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SHORT COMMUNICATION

Protein and amylase profile- an aid in cultivar identification in some date palm (*Phoenix dactylifera* L.) cultivars

Rajesh Kumar Gothwal[1], R. Bhargava[2], P. K. Yadav[1] and R. S. Singh[2]
[1]SKRAU, Beechwal, Bikaner-334006, Rajasthan, India
[2]CIAH, Beechwal, Bikaner-334006, Rajasthan, India

Morphological and anatomical characteristics of plants have been extensively exploited in plant systematics. However, at infra- specific level, these parameters become limiting since varieties/ cultivars are distinguished from each other on the basis of one or two floral or fruit characters. These at times are so overlapping that it becomes difficult to identify the cultivars. In a situation such as this, when traditional methods of taxonomy becomes limiting for variety/ 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 are specific and are not affected by environmental factors. Among these, protein and amylase profiles of a cultivar have gained reputation to be an ideal markers for delimitation of taxonomic entity at intra specific level (Wang-Xiao *et al.*, 2007; Emari *et al*., 2006; Mohamed , 2006; Sharma, *et al*. 2006; Abel *et a*l.,2005; and Kende *et al*., 2004).

Date palm is represented by a large number of cultivars which have been identified/ developed for specific purpose. In this 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 among the cultivars is not certain which is utmost required under pre- breeding programme.

Accordingly, the present investigation was under taken using five date palm cultivars to develop phyto-chemical markers for them using foliar protein and amylase profiles and the data thus generated was used to assess the phylogenetic relationships among them.

Five genotyps of date palm (*Phoenix dactylifera* L.) viz. Halawy, Khalas, Sewi, Khuneizi and Dayari constituted the material for present investigation. The foliar protein and amylase profiles of these cultivars were studied as described below:

Extraction of foliar protein and amylase

Extraction of proteins as well as isozymes was performed in cold. 10 gm of leaf base material was crushed in ice chilled all glass pestle and mortar containing 5 ml of 0.05 M phosphate buffer, (pH 7.3). The mixture was left undisturbed for two hours at low temperature and then centrifuged at 8000 rpm for 10 minutes. The supernatant was collected in a separate tube and supplemented with dry sucrose (about 10% by volume). The extract thus obtained, was utilized subsequently for analysis.

Separation of protein and isozyme through electrophoresis

The gel- electrophoresis technique of Ornstein and Davis (1964) was employed for separating anodic proteins and isozymes. All separations were achieved on 7.5% polyacrylamide gel columns in electrophoresis apparatus. The steps involved in electrophoresis are:

Polymerization of gel

For preparing gels three solutions viz. A, B and C were mixed in a proportion of 1:1:2, by volume. This mixture was poured, with the help of glass dropper, into 10 cm long corning glass gel tubes, closed at one end by rubber bungs. The tubes were left undisturbed at room temperature for two hours to allow for polymerization.

For separation of amylases, gels were polymerized in the same way as described above, except for addition of starch (0.07%) to the final volume of the polymerizing solutions. This was done to facilitate detection of amylases on gel columns.

Loading the extract

Once polymerization was achieved, for separating proteins, each tube was loaded with 200l of the extract. The same volume of the extract was utilized for purpose of amylases separation. Loading was completed, by adding a drop of 0.01% bromophenole blue to each gel tube. The remaining space therein, was filled with Tris-glycine electrode buffer of pH 8.3

Running the apparatus

The reservoirs of the electrophoresis apparatus containing loaded gel tubes were filled with Tris- glycine electrode buffer maintained at pH 8.3. Current at the rate of

5 mA per gel tube was applied to the apparatus. The whole operation was performed inside a refrigerator at 8 ^{0}C. Once the tracking dye reached the anodic end, the current was switched off and gel columns were removed from the containers by forcing water along the inner wall of the tubes with the help of hypodermic syringe. The gels were transferred into bigger glass vials for further processing.

Detection of protein bands

For detection of proteins, gels were strained at room temperature, in coomassie brilliant blue solution for 15 hours and differentiated in a mixture of methanol, acetic acid and water. The differentiating medium was changed daily till such time the inter-band region got destained and band become distinct. These gels were stored at low temperature in 7.5% acetic acid and scrutinized for band pattern and characteristics.

