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Research Article

Genetic diversity assessment and *in vitro* propagation of some date palm (*Phoenix dactylifera* L.) varieties

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Abstract

The evaluation of genetic diversity is crucial for breeders to develop strategies and improve the resilience, quality, and adaptability of the date palm. In this study, the genetic diversity of three date palm varieties was performed using ISSR-PCR molecular markers to determine its relationship with *in vitro* propagation response of these varieties. The molecular profiling was performed using ISSR-PCR. A total of 49 loci were produced by the PCR reactions, 38 of which were polymorphic while 11 were monomorphic. The level of polymorphism revealed by ISSR-PCR varied from 33.33% to 100%. The three date palm varieties were grouped into two clusters based on the results of cluster analyses that used morphological data and molecular profiles. Cluster I comprised the 'Barhy' variety and Cluster II included 'Magdoul' and 'Amri' varieties. The clustering analyses revealed the independence of the 'Barhy' variety in its characteristics from the other varieties based on either morphological or molecular data. The results of *in vitro* propagation showed that the 'Amri' variety exhibited the highest callus induction frequency (86.28%), callus weight (2.33 g), number of somatic embryos (9.32), number of shoots (14.62), number of roots (4.11), root length (4.63 cm), shoot length (13.61 cm) followed by 'Magdoul' and 'Barhy' varieties. The 'Amri' variety had the shortest callus induction period, at 23.26 days while the 'Barhy' variety exhibited the longest period of callus induction (28.55). It was deduced from the study that the ISSR marker reproduced trustworthy patterns of bands to determine the genetic diversity among different date palm varieties that are considered the cornerstone for the genetic improvement of date palms. The

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understanding of the relationship between genetic diversity and *in vitro* propagation response of date palm is essential for ensuring the long-term sustainability of its crop. This will facilitate better conservation and development of new date palm varieties that fulfil the needs of farmers and consumers.

Keywords: date palm; diversity assessment; genetic polymorphism; ISSR-PCR; micropropagation; molecular markers; regeneration

Introduction

Date palm (*Phoenix dactylifera* L.) is a long-lived, diploid (2n = 36), dioecious, and heterozygous perennial plant that is a member of the Arecaceae family (Vardareli *et al*., 2019). It is the major fruit crop grown in warm climates due to its economic and dietary importance, notably in the Middle East and North Africa (Krueger, 2021). The fruit of the date palm, known as dates, is a sweet and nutritious food that is high in fiber, vitamins, sugar and minerals. Dates are also a good source of energy and can be used as a part of a meal. In addition to its nutritional value, date palm is also an important source of income for many people. The date palm tree is a hardy plant that can grow in arid and semi-arid regions, where other crops may not grow. This makes it an important source of food and income for people living in these regions (Aleid *et al*., 2015).

Date palm industry is a major contributor to the economies of many countries. In Saudi Arabia, for example, date palm production is worth millions of dollars each year (Aleid *et al*., 2015). Date palm has conventionally been propagated through offshoots, but this method is inefficient. Due to either the inability of date palm to produce offshoots (Gurevich *et al*., 2005) or the limited offshoots number produced by each tree and the duration needed for each offshoot to bear fruit (Gantait *et al*., 2018). Tissue culture is a highly efficient and effective technique to produce high-quality date palm plants that are genetically identical to the original plant (Abdalla *et al*., 2022). This process typically involves four stages: (1) culture initiation and establishment. In this stage, a small piece of tissue called an explant, is taken from a healthy plant and placed on a sterile culture medium. The explant is allowed to grow and develop in the culture medium until it forms a callus, which is a mass of undifferentiated cells. (2) shoot multiplication. Once the callus has formed, it is induced to produce shoots. This is typically done by manipulating the culture conditions, such as the light intensity, temperature, and composition of the culture medium. (3) rooting of the shoots. Once the shoots have reached a certain size, they are induced to form roots. This is done by transferring the shoots to a different culture medium that contains a plant hormone that promotes root growth. (4) transfer to the greenhouse. Once the shoots have rooted, they are transferred to the greenhouse for acclimatization. In the greenhouse, the plants are gradually exposed to ambient conditions until they are ready to be transplanted into the field (Al-Khayri, 2007). Also, date palm micropropagation depending on the somaclonal variation is a promising technology that has the potential to revolutionize the date palm industry. It can be used to produce high-quality date palm trees with improved characteristics, such as higher yields, better fruit quality and resistant to pests and diseases (El Hadrami and Baaziz, 1995).

