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PHYTOCHEMICAL CONTENTS AND ANTIOXIDANT ACTIVITIES OF SOME TUNISIAN FABA BEAN POPULATIONS

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ABSTRACT

Faba bean (*Vicia faba* L.) as the most cultivated leguminous species in the world, is an excellent source of protein, dietary fibre, micronutrients and phytochemicals. Seeds of eight Tunisian and two commercial varieties "Aguadulce" and "Seville" were analysed. Protein content (determined by Kjeldahl assay), potassium, sodium, phosphorus and calcium /magnesium were analyzed. Total phenolics ranged from 19.63 to 28.20 mg gallic acid equivalent 100 g^{-1} of dry weight (DW). Total flavonoids ranged from 3.40 to 7.27 mg rutin equivalents 100 g^{-1} DW. The free radical scavenging activity, determined by DPPH, varied from 0.374 to 0.578 mM TEAC, and the values determined by ABTS ranged from 0.095 to 0.271 mM TEAC. Furthermore, all genotypes exhibited a ferric-reducing power. Most Tunisian genotypes had a comparable micronutrients and phytochemicals composition to the commercial varieties. Particularly the two genotypes coded P12 and P37, with morphological and chemical characteristic comparable to commercial «Aguadulce» and «Seville» should be subjected to a selection program. These findings imply that the improvement of Tunisian Faba bean genotypes tolerant to arid condition must be taken into consideration, not only based on their nutritional and morphologic traits, but also based on their mainly natural antioxidants, as functional foods.

Key words: *Vicia faba L.,* faba bean components, polyphenols, flavonoids, DPPH, ABTS, reducing power.

INTRODUCTION

Due to the high protein contents of their seeds or leaves (Iantcheva et al., 2009), legumes play an important role in the traditional diets of many regions throughout the world. They are excellent sources of protein, dietary fibre, micronutrients and phytochemicals (Anderson et al., 1999; Messina, 1999). Faba bean (*Vicia faba* L.) is the most cultivated leguminous species in the world. It is considered one of the oldest legumes grown by man, providing high-protein seeds for human and animal nutrition (Gutierrez et al., 2006).

The world production of faba beans is 4 Mt from 2.5 Mha (FAOSTAT, 2009). In the Mediterranean basin, like in Europe, the faba beans is a traditional crop used as Weld beans (*minor* and *equina* types) for animal feeding and human food and as broad beans (*major* type) for direct human consumption. In Tunisia, this plant is still in a fairly traditional culture especially in southern regions and is characterized by its instability (Khaldi and Zekri, 2002). The national production quantity is 60,000 t grown on a surface of 58,000 ha (FAOSTAT, 2009). Faba beans are consumed dry, fresh, frozen or canned.

Faba beans contain a large amount of proteins, carbohydrates, B-group vitamins and minerals. Their protein content ranged from 19 to 40% (Duc et al., 2008). The content of carbohydrate ranged from 51 to 68%, the starch ranged from 41 to 53%. The main soluble sugars in faba beans are the antinutritional factors α-galactosides, including raffinose, stachyose and verbascose. Faba bean seeds are particularly rich in verbascose and stachyose (Sosulski and Cadden, 1982). Faba beans are a good source of dietary minerals, such as phosphorus, potassium, calcium, sulphur and iron. Calcium content ranged from 120 to 260 mg 100 g^{-1}

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dry weight (Vidal-Valverde et al., 1998). More than 40-60% of the phosphorus present is unavailable, being in the form of phytates. Moreover, phytic acid in legumes has been reported as reducing the bioavailability of minerals (Deshpande and Cheryan, 1984) and inhibiting the activity of several enzymes (Knuckles at al., 1989). The ability of phenolic substances, in legumes, including flavonoids and phenolic acids as antioxidants, has been extensively investigated (Rice-Evans et al., 1996). The heterogeneity in the varieties of legumes and their complexity in the different phytochemical compositions lead to further research on their bioactive compounds and biological properties, because the mechanisms by which polyphenolic compounds present in legumes exert their biological properties have just started to be evaluated.

