Sugar and Free Amino Acid Composition of Five Cultivars of Dates from Offshoots or Vitroplants in Open Fields

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Morphological and chemical analyses of mature dates related to the propagation method (traditional vegetative propagation and two different methods of *in vitro* multiplication) of the date palm are presented. No significant differences were seen among Bou Sthammi noire, Mejhool, Thoory, and Zahidi cultivars propagated by traditional methods and by the French Group on Date Palm Research *in vitro* multiplication technique, based on axillary budding. These results could indicate that the vitroplants conform to mother trees. Of the five cultivars studied, only the Deglet Nour dates, obtained from callus (somatic embryogenesis), showed divergences in sugar and amino acid composition. In this case, Deglet Nour vitroplants contain no sucrose, more glutamic acid, glutamine, γ-amino-butyric acid, and arginine, and less alanine. We could suppose that variations were introduced by the micropropagation technique.

INTRODUCTION

The date palm (*Phoenix dactylifera* L.) is the subject of intensive exploitation in Mediterranean Africa, the Middle East, West Asia, and the United States. In exporting countries as in self-consumer ones, it is advantageous to quickly replace aged or diseased trees. This replacement should be done with shoots offering identical characteristics of fruit quality, flavor, and productivity with the selected cultivars. Seedlings show a great genetic variation, even if they are from the same mother tree, so the date palm is traditionally propagated by separating and independently establishing the offshoots produced by the same tree. They are often limited in number because the date palm only produces offshoots during the vegetative development phase of its life (Toutain, 1973) and also because the number of offshoots produced is dependent on the cultivar. A date palm tree produces an average of 20 offshoots during its life (approximately 80 years), of which perhaps 50% are suitable for producing a mature tree. Thus, research on *in vitro* propagation of date palm buds was undertaken to rapidly satisfy the needs of planters and of the groves' renewal. Date palm micropropagation is developed by the French Group on Date Palm Research (GRFP; Ferry et al., 1987) on Martin basic principles (Martin, 1980) and by industrial laboratories on Tisserat technique (Tisserat, 1981). It is essential that dates produced by acclimatized vitroplants or by offshoots offer identical characteristics. Morphological observations might be difficult or even inaccurate, so we have studied the chemical composition of mature dates, especially moisture, sugar, and free amino acid contents.

Sugars are the most prevalent compounds in dates and have been widely studied (Cook and Furr, 1952; Ashmawi et al., 1956; Dowson and Aten, 1973; Munier, 1973; Hussein et al, 1974; Vandercook et al., 1979; Peyron and Gay, 1988). Most reports, however, have been limited to total and invert sugars, their ratio, and changes with maturity. There is, as yet, only a small amount of information available on the amino acid content of dates (Grobbelaar et al., 1955; Al-Rawi et al., 1967; Auda et al., 1976; Nour and Magboul, 1985). Thus, this study was conducted to compare sugar and amino acid composition of dates harvested on trees obtained by the traditional vegetative method of propagation and by *in vitro* culture.

MATERIALS AND METHODS

Microprogation Techniques. The basis of the technique used by the GRFP was developed by Poulain et al. (1979). It consists in bud proliferation from the apex and the axillary buds taken from small offshoots. Another technique was used for the obtention of DN cultivars, based on the Tisserat (1981) procedure. Date palm plantlets have been initiated from clonal explants via callus. The plantlets produced by these methods are gradually acclimatized. They are set in "Melfert" and go through four phases: misting according to the procedure of Saaidi et al. (1979), a period in a heated greenhouse, outside plantation, and behavior trials in open-field culture in arid areas. They are grown in five locations: Saudi Arabia (Hofuf, Al-Hassa), United Arab Emirates (Al-Ayn), Qatar (Al-Utorieh), Mali, and Algeria. Five cultivars were studied: Bou Skri (BSK), Deglet Nour (DN), Mejhool (MJH), Thoory (THR), and Zahidi (ZHD). All of the vitroplants of BSK, THR, and ZHD cultivars and some MJH trees were produced by the GRFP laboratory.