The out-crossing breeding system of date palm is responsible for the high genetic diversity of the species, which allows it to grow under a variety of climates. The Middle East and North Africa are the main centers of this diversity (Krueger, 2021). The assessment of genetic diversity in date palm is important for several reasons, including; the identification and conservation of the genetic resources. Date palm is a highly heterozygous plant, meaning that each plant has a unique genetic makeup. This genetic diversity is important for the long-term sustainability of the date palm industry, as it provides a buffer against pests, diseases, and environmental stresses. By identifying and conserving genetic resources, we can ensure that there is a diversity of date palm cultivars available for future generations. Also, the assessment of genetic diversity resulting from somaclonal variation is crucial to conserve endangered species and develop new cultivars (Slazak *et al*., 2015; Żabicki *et al*.,

2021; Żabicki *et al*., 2019). The assessment of genetic variation can be used to identify date palm cultivars with desirable traits, such as high yield, good fruit quality, and resistance to pests and diseases (El Hadrami *et al*., 2011). This information can be used to develop new cultivars of date palm that are better suited to the needs of farmers and consumers and to understand the evolution of date palm. The assessment of genetic variation can be used to track the evolution of date palm over time.

Several studies discussed the different methods for the assessment of the genetic variation of date palm, including proteomics (de Carvalho Silva *et al*., 2014; Tan *et a*l., 2016); cytogenetics (Soliman *et al*., 2009); epigenetics (Ho *et al*., 2013); genomics (Ting *et al*., 2013; Tranbarger *et al*., 2012); and biochemical methods were used to distinguish between regenerants (Khierallah *et al*., 2014). Studies using peroxidase and isozyme analysis did not reveal genetic diversity among date palm varieties (Khierallah *et al*., 2014). In addition, a range of molecular markers has been used to measure genetic diversity. These markers include ISSR markers (Ma *et al*., 2008; Zehdi *et al*., 2002), SRAP markers (Yan Jia-jun, 2010), SSR markers (Zehdi *et al*., 2004), RAPD (Al-Khalifah and Askari, 2003), AFLP (Rhouma *et al*., 2007), and RFLP (Sakka *et al*., 2003).

The morphological characteristics are not only affected by environmental conditions and developmental stages, but they also require a large set of phenotypic data that can be difficult to collect and interpret. Consequently, the ISSR molecular marker has been effectively used to measure genetic variation because it offers a virtually limitless pool of markers that can be used to identify differences at the molecular level. ISSR is a versatile technique that can be used to identify specific genomes and estimate phylogenetic relationships among the different date palm varieties (Munshi and Osman, 2010). It can also be used to identify molecular markers that are associated with traits that are valuable to farmers or consumers (Younis *et al*., 2008).

In this study, we studied the relationship between genetic diversity and *in vitro* propagation response of three elite date palm varieties with high economic and dietary value in Egypt through ISSR molecular marker.

Materials and Methods

Plant materials

Three date palm varieties: 'Amri', 'Magdoul' and 'Barhy' were used in this study that are native to the Middle East and North Africa. They are all popular for their sweet and delicious fruit, which can be eaten fresh, dried, or used in a variety of culinary preparations.

Genomic DNA extraction

Genomic DNA from the initial plants of the three varieties was isolated from 5 grams of fresh young leaves using the CTAB method (Scobeyeva *et al*., 2018). The concentration of the isolated DNA was measured using a Nano Drop 2000 (Thermo Scientific™, Waltham, MA, USA). The quality and quantity of the extracted DNA was verified using electrophoresis on a 1.5% agarose gel. The DNA was diluted to a final concentration of 50 ng/µL and stored at -20 °C for future use.

ISSR-PCR amplification

To detect polymorphism among date palm varieties, a set of primers were used (Table 1). The reactions were performed according to (Moreno *et al*., 1998). The PCR mixture contained 20 ng/µL of template DNA, 2 µL of 5X buffer, 2 µL of MgCl2 (25 mM), 2 µL of dNTPs (200 µM), 2 µL of primer (10 pmol), and 1 unit of Taq DNA polymerase (Promega). The ISSR-PCR-based amplification was performed using a 96 well plate thermal cycler (Applied Biosystem) as the following as follows: an initial denaturation step at 94 °C for 5 minutes, followed by 35 cycles of denaturation at 94 °C for 1 minute, annealing at the annealing temperature for the primer used, extension at 72 °C for 1 minute, and a final extension at 72 °C for 5 minutes.

Table 1. The nucleotide sequences, Tm °C, molecular weight g/mol, primer length, and GC content (%) of ISSR primers

Primers	Sequences (5′-3′)	Tm (°C)	Molecular weight g/mol	Primer length	GC content (%)
UBC825	ACACACACACACACT	54.9	4459.0	15	46.67
UBC835	AGAGAGAGAGAGAGAGYC	56.3	5366.6	18	52.94
UBC814	CTCTCTCTCTCTCTCAT	53.5	4998.3	17	47.06
UBC826	ACACACACACACACACC	60.3	5046.3	17	52.94
UBC827	ACACACACACACACACG	60.6	5086.4	17	52.94
UCB840	GAGAGAGAGAGAGAGATT	54.3	5685.8	18	44.44
UCB808	AGAGAGAGAGAGAGAGC	56.3	5366.6	17	52.94
UCB811	GAGAGAGAGAGAGAGAC	54.2	5366.6	17	52.94
UCB868	GAAGAAGAAGAAGAAGAA	51.8	5671.8	18	33.33
UCB901	CACACACACACACACARY	57.2	4757.2	18	50.00

