INDIAN JOURNAL OF ARID HORTICULTURE
2008, Vol. 3 (1) : 54-56

Phylogenetic relationship among some cultivars of date palm (*Phoenix dactylifera* L.) on the basis of flavonoid spectrum

Rajesh Kumar Gothwal, R. Bhargava, P. K. Yadav and R. S. Singh
Central Institute for Arid Horticulture, Beechwal, Bikaner-334006, Rajasthan, India

Abstract

In order to develop phyto-chemical markers for delimitation of date palm cultivars, a study was undertaken using five cultivars. The foliar flavonoids were extracted and separated on TLC plates coated with cellulose. The spots thus developed were compared and subjected to cluster analysis using computer software. The results demonstrated that date palm cultivars can be identified using either specific spot or spot combinations. The results of the cluster analysis demonstrated that the selected five cultivars can be grouped into three distinct groups on the basis of their genetic distances. The group A was represented by cultivars Khuneizi and Dayari were has closer affinity with Halawy. The Group B1 was represented by Sewi and the group B2 by cvs. Khuneizi , Dayari and Halawy.

Key words: *Date palm, Phoenix dactylifera, Phylogeny, flavonoid, TLC*

Introduction

Morphological and anatomical characteristics of plants have been extensively exploited in plant systematics. However, at infra- specific level, these parameters become limiting. In a situation such as this, when traditional methods of taxonomy becomes limiting for cultivar identification, recourse to more sophisticated methods such as phyto- chemical markers are adopted. For this, secondary metabolites have gained reputation to be used as taxonomic markers. Since, their constitution in plants is specific and is not affected by environmental factors. Among these flavonooid spectrum of a cultivar have gained reputation to be an ideal marker for delimitation of taxonomic entity at intra specific level (Das et al., 1977; Koul et al., 1984; Garcia and Oieda, 2004 and Rusak et al., 2005).

Date palm is represented by a large number of cultivars which have been identified/ developed for specific purpose. In these species too, the morphological and anatomical parameters are insufficient to clearly distinguish the cultivars. Moreover, majority of cultivars have come up either due to natural selection or through deliberate breeding. The genetic variability once generated has been fixed on account of vegetative propagation which is commonly practiced in this taxon. Hence, the phylogenetic kinship between the cultivars is not certain which is utmost required under pre- breeding programme.

Accordingly, the present investigation was undertaken using five date palm cultivars to develop phychemical markers for them using foliar flavonoid and the data thus generated was used to assess the phylogenetic relationship among them.

Materials and methods

Five genotypes of date palm viz. Halawy, Khalas, Sewi, Khuneizi and Dayari constituted the material for present investigation. Thefoliar flavonoid spectrum of these cultivars was studies as described below:

Extraction of foliar flavonoids

Two grams of foliar sample was fixed in 10 ml of methanol containing 1% HCl . The fixed samples were stored at room temperature and before analysis they were macerated in mortar and pestle. The whole content was centrifuged at 10000 rpm for 20 minutes at room temperature. The supernatant was taken and evaporated to dryness in an oven maintained at 60°C. Finally the sample was taken in 1 ml methanol.

Analysis of flavonoids

The flavonoids were separated on TLC plates coated with 0.6mm thick layer of cellulose. An aliquot of 10 µl of sample as prepared above was loaded on one corner of the plate. The plate was first developed with 2% formic acid and later, after rotating at 90°, in solvent containing amyl alcohol: acetic acid and water in the ratio of 10:6:5. the

plates after air drying were viewed for flavonoid spots as under:

1. without any spray
2. after spraying with 1% methanolic $AlCl_3$ under UV
3. after spraying with 1% mehanolic NaOH under UV

The spots were marked and pooled chromatogram of each cultivar was prepared. They were then numbered and master chromatogram was prepared for comparison of cultivars. The phylogenetic relationship was ascertained by using computer software.

Results and discussion

Foliar flavonoid patterns have been worked out for five date palm (*Phoenix dactylifera* L.) cultivars. A total of 47 flavonoid spots were observed in these cultivars. The spots have been characterized on the basis of their respective fluorescence properties and position on the composite chromatograms. Fluorescence was checked under UV-light before and after spraying enhancing chemicals.

Perusal of table1 revealed that spot number 10, 14 and 25 represent commonly occurring flavonoids, as these spots were common in all the cultivars. The other spots were presented in either one or more cultivars. This is illustrated by the fact that spot number 1, 2, 3, 6, 7, 11, 13, 17, 19, 21, 26, 31, 33, 34, 35, 36, 37, 38, 40, 42 and 43 were present in 20 per cent cultivars, spot numbers 4, 5, 9, 12, 18, 22, 23, 26, 27, 29, 30, 32, 39 and 47 were present in 40 per cent cultivars and spot number 15, 16, 20, 24 and 46 were present in 60 per cent cultivars.

The cultivars can be identified using either typical spots or spot combinations as presented in table 1. Cultivar Halawy can be identified based on typical spot or spot combinations 5, 9, 21, 22, 27, 39 and 47; cultivar Khalas by 1, 2, 3, 4, 5, 11, 13, 17, 18, 19, 28, 29, 33, 43 and 47; cultivar Sewi by 6, 9, 12, 26, 35, 37, 38, 39, 40 and 42; cultivar Khuneizi by 4, 7, 12, 23, 26, 27, 30, 31 and 32 and cultivar Dayari by 18, 23, 29, 30, 32 and 34.

