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Original Research

Biochemical Characterization of Local Onion Genotypes (*Allium cepa* L.) in the Arid Regions of Tunisia

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Abstract

Nineteen Tunisian onion genotypes were characterized based on phytochemical composition, sugar content, and antioxidant activity. The studied onions showed a great diversity on biochemical contents and composition. It seemed that there were differences between genotype categories (local landraces or local breeding lines) and within the same category. Quercetin and quinic acid were, respectively, the main flavonoid (flavonol) and organic acid (cyclitol) identified and quantified by HPLC. In all studied onions, quercetin was the predominant flavonoid, with the highest content in the local breeding line OP2-w (1142.19 mg 100 g^{-1} DW). OP3-w showed the highest value of total phenolic as well as total flavonoid content at 12.52 mg GAE g^{-1} DW and 48.28 RE g^{-1} DW respectively. Fructose and sucrose were the most abundant sugars in all the genotypes. Clustering and PCA analysis showed a great dispersion of these genotypes which were classified into 3 major groups. The chemical and nutritional composition found highlights the great value of this onion germplasm which can be used for the sustainable conservation and management of Tunisian onion genetic resources.

Keywords: diversity, local landraces, onion, phenolic compounds, phytochemicals

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Introduction

Onion (*Allium cepa* L., 2n = 2x = 16) is a morphologically diverse and an outcrossing crop of economic importance that belongs to the genus *Allium* in Alliaceae family. It is one of the most important vegetable crops worldwide, with an annual production of 100 million tons of dry bulbs [1].

This crop is recognized as a neccessary component of the Mediterranean diet and it is consumed in almost all savory dishes. In addition, this vegetable is a worthy natural source of bioactive compounds which provide multiple health benefits since it is anti-allergic [2], anti-cancerous [3], and provides cardiovascular support [4, 5]. Quercetin is the major flavonoid compound in onion bulbs, representing more than 95% of the total flavonoids [6, 7].

Quercetin has an important antioxidant property, is a free radical scavenger and has a good effect on human health [6], due to its anti-inflammatory activity, antihistamine effect, anti-allergy, anticancer and antivirus activities [8]. The onion bulb is one of the crops that contain high levels of quercetin. As well, onion is a good source of carbohydrates that represent about 80% of the dry weight of the onion bulb. The main carbohydrates include glucose, fructose, sucrose, and low-weight fructo-oligosaccharides (FOS) [9]. Onion FOS include inulin, kestose, nystose, and fructo-furanosylnystose. These compounds have prebiotic effects that enhance health and well-being [10].

In Tunisia, the onion crop is facing many problems such as the lack of quality of seeds which have led to a low country-wide production of 188,473 tons [1]. Indeed, most farmers use their self-regenerated onion seeds. Under intensive modern agriculture, growers tend to abandon local landraces [11, 12]. In consequence, the Tunisian onion is threatened by genetic erosion and biodiversity loss. Therefore, preserving our genetic resources is very important to conserve and regenerate onions in Tunisia.

Onion is considered as a healthy food that provides health benefits, including prevention of diseases which has attracted the attention of consumers. The selection of food plants with a high content of nutraceuticals is one of the research fields directed to disease prevention rather than cure [13, 14].

In front of the enormous demand of onion for consumption and medical purpose it has become a serious necessity to breeding new cultivars with high productivity and a good quality for specific production areas [14].

In Tunisia, there are no published data about the chemical composition of onion landraces collected from different regions of the country. The characterization of onion genotypes was still scarce. For this purpose, this research aimed to characterize onion genotypes (local breeding lines and local landraces) for the main quality parameters (sugars, ascorbic acid, flavonoids, polyphenols and antioxidant activity) specially to identify and quantify the phenolic compounds in order to understand their bioactivities such as antioxidant potentialities for further applications and also for the identification of promising genotypes. This characterization will be useful for the sustainable conservation and management of Tunisian onion genetic resources.

Experimental

Plant Material

Nineteen Tunisian onion genotypes, made up of 11 local landraces and 8 local breeding lines, were evaluated (Table 1). Landraces were collected as seeds from local farmers in the arid region of South Tunisia and 8 local breeding lines were selected in the Regional Research Center on Horticulture and Organic Agriculture (CRRHAB, Chott-Mariem, Tunisia) by Dr. Rafika Sta-Baba. The onion seeds were sown in mid-October in the arid region at the Experimental Station of Arid Lands of Chenchou, Tunisia (latitude 33º61'22''N, longitude 10º23'47''E). The soil of the experimental field was sandy. The crop was irrigated with a drip-irrigation system. Fertilization was carried out according to the need and recommendations for the crop. Weeds were controlled manually throughout the season. The onion plantlets were transplanted to the field in February in a randomized complete block design (RCBD) with three replications, the unit plot size was 3 × 2 m^2 spaced 15 cm between rows and 10 cm between plants. Bulb harvest took place from July to August, whenever 50% of the leafy tops of each genotype fell over. Onion bulbs were divided into three batch samples for further morphological, phytochemical, and nutritional analyses.

Preparation of Extracts

Ten fresh onion bulbs from each genotype were sliced and air dried at 40ºC for five consecutive days until a constant weight was reached. The resulting onion dry matter was then ground into powder. The powdered samples were conserved in a sealed plastic bottles at -20ºC until analysis

Phenolic compounds were extracted as reported by Santas et al. [15] with some minor modifications. Onion powder (5g) was dissolved in 50 ml of methanol. After mixing well, the extract was placed in a room temperature of 30ºC for one night. The centrifugation of samples was done at 3000 rpm for 15 min. The supernatant was recovered and the extract was filtered through Whatman no.1 filter paper. Extracts were preserved in the dark at −20ºC until further analysis. These extracts were used to determine total phenolics, total flavonoids and phenolic compounds

Table. 1. Onion local landraces and local breeding lines used in our study; original geographical localities and skin color.