Y indicates C or T; R indicates A or G

Gel electrophoresis

1.5% agarose gel electrophoresis was performed in TBE buffer according to Maniatis (1989) to separate the results of the ISSR reactions. The size of the DNA bands on the gel was determined using a 1Kb plus DNA ladder. Ethidium bromide (MP Biomedicals, Goddard Irvine, CA, USA) was used to fluorescently stain the gel, The gel was then visualized under a UV illuminator (VilberLourmat, France). The frequency of polymorphisms and the number of bands were calculated for each primer individually.

Preparation and sterilization of explants

The spathes were washed with running water to remove any contaminants. Then, they were surface sterilized with 60% Clorox for 30 minutes to kill any microorganisms that could potentially contaminate the spathes. After the sterilization, the spathes were rinsed with sterilized distilled water for 5 minutes, 10 minutes, and 15 minutes, respectively, to remove any residual Clorox. The sheath of the spathe was then opened and the spikelet explants (2-3 florets) were carefully removed from the sheath. The explants were then cultured in sterile conditions on starting growth media.

Culture media preparation

The current investigation used Murashige and Skoog (MS) nutrient medium (Murashige and Skoog, 1962). Full-strength MS media were used in starting medium (4.4 g L^{-1} MS +2 mg L^{-1} 2, 4-D + 1 mg L^{-1} IAA + 5.0 mg L^{-1} NAA + 30 g L^{-1} sucrose + 7 g L^{-1} agar), maturation medium (4.4 g L^{-1} MS + 1 mg L^{-1} 2, 4-D + 2 mg L^{-1} 2 iP + 30 g L^{-1} sucrose + 7 g L^{-1} agar + 1.5 g L^{-1} AC) and multiplication medium (4.4 g L^{-1} MS + 1 mg L^{-1} BA + 2 mg L^{-1} 2-iP+ 30 g L^{-1} sucrose + 7 g L^{-1} agar) while three-quarter-strength MS medium in rooting medium (3.3 g L^{-1} MS + 0.1 mg L^{-1} NAA + 0.25 mg L^{-1} IBA + 50 g L^{-1} sucrose + 6 g L^{-1} agar + 3 g L^{-1} AC). The pH adjustment using 0.1 M KOH and 0.1 M HCl was done before autoclaving of the medium to 5.7 ± 0.1. Then the agar (PTC agar, sigma) (7 g L^{-1}) was added followed by autoclaving the medium at 121 °C for 20 minutes. In a laminar flow hood, 50 milliliters of sterilized media were poured into sterilized screw-top jars. The media were then allowed to solidify.

Culture conditions

Four spikelets explants with 3 cm length were cultured on a starting medium in three replicates and then were incubated in darkness at 27 °C. Thereafter, they were transferred to a new culture medium twice every three weeks for 8 weeks. Responded explants were transferred to a maturation medium for two subcultures on the same medium in darkness. Explants that had differentiated in the dark were transferred to a multiplication medium under light conditions (8 h darkness and 16 h light) for three subcultures for one month each. The

developed shoots were the transferred to a rooting medium (3/4 strength MS) for 24 weeks. The incubation conditions of the cultures were at 27±2 °C with a light intensity of 3,000 lumens per square meter, for 18 weeks and 4000 lumens per square meter for 6 weeks.

Data analyses

The morphological data, including callus induction frequency, callus weight, callus induction days, the number of somatic embryos and shoots per jar, the number of roots, root length, and shoot length were analyzed using Microsoft Excel. Significant differences between groups were evaluated using Student t-tests at a significance level of $p \leq 0.05$.

The bands resulting from ISSR-based PCR reactions were scored as either absent (0) or present (1). The presence or absence of each locus was treated as an independent event. Genetic diversity was identified by comparing the banding patterns of the three varieties. The polymorphism (%) was measured by dividing the number of polymorphic loci by the total number of loci that were scored. The genetic similarities between the varieties were calculated using the Dice coefficient (Dice, 1945). The calculation was performed using IBM SPSS statistics software (Norušis, 1993). The clustering analysis method was then used to generate a phylogenetic dendrogram (Berk, 2010) using STATISTICA 8 software. (Weiß, 2007).

Results

Genetic diversity analysis

The genetic variation among the three date palm varieties was investigated using ISSR-PCR amplification using 10 primers. This method allowed for the identification of molecular polymorphisms among the three varieties. Finally, the ten primers produced reliable banding profiles that were polymorphic among the studied varieties as shown in Table 2 and Figure 1). The PCR reactions produced a total of 49 loci, 38 of which were polymorphic, while 11 were monomorphic (Table 2). The level of polymorphism revealed by ISSR-PCR varied from 33.33% with primer UBC 835 to 100% with primers; UBC 814, UBC 826 and UBC 868 with an average of 74.99% per primers (Table 2).

