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RESEARCH ARTICLE

Functional quality traits of snake melon (*Cucumis melo* var. *flexuosus* L.) fruits as affected by genotypic differences

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ABSTRACT

This study reports the variability affecting the main antioxidant components and the antioxidant activities of three F1 hybrids (KP7, LP5 and XP12), six breeding lines (L1P3, L2P4, L4P18, L5P10, L6P4, L9P1) of snake melon (*Cucumis melo* var. *flexuosus* L.) and the widely grown and consumed cultivar Mornagui grown under greenhouse during the seasons 2014 and 2015. The functional quality varied significantly ($P < 0.05$) depending on the cultivar. The F1 hybrid LP5 was the richest cultivar in total phenols (47.6 mg GAE/kg fw). The breeding line L4P18 was characterized by a peak of total carotenoids (36.1 mg β-Ca. E/kg fw) and flavonoids (231.6 mg RE/kg fw), while L9P1 ranked first for ascorbic acid (AsA) (146.0 mg/kg fw), dehydroascorbic acid (DHA) (231.2 mg/kg fw) and total vitamin C (377.2 mg/kg fw). The antioxidant activity of the hydrophilic and lipophilic fractions were greater in XP12 and L2P4 (>215 μM Trolox/100 g fw) and L1P3 (458.2 μM Trolox/100 g fw) than all other genotypes. These findings are useful for 1) researchers targeting the development of snake melon cultivars with satisfying functional quality and 2) consumers searching for alternative sources of natural antioxidants.

Keywords: Fakous, functional quality, hydrophilic and lipophilic antioxidant activity, snake melon

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INTRODUCTION

Snake melons (*Cucumis melo* var. *flexuosus* L.), are heirloom plants belonging to the Cucurbitaceae family broadly distributed and consumed since the antiquity and across large geographical area (Pandey et al., 2010; Paris, 2012). The *flexuosus* group has a green rind covers a creamy white or pale green flesh (mesocarp and endocarp) that contains

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numerous whitish edible seeds, deliquescent at maturity. Because the stem is rather thin, the plant is usually supported on trellises, where the snake-like fruits grow on vines up to 120 cm lengths (Burger et al., 2010) (Fig. 1).

Figure 1: Snake melon vines of the cultivar Mornagui grown under greenhouse conditions during the production season 2014, in the experimental fields of the National Agricultural Research Institute of Tunisia (INRAT) at Teboulba in the coastal zone of Tunisia.

Snake melon fruits are known all around the world with different trivial names: alficoz in Spain, tortarello o cetrangolo in Italy, fakous or fegous in Arabic Maghreb countries, agoor in Soudan, acur, hıtta or hıtı in Turquie, kakri in India, uri in Japan and Armenian cucumber, yard-long melon, serpent-melon or Gutah elsewhere (Solmaz *et al*., 2016). They are generally consumed raw at the immature stage of ripening with a preference for straight long and thick green fruits in the Mediterranean region.

In the last decade, various authors reported the profiles of valuable bioactive compounds in other species of the Cucurbitaceae family including watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) (Tlili *et al*., 2011a, b, 2013), melon (*Cucumis melo* L.) (Henan *et al*., 2013), winter melon (*Benincasa hispida* (Thunb.) Cogn.) (Mandana *et al*., 2012), pumpkin (*Cucurbita moschata* Duchesne ex Poir.) (Durante et al., 2014) and largely in cucumber (*Cucumis sativus* L.), a crop often confused with snake melon (Ibrahim, 2017). Though, data regarding the functional quality of Fakous genotypes are scarce and incomplete.

Recently, nutritious, healthy and top-quality foods are becoming a priority for the consumer. Moreover, due the warning of World Health Organization on the 1.7 million deaths (2.8%) attributable worldwide to low fruit and vegetable consumption, governments are investing funds to help prevent cardiovascular diseases and cancer (http://www.who.int/dietphysicalactivity/fruit/en/) and promote the integration in the daily diet of some still underestimated sources of functional compounds (Durante *et al*., 2017; 2018).