Morphological Analyses. Because we no longer have control of true date palm culture areas, we could not have at the same time and in the same experimental plots the standard plants from offshoots more similar to vitroplants. Nevertheless, at Al-Hassa, we dispose of a varietal collection of offshoots for the cultivars investigated, similar to our experimental plot stations. These offshoots were imported from California (THR, ZHD) or from their native countries (Maghreb for BSK, MJH, and THR cultivars). The vitroplant plots analyzed are at the same time behavior tests with limited repetitions, adaptability assays (the trees tested are foreign cultivars for Qatar, Saudia Arabia, and United Arab Emirates), and demonstration tests, because they are planted in extensive areas. To facilitate the comparative observation of intervarietal silhouettes (shape of the trees) and between individual vitroplants, trees of the same clone were planted in straight lines. Since their plantation, the vitroplants planted for the comportment tests are followed by regular growth records, in the greenhouse and in the plantation: phyllotaxy,

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Table I. Morphological Observations on Offshoots or Vitroplants of Five Date Palm Cultivars of Similar Ages (Toutain, Personal Communication) and Moisture, Sugar, and Azote Content of Mature Dates[a]

	MJH v	MJH r	BSK v	BSK r	DN v	DN r	ZHD v	ZHD r	THR v	THR r
tree height (m)	2.60 ± 0.20	2.30 ± 0.35	3.20 ± 0.15	3.50 ± 0.15		2.60 ± 0.15	2.30 ± 0.10	2.50 ± 0.05	3.10 ± 0.50	3.80 ± 0.20
stipe diameter (m)	0.33 ± 0.01	0.28 ± 0.05	0.40 ± 0.02	0.43 ± 0.01		0.40 ± 0.10	0.45 ± 0.05	0.42 ± 0.05	0.45 ± 0.02	0.43 ± 0.03
palm range	50	sim	52	sim			25	sim	40	sim
palm length	1.80 ± 0.20	2.08 ± 0.05	1.97 ± 0.70	2.86 ± 0.20		2.30 ± 0.05	1.64 ± 0.30	1.97 ± 0.25	2.37 ± 0.20	2.24 ± 0.35
% of leaflets parts	65 ± 0	70 ± 0	69 ± 0	67 ± 0		71 ± 0	73 ± 0	73 ± 0	65 ± 0	68 ± 0
leaflet density on 10 cm	5.20 ± 0.75	6.90 ± 0.10	6.50 ± 0.65	7.40 ± 0.05		6.75 ± 0.55	6.40 ± 1.20	8.40 ± 0.05	3.50 ± 0.85	4.20 ± 0.10
thorn density on 10 cm	4.70 ± 0.10	4.90 ± 0.10	3.80 ± 0.10	2.60 ± 0.50		5.96 ± 0.20	4 ± 0.80	5.10 ± 0.10	3.30 ± 0.20	3.60 ± 0.20
leaflet gauge (cm)	13 ± 1.50	10 ± 0.55	8.70 ± 1.50	11.70 ± 0.95		13.35 ± 1.25	9.70 ± 0.20	8.50 ± 0.85	10.50 ± 0.05	10.60 ± 0
rachis gauge (cm)	4.90 ± 0	4.70 ± 0	3.85 ± 0	3.75 ± 0		5 ± 0	2.70 ± 0.10	2.20 ± 0.55	3 ± 0	3 ± 0
number of bunches	2 ± 5	10 ± 1	5 ± 0	4 ± 0		4 ± 0	11 ± 0	7 ± 2	5 ± 0	5 ± 0
seed gauge (cm)	1.80 ± 0	1.60 ± 0	1.29 ± 0	1.40 ± 0	1.50 ± 0	1.50 ± 0	1.40 ± 0	1.60 ± 0	1.70 ± 0	1.90 ± 0
date gauge (cm)	3.50 ± 0.50	2.30 ± 1.25	1.80 ± 0	1.80 ± 0	2 ± 0.45	1.75 ± 0.15	2.30 ± 0.50	3 ± 0.20	2.10 ± 0	2.30 ± 0
date weight (g)	8.30 ± 3.25	12.75 ± 2.65	7.30 ± 0.20	6 ± 1.20	5.60 ± 1.05	6.58 ± 0.85	4.70 ± 2.55	6.60 ± 1.35	4.23 ± 1.65	5.94 ± 0.55
% flesh	87.30 ± 3.67	91.40 ± 0.84	81.50 ± 2.97	80.10 ± 1.95	82.80 ± 4.80	91.10 ± 1.25	86.30 ± 1.26	84.70 ± 1.07	84.20 ± 3.53	81.50 ± 2.90
% moisture in ripe stage	28.70 ± 2.30	28.80 ± 3.50	33.10 ± 3.10	35.00 ± 1.30	29.35 ± 2.34	24.90 ± 0.54	22.00 ± 3.80	28.00 ± 0.84	18.60 ± 3.18	22.00 ± 1.52
% sugars in ripe stage	77.60 ± 6.84	72.60 ± 0.88	73.30 ± 0.16	71.00 ± 1.65	66.30 ± 7.84	79.10 ± 2.80	73.70 ± 1.47	72.20 ± 0.80	71.60 ± 0.30	73.50 ± 0.30
% azote in ripe stage	0.536 ± 0.07	0.560 ± 0.08	0.503 ± 0.06	0.503 ± 0.08	0.441 ± 0.00	0.485 ± 0.04	0.444 ± 0.03	0.424 ± 0.15	0.416 ± 0.05	0.440 ± 0.07

