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SCoT MARKER FOR THE ASSESSMENT OF GENETIC DIVERSITY IN SAUDI ARABIAN DATE PALM CULTIVARS

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Abstract

Different types of molecular markers based on DNA have been used for the assessment of genetic diversity in the plant species. Start Codon Targeted Polymorphism (SCoT) marker has recently become the marker of choice in genetic diversity studies. SCoT marker was used for the assessment of genetic diversity in Saudi Arabian date palm cultivars. The percentage of polymorphic loci (PPL) at population level ranged from 3.28 to 13.11 with an average of 7.10. The Nei's gene diversity (h) and Shannon's Information index (I) were 0.033 and 0.046, respectively. However, at cultivar level, PPL, Nei's gene diversity (h) and Shannon's Information index (I) were 42.62, 0.090 and 0.155, respectively. Analysis of molecular variance (AMOVA) showed 48% of variation within the populations, whereas 52% was found among the populations. A hierarchical analysis of molecular variance revealed level of genetic differentiation among populations (52% of total variance, P = 0.001), consistent with the gene differentiation coefficient (Gst = 0.631). Unweighted pair group method of arithmetic averages (UPGMA) cluster analysis of the SCoT marker data divided the six cultivars and their populations into five main clusters at 0.95 genetic similarity coefficient level.

Keywords: Genetic diversity, DNA markers, Conservation, UPGMA.

Introduction

The date palm (*Phoenix dactylifera* L.), (family Arecaceae) is a dioecious, perennial, heterozygous, monocotyledonous plant. The genome size is estimated to be approximately 658-Mbp long (Al-Dous *et al*., 2011). It is the most important fruit crop in the Middle East and North African countries. The annual world production of dates has reached 6-8 million mt (metric tons). This plant is predominating in the arid regions and has high tolerance to various environmental stresses such as drought and salinity (Zohary & Hopf, 2000; Johnson, 2011: Al-Mulla *et al*., 2013; Ebert, 2000).

The Kingdom of Saudi Arabia has more than 350 cultivars and approximately 2,000 cultivars around the world (Al-Mssallem, 1996). Each cultivar has different morphological characteristics and nutritional value (Fayadh & Al-Showiman, 1990; Anon., 1982). According to data from the Food and Agriculture Organization (FAO) of the United Nations, Saudi date production represents approximately 12 to 13 percent of world production (http://www.saudigazette.com.sa). This species was probably domesticated around 4,000 B.C. in the Mesopotamia-Arabic Gulf area (Nesbitt, 1993; Zohary & Hopf, 2000; Tengberg, 2012) and nowadays is distributed worldwide. Dates are a good source of vitamins, energy and various elements viz., iron, phosphorus, calcium and potassium (Anwar-Shinwari, 1987; Gamil-Abdel-Hafez *et al*., 1980). Besides nutritional value, date fruits are rich in phenolic compounds which shows antioxidant activities (Zineb *et al*., 2012).

Molecular markers based on DNA have been developed in *P. dactylifera* for their genetic diversity assessment (Bahraminejad & Mohammadi-Nejad, 2015), SSR (Khierallah *et al*., 2011; Racchi *et al*., 2014) and AFLP (Jubrael *et al*., 2005), sex determination (Elmeer & Mattat, 2012) and phylogenetic study (Al-Qurainy *et al*., 2011). Each marker has its own advantages and disadvantages. In the past, the cultivar identification depends on size and colour of the fruit and morphology of the leaves (Sedra *et al*., 1998). However, these morphological markers, sometimes deviated to identify accurate cultivar as markers may be vary at different developmental stages and under various environmental conditions (Elhoumaizi *et al*., 2002). Biochemical markers have limitations for the assessment of genetic diversity due to low polymorphism and difficult to discriminate the closely related cultivars (Al-Jibouri & Adham, 1990). Now a days, the molecular markers based on DNA, have polymorphism and have been used for the assessment of genetic diversity within and among the cultivars.

The genetic diversity was assessed among the cultivars of date palms using the DNA markers such as random amplified polymorphic DNA (RAPD), inter simple sequence repeat (ISSR) (Srivashtav *et al*., 2013; Marsafari and Mehrab, 2013), simple sequence repeat (SSR) (Elshibli & Korpelainen, 2008), random amplified microsatellite polymorphism marker (RAMPO) (Rhouma *et al*., 2008) and amplified fragment length polymorphism (AFLP) (Khierallah *et al*., 2011a).