Table 2. The numbers of total, monomorphic, polymorphic, and unique loci generated by ten primers of ISSR-PCR reactions in three date palm varieties, and the associated polymorphism

Primer	Number of Loci	Monomorphic Loci	Polymorphic Loci	Unique Loci	Polymorphism (%)
UBC 825	2	1	1	1	50%
UBC 835	3	2	1	0	33.33%
UBC 814	4	0	4	3	100%
UBC 826	6	0	6	5	100%
UBC 827	4	1	3	3	75%
UBC 840	4	1	3	1	75%
UBC 808	6	2	4	1	66.66%
UBC 811	9	3	6	5	66.66%
UBC 868	5	0	5	4	100%
UBC 901	6	1	5	3	83.33%
Average	4.9	1.1	3.8	2.6	74.99%
Total	49	11	38	26	

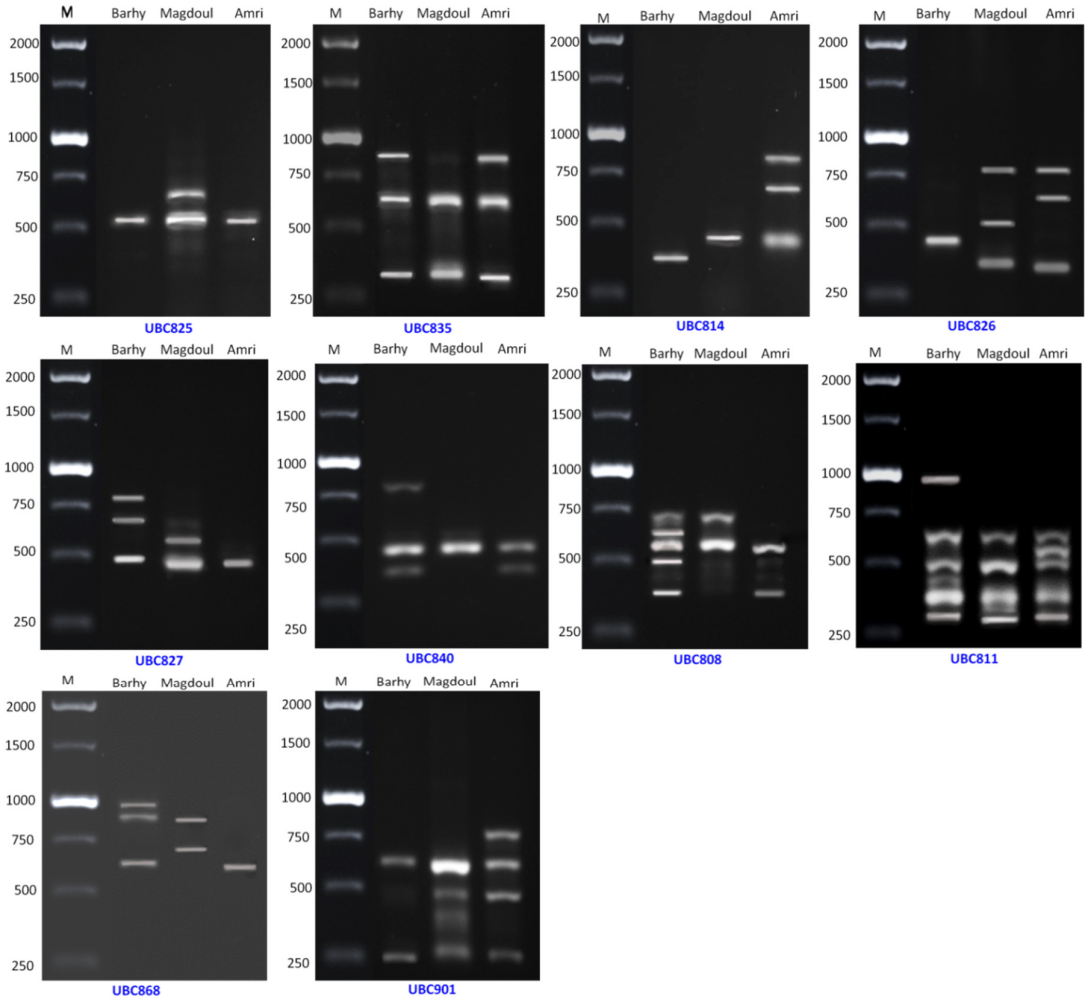

Figure 1. The patterns of DNA fragments produced by ISSR-PCR amplification of three date palm varieties. (**A**). M = 1kb Plus DNA Ladder

In vitro propagation responses

Explants from the three date palm varieties, 'Magdoul', 'Amri', and 'Barhy', were cultured on a starting medium (4.4 g L^{-1} MS +2 mg L^{-1} 2, 4-D + 1 mg L^{-1} IAA + 5.0 mg L^{-1} NAA + 30 g L^{-1} sucrose + 7 g L^{-1} agar) for induction of calli (Figure 2A). The data collected on the explants' initial responses after 21-30 days of culture varied significantly among the three varieties. The highest callus induction percentage (86.28%) was scored by the 'Amri' followed by 'Magdoul' and 'Barhy' with 72.21%, and 49.99%, respectively. These results indicate a significant difference in callus induction among the three varieties (Figure 3A). 'Amri' and 'Magdoul' varieties took the shortest time to induce callus (~23 days), while 'Barhy' significantly differed where it induced callus after 28 days (Figure 3B).