[a] BSK, Bou Skri; DN, Deglet Nour; MJH, Mejhool; THR, Thoory; ZHD, Zahidi; r, offshoots; v, vitroplants.

Table II. Sugar Composition of Mature Dates of Five Date Palm Cultivars from Offshoots or Vitroplants[a]

cultivar	fructose	glucose	sucrose	nonsugars
MJH v	38.15 ± 3.00	39.25 ± 4.40	0.15 ± 0.30	22.45 ± 1.84
MJH r	35.30 ± 0.20	38.95 ± 0.35	0.25 ± 0.35	23.50 ± 0.90
BSK v	36.75 ± 1.30	39.80 ± 2.15	0 ± 0.00	23.45 ± 0.20
BSK r	37.40 ± 0.45	37.40 ± 0.85	0.6 ± 1.00	24.60 ± 1.65
DN v	36.00 ± 5.95	36.55 ± 4.20	0.95 ± 1.10	26.50 ± 7.85
DN r	7.95 ± 3.25	8.10 ± 2.85	62.90 ± 2.50	21.05 ± 2.80
ZHD v	29.20 ± 1.82	30.55 ± 1.50	13.55 ± 4.15	26.70 ± 1.50
ZHD r	32.40 ± 1.05	32.25 ± 3.40	9.45 ± 0.40	25.90 ± 0.80
THR v	21.30 ± 2.55	22.90 ± 1.90	26.25 ± 1.80	29.55 ± 0.30
THR r	21.35 ± 3.70	22.40 ± 3.20	27.00 ± 0.95	29.25 ± 0.30

[a] BSK, Bou Skri; DN, Deglet Nour; MJH, Mejhool; THR, Thoory; ZHD, Zahidi, r, offshoots; v, vitroplants.

number of palms, measurement of palm fronde growth, morphological states, size of vitroplants, fruit size, weight and color of fruit and of seed, percentage of flesh, astringency, texture, and taste. Unlike vitroplants, the standard trees from offshoots were not biologically recorded since their plantation. The age of an offshoot being difficult to establish, only criteria of size and form are recorded for comparison with vitroplants. Works of date palm variety recognition were based on studies by Perreau-Leroy (1952), Nixon (1960), and Toutain (1966–1977). They have led to the establishment of detailed phenological data, providing different recognition criteria.

The data shown are the result of many years of experiment, carried out on all of our vitroplants, i.e., in 1989, 15 males and 134 females. For example, we studied the following cultivars: 9 trees of BSK, 10 of THR, 12 of MJH, and 36 of ZHD. Pollination was carried out mechanically and was controlled. The date palm studies and varietal identity works on vitroplants concerned 41 subjects having borne fruits, which corresponded with 228 inflorescences and 130 bunches of dates. Each bunch studied comprised an average of 32 spikes of 10 dates, i.e., a little more than 300 fruits per bunch. One hundred mature dates were harvested on different bunches of the same tree for each cultivar studied. Identical analyses were carried out in 1990. All of the results are reported in percent of dry matter.