The main objective of this research was to quantify total phenols and flavonoid contents from ten faba bean genotypes collected from five Oases (South of Tunisia) and two commercial varieties "Aguadulce" and "Seville" as a reference. The chemical diversity was investigated and compared between faba bean genotypes. Ferric reducing power, and radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) were also compared between cultivars.

This information related to the genotypes characteristics, may be of the both agronomic and nutritional interest.

MATERIAL AND METHODS

Chemicals and reagents

All solvents were of reagent grade without any further purification. Gallic acid, rutin and Folin-Ciocalteu's phenol reagent were purchased from Sigma Chemical Co. (USA). The analytical reagent grade methanol was obtained from Lab-Scan (Labscan Ltd, Dublin, Ireland). The water used in sampling was prepared with a Millipore Simplicity (Millipore S.A.S., Molsheim, France). 2,2-diphenyl-1-picrylhydrazyl radical was purchased from Sigma Chemical Co. (Poole, Dorset). Spectrophotometric measurements were performed with Shimadzu UV-1600 spectrometer (Shimadzu, Kyoto, Japan).

Plant materials

From a collection of 42 populations of broad bean grown in south of Tunisia, eight populations were selected, based on morphological characterization (Table 1). Both commercial varieties "Aguadulce" and "Seville" were investigated as reference. Germplasm collection missions to all oases of southern Tunisia were undertaken in 2006 from five provinces: Gabes, Gafsa, Medenine, Tataouine and Tozeur. All samples were obtained from Randomised Complete Block experiments established under homogenous conditions, without any special management, at Arid Land Institute in Medenine, Tunisia (33°30′ N, 10°38′ E). Seeds were stored at room temperature (+18°C) for few days until used for analyses.

Table 1. Locations and characteristics of seeds of *Vicia faba L.* populations collected from the oases of southern Tunisia

Population code	Oasis	Province	Latitude (N)	Longitude (E)	Altitude (m)
P01	Mareth	Gabes	33°37'	10°16'	48
P12	Medenine	Medenine	33°20'	10°29'	48
P30	Tafartassa	Gafsa	34°24'	8°46'	294
P31	Tafartassa	Gafsa	34°24'	8°46'	294
P32	Ferch	Tataouine	32°55'	10°27'	235
P33	Beni Khedache	Medenine	33°15'	10°11'	506
P37	El Hamma	Gabes	33°52'	9°47'	64
P41	Tozeur	Tozeur	33°55'	8°08'	43

Morphological characteristics

Morphological characterization was taken using the faba bean Descriptors «Bioversity international» and UPOV (2003). Three qualitative morphological traits were examined in the present study:

i) Shape of median longitudinal section of dry seed,
ii) green testa colour of dry seed (immediately after harvest), and
iii) black pigmentation of hilum of dry seed. One quantitative trait was determined (weight of dry seed). The 1000 seed weight has been traditionally used to classify faba bean populations into *Vicia faba minor*, *Vicia faba equina* and *Vicia faba major*.

Kjeldahl assays

The Kjeldahl technique is the method commonly used for protein analysis in food products (AOAC, 1995). The amount of protein present in Broad Bean Seeds was determined by analysis of total nitrogen (TN) by the Kjeldahl method (Kjeldahl, 1883), reported previously by Elfalleh et al. (2009).

Minerals contents

Minerals in Broad Bean Seeds were determined using the method reported by Elfalleh et al. (2009). Briefly, plant material was dried at 70°C, until maximal dehydratation. One gram of sample powdered dry seed, placed in a porcelain capsule and was calcinated by the muffle furnace at 550°C.

After cooling, ashes were attacked by 5 ml of deionised water and 1 ml of hydrochloric acid and were boiled. The capsule content was filtered. The filtrate was adjusted by deionised water at 100 ml volume. This solution was used for mineral analysis. The combined concentration of calcium and magnesium ions was determined by complexometric titration.