In the maturation stage, 'Amri' produced significantly higher callus weight (2.33 g) than 'Magdoul' and Barhy varieties (2.04 g and 1.58 g, respectively), as shown in Figures 2B and 3C. These results indicate that there is a significant difference in callus weight among the three varieties on the maturation medium (4.4 g L^{-1} MS + 1 mg L^{-1} 2, 4-D + 2 mg L^{-1} 2-iP + 30 g L^{-1} sucrose + 7 g L^{-1} agar + 1.5 g L^{-1} AC).

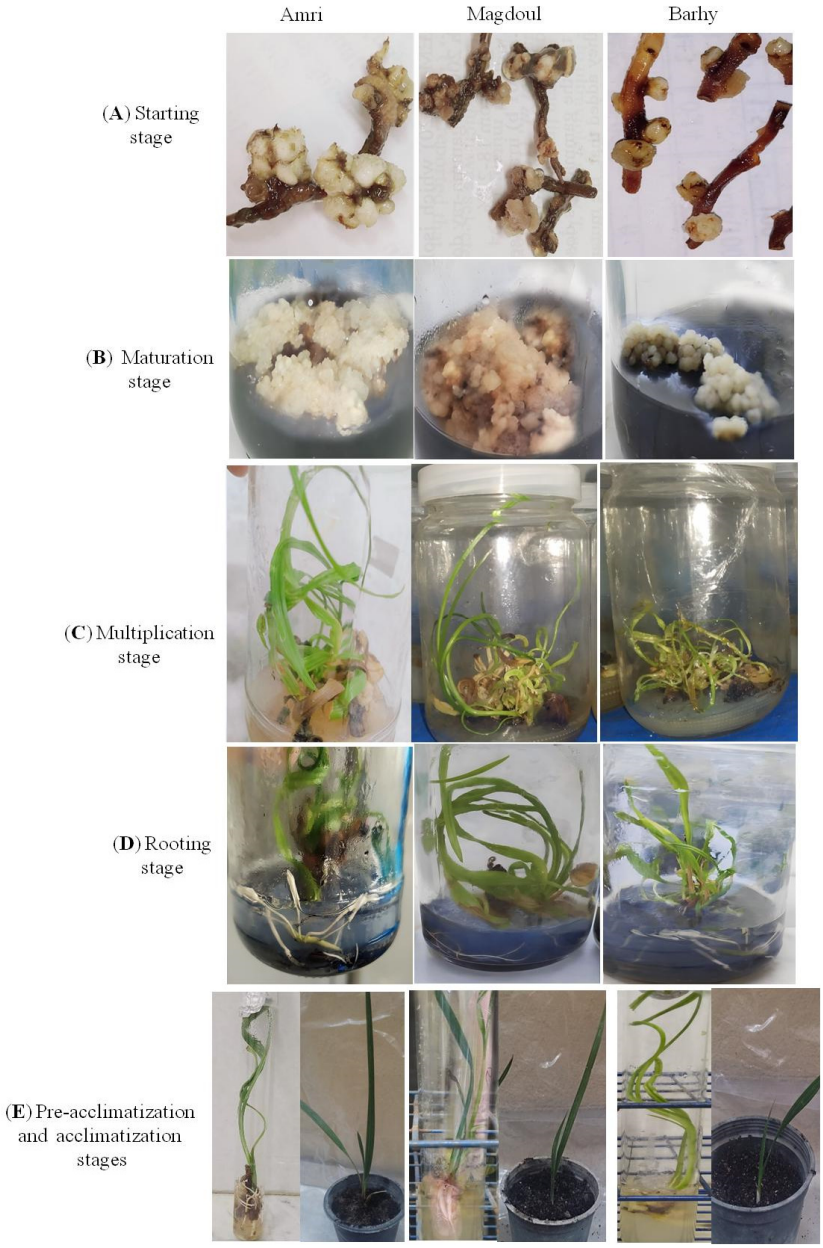

Figure 2. *In vitro* propagation of three date palm varieties using immature female inflorescences. (**A**). Formation of calli on starting media (**B**). Matured embryogenic calli on maturation media(**C**). Shoot multiplication (**D**). Rooting stage (**E**). Pre- acclimatization and acclimatization stages.