Based on the presence or absence of spots, the cultivars were assessed for their phylogenetic distance. Perusal of data indicates that cultivar Khuneizi and Dayari are most closely related as demonstrated by a similarity index of 42%. This is followed by cultivars Sewi with Khuneizi (34%) and Sewi with Dayari (30%). All other cultivars show poor relationship.

The present results are in line with those reported earlier by Rusak et al.(2005) in Iris., Garcia and Oieda (2004) in *Morus alba*, Sanches- Yelano (2004) in *Erucastris* and *Brassica*, Koul et al. (1984) in *Narcisssus* and Das et al. (1977) in *Citrus*.

The data generated in the present study was further subjected to cluster analysis using NTSYS Software. The Jaccard's similarity indeces among the cultivars are presented in Table 2 and the dendrogram developed is depicted in Fig. 1. Perusal of Table 2 and Fig. 1 demonstrates that the five genotypes of date palm under investigation could be grouped into three groups based on their phylogenetic relationships. Group "A" consisted of one cultivars viz. Khalas which shows distant relationships with other cultivars. Similarly the group B is sub-divided into B1 and B2. The group B1 is represented by one cultivar viz. Sewi whereas group B2 consists of 3 cultivars. Among these cvs. Khuneizui and Dayari shows the closer affinity as compared to Halawy.

Table 1. Foliar flavonoid profiles of different date palm cultivars

Spot numbers	Halawy	Khalas	Sewi	Khuneizi	Dayari
1		+			
2		+			
3		+			
4		+		+	
5	+	+			
6			+		
7				+	
8		+	+	+	+
9	+		+		
10	+	+	+	+	+
11		+			
12			+	+	
13		+			
14	+	+	+	+	+
15			+	+	+
16		+	+	+	
17		+			
18		+			+
19		+			
20	+	+			+
21	+				
22	+			+	
23				+	+
24	+		+		+
25	+	+	+	+	+
26			+	+	
27	+			+	
28		+			
29		+			+
30				+	+
31				+	
32				+	+
33		+			
34					+
35			+		
36			+		
37			+		
38			+		
39	+		+		
40			+		
41					
42			+		
43		+			
44		+	+	+	+
45	+		+	+	+
46	+	+		+	
47	+	+			

Table 2 Similarity coefficient based on the flavonoid spots

	Halawy	Khalas	Sewi	Khuneizi	Dayari
Halawy	1.00				
Khalas	0.23	1.00			
Sewi	0.26	0.16	1.00		
Khuneizi	0.27	0.24	0.34	1.00	
Dayari	0.26	0.27	0.30	0.42	1.00

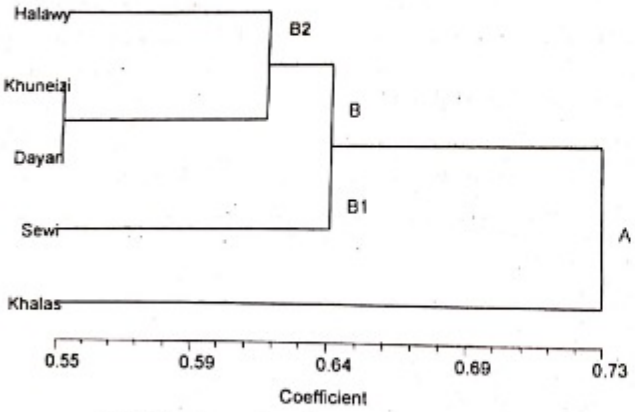

Fig. 1. Dendrogram based on foliar flavonoid spots

Our results further demonstrated that the Jaccard's similarity index between two cultivars is 0.42 showing thereby that the genotypes are phylogenetically similar.

Thus, from the foregoing results it is evident that flavonoid spectrum can be used for cultivar identification as well as for assessing phylogenetic relationship among taxa in date palm.

Reference

Bhargava, R. (1983). Metabolites as an indicator of polyploidy and hybridity in bulbous angiosperms. Ph. D. Thesis, University of Jammu, Jammu.

Dass, H. C., Randhawa, G. S. and Prakash, D. (1977). Chemosystematic studies in relation to variability in *Citrus limon*. *Indian Jour. of Experimental Biology.*, 15(2): 158-160.

Frost, S., Holm,G. and Askar, S. (1975). Flavonoids patterns and the phylogeny of barley. *Hereditas* 79: 133-142.

Garcia, D. E. Ojeda, F. (2004). Evaluation of the main factors that influence the phytochemical composition of *Morus alba* (Linn.). III. Total flavonoids. *Pastos y. Forrajes.*, 27(3): 267-272.

Koul, A. K., Sharma, M. C. and Bhargava, R. (1985). Impact of polyploidy on flavonoid constitution in *Narcissus tazetta* L. *Proc. Indian Aad. Sci.*, 95: 1-5.

Rusak, G., Mitic, B. and Liber, Z. (2005). Leaf flavonoid patterns in the Croatian endemic species *Iris illyrica* Tomm. and *Iris pseudopallida* Trinajstic (Iridaceae). *Periodicum Biologorum.*, 107(1): 45-49.

Sanchez-Yelamo, M. D. (2004). Taxonomic relationships among *Erucastrum* and *Brassica* species based on flavonoid compounds. *Cruciferae Newsletter.* 25: 13-14.