Sugar Analysis. A 3-g sample was ground and homogenized. It was mixed in 100 mL of ethanol and distilled water (80:20) and heated to boiling point twice for an hour. The filtrate was collected, evaporated, adjusted to 50 mL with distilled water, and centrifuged for 5 min at 8000*g*. Before injection, this solution was filtered through 0.45-μm Sartorius filters. HPLC apparatus was used (Beckman M332). The separation was carried out with a Brownlee column (Amino-Spheri 5). The eluant phase was obtained by the use of acetonitrile–water (80:20) solution at a flow rate of 1.2 mL/min. The pressure was 1000 psi, and the column was controlled at 35 °C. Detection was realized by a differential refractometer (Jobin-Yvon, IOTA), and the peak area was obtained using a Hewlett-Packard 3390 A integrator, after comparison with external standards (fructose, glucose, and sucrose from Merck, mixed to obtain a synthetic solution of standards at 10 g/L; the standards analysis was carried out on one in every eight samples). The standard deviation obtained for the data was between 1.5 and 4%.

Amino Acid Analysis. A 35-g pulp sample was homogenized in a Sorvall high-speed homogenizer in 100 mL of distilled water. The homogenate was centrifuged for 15 min at 5000*g* and then a 2-mL aliquot for 20 min at 12500*g*. One milliliter of supernatant was diluted with sodium borate solution (pH 9.5, 0.4 M). Amino acid derivatives were made in an automatic injector (Sedere 100) using *o*-phthaldialdehyde (OPA). Analyses were performed using a liquid chromatograph (Beckman 420) equipped with a fluorescence detector (Shimadzu RS 530). The column was a 125 × 4.7 mm Shandon Hypersyl C_{18} with 3-μm particle size. A discontinuous elution gradient was obtained with sodium acetate (0.02 M), 1% tetrahydrofuran (THF), and methanol (HPLC grade; 12.5% at the beginning, going to 100% in 30 min). Standards from Pierce (ref 20089) were diluted 100 times to have 250 pmol of each amino acid. The standard analysis was established on one in every five analyses. The standard deviation obtained for the data was between 3 and 5%.

Statistical Studies. A principal component analysis (PCA) was carried out using the content of each amino acid to visualize the relationship between their molecules and to test their usefulness as biological markers for dates (Sanier et al., 1991).

We introduced in a PCA the amino acid values of each cultivar, related to the geographical origin and the mode of propagation.

RESULTS AND DISCUSSION

Morphological Observations. Because we have no access to the corresponding experimental plots, morphological observations of DN vitroplants were not possible.

On the basis of existing date palm data, we have established tables showing the varieties of plants studied, so that we could make comparisons of vitroplants and offshoots of identical cultivars and also between vitroplants. For the cultivars obtained by axillary budding (BSK, MJH, THR, ZHD) these observations allowed us to clearly establish a great homogeneity among vitroplants of the same cultivar and to insist on the fact that no anomalies were found in the vegetative comportment, the pollination, and the fructification aspects of vitroplants related to trees from offshoots (Toutain and Ferry, 1989; Ferry et al., 1988, 1990). This could be an indication that vitroplants conform to mother trees.

Morphological observations of these cultivars gave similar data for offshoots and vitroplants (Table I). The differences found in tree height and number of bunches produced are not significant. Indeed, tree height is not related to age. Thus, for example, if we compare two trees of different sizes, the smaller one could produce more dates than the bigger one because the smaller one is older. This seems to be the case of MJH and ZHD cultivars.

In the first case, MJH vitroplants produced 2 bunches and MJH offshoots 10. Even if the offshoot size is 2.30 m instead of 2.60 m for the vitroplant, the offshoot should be older and thus more productive. In the case of ZHD cultivar, vitroplants produced 11 bunches and ZHD offshoots only 7. Vitroplant size is greater than that of offshoots. Because the vitroplants' productivity was comparable to that of the offshoots, they seem to be older.

The differences found for the five cultivars studied in date gauge for DN, MJH, and ZHD cultivars (Table I), in date weight for the five cultivars studied, and in seed gauge (especially for BSK cultivar, Table I) are certainly the result of environmental factors.

The variations observed in the percentage of moisture, sugar, and nitrogen (Table I) in mature stage will be explained below.

Sugar Analysis. We studied the invert sugar [fructose (F) and glucose (G)] and sucrose (S) contents of mature dates. The results are figured from offshoots and vitroplants of different geographical origins.

BSK and MJH are characterized by the absence of sucrose at the mature stage (Figure 1). These observations are in accordance with those of Munier (1973) and Dowson and Aten (1973). No significant differences were found between fruits from offshoots or acclimatized vitroplants (Table II).