In the multiplication stage (4.4 g L^{-1} MS + 1 mg L^{-1} BA + 2 mg L^{-1} 2 iP+ 30 g L^{-1} sucrose + 7 g L^{-1} agar), according to the LSD 0.05 test, a significant difference was observed between the three varieties in the number of shoots and somatic embryos (Figure 3D and E). The number of somatic embryos and shoots produced by Amri was significantly higher than 'Barhy' and 'Magdoul' varieties. Amri produced 9.32 somatic embryos and 14.62 shoots, while 'Magdoul' produced 6.33 and 6.77, respectively. While 'Barhy' revealed the fewest somatic embryos (4.66) and shoots (6.77) (Figures 2C and 3D and 3E).

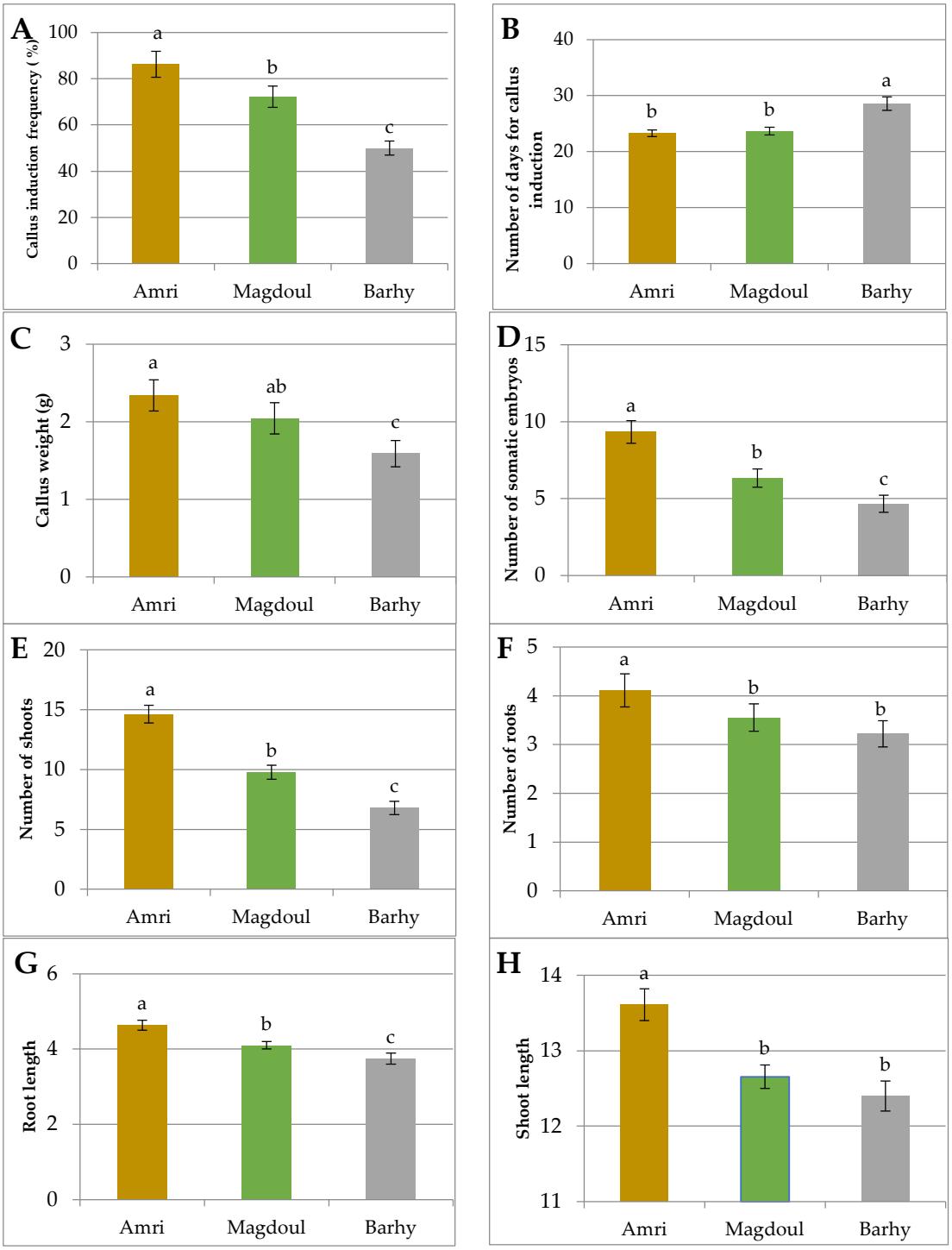

Figure 3. *In vitro* propagation responses of date palm varieties (Amri, Magdoul and Barhy). (**A**). Callus induction frequency (%). (**B**). The number of days for callus induction. (**C**). Callus weight. (D). Number of somatic embryos. (E). Number of shoots. (F). Number of roots. (G). Root length. (H). Shoot length. Note: Charts with the same letters are not significantly different from each other at the 5% significance level. Different letters denote a significant difference between culture media at the 5% significance level.