Genotypic differences greatly influence quality traits in horticultural crops (Ilahy *et al*., 2018; Siddiqui *et al*., 2015a), as well as pre- and post-harvest treatments and manipulations (Siddiqui *et al*., 2015a, b). Thus, this study investigates the variability affecting the main antioxidant compounds and their resulting activity of different fakous genotypes (three F1 hybrids, six breeding lines and a traditional cv Mornagui) grown under greenhouse condition.

MATERIALS AND METHODS

Plant material

Ten powdery mildew resistant (PMR) snake melon genotypes were used in our study (Three hybrids; KP7, LP5 and XP12), six breeding lines; L1P3, L2P4, L4P18, L5P10, L6P4 and L9P1) and the largely grown and consumed snake melon cultivar Mornagui (Fig 2). The breeding lines were heirlooms landraces conserved for their higher productivity and powdery mildew resistance levels as well as desirable agricultural traits generally appealed by the consumer (length and skin colour).

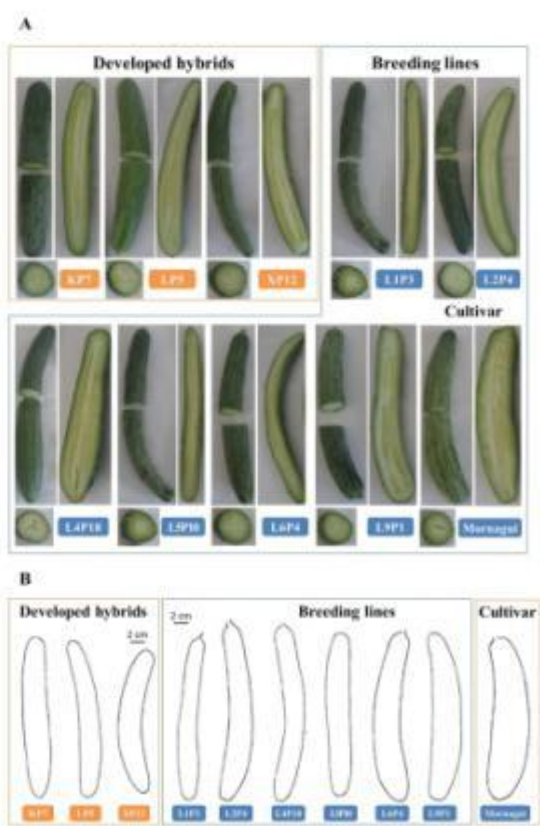

Figure 2: External appearance, longitudinal and cross sections (A), and schematic representation of the morphology (B) of the peponides of the different snake melon genotypes under analysis (hybrids, breeding lines and the traditional cultivar Mornagui).

The (F1) hybrids were developed in a line X line mating design, in which parents characterized by high levels of powdery mildew resistance (L2 and Mornagui), were crossed with lines (characterized by good powdery mildew resistance coupled with high productivity and desirable phenotypic traits (L1, L5 and L6). The breeding lines and the obtained lines were exchanged with different laboratory working on the same topics in Italy, Hungary and India.

Plug trays were used to grow Fakous seedlings at the beginning of December 2013 and 2014. Four weeks later seedlings were transferred into a sandy soil under unheated water-impermeable plastic screens greenhouse, recommended for growing vegetable crops particularly cucurbits during winter cycles, using 125 and 150 cm as in- and between-row separations respectively at the region of Teboulba (latitude 35.638057N, longitude10.955858E) in the coastal zone of Tunisia. Three blocks were used with 10 plants per genotype. In the experiments 10 kg N, 10 Kg P_2O_5, 12 kg (13/00/46 NPK) mixed soluble fertilizers and 1 l Actiphos Phosphoric acid were used.

Hand weeding control was applied and plant-pathogen control with chemical pesticides Sabero (Atlas Agricole, Tunis, Tunisia, 250 g/hl) was used to prevent mildew, Decis (El Moussem Agricole, Tunis, Tunisia, 57 cc/hl) was used to reduce aphids and whitefly and Agrocuivre (Agro-systemes, Tunis, Tunisia) was used to prevent bacterial infection.