Recently, new marker has been developed for the assessment of genetic diversity, called Start Codon Targeted (SCoT) marker (Collard & Mackill, 2009). SCoT marker is reproducible as compared to the RAPD as it has longer primer sequence. It is a type of targeted molecular marker technique. Start codon (ATG) and flanking sequences are highly conserved in the plant genes. SCoT marker is based on the conserved regions which is surrounded by the start codon ATG (Sawant *et al*., 1999). However, SCoT markers can be developed from the transcribed regions and might be linked to the gene function as studied in *Mangifera indica* L., sugarcane etc. (Luo *et al*., 2014; Wu e*t al*., 2013). Like RAPD and ISSR markers, SCoT marker is developed using single primer in a PCR amplification reaction. The SCoT marker has been used in many crop plant species such as rice (Collard & Mackill, 2009), longan (Chen *et al*., 2010), grape (Guo *et al*., 2012), potato (Gorji *et al*., 2011), orange (Jiang *et al*., 2011), peanut (Xiong *et al*., 2011), *Cicer* species (Amirmoradi *et al*., 2012) and sugarcane (Que *et al*., 2014).

Environmental stresses have great impact on genetic diversity of plant species. Therefore, assessment of genetic diversity is very important to see the genetic variations within and among the Saudi Arabian date palm cultivars which is necessary for their survival under different environmental stresses.

Material and Methods

Date palm collection: The cultivars were collected from the Al-Rajhi farm (Al-Qassim) (Pop IV and VI), Al-Hasa (Pop II and III) and Al-Kharj (Pop V and I), Saudi Arabia (Table 1). The cultivars were identified by date palm expert available at farm. The leaves of cultivars were collected for the genomic DNA isolation. The fruits were collected for the comparison among the cultivars based on morphology. The genomic DNA was isolated using the modified CTAB method (Khan *et al*., 2007).

SCoT PCR reaction and amplification conditions: The reaction mixture (25µL) with all components was taken for the amplification and further development of SCoT marker. PCR reaction was performed on Veriti 96-well Thermal Cycler (Applied Biosystem, Singapore). Initial denaturation was carried out at 94°C for 4 min, followed by 40 cycles at 94°C for 1 min, 51°C for 1 min, 72°C for 1 min, and final extension at 72°C for 5 min. The amplification products were separated on 1.3% agarose gel containing 0.5 µg/mL of ethidium bromide. The electrophoresis was performed in 1X TBE buffer solution at 5 V/cm. The agarose gel was visualized under a UVP ultraviolet SYNGENE transilluminator (UK).

SCoT marker profiling and statistical analysis: Thirty primers were used for the amplification of the genomic DNA to assess the genetic diversity. The primers which gave prominent and reproducible bands were selected for the final amplification and data analysis. The primers which gave lesser number of loci were not included in final data analysis. The prominent band was counted as (1) for present, whereas, for the absent of the band counted as (0) for the phylogram reconstruction. The faint bands were excluded from the final data analysis. Nei's genetic diversity index (h), percentage of polymorphic bands (PPB), genetic diversity index within series (Hs), Shannon's information index (I), total genetic diversity index (Ht) and coefficient of genetic differentiation (*G*st) were calculated using POPGENE 1.31 (Yeh *et al*., 1999). Analysis of molecular variance (AMOVA) was performed using Arlequin version 3.01 to examine differences within and among populations (Excoffier *et al*., 2005). Unweighted pair group method of arithmetic averages (UPGMA) was used for cluster analysis using NTSYSpc (Rohlf, 2000).

Results

Date palm is an important commercial crop in the Middle Eastern countries and North Africa. Its fruits provide staple food to many countries across the world. Several propagation technique has been used for the female plant production. The vegetative propagation is commonly used to raise more female plants. However, in vegetative propagation method, the genetic diversity decreases as compared to the ancestor plant species. Date palm tree withstand adverse environmental changes such as high and low temperature, drought, rain etc. Such adverse changes affect the genetic diversity of date palm. First time, we have developed SCoT marker for Saudi Arabian date palm cultivars for the identification of genetic diversity.