Detection of amylase bands

Starch impregnated gels were used to facilitate detection of amylases. These were incubated for two hours at room temperature in 0.02 M-phosphate buffer at pH 6.9 and then transferred to 0.004 M iodine solution for about 10 minutes. After a while, transparent bands and dark inter-bands differentiated on the gels. The gel columns were subsequently scrutinized for band number and characteristics.

Proteins

Anodic protein profiles of the five cultivars of date palm contained a total of 13 protein bands. The individual bands in each cultivar are summarized in table1. Perusal of table revealed that the number of protein bands varied from 6 to 9 in different genotypes; maximum in cv. Halawy and minimum in cv. Khuneizi. A wide variation was observed for their distribution among the genotypes investigated. For instance, band numbers 5 and 13 had the highest representation as it is present in 100% of genotypes. Band number 2 illustrated a representation of 80% of genotypes. Similarly, band numbers1, 4 and 11 were represented in 60 of the genotypes. On the contrary few bands have restricted distribution as illustrated by band numbers3, 6, 7, 8, 10 and 12 were found only in 40% of genotypes and used as phyto-chemical markers.

The distribution of the protein bands also indicated qualitative variation among the genotypes. Single band or band combinations were typical for individual cultivar which is found in particular genotype. For instance band combination 7, 9 and 12 were specific bands for cv. Halawy, bands 7, 8, 10 and 12 were typical for cv. Khalas, bands 3, 6 and 8 were found special for cv. Sewi, bands 3, 6 and 10 were typical for cv. Khuneizi and band number 9 was typical for cv. Dayari.

A role of protein in cultivar identification at species or sub species level has been demonstrated earlier too (El-Akkad *et al*., (2002), Kende *et al*. (2004), Abou-El-Enain, (2004), Yan-Xue Bing *et al*. (2005), Signor, *et al.* (2005), Jalilion, *et al.* (2005), Abel *et al*. (2005), Sharma *et al.* (2006), Mohamed (2006), Emre *et al*. (2006) and Wang-Xiaofei *et al.* (2007)

Table 1. Banding pattern for Proteins in different date palm cultivars

Cultivars	Bands												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Halawy	+	+		+	+		+		+		+	+	+
Khalas	+				+		+	+		+		+	+
Sewi		+	+	+	+	+		+			+		+
Khuneizi		+	+		+	+				+			+
Dayari	+	+		+	+				+		+		+

Table 2. Banding pattern for amylase in different date palm cultivars

Cultivars	Bands				
	1	2	3	4	5
Halawy	+	+			
Khalas	+		+		+
Sewi	+	+		+	
Khuneizi	+		+		
Dayari	+		+		

Amylase

Like other secondary metabolites, isozyme plays an important role to assess phylogenetic relationship between species, sub-species, genera and cultivars. A total of 5 bands were depicted for amylase during the study. The banding pattern is illustrated in Table 3. Perusal of data revealed that the number of bands varied from 2 to 3 in different genotypes, maximum in cv. Khalas and Sewi and minimum in Halawy, Khunezi and Dayari. Band number 1 was universally distributed (100 per cent) in all the

cultivars. Similarly band number 3 had 60 per cent representation and band number 2 has 40 percent distribution. Contrary to this, band numbers 4 and 5 had least distribution as these were found in only one of the cultivars and represented 20 percent distribution.

Like other secondary metabolites, isozyme plays an important role to assess phylogenetic relationship between species, sub-species, genera and cultivars. This has been demonstrated by many researchers viz. Staub and Frederick, (1985), Ramirez and Pisabarro, (1985), Jarret and Litz, (1986), Kesseli and Michelmore, (1986), Tao and Sugiura, (1987), Yang and Xi (1989), Oliva-Tajera *et al*., (2004), Bessage, et al. (2005), Karam, *et al*., (2006).

Thus from the foregoing account, it can be concluded that proteins and amylase profile can be used for cultivar identification in date palm.

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