Fruit sampling

Spaced harvests were applied to different snake melon genotypes from April 2014 and 2015 to June 2014 and 2015. Fruits were harvested from the selected plants at the immature ripening stage. For the biochemical characterization three independent samples of at least 6 healthy snake melon fruits per cultivar were arbitrarily picked during the first harvest. Snake melon fruits were carefully transported to the laboratory. Snake melon fruits were 5 min blended using a laboratory mixer (Waring Laboratory & Science, Torrington, CT, USA) and further homogenates were kept at -82 °C for further analysis.

Total carotenoid content determination

The method of carotenoid extraction was followed according to Lee (2001) and measured as detailed in Tlili et al (2011a, b) and the content was expressed as mg β-carotene equivalents/kg of fw (mg β-CaE/kg fw).

Total phenols determination

The extraction of total phenols was performed as outlined in Tlili et al (2011a, b) in triplicates (0.3 g) sample extracts. The Folin–Ciocalteu reagent was used for concentration determination and external calibration with gallic acid. The absorption was detremined using 750 nm as wavelength and a PG60 spectrophotometer (PG Instruments Limited, Woodway lane, Alma Park, Leicestershire, United Kingdom). Results were expressed in mg GAE/kg fw. Results were corrected for possible sugars interference with the total phenolic content as outlined in Asami *et al*. (2003).

Total flavonoid content determination

The total flavonoid content was determined using the $AlCl_3$ as outlined in Tlili et al (2011a, b). The wavelength 510 nm and a PG60 spectrophotometer were used for flavonoid detection. However quantification was performed using external calibration with rutin (mg RE/kg fw).

Ascorbic acid and dehydroascorbic acid determination

The extraction of Ascorbic acid (AsA) and dehydroascorbic acid (DHA) was accomplished by means of the metaphosphoric acid method and further detection at 525 nm in PG60 spectrophotometer (Kampfenkel *et al*. 1995). Results were expressed in mg/kg of fw (mg/kg fw).

Antioxidant activity determination

The ABTS method was used for the determination of the antioxidant activity followed by Trolox external calibration (Miller and Rice-Evans, 1997). The extraction was performed using 50% methanol or 50% acetone for hydrophilic and lipophilic extracts, respectively. The absorbance values were detected at 734 nm in a PG60 spectrophotometer and Trolox equivalents were used to express the obtained results (µM Trolox/100 g fw).

Experimental design and Statistical analysis

The effect of genotypic differences on the functional quality of snake melon was assessed by analysis of variance (ANOVA). When a significant difference was detected, means were compared using the least significant difference (LSD) test ($p < 0.05$). All statistical analysis was performed using SAS Version 6.1 software (SAS Institute, Cary, NC, USA).

RESULTS AND DISCUSSION

The amount of total carotenoids, total phenols, total flavonoids, ascorbic acid, dehydroascorbic acid and total vitamin C of the studied fakous genotypes are presented in Table 1. The results display significant differences ($P<0.05$) between the studied genotypes. L4P18 ranked first for total carotenoids (36.1 mg β-CaE/kg fw), followed by both L9P1 (35.8 mg β-CaE/kg fw) and XP12 (34.3 mg β-CaE/kg fw). L6P4 (31.4 mg β-CaE/kg fw) and KP7 (31.1 mg β-CaE/kg fw) genotypes exhibited middle levels, while the lowest value was recorded in L1P3 (20.9 mg β-Ca E/kg fw). The data regarding carotenoids are much higher than those of Henan et al. (2013) who reported a value attaining 4 mg/kg fw, higher than the values obtained by the two muskmelon (*C. melo*) cultivars Stambouli and Maazoul, widely grown and consumed in Tunisia. Nevertheless, the results are in line with those of Henan et al. (2017) for different local varieties of muskmelon grown in Tunisia ranging from 5.16 mg/kg fw in the green-fleshed cultivar Trabelsi to 54.25 mg/kg fw in the orange-fleshed cultivar Galaoui. The higher consumption of snake melon (fresh or processed) in the Mediterranean basin, suggests that the fruits can be considered a relevant source of carotenoids, similarly to other fruits and vegetables. Provesi et al. (2011) assessing the carotenoid content of fresh and stored pumpkins puree, reported that carotenoid content ranged from 27.62 µg/g in *Cucurbita maxima* cv. Exposição to 38.45µg/g in *Cucurbita moschata* cv. Menina Brasileira. The carotenoid profile were dominated by β-carotene and α-carotene in *C. moshata* cv and by β-carotene and lutein in *C. maxima*.