THR and ZHD have a higher concentration of sucrose than the previously discussed cultivars (Figures 2A and 3). Fruits from THR cultivar, characterized by a high percentage of sucrose (Figure 3), showed no significant differences related to the fruits' origins (Table II). On the contrary, small differences between vitroplant and offshoot sucrose content were found in ZHD cultivar (Table II). These could be explained by differences in maturity stages of the fruits. Indeed, we ascertain the storage of sucrose at green stages, which underwent an essentially complete hydrolysis to reducing sugars (fructose plus glucose) at the mature stage. Thus, dates from offshoots containing less sucrose for the same total sugar content (Table II) should be at a more mature stage than those from vitroplants.

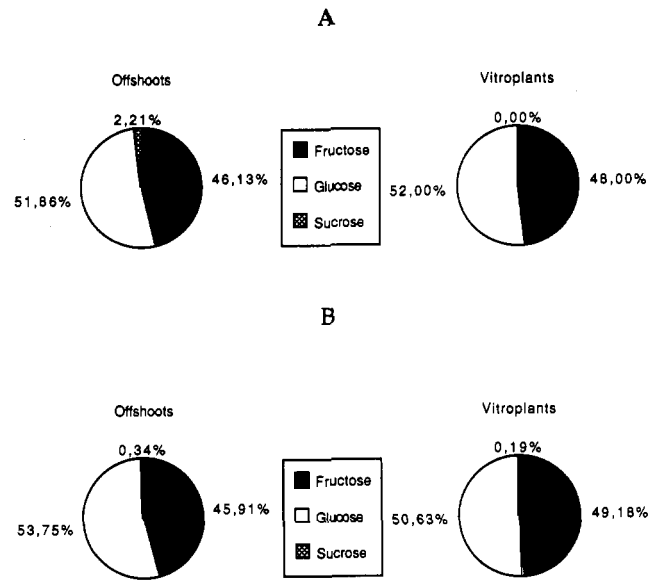

Figure 1. Sugar composition of two soft date cultivars, (A) Bou Skri and (B) Mejhool, from offshoots or vitroplants (produced by axillary budding).

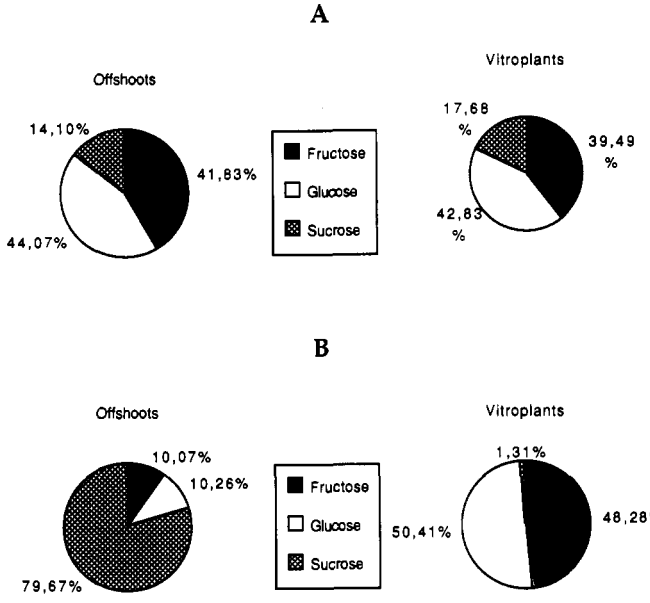

Figure 2. Sugar composition of two semisoft date cultivars, (A) Zahidi and (B) Deglet Nour, from offshoots or vitroplants (produced by axillary budding for Zahidi cultivar and by somatic embryogenesis for Deglet Nour cultivar).

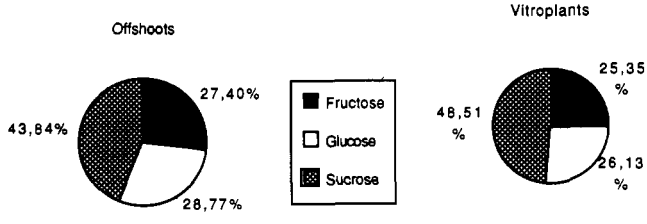

Figure 3. Sugar composition of a dry date cultivar, Thoory, from offshoots or vitroplants (produced by axillary budding).