Six potential cultivars viz., Ruthana, Khalas, Sukary, Salag, Nabtat sef, Rozaizi and their individuals were collected from three different region of Saudi Arabia. All dates have good nutrition value and taste. The morphological markers such as fruit and seed size, length, width and color were varied among these cultivars (Fig. 1). The variations in these morphological markers might be assume genetic and environmental factors. The genomic DNA was isolated and purified from the leaves of these cultivars. The yield and quality of genomic DNA was varied among the cultivars because of the complex secondary metabolites present in the leaves. The isolated DNA was diluted for the PCR amplification and development of SCoT marker.

Thirty six SCoT primers were selected and designed **(**Collard & Mackill, 2009) for the amplification of the genomic DNA of these potential cultivars**.** Length of SCoT primer and their annealing site on genomic DNA have important role for more reproducibility. Out of thirty six primers used, eleven primers gave bright and reproducible bands which were processed further in data analysis. All primers were selected from the literature of monocot plant **(**Collard & Mackill, 2009). A SCoT marker profile generated with primer-2 was given in (5′-CAACAATGGCTACCACCC-3′) (Fig. 2). The populations of these cultivars (Table 1) were collected from three regions including, Al-Qassim, Al-Hasa and Al-Kharj of Saudi Arabia. All populations were clustered according to the region except populations I and VI which were collected from the Al-Kharj. The percentage of polymorphic loci was ranged from 3.28-13.11 (Table 2) and highest polymorphic loci were found to be in the population (III). The Nei's gene diversity (h) and Shannon's Information index (I) were ranged from 0.015-0.061 and 0.021-0.086, respectively.

Table 1. Date palm cultivars and their collections from Saudi Arabia

Populations	Accessions	Population size	Collection place
Ruthana (I)	Rut1, Rut2, Rut3, Rut4	4	Al-Kharj
Khalas (II)	Kha1, Kha2, Kha3, Kha4	4	Al-Hasa
Sukary (III)	Suk1, Suk2, Suk3, Suk4,	4	Al-Hasa
Salag (IV)	Sal1, Sal2, Sal3, Sal4	4	Al-Qassim
Nabtat sef (V)	Nab1, Nab2, Nab3, Nab4	4	Al-Kharj
Rozaizi (VI)	Roz1, Roz2, Roz3, Roz4	4	Al-Qassim

Fig. 1. Fruits and seeds of collected various date palm cultivars.

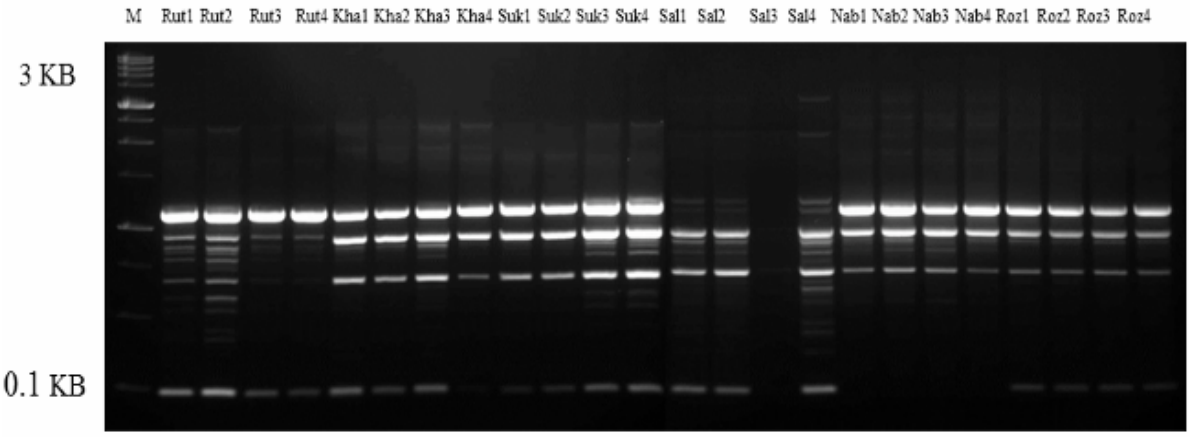

Fig. 2. SCoT marker gel profile was generated using the ScoT primer-2.

Table 2. Genetic diversity within Saudi Arabian date palm populations using the SCoT marker.