In the rooting stage, 'Amri' had the highest number of roots, the longest roots, and the longest shoots, outperforming the other two varieties. It produced an average of four roots with about 4.63 cm each, and shoots that were 13.61 cm long. 'Barhy' showed the lowest response, with an average of 2.92 roots, each 3.71 cm long, and shoots that were 11.82 cm long (3.3 g L^{-1} MS + 0.1 mg L^{-1} NAA + 0.25 mg L^{-1} IBA + 50 g L^{-1} sucrose + 6 g L^{-1} agar + 3 g L^{-1} AC) (Figures 2D and 3F, 3G and 3H) indicating no significant difference between 'Magdoul' and 'Barhy' varieties.

In pre-acclimatization and acclimatization stages (Figure 2E), Amri showed the highest growth vigor, followed by 'Magdoul' and 'Barhy', according to the visual description of Bottino (1981). Amri had the highest frequency of acclimatized plantlets, at 85.5% followed by 'Magdoul' (82.1%) and 'Barhy' (75.31%).

Phylogenetic analyses

Phylogenetic relationships among the three date palm varieties were inferred using data from ISSR molecular marker profiles and also based on *in vitro* propagation responses criteria. The clustering analysis based on *in vitro* propagation responses criteria grouped the three date palm varieties into two groups (I, II) (Figure 4A). Cluster I comprised 'Barhy' and Cluster II included 'Magdoul' and 'Amri' varieties. On the other hand, the same analysis was conducted among the three date palm varieties based on the ISSR-PCR molecular marker profiles. Also, the phylogenetic analysis (Figure 4B) grouped the three varieties into two clusters. 'Barhy' independently formed cluster I, while 'Magdoul' and 'Amri' formed Cluster II.

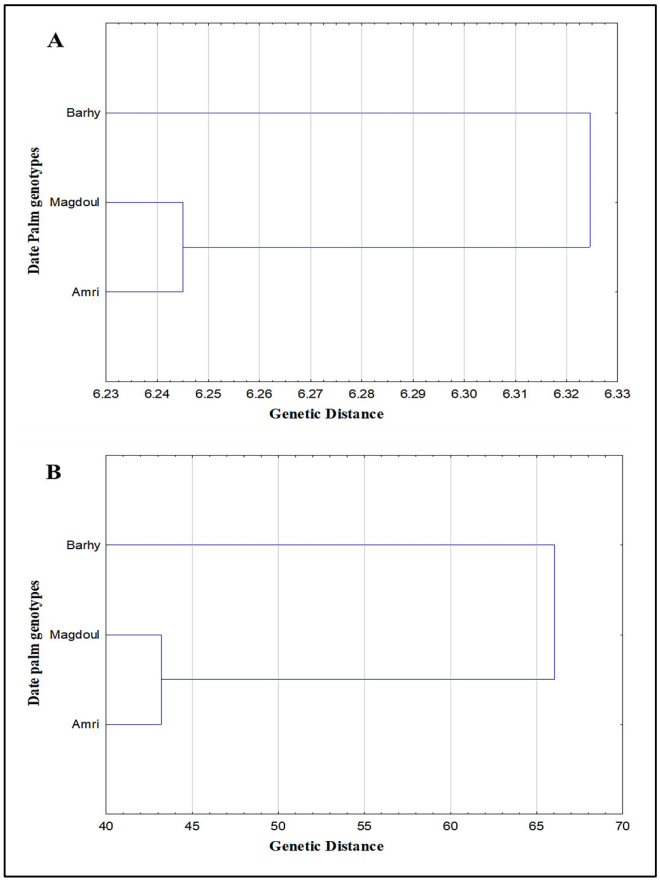

Figure 4. A. The phylogenetic tree of the three date palm varieties was revealed based on *in vitro* propagation responses criteria. B. The phylogenetic tree of the three date palm varieties was revealed based on ISSR banding patterns. The trees show the relationships among the three varieties, with the most closely related varieties being grouped

Discussion

This study was conducted to identify the relationship of molecular differences among three date palm varieties with the responses of the explants during the different *in vitro* propagation stages.

ISSR marker is a widely used molecular marker (Sadhu *et al*., 2020). The ISSR primers generated reproducible and scorable banding patterns, which allowed for the determination of genetic polymorphism among the date palm varieties. These primers generated an appropriate amplification pattern and produced varied polymorphism ratios ranging from 33.33% with UBC835 primer to 100% with UBC814, UBC826 and UBC868 primers. These results indicated the efficiency of UBC814, UBC826 and UBC868 primers in identifying the genetic diversity and the relationship among the varieties based on the ISSR-PCR polymorphic banding profiles. Several studies applied the ISSR primers to determine the genetic polymorphism among date palm varieties (Mitra *et al*., 2011; Zehdi *et al*., 2002) or other plant species such as tomato (Al-Khayri *et al*., 2023) and Kalanchoe (Al-Khayri *et al*., 2022). Similar results were found by Hamza *et al.* (2012) where a set of different ISSR primers were used to amplify DNA from 26 different date palm varieties. They concluded that the ISSR primers generated a total of 43 DNA fragments, of which 34 were polymorphic. The polymorphism levels ranged from 60% to 100%, with an average polymorphism level of 78.91%. The researchers concluded that ISSR primers are a reliable and effective tool for detecting genetic polymorphism in date palm varieties and could be used to study the genetic diversity of date palm varieties and to identify varieties with desirable traits.