The calcium + magnesium, sodium and potassium were determined by flame photometer (Sherwood 410, Sherwood Scientific Ltd, Cambridge, UK). Total phosphorus was determined by a Spectrophotometer (Secomam 1000, French).

Determination of total polyphenol content (TPP)

Total phenols were estimated by the Folin-Ciocalteu method reported in Elfalleh et al. (2009). From each sample, 0.5 ml of methanolic solution and 0.5 ml of Folin-Ciocalteu (Prolabo) reagent were added. Then, 4 ml of sodium carbonate solution 1M was added. The tubes were laid for 5 min in a water bath at 45°C and then put in a cold water bath. The reading of the absorbance was made at 765 nm using a Shimadzu 1600-UV spectrophotometer. Total phenolic contents of each fraction were converted into mg gallic acid equivalents per g dry weight (mg GAE g^{-1} DW).

Determination of total flavonoids content (TF)

The amount of total flavonoids in the extracts was measured spectrophotometrically following the method reported by Nasri et al. (2011). This method is based on the formation of a complex flavonoid-aluminium, having the maximum absorbance at 430 nm. Rutin was used to make a calibration curve. 1 ml of methanolic extract was mixed with 1 ml of 2% $AlCl_3$ methanolic solution. After incubation at room temperature for 15 min, the absorbance of the reaction mixture was measured at 430 nm using a Shimadzu 1600-UV spectrophotometer. The flavonoids content was expressed as rutin equivalents in mg per g dry weight (mg RE g^{-1} DW).

DPPH radical scavenging activity

The DPPH radical scavenging activity of methanolic extracts was determined following the method reported by Okonogi et al. (2007). A methanolic test solution of different concentrations was prepared from a stock solution of faba bean seed extracts (2.5 g of dry powder per 25 ml). DPPH (100 µM) was dissolved in methanol and mixed with an aliquot of 100 µl of each dilution.

The mixture was shaken vigorously and left to stand for 30 min in the dark at room

temperature. After the reaction was allowed, the absorbance at 517 nm was recorded to determine the concentration of remaining DPPH. Results were expressed as Trolox equivalent antioxidant capacity (TEAC).

ABTS radical scavenging activity

The ABTS radical-scavenging activity values were estimated by the Trolox equivalent antioxidant capacity (TEAC) test as reported by Elfalleh et al. (2009). In this test, we measured the relative capacity of antioxidants to scavenge the $ABTS^+$ radical, compared to the antioxidant potency of Trolox, which is used as a standard. $ABTS^+$ was generated by oxidation of ABTS with potassium persulfate. The $ABTS^+$ radical was generated by mixing 7 mM ABTS solution with 2.45 mM K_2S_2O8 in the dark for 24 h, at room temperature. Before usage, the $ABTS^+$ solution was diluted with methanol to get an absorbance of 0.700±0.020 at 734 nm. 25 µl of antioxidant sample was added to 1 ml of the diluted $ABTS^+$ solution. The reaction mixture was vortexed for 20 s and then the absorbance was recorded at 734 nm for 5 min. The final TEAC value of the antioxidant compound was calculated by comparing $ABTS^+$ decolourisation with Trolox, which gives a useful indication of the antioxidant potential of the plant extracts. Measurement was performed in triplicate.

Reducing power assay

The reducing power of methanolic extracts was quantified according to the method cited by Ferreira et al. (2007) and previously by Singh and Rajinia (2004). 1 ml of reaction mixture, containing various concentration of samples (5, 10, 20, 50 and 100 mg ml^{-1}) in phosphate buffer (0.2 M, pH 6.6), was incubated with potassium ferricyanide (1% w/v) at 50°C for 20 min. The reaction was terminated by adding TCA solution (10% w/v) and the mixture was centrifuged at 3000 rpm for 10 min. The supernatant was mixed with distilled water and ferric chloride (0.1% w/v) solution and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. The reducing power was calculated and EC_{50} values denote the effective concentration at which the absorbance is 0.5.