Table 1: Total carotenoids, total phenolics, flavonoids, ascorbic acid, dehydroascorbic acid and total vitamin C content of the studied snake melon hybrids, breeding lines and cultivar Mornagui grown under greenhouse during the seasons 2014 and 2015. Values represent the mean of six replicates ± standard deviation. Mean followed by the same letters do not differ significantly (LSD test, P < 0.05).

Genotypes	Total carotenoids (mg β-Ca E/kg fw)	Total phenolics (mg GAE/kg fw)	Total flavonoids (mg RE/kg fw)	AsA (mg/kg fw)	DHA (mg/kg fw)	Total vitamin C (mg/kg fw)
Developed hybrids (Line X Line)						
KP7 (Mornagui X L5P10)	31.0±1.4c	37.0±1.2b	182.6 ±6.0bc	86.3±5.5cd	69.4±7.5g	155.7±3.4e
LP5(L2P4 X L1P3)	25.9±0.8e	47.6±1.8a	136.6±11.0def	91.3±5.0c	58.3±5.7g	151.1±6.6e
XP12(Mornagui X L6P4)	34.3±0.8ab	25.8±1.2cd	89.5±5.2g	134.7±7.9ab	72.1±6.6g	206.7±14.4cd
Breeding lines						
L1P3	20.8±0.6f	29.6±1.3c	159.4±7.0cde	125.2±5.0b	95.7±11.4ef	221±8.8cd
L2P4	30.7±0.8c	21.5±0.7de	100.1±6.0fg	121.0±4.2b	105.6±2.6e	226.6±5.0c
L4P18	36.1±0.8a	39.6±2.8b	231.6±8.6a	127.4±6.5b	158.0±4.2c	285.5±8.42b
L5P10	29.1±1.2cd	39.8±2.4b	179.4±3.5bc	70.9±3.9cd	134.7±9.5d	201.1±11.1d
L6P4	31.4±0.8bc	39.0±1.1b	207.2±32.2ab	22.1±3.6f	74.6±3.4g	96.7±3.8f
L9P1	35.8±1.0a	15.7±0.9f	174.5±12.5bcd	146.0±7.8a	231.2±8.6a	377.2±9.7a
Cultivar						
Mornagui	26.5±2.4de	18.0±1.0ef	122.4±13.1efg	41.2±3.0c	181.2±11.1b	222.4±8.5cd

Regarding total phenolics, the hybrid LP5 showed the highest value (47.6 mg GAE/kg fw), followed by the breeding lines L5P10 (39.8 mg GAE/kg fw), L4P18 (39.6 mg GAE/kg fw) and L6P4 (39.0 mg GAE/kg fw) which did not differ statistically each other (Table 1). The higher concentration of total flavonoid was found in both L4P18 and L6P4 with 231.6 and 207.3 mg RE/kg fw, respectively. The lowest total phenolic content was recorded for the breeding line L9P1 (15.7 mg GAE/kg fw) showing, however, a substantial amount of total flavonoids (174.5 mg RE/kg fw). The lowest content of total flavonoids (89.5 mg RE/kg fw) was recorded in the hybrid XP12. As far as we know, this is the first data on total phenolics and total flavonoids in fakous genotypes, so that it provides evidence that these fruits constitute a significant source of these molecules similarly to other cucurbits. In fact, the amounts of total phenolics measured in snake melon hybrids and breeding lines under analysis are in the range of those reported for watermelon (92.3-147.3 mg GAE/kg fw) by Tlili *et al*. (2011b) and for four muskmelon cultivars, including a snake melon variety (338.1-477.1 mg GAE/kg fw) by Henan *et al*. (2013b).