For DN cultivars, unexpectedly, dates from vitroplants are unlike dates from offshoots, containing almost no sucrose (Table II; Figure 2B). These differences could not be related to the maturity stages of the fruits. We suspect that *in vitro* multiplication had introduced variations in sugar composition of DN dates. These variations

Table III. Free Amino Acid Composition of Mature Dates of Five Date Palm Cultivars from Offshoots or Vitroplants

	MJH v	MJH r	BSK v	BSK r	DN v	DN r	ZHD v	ZHD r	THR v	THR r
Asp	8.46 ± 4.73	9.31 ± 2.32	3.78 ± 0.32	3.58 ± 0.10	5.57 ± 0.75	4.60 ± 0.32	4.91 ± 0.61	5.03 ± 0.85	5.77 ± 0.85	5.13 ± 0.52
Glu	3.66 ± 1.61	3.06 ± 1.02	1.78 ± 0.08	1.82 ± 0.05	2.55 ± 0.47	0.40 ± 0.25	0.79 ± 0.19	0.60 ± 0.26	0.65 ± 0.09	0.80 ± 0.20
Asn	9.80 ± 2.85	8.49 ± 1.50	32.94 ± 0.07	35.01 ± 1.02	6.31 ± 2.02	8.65 ± 1.02	26.11 ± 4.79	26.44 ± 3.09	39.20 ± 3.41	41.58 ± 5.36
Ser	3.13 ± 0.02	3.35 ± 0.07	4.42 ± 0.50	4.85 ± 0.48	8.78 ± 1.65	8.42 ± 2.05	5.14 ± 0.03	6.55 ± 2.00	8.32 ± 1.35	8.76 ± 1.02
Gln	6.95 ± 1.59	5.75 ± 1.44	12.43 ± 1.41	12.61 ± 0.95	6.25 ± 0.47	23.46 ± 0.07	9.14 ± 0.18	8.93 ± 0.20	16.35 ± 0.43	14.01 ± 3.89
His	2.10 ± 0.61	1.89 ± 0.44	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Gly	5.79 ± 0.76	5.87 ± 0.35	4.46 ± 0.08	4.21 ± 0.12	6.81 ± 3.21	4.44 ± 1.58	5.70 ± 0.92	4.65 ± 0.54	2.62 ± 0.64	2.18 ± 0.85
Thr	0.85 ± 0.08	0.85 ± 0.15	1.65 ± 0.07	1.74 ± 0.02	0.89 ± 0.23	1.34 ± 0.14	1.15 ± 0.15	1.14 ± 0.10	0.56 ± 0.03	0.56 ± 0.04
Arg	2.36 ± 0.60	2.24 ± 0.82	3.20 ± 0.01	3.04 ± 0.13	2.97 ± 0.97	7.34 ± 0.05	2.90 ± 0.03	2.37 ± 0.04	0.88 ± 0.18	0.77 ± 0.07
Ala	19.68 ± 5.61	15.71 ± 0.19	8.00 ± 0.63	7.33 ± 0.31	32.08 ± 8.46	12.64 ± 1.54	25.40 ± 8.11	23.61 ± 6.05	14.39 ± 1.13	16.40 ± 0.96
Gab	32.70 ± 8.00	35.32 ± 13.33	23.38 ± 1.12	22.86 ± 0.46	22.12 ± 0.41	26.67 ± 1.02	23.68 ± 2.53	21.60 ± 1.21	12.01 ± 0.54	10.69 ± 0.03
Tyr	1.30 ± 0.66	1.40 ± 0.76	0.82 ± 0.02	0.84 ± 0.01	1.15 ± 0.05	1.14 ± 0.03	0.61 ± 0.11	0.53 ± 0.08	0.51 ± 0.11	0.49 ± 0.09
Met	0.46 ± 0.17	0.41 ± 0.14	0.36 ± 0.00	0.47 ± 0.08	0.25 ± 0.07	0.26 ± 0.05	0.31 ± 0.03	0.35 ± 0.06	0.00 ± 0.00	0.00 ± 0.00
Val	1.84 ± 0.69	1.10 ± 0.33	0.97 ± 0.00	0.97 ± 0.00	1.24 ± 0.12	1.22 ± 0.08	0.75 ± 0.11	0.84 ± 0.04	1.02 ± 0.16	1.48 ± 0.28
Phe	0.40 ± 0.00	0.52 ± 0.19	0.40 ± 0.01	0.35 ± 0.07	0.53 ± 0.23	0.37 ± 0.19	0.45 ± 0.05	0.41 ± 0.09	0.34 ± 0.01	0.27 ± 0.00
Ile	0.33 ± 0.02	0.44 ± 0.12	0.29 ± 0.03	0.29 ± 0.02	0.49 ± 0.22	0.30 ± 0.20	0.21 ± 0.02	0.25 ± 0.04	0.24 ± 0.06	0.18 ± 0.03
Leu	0.78 ± 0.10	0.70 ± 0.18	0.33 ± 0.02	0.35 ± 0.00	0.57 ± 0.36	0.22 ± 0.15	0.28 ± 0.08	0.26 ± 0.05	0.12 ± 0.04	0.12 ± 0.03
Lys	1.63 ± 0.31	1.30 ± 0.54	0.76 ± 0.02	0.65 ± 0.09	1.38 ± 0.13	1.53 ± 0.05	0.60 ± 0.02	0.60 ± 0.01	0.47 ± 0.07	0.44 ± 0.05