Statistical analysis

All samples were measured in triplicate. Statistical analyses were performed using XLSTAT 2009. Data were expressed as mean ± SD (Standard deviation) using ANOVA. Differences at $p<0.05$ were considered statistically significant by Duncan's new multiple range test.

In addition, to evaluate the information contained in experimental data, principal component analysis (PCA) was applied (Miloun et al., 1992). PCA generates a set of new orthogonal variables (axes), linear combinations of the original ones, so that the maximal amount of varian ce contained in the dataset (information) is concentrated in the first principal components (Armanino et al., 2002).

RESULTS AND DISCUSSION

Morphological proprieties and protein contents of faba bean seeds

The results of some morphological seeds characteristics and protein content of faba bean populations are shown in Table 2. Based on 1000 seed weight, faba bean populations were divided into *Vicia faba minor*, *Vicia faba equina* and *Vicia faba major*.

This characterization and the limits proposed by Henelt (cited by Lawes et al., 1983), grouped five populations (P31, P33, P37, "Aguadulce" and "Seville") into the *Vicia faba equina* category. The rest of populations (P01, P12, P30, P32 and P41) were included in *Vicia faba major* category.

Two populations coded P31, P37 and "Seville" variety had elliptic shape of median longitudinal section seeds. The rest of populations, and the "Aguadulce" variety had broad elliptic seeds. Most of populations (P01, P12, P30, P33, P41, and "Aguadulce") had green testa colour. The testa of P31 and P32, and "Seville" and P37 were respectively beige and violet.

Protein content of studied populations and of both commercials varieties was in

agreement with content reported by other authors. Protein content of *Vicia faba* L. seeds ranged from 20.35±1.97 (P41) to 23.02±3.61 % (P32) on a dry weight basis, with an average of 21.73±2.70 %. In general the protein content of faba beans varies from 19 to 40 % (Duc et al., 2008), with percentage depending on the variety (Vidal-Valverde et al., 1998).

Table 2. Some morphological and chemical characteristics of 10 faba bean samples used in the current study

Code	Shape of median longitudinal section	Colour of testa	Black pigmentation of hilum	1000 seeds weight (g)	Protein (%)
P01	Broad elliptic	Green	Present	1888.50 ± 747.62 ^{E}	22.59 ± 3.35 ^{A}
P12	Broad elliptic	Green	Present	2140.73 ± 1294.64 ^{F}	22.59 ± 3.53 ^{A}
P30	Broad elliptic	Green	Present	1678.68 ± 534.77 ^{CD}	22.02 ± 3.81 ^{A}
P31	Elliptic	Beige	Present	1175.14 ± 452.52 ^{B}	22.08 ± 2.26 ^{A}
P32	Broad elliptic	Beige	Present	1827.02 ± 370.78 ^{E}	23.02 ± 3.61 ^{A}
P33	Broad elliptic	Green	Present	1425.28 ± 348.84 ^{C}	20.62 ± 2.13 ^{A}
P37	Elliptic	Violet	Present	1263.86 ± 329.68 ^{BC}	22.71 ± 2.33 ^{A}
P41	Broad elliptic	Green	Present	1641.14 ± 502.14 ^{CD}	20.35 ± 1.97 ^{A}
AG	Broad elliptic	Green	Absent	1438.20 ± 354.37 ^{C}	20.50 ± 1.84 ^{A}
SE	Elliptic	Violet	Present	1004.90 ± 287.94 ^{A}	20.88 ± 2.19 ^{A}
Mean				1548.345 ± 522.33	21.73± 2.70

Protein was quantified using Kjeldahl, Each value in the table is represented as mean ± SE (n=3).
Different superscript letters in the same column indicate significant difference (P<0.05) by Duncan's multiple range test.

Minerals contents

Quantified minerals in faba bean seeds are shown in Table 3. In this investigation of broad bean populations, the average of ash content was 5.04±0.14 %. Tunisian broad bean populations had higher ash, potassium, sodium and phosphorus content compared to both commercials "Aguadulce" and "Seville" varieties.