Populations	No of polymorphic loci	PPL	h	I
PopI	5	8.20	0. 039 (0.133)	0. 055(0.187)
PopII	4	6.56	0.030(0.144)	0.042(0.162)
PopIII	8	13.11	0.061(0.160)	0.086(0.225)
PopIV	3	4.92	0.023(0.103)	0.032(0.145)
PopV	4	6.56	0.030(0.144)	0.042(0.162)
PopVI	2	3.28	0.015(0.082)	0.021(0.166)
Average	4.33	7.10	0.033	0.046

Standard deviations are in parentheses. PPL, Percentage of polymorphic loci; h, Nei's gene diversity; I, Shannon's Information index.

A dendrogram was constructed to represent the genetic relationship among the populations of these cultivars (Fig. 3). The populations of all cultivars were clustered according to the regions and individual genetic similarity**.** The accessions of cultivar collected from the one location almost were similar and all clustered in one. The accessions from population (Pop III) of cultivar Sukary showed 100 % similarity (Suk4 and Suk 2) and this was the highest similarity among the individuals of this cultivars and also from other cultivars. The individuals from population (I, II, III, V and VI), except of individual of the Pop IV showed minimum 88.5% similarity to the other individuals of the populations (Table 3). Thus, within the individuals of same cultivars was found high similarity as all cultivars were produced from offshoots. However, more genetic diversity was found among the populations of other cultivars (Table 4). All populations were clustered into five main cluster. The second cluster had two populations which were collected from a same region (Al-Qassim).

Table 3. Similarity matrix values generated using the NTSYSpc software for the data produced from SCoT marker for the date palm cultivars.

	Pop I (Ruthana)				Pop II (Khalas)				Pop III (Sukary)				Pop IV (Salag)				Pop V (Nabtat saif)				Pop VI (Rozaizy)			
	Rut1	**Rut2**	**Rut3**	**Rut4**	**Kha1**	**Kha2**	**Kha3**	**Kha4**	**Suk1**	**Suk2**	**Suk3**	**Suk4**	**Sal1**	**Sal2**	**Sal3**	**Sal4**	**Nab1**	**Nab2**	**Nab3**	**Nab4**	**Roz1**	**Roz2**	**Roz3**	**Roz4**
Rut1	1.000																							
Rut2	0.950	1.000																						
Rut3	0.966	0.950	1.000																					
Rut4	0.950	0.966	0.950	1.000																				
Kha1	0.918	0.901	0.918	0.901	1.000																			
Kha2	0.934	0.918	0.934	0.918	0.950	1.000																		
Kha3	0.901	0.885	0.901	0.885	0.982	0.966	1.000																	
Kha4	0.934	0.918	0.934	0.918	0.950	0.966	0.933	1.000																
Suk1	0.934	0.918	0.934	0.918	0.918	0.934	0.901	0.934	1.000															
Suk2	0.918	0.901	0.918	0.901	0.901	0.918	0.885	0.918	0.950	1.000														
Suk3	0.934	0.918	0.934	0.918	0.918	0.934	0.901	0.934	0.966	0.983	1.000													
Suk4	0.918	0.901	0.918	0.901	0.901	0.918	0.885	0.918	0.950	1.000	0.983	1.000												
Sal1	0.950	0.934	0.950	0.934	0.934	0.9508	0.918	0.950	0.950	0.934	0.950	0.934	1.000											
Sal2	0.950	0.934	0.950	0.934	0.934	0.9508	0.918	0.950	0.950	0.934	0.950	0.934	0.967	1.000										
Sal3	0.950	0.934	0.950	0.934	0.934	0.9508	0.918	0.950	0.950	0.934	0.950	0.934	0.967	0.967	1.000									
Sal4	0.950	0.934	0.950	0.934	0.934	0.9508	0.918	0.950	0.918	0.934	0.950	0.934	0.967	0.967	0.967	1.000								
Nab1	0.918	0.901	0.918	0.901	0.901	0.9180	0.885	0.918	0.934	0.901	0.918	0.901	0.934	0.934	0.934	0.934	1.000							
Nab2	0.934	0.918	0.934	0.918	0.918	0.9344	0.901	0.934	0.934	0.918	0.934	0.918	0.950	0.950	0.950	0.950	0.950	1.000						
Nab3	0.934	0.918	0.934	0.918	0.918	0.9344	0.901	0.934	0.918	0.918	0.934	0.918	0.950	0.950	0.950	0.950	0.950	0.966	1.000					
Nab4	0.918	0.901	0.918	0.901	0.901	0.9344	0.885	0.918	0.934	0.901	0.918	0.901	0.934	0.934	0.934	0.934	0.966	0.950	0.983	1.000				
Roz1	0.934	0.918	0.934	0.918	0.918	0.9180	0.901	0.934	0.918	0.918	0.934	0.918	0.950	0.950	0.950	0.950	0.918	0.934	0.934	0.918	1.000			
Roz2	0.950	0.934	0.950	0.934	0.934	0.9508	0.918	0.950	0.950	0.934	0.950	0.934	0.967	0.967	0.967	0.967	0.934	0.950	0.950	0.934	0.983	1.000		
Roz3	0.934	0.918	0.934	0.918	0.918	0.9344	0.901	0.934	0.934	0.918	0.934	0.918	0.950	0.950	0.950	0.950	0.918	0.934	0.934	0.918	0.983	0.983	1.000	
Roz4	0.918	0.901	0.918	0.901	0.901	0.9180	0.885	0.918	0.918	0.901	0.918	0.901	0.934	0.934	0.934	0.934	0.901	0.918	0.918	0.901	0.966	0.966	0.983	1.000

