Such molecular markers, such as the 150 bp fragment, identified in specific cultivars such as Zaghloul and Siwi, can have great potential in cultivar-specific breeding strategies (Wang *et al*. 2014). These markers not only offer a reduced number of markers for selection but also prevent the dissolution of genetic diversity during breeding processes.

The markers PR-4 and NPR1-like identified in the present study have the greatest significance since they can be used for marker-assisted selection (MAS) in breeding programs. Such markers are linked to important traits that are more and more important for climate change and sustainable agriculture, including disease resistance and stress tolerance traits. PR-4 is involved in the defense response to certain fungal pathogens in plants and therefore an important marker for breeding plants with disease resistance (Jiang 2013). Likewise, the NPR1-like marker is linked to enhanced systemic acquired resistance, enabling the plant to be more resistant to systemically against a number of biotic stresses (Jiang 2013). These markers are used in MAS (Marker Assisted selection) and enable the breeders to select at the seedling stage for the characters that are needed, thereby minimizing the time and resources required for the identification of new cultivars (traditional phenotypic selection takes more time/takes resources) (Tharun 2024).

In addition, the incorporation of these markers into breeding programs can help produce high-yielding, stress-tolerant crops. This is especially important when you consider the increasing global demand for resilient crop varieties as it pertains to food security. These markers can be used in the application of MAS allowing for selection of plants which harbor favorable alleles associated with enhanced traits, thus shortening the breeding cycle (Goswami *et al*. 2022). Molecular markers can also be used in combination with quantitative trait loci (QTL) mapping to increase the effectiveness of breeding programs by identifying specific genetic areas linked with desirable agronomic traits (Rutkoski *et al*. 2014). Thus, the qrPR-4 and qrNPR1-like markers identified in this study are a great contribution to the knowledge about the genetic foundation of important traits, but there are also practical breeding tools for developing resilient and high-yielding crops in future. In contrast, the genetic differentiation detected between soft and dry cultivars suggests that preserving genetic resources will be crucial to retaining the remaining adaptive potential of the species (Rajkumar *et al*. 2016; Jaouni *et al*. 2019). Therefore, clustering cultivars based on their genetic similarity may assist breeding strategies to combine desired traits from various cultivars and improve yield and adaptiveness (Hansen *et al*. 2021).

While this study lays the groundwork to understand the genetic foundation for stress tolerance in date palms, it should also prompt more broad genomic studies. The application of a larger range of cultivars and stress conditions will further inform the genetic pathways governing resiliency. Molecular functional analysis of the detected markers would help to reveal their role in stress adaptation and based on that, we can target to develop genetic manipulation. Genomic information, together with phenotyping data, will also be important for developing breeding programs. The combination of the molecular markers with high-throughput phenotyping techniques would be beneficial for developing predictive models of traits to be selected, which could accelerate the breeding of date palm cultivars with improved resistance to climate change. Moreover, date palms have a large number of

cultivars, and it is important to develop a robust DNA barcoding system for their identification and authentication to promote the integrity of their breeding programs and also commercial production.

Conclusion

Here we reveal the genomic basis of date palm resilience and performance that can inform breeding and conservation. These molecular markers can be utilized to develop new genetic resources for the improvement of stress tolerance in date palms, ensuring the sustainability of agroecosystems in drylands. Using these genetic insights, better approaches could be developed by breeders and researchers to confront the increasing demand for resilient crops from a food and economic security perspective in a changing climate.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability

Data will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper.

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