Table 3. Some minerals (mg 100 g^{-1}) quantified in faba bean seed

Code	Ash (%)	Potassium	Sodium	Phosphorus	(Calcium + Magnesium)
P01	5.16 ± 0.34^{A}	367.50 ± 74.25^{B,C}	31.45 ± 2.62^{C}	127.72 ± 0.09^{G}	62.75 ± 3.18^{A,B}
P12	5.05 ± 0.34^{A}	430.50 ± 14.85^{A}	37.00 ± 0.00^{B}	**200.28 ± 0.09^{A}**	56.48 ± 2.86^{B}
P30	4.85 ± 0.32^{A}	367.50 ± 14.85^{B,C}	**48.10 ± 0.00^{A}**	136.76 ± 0.07^{E}	50.20 ± 2.55^{C}
P31	5.03 ± 0.33^{A}	**451.50 ± 14.85^{A}**	35.15 ± 2.62^{B}	133.16 ± 0.15^{F}	50.20 ± 2.55^{C}
P32	5.22 ± 0.35^{A}	357.00 ± 0.00^{B,C,D}	38.85 ± 2.62^{B}	119.19 ± 0.16^{H}	56.48 ± 2.86^{B}
P33	**5.30 ± 0.35^{A}**	430.50 ± 14.85^{A}	31.45 ± 2.62^{C}	112.57 ± 4.86^{I}	62.75 ± 3.18^{A,B}
P37	4.94 ± 0.33^{A}	388.50 ± 14.85^{B}	35.15 ± 2.62^{B}	151.13 ± 0.16^{C}	50.20 ± 2.55^{B,C}
P41	4.91 ± 0.33^{A}	325.50 ± 14.85^{D,E}	37.00 ± 0.00^{B}	101.86 ± 0.07^{J}	**69.03 ± 3.50^{A}**
AG	4.98 ± 0.33^{A}	336.00 ± 0.00^{C,D,E}	33.30 ± 0.00^{B,C}	142.15 ± 0.09^{D}	**69.03 ± 3.50^{A}**
SE	4.99 ± 0.33^{A}	304.50 ± 14.85^{E}	25.90 ± 0.00^{D}	164.86 ± 0.16^{B}	62.75 ± 3.18^{A,B}
Mean	5.04 ± 0.14	375.90 ± 48.95	35.34 ± 5.81	138.97 ± 28.32	58.99 ± 7.37

Each value in the table is represented as mean ± SE (n=3).
Different superscript letters in the same column indicate significant difference (P<0.05) by Duncan's multiple range test.

Potassium was the most abundant mineral in dry Faba bean seed, with an average of 375.90±48.95 mg 100 g^{-1}. Population P31 contained the highest Potassium content (451.50±14.85 mg 100 g^{-1}). The average content of sodium was 35.34 mg 100 g^{-1} of dry seed; P30 had the highest content (48.10 mg 100 g^{-1}). The average

content of Phosphorus was 138.97±28.32 mg 100 g^{-1}, and the values ranged from 101.86±0.07 mg 100 g^{-1} (P41) to 200.28±0.09 mg 100g^{-1} (P12). The phosphorus in many plant foods, such as beans, whole-grain cereals, and nuts, is found in the form of phytic acid. Phosphorus plays a critical role in bone formation, as it is a part of the mineral complex of bones. About 85% of the body's phosphorus is stored in bones, with the rest stored in the soft tissues such as muscles and organs (Thompson et al., 2011).

The calcium and magnesium level (Ca^{2+}/Mg^{2+}) seemed to be similar in all populations. The average was 58.99±7.27 mg 100 g^{-1} and the highest value was detected in P41 and "Aguadulce" variety (69.03±3.5 mg 100 g^{-1}).

Compared to the results reported previously by Apata and Ologhobo (1994) and Waldemar et al. (2000), the studied plants contained higher ash, potassium, phosphorus content and similar calcium and magnesium content. Nevertheless, in our study we obtained lower minerals values than those reported by Hacıseferogulları et al. (2003) and Jensen et al. (2010). The study of nutrients and minerals in rice and legumes conducted by Montira et al. (2010) showed that the differences in mineral contents can be due to many factors such as soil, fertilizer and agricultural chemicals (herbicides, fungicides, wood preservatives, insecticides, rodenticides and sheep-dips). Apata and Ologhobo (1994) suggested that such variation in the content of minerals for the legume species is related to genetic origin, geographical source and the level of soil fertility.