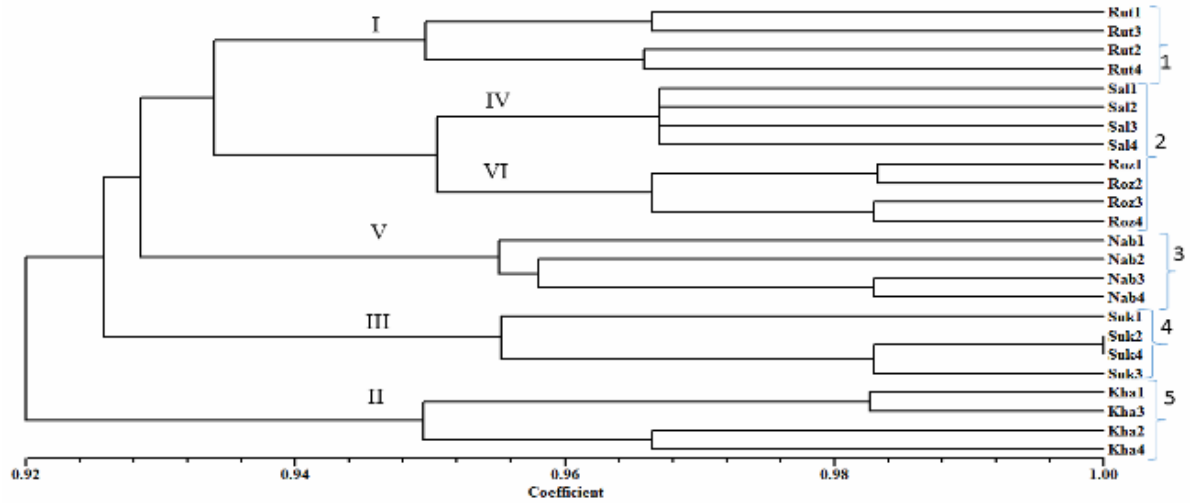

Fig. 3. Dendrogram showing the clustering among cultivars.

Table 4. Analysis of molecular variance (AMOVA) for the populations of date palm cultivars

Source of variance	df	SS	MS	% total of variance	P-value[a]
Among Regions	2	11.250	5.625	0%	
Among Pops	3	16.875	5.625	52%	p<0.001
Within Pops	18	19.250	1.069	48%	p<0.001
Total	23	47.375		100%	

d.f., Degree of freedom; SS, sum of squares; MS, expected mean squares; P-value, probability of null hypothesis
[a] Significance tests after 1000 permutations

Table 5. Genetic diversity among date palm cultivars estimated using SCoT marker.

h	I	Ht	Hs	Gst	Number of polymorphic loci	PPL
0.090 (0.112)	0.155(0.188)	0.090(0.012)	0.033(0.002)	0.631	26	42.62

Standard deviations are in parentheses. PPL, Percentage of polymorphic loci; h, Nei's gene diversity; I, Shannon's Information index; Ht, total gene diversity; Hs, population diversity; Gst, Coefficient of genetic differentiation

Table 6. Nei's Unbiased measures of genetic identity and genetic distance.