Total vitamin C in the snake melon fruit oscillated between 96.7 mg/kg fw (L9P1) and 377.2 mg/kg fw (L6P4), indicative of the importance of genotypic pressure in significant in such quality attribute. The AsA and DHA input to total vitamin C content varied also depending on the considered genotype and were respectively 55-56% and 34-44% in hybrids, 22-56% and 43-77% in the breeding lines and 18 and 61% in the traditional cultivar Mornagui. To our knowledge, no literature data on the genotypic variation of AsA, DHA and total vitamin C in snake melons is currently available. Nevertheless, our values were in the range (7-353 mg/kg fw) reported by Burger et al. (2006) for AsA in a selection of representative accession of different *C. melo* groups, including the *flexuosus* line PI149149 in which AsA content was 28 mg/kg fw, close to the lowest value registered in this study in the breeding line L6P4 (21.1 mg/kg fw). Our total Vitamin C values were also in line with those reported in different red watermelon cultivars during ripening and at full maturity, ranging from 76.1 to 304.5 mg/kg and 105.3 to 239.8 mg/kg fw, respectively (Tlili et al., 2011a, b).

The radical scavenging activity, of the different snake melon genotypes are shown in Table 2. The data revealed significant variability ($P<0.01$) among genotypes. HAA was highest in L2P4 and the hybrid XP12 (215.8 and 215.6 µM Trolox/100 g fw respectively), however, it was lowest the line L9P1 (132.3 µM Trolox/100 g fw). Regardless genotypes, LAA was higher than HAA by 1-2.2-fold in hybrids, 0.7-2.1-fold in breeding lines and 1-fold in cultivar Mornagui, respectively and varied between 458.2 µM Trolox/100 g fw in L1P3 to 356.2 µM Trolox/100 g fw in Mornagui. As far as we know no literature data on HAA, LAA and TAA in snake melon genotypes was reported. Nevertheless, it can be highlighted that snake melon fruits have similar or higher antioxidant activity with respect to other Cucurbits, such as watermelons in which hydrophilic and lipophilic antioxidant activity ranged from 182.4-546.5 and 210.7-467.9 µM Trolox/100 g fw respectively (Tlili *et al*., 2011a, b). This data highlights the importance of the snake melon as a source of natural bio-actives endowed with high functional quality. Nawirska-Olszańska *et al*. (2013) have reported that hydrophilic and lipophilic antioxidant activities were from 0.44 to 1.22 and from 0.14 to 0.62 µM Trolox/g fw in the seeds of pumpkin cvs Danka and Karowita respectively. The author revealed also that the varieties of the *Cucurbita maxima* species had higher hydrophilic antioxidant levels whereas cultivars of the species *Cucurbita pepo* were more rich in lipophilic antioxidants.

Table 2: Antioxidant activity of the studied snake melon hybrids, breeding lines and cultivar Mornagui grown under greenhouse during the seasons 2014 and 2015. Values represent the mean of six replicates ± standard deviation. Values within column and ripening stage followed by the same letters do not differ significantly (LSD test, P < 0.01).

	HAA	**LAA**
Genotypes	**(µM Trolox/100 g fw)**	
Developed hybrids (Line X Line)		
KP7 (Mornagui X L5P10)	143.9±8.4cd	432.2±10.5b
LP5 (L2P4 X L1P3)	191.6±6.2ab	388.3±5.0c
XP12 (Mornagui X L6P4)	215.6±2.5a	446.3±19.3ab
Breeding lines		
L1P3	170.5±6.3bc	458.2±9.4a
L2P4	215.8±22.4a	375.4±5.0cd
L4P18	135.7±1.9d	425.1±9.5b
L5P10	182.8±8.7b	363.2±14.5cd
L6P4	161.7±9.0bcd	373.2±8.7cd
L9P1	132.3±10.0d	377.9±5.7cd
Cultivar		
Mornagui	174.0±16.9bc	356.2±8.5d

CONCLUSION

This study highlights the genotypic differences on the functional quality and as *in vitro* antioxidant activity of snake melons. Snake melon could contribute substantially to the hydrophilic and lipophilic antioxidant levels of human diets and is far from being considered as an underutilized fruit. However, the *in vivo* health benefit of such molecules requires further focus. The variability detected in the different snake melon genotypes (F1 hybrids and breeding lines) can be useful for conventional breeding programs aiming to improve their nutritional value.

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