Total phenol and flavonoid contents of broad bean seeds

Polyphenol and flavonoid contents of eight broad beans populations and the two commercial varieties are presented in Table 4. Total seed polyphenols ranged from 19.63±1.53 (P31) to 28.20±2.56 mg Gallic Acid Equivalent g^{-1} DW (P37); the average was 23.61±2.47 mg g^{-1} GAE. The total flavonoids in seeds varied from 3.40±0.23 (P31) to 7.27±27 mg rutin g^{-1} DW (Seville) and the average was 4.81±1.19 mg rutin g^{-1} DW. Lower total polyphenol contents, ranging from 4.4 to 7.4 g GAE 100 g^{-1} DW were reported in Poland broad bean seeds (Wolosiak et al., 2010).

Table 4. Mean values of total polyphenols, flavonoids, DPPH/ $ABTS^{+}$ radical scavenging activities and reducing power of faba bean seed

Code	Polyphenols mg g^{-1} GAE	Flavonoids mg g^{-1} RE	DPPH TEAC mM	ABTS TEAC mM	Reducing power[a] EC 50%
P01	22.59±0.62^{A}	6.22±0.67^{A,B}	0.388±0.062^{C,D}	0.227±0.021^{B}	26.67±1.53^{H}
P12	23.24±0.82^{A}	3.86±0.63^{D,E}	0.413±0.083^{C,D}	0.095±0.005^{G}	**67.00±1.00^{A}**
P30	22.42±0.31^{A}	4.08±0.95^{C,D,E}	0.394±0.025^{C,D}	0.176±0.003^{C}	61.33±2.08^{B}
P31	19.63±1.53^{A}	3.40±0.23^{E}	0.433±0.012^{B,C,D}	0.106±0.017^{F,G}	46.67±0.58^{C,D}
P32	22.87±1.15^{A}	3.83±0.88^{D,E}	0.438±0.008^{B,C,D}	0.161±0.010^{C,D}	42.00±2.00^{E}
P33	25.66±0.43^{A}	4.70±0.97^{C,D,E}	0.533±0.085^{A,B}	0.142±0.011^{D,E}	41.00±1.00^{E}
P37	**28.20±2.56^{A}**	5.04±0.51^{C,D}	0.374±0.126^{D}	0.128±0.001^{E,F}	45.33±0.58^{D}
P41	25.91±4.37^{A}	5.32±0.27^{B,C}	0.503±0.002^{A,B,C}	0.135±0.019^{D,E}	31.00±1.00^{G}
AG	24.23±0.96^{A}	4.42±0.84^{C,D,E}	0.422±0.056^{B,C,D}	0.148±0.009^{D,E}	48.00±1.00^{C}
SE	21.38±5.37^{A}	**7.27±0.49^{A}**	**0.578±0.019^{A}**	**0.271±0.025^{A}**	37.67±1.53^{F}
Mean	23.61±2.47	4,81±1,19	0.450± 0.070	0.160± 0.050	44.67±12.35

[a]EC_{50} (µg ml^{-1}): effective concentration at which the absorbance is 0.5.
Each value in the table is represented as mean ± SE (n=3).
Different superscript letters in the same column indicate significant difference (P<0.05) by Duncan's multiple range test.

For the *Vicia faba* species, Jansen et al. (2001) and Chaieb et al. (2011) reported that the vegetative stage showed the highest TPC and TFC due to the need of a protective

physicochemical barrier against the ultra violet radiation. For the productive stage, Chaieb et al. (2011) found that pod had higher TPC and TFC than seeds coat, whole seeds and cotyledons. Similarly, Marinova et al. (2005), studying the total phenolics and total flavonoids of some fruits and vegetables, concluded that their concentrations depend on the stage and the parts of the plant.