Population ID	Pop I	Pop II	Pop III	Pop IV	Pop V	Pop VI
Pop I	****	0.9198	0.9164	0.9452	0.9198	0.9322
Pop II	0.083	****	0.9285	0.9532	0.9198	0.9318
Pop III	0.087	0.0742	****	0.9532	0.9407	0.9289
Pop IV	0.056	0.0479	0.0598	****	0.9447	**0.9567**
Pop V	0.083	0.0839	0.0611	0.0568	****	0.9318
Pop VI	0.070	0.0839	0.0737	0.0443	0.0706	****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal)

Genetic differentiation within and among the date palm cultivars: Nei gene diversity statistics was used to assess the genetic variation among and within populations of date palm cultivars (Nei, 1973). The Nei's total genetic diversity (Ht), population genetic diversity (Hs), and coefficients of genetic differentiation (Gst) were found to be 0.090, 0.033 and 0.631, respectively (Table 5). The number of polymorphic loci was found to be 26 among the populations and the percentage of polymorphic loci was 42.62. However, the polymorphism was detected to be low using the SCoT marker and it might be due to the more conserved regions in the clonally propagated date palm cultivars.

Coefficient of genetic differentiation (Gst) was found to be high (0.631) and it might be assume due to the tree production through offshoot (vegetative propagation). Similarly, the high coefficient of genetic differentiation (Gst) was found in *Curculigo latifolia* (Gst=0.48) (Ranjbarfard *et al*., 2014), *Torreya jackii* Chun (Gst=0.63) (Li & Jin, 2007) and *Commiphora wightii* (Gst=0.58) (Harish *et al*., 2014) using the SCoT, ISSR and RAPD markers. The results of AMOVA showed genetic differences among (52%) and within populations (48%) (Table 4). The highest genetic distance was found between the populations I and III, whereas highest genetic similarity was found between IV and VI populations (Table 6). The reason behind this high and low genetic diversity in these populations might be assume due to geographical isolation and gene flow. The gene flow occurs in date palm cultivars via pollens (Balthazard, 2013). However, the eco-geographical factors such as rainfall, temperature and soil type might be consider for this genetic diversity among the populations.

Discussion

Genetic diversity is very important for the conservation of plant species in their natural habitat as it protect the plants from various environmental stresses. The impact of these environmental stresses may vary on same or different plant species and depend on various morphological and physiological characteristics of the plant.

The different marker system has been developed for the assessment of genetic diversity in *Phoenix dactylifera* as discussed above. However, these markers have advantages and disadvantages. We used more reproducible molecular marker named "SCoT" for Saudi Arabian date palm trees for the assessment of genetic diversity. It involves the single primer as forward as reverse as in RAPD and ISSR markers. The primers for this marker were deigned from target regions surrounding the ATG initiation codon. The developed SCoT marker is reproducible as the designed primers amplified the genomic segment started from the conserved region, initiation codon (ATG).

The SCoT marker has potential applications viz., genetic diversity studies, QTL mapping and bulked segregant analysis (Collard & Mackill, 2009). However, the simplicity and reproducibility of this method has been successfully applied to the assessment of genetic diversity and taxonomic study of *Citrus* (Han *et al*., 2011), *Arachis* (Xiong *et al*., 2010, 2011), *Dimocarpus longan* (Chen *et al*., 2010), and *Mangifera indica* Linn, (Luo *et al*., 2010, 2011). The polymorphism detected by this marker was low as compared to other markers such as RAPD (Bahraminejad & Mohammadi-Nejad, 2015), SSR (Khierallah *et al*., 2011; Racchi *et al*., 2014) and AFLP (Jubrael *et al*., 2005).

Thus, marker used in this study was found to be useful for estimating the genetic diversity within and among the cultivars. However, low genetic diversity was found using the SCoT marker. The more genetic diversity was found among the cultivars than within the cultivars. Thus, assessed genetic diversity among cultivars and within cultivars would be beneficial for their conservation in the Kingdom of Saudi Arabia.

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