Previous studies have shown that molecular markers are a powerful tool for assessing genetic diversity in date palm varieties. Moreover, some studies investigated the relationship between phenotypic and molecular markers. Mitra *et al*. (2011) highlighted the ability to use ISSR markers to distinguish between male and female date palm cultivars. This study confirmed that molecular markers are a powerful tool for exploring the genetic diversity of plants (Agarwal *et al*., 2008).

The responses of date palm explants during the different micropropagation stages varied depending on the genotype of the plant. Some genotypes are more responsive to micropropagation than others. In the culture initiation and establishment stage, date palm explants from genotypes that are known to be responsive to *in vitro* propagation are more likely to form calli and shoots. These genotypes typically have a high concentration of undifferentiated cells which are more easily manipulated to form callus and shoots (Bekheet, 2011). Amri had the highest 'Amri' had the highest performance for all measured followed by 'Barhy' and 'Magdoul'. These findings suggest that the responses of date palm varieties to different micropropagation stages are not the same. 'Barhy' required the fewest days for callus induction, while the 'Magdoul' and 'Amri' showed no significant difference in the number of days required for callus induction. Similar findings were reported by Solangi *et al*. (2020) who found that the Aseel variety produced a higher amount of calli on a medium containing NAA (2 mg L^{-1}), while the 'Dhakki' variety produced a higher amount of calli on a medium containing 2, 4-D (2 mg L^{-1}).

In the shoot multiplication stage, the response of date palm explants to the composition of the culture medium can also vary depending on the genotype. Some genotypes require a higher concentration of growth hormones than others to promote shoot growth and development (Davies, 1995). The Amri variety had a completely different response to callus weight than the other varieties. This was also the same for the number of shoots, somatic embryos. In the same context, in the rooting stage, the response of date palm explants to the concentration of auxin in the culture medium can also vary depending on the genotype. Some genotypes require a higher concentration of auxin than others to form roots. The Amri variety had a higher response to the number of roots, root length, and shoot length than the other varieties. These results suggest that the three varieties have significant differences in their responses to micropropagation.

The responses of date palm calli to growth regulators can vary depending on the variety of date palm. Due to genetic variation which can affects their response to hormones. Several studies have documented

different interactions between date palm varieties and the growth regulators resulting in varied responses in the embryogenesis of calli (Eshraghi *et al*., 2005; Fki *et al*., 2003). Metwali *et al*. (2020) found that the response to root formation and the lengths of shoots/roots of Magdoul variety improved by using IBA (0.5 mg L^{-1}) in combination with NAA at 2.0 mg L^{-1}.

Phylogenetic analyses are a set of methods used to infer the evolutionary relationships between organisms. These methods use similarities and differences in heritable traits, such as DNA sequences, protein sequences, or morphology to construct phylogenetic trees (*Hassanin et al., 2022b*; *Raza et al., 2021*). Also, phylogeny is an important analysis to emphasize genetic diversity (Fang *et al*., 2010; Farrag *et al*., 2019; Fathy *et al*., 2021; Fei Fang *et al*., 2011; Hassanin *et al*., 2022a; Hassanin *et al*., 2020; Raza *et al*., 2021). Based on the results of ISSR profiles and *in vitro* propagation response data, two phylogenetic trees were generated. Phylogenetic analyses of date palm varieties based on *in vitro* propagation responses and molecular profiles yielded similar results. These results provide precise insights into the genetic relationships among date palm varieties and confirm the distinctiveness of the 'Barhy' variety.

Conclusions

This study investigated the genetic diversity among three date palm varieties based on ISSR molecular marker profiles and its relationship with the response of these elite varieties to different tissue culture stages. The study found that the ISSR molecular marker generated consistent and reproducible bands to detect the genetic diversity in different date palm varieties. Overall, the ISSR molecular marker is a reliable, inexpensive and effective tool for detecting genetic polymorphism in date palm varieties. Amri variety exhibited the highest performance in all studied followed by 'Magdoul' and 'Barhy', while the 'Barhy' variety showed the highest number of days for callus induction. Various responses of date palm varieties to *in vitro* propagation are genotype-dependent process. This information can be used to improve the efficiency of date palm breeding programs and to develop new date palm varieties that are better suited to specific environments and markets.

Authors' Contributions

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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