Antioxidant capacity of broad bean seeds

The antioxidant capacity of broad bean seeds extracts was evaluated with the DPPH and ABTS tests. Results are reported in Table 4. The free radical scavenging activity determined by DPPH varied from 0.578 (Seville) to 0.374 mM TEAC (P37), with an average of 0.450 mM TEAC and the values determined by ABTS ranged from 0.095 (P12) to 0.271 (Seville) mM TEAC, with an average of 0.160 mM TEAC. Furthermore, the ferric reducing power was determined and expressed as the EC_{50} value (Effective concentration at which the absorbance is 0.5). A lower value of EC_{50} indicates a higher antioxidant activity. For broad bean seeds the EC_{50} values ranged from 26.67±1.53 (P01) to 67.00±1.00 (P12) mg ml^{-1}, and the average value was 44.67±12.35 (mg ml^{-1}) (Figure 1).

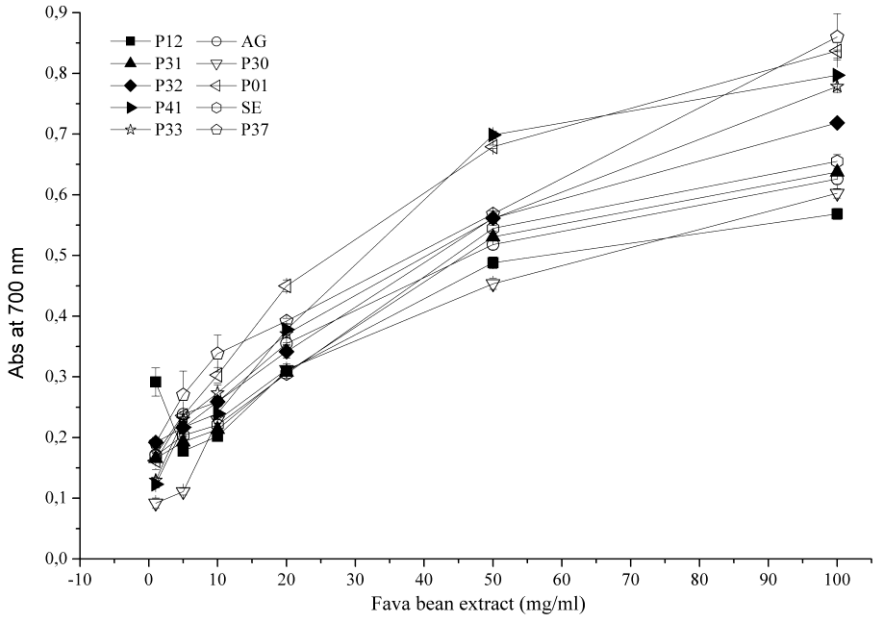

Figure 1 Reducing power of methanolic extracts (mg/ml) of faba bean seed from 10 populations. Each value is expressed as mean ± standard deviation (n = 3).

The antioxidant activity of faba bean seeds was possibly due to the redox properties of phenolic compounds, which make them act as reducing agents, hydrogen donors, and single oxygen quenchers and also may have a metallic chelating potential. Moreover, synergism between the antioxidants in the mixture makes the antioxidant activity not only dependant on the concentration, but also on the structure and the interaction between the antioxidants (Elfalleh et al., 2011).

Broad beans exhibited very good antioxidant properties, in comparison to related vegetables. In fact, Dastmalchi et al. (2008) suggested that this scavenging ability can be beneficial in the preservation of foodstuffs, drug products and cosmetics, where free-radical-mediated chain reactions result in lipid oxidation and subsequent deterioration of the products.

Principal component analysis

The plot of PCA identified two principal components (PC) that explained 82.37% of the total variance (Figure 2).

The first axis can be interpreted as an expression of chemical characters, which were well correlated with the morphological characters “colour of testa of dry seeds”; it accounted for 66.55% of total variation.

The highest loadings were CTF, CTP, DPPH, phosphorus and Potassium content with positive sign and correlated negatively to seed weight.