[a] BSK, Bou Skri; DN, Deglet Nour; MJH, Mejhool, THR, Thoory; ZHD, Zahidi; r, offshoots; v, vitroplants.

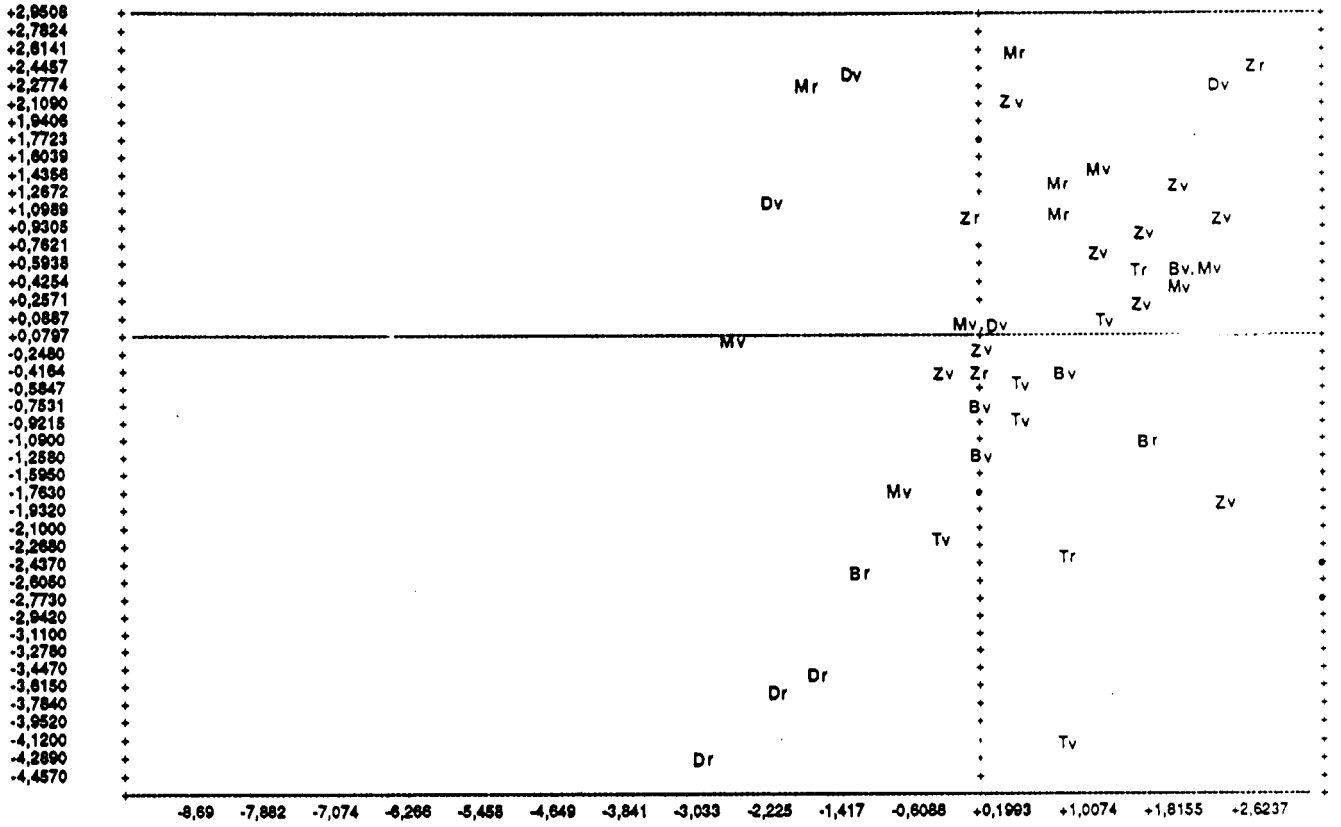

Figure 4. Principal component analysis: free amino acid composition of five cultivars of mature dates (B, Bou Skri; D, Deglet Nour; M, Mejhool; T, Thoory; Z, Zahidi) from offshoots (r) or acclimatized vitroplants (v). Horizontal axis (axis 1) is principally determined in the negative direction by isoleucine, leucine, lysine, phenylalanine, tyrosine, and valine. Vertical axis (axis 2) is principally determined in the negative direction by glutamine, arginine, and γ-aminobutyric acid.

could be explained by differential activities of the invertase, avoiding the accumulation of sucrose in vitroplants fruits during their maturation, and by hydrolysis of the sucrose synthetized. It should be noted that DN vitroplants have been produced by somatic embryogenesis.

Amino Acid Composition. Eighteen free amino acids were studied: alanine (Ala), asparagine (Asn), aspartic acid (Asp), arginine (Arg), glutamine (Gln), γ-aminobutyric acid (Gab), glutamic acid (Glu), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), serine (Ser), threonine (Thr), tyrosine (Tyr), and valine (Val). Only the 10 following were significant: Gab, Glu, Gly, Ser, Ile, Leu, Lys, Met, Phe, and Val.

Regarding the variables from PCA for which $\cos^2 > 0.5$, that is to say the representative point is less than 45° from the principal axis, the PCA showed that axis 1 is principally determined in the negative direction by the followihg amino acids: Ile, Leu, Lys, Phe, Tyr, and Val. Axis 2 is principally determined in the negative direction by Gln, Arg, and Gab. Regarding the additional variable analyzed, the content of total amino acids is well represented by axis 2. To conclude this PCA, and to simplify, we can say that axis 1 is characterized by the essential amino acid content and axis 2 is better correlated to the total amino acid content. Overall, the contribution to the total variability explained by the two first axes is 53%, with 35% for axis 1 and 18% for axis 2.