The second axis explained 15.82% of total variance and was related to morphological traits, which were well correlated positively with ABTS radical scavenging activity, expressed in terms of colour of testa of dry seeds, and correlated negatively with black pigmentation of hilum of dry seed and shape of median longitudinal section of dry seed (BHDS). The plot of principal components (Figure 2) showed a high dispersion of populations without correlation with their geographical origin. Four population groups were differentiated at a dissimilarity level of 0.2. These groupings can also be identified on the left, central and right part of the graph, respectively, with positive and negative values of the first component.

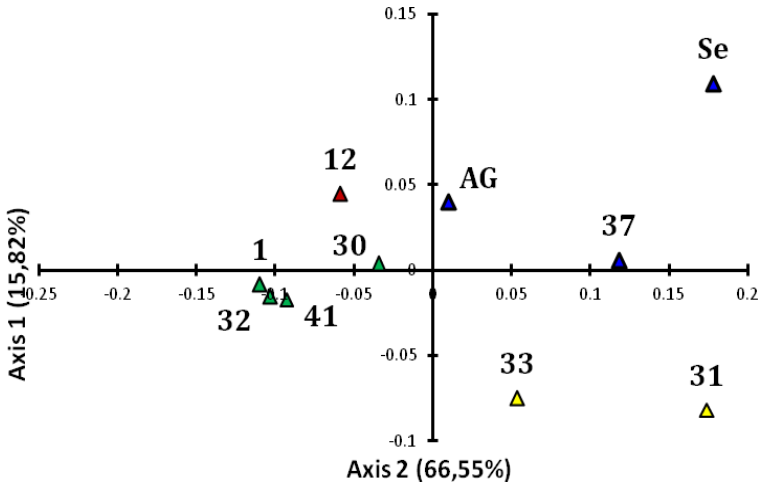

Figure 2: The Principal Components Analysis based on morphological and chemical characteristics in eight populations of broad bean from south of Tunisia and two commercial varieties "Aguadulce" and "Seville"

The first group included both varieties and population P37. They were positively correlated to PC1 and PC2, and characterized by a high content total polyphenol and flavonoid, high antioxidant activity (DPPH, ABTS and RP) and the violet colour of testa of dry seeds, especially for "Seville" variety and P37.

The second one grouped two populations (P31 and P33), located in the right part of the graph. They were positively correlated to PC1, characterized by high potassium and ash content and lower protein than group 1.

The third group contained four populations P01, P30, P32 and P41. They were negatively correlated to PC1, characterized more by morphological than chemical identity. Finally, population P12 represented alone the last group; it differed from the first group by an important ABTS radical scavenging activity and by seed weight.

CONCLUSIONS

The current studies confirm the high contents of proteins and minerals in Faba beans. The protein content ranged from 20.35 to 23.02%. High average content of ash (5.04%), potassium (375.9 mg 100 g^{-1}), sodium (35.34 mg 100 g^{-1}), phosphorus (138.97 mg 100 g^{-1}) and calcium /magnesium (58.99 mg 100 g^{-1}) were recorded. In arid conditions, these populations were cultivated under abiotic stress: high salinity and low precipitation, and were able to compete with the commercial « Aguadulce» and « Seville » varieties. Particularly two populations P12 and P37, with morphological and chemical characteristics comparable to commercial «Aguadulce» and «Seville» aba bean, should be subjected to a selection program.

These results suggest the importance of preserving the genetic resources of broad bean and could be a starting point for further

studies, with the aim of chemical selection for highest antioxidant activity and minerals content for intensive cultivation, or with particular qualitative and morphological characteristics for improved industrial uses.

Moreover, the development of varieties more adapted to present and future demands must be taken in consideration as a strategy that prevents genetic erosion, having in mind that biodiversity reduction of these species in the region is a real risk. Therefore, evaluation of genetic resources of broad bean all over the south Mediterranean region constitutes a fundamental step of conservation and amelioration strategy, in a crop of nutritional importance and pharmaceutical value.

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