The mature dates of the five cultivars studied are poor in Ile, Leu, Lys, Phe, Met, Ser, Tyr, and Val (Table III; Figure 4). THR mature dates seem to have higher content of Gln and Asn and contain no Met. MJH dates contain more Asp, Gab, Glu, and Tyr and are the only ones to contain His. ZHD dates have higher content of Ala and BSK dates of Arg (Table III). Thus, free amino acid content could be useful to varietal identification of date palm cultivars.

No differences in amino acid content of dates from offshoots or from acclimatized vitroplants were found for BSK, MJH, THR, and ZHD cultivars (Table III; Figure 4). However, DN dates from offshoots have higher levels of Gln, Glu, Gab, and Arg and lower levels of Ala than those from acclimatized vitroplants (Table III). Again, we suspect that *in vitro* multiplication had introduced variations in free amino acid composition of DN dates.

The *in vitro* technique used for the multiplication of DN cultivars (somatic embryogenesis) has perhaps not allowed the maintenance of their physical and chemical properties, at least for the chemical composition of fruits (sugars, free amino acids). On the other hand, results from morphological and chemical analyses for BSK, MJH, THR, and ZHD cultivars should indicate that micropropagated date palms are the same as offshoots of identical cultivars. In this case, the *in vitro* multiplication method should have maintained the characteristics of the fruits. It should be a good indication that this method produces plants which conform to the mother tree.

These hypotheses need to be verified by new analyses, based on experimental protocol by which vitroplants and offshoots will be in sufficient number and planted in a random manner on the same fields. This is in preparation at the experimental station of Elche, Spain.

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