	Code	Skin Color	Origin	Latitude	Longitude
Local Landraces	LL1-r	Red	Kelwamen, Kebeli	33°12’	8°84’
	LL2-r	Red	Chouba, Gafsa	34°43’	8°77’
	LL3-r	Red	Chenchou, Gabes	33°53’	9°53
	LL4-p	Purple	Chenchou, Gabes	33°53’	9°53
	LL5-p	Purple	Ghannouch, Gabes	33°56’	10°03’
	LL6-p	Purple	Gabes	33°88’	10°05’
	LL7-p	Purple	Gabes	33°88’	10°05’
	LL8-y	Yellow	Katana, Gabes	33°88’	10°05’
	LL9-w	White	Mzaraa Naji, Kebili	33°12’	8°84’
	LL10-w	White	Tozeur	33°92’	8°12’
	LL11-w	White	Sidi Bouzid	35°03’	9°48’
Local Breeding Lines	BL1-r	Red	CRRHAB	35° 82’	10°60’
	BL2-y	Yellow	CRRHAB	35° 82’	10°60’
	BL3-y	Yellow	CRRHAB	35° 82’	10°60’
	BL4-w	White	CRRHAB	35° 82’	10°60’
	BL5-w	White	CRRHAB	35° 82’	10°60’
	OP1-y	Yellow	Open pollinated CRRHAB	35° 82’	10°60’
	OP2-w	White	Open pollinated CRRHAB	35° 82’	10°60’
	OP3-w	White	Open pollinated CRRHAB	35° 82’	10°60’

by LC-MS-2020 (Liquid Chromatography-Mass Spectrometry UFLC XR system).

Chemicals and Reagents

HPLC grade methanol, formic acid, gallic acid and Folin-Ciocalteu reagent were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Chemical standards (quinic acid, salviolinic acid, protocatechuic acid, p-coumaric acid, trans-ferulic acid, cirsiliol, quercetin, luteolin, glucose, fructose and sucrose) were boreyht from Sigma Chemical Co. (St. Louis, MO, USA).

Determination of Total Phenolic Content (TPC)

For the TPC, samples were extracted in triplicate according to Elfalleh et al. [16]. 0.5 ml of methanolic solution was mingled with 0.5 ml of Folin-Ciocalteu (Sigma Chemical, Co, St-Louis, MO, USA) and 4 ml of sodium carbonate solution 1M. The mixtures were incubated for 90 min in darkness at room temperature. Then the absorbance was measured at 765 nm using a UV spectrophotometer (Tecan Infininte M200, Männedorf, Switzerland). The results were expressed as mg Gallic acid equivalents per g of dry weight (mg GAE g^{-1} DW).

Determination of Total Flavonoid Content (TFC)

The TFC in the extracts was determined spectrophotometrically according to Elfalleh et al. [16]. Rutin (Sigma Chemical, Co, St-Louis, MO, USA) was used to make a calibration curve. 1 ml of methanolic extract was added to 1 ml of 2% AlCl3 (Sigma Chemical Co., St Louis, MO, USA) methanolic solution. The mixture was incubated for 15 min at room temperature. The absorbance against a blank was measured at 430 nm using a visible UV spectrophotometer (Tecan Infininte M200, Männedorf, Switzerland). The flavonoids content was reported as mg of rutin equivalents per g dry weight (mg RE g^{-1} DW).

HPLC Analysis of Flavonoids and Phenolic Acids

The polyphenol extracts were filtered through a 0.45 µm filter syringe before injection into the HPLC system and injected into Shimadzu UFLC XR system (Kyoto, Japan). The phenolic compounds were separated by using an LC-MS-2020 equipped with an electrospray ionization source (ESI). An Aquasil C18 column (250 × 4.0 mm I.D., particle size 5 µm) was utilized for analysis. The column temperature was set at 40ºC

and the injection volume was 5 µl with a flow rate of 0.4 ml/min. Water/formic acid (0.1%) and methanol/formic acid (0.1%) were used as mobile phases A and B, respectively. The analysis was performed using a linear gradient programmed as follows: 0-14 min, from 10% to 20% B; 14-27 min, from 20% to 55% B; 27-37 min, from 55 % to 100 % B; 37-45 min, 100% B; 45-50 min 10%B. High-purity nitrogen was used as the nebulizer and auxiliary gas. The mass spectrometer was operated in negative ion mode with a nebulizing gas flow of 1.5 L/min, a dry gas flow rate of 15 L/min, a dissolving line temperature of 280ºC and a block source temperature of 450ºC.

Determination of ABTS Radical Scavenging Activity

The antioxidant activity was determined by ABTS (2,2′-Azinobis-(3-Ethylbenzthiazolin-6-Sulfonic Acid)) following the procedure by Re et al. [17]. Briefly, ABTS solution (7 mM) and the potassium persulfate (2.45 mM) were mingled at room temperature for 12 h in dark. This solution was then diluted with methanol to set the initial absorbance of 0.70±0.02 at 734 nm. The ABTS diluted solution (3 ml) was mixed with 30 µl of blank, samples were mixed and the absorbance was read at 734 nm for 6 min using a spectrophotometer. The results were expressed in µmol Equivalence Trolox (µmol TE 100 g^{-1} DW).

Ascorbic Acid Analysis by LC-ESI-MS

Ascorbic acid content was determined by the liquid chromatographic method [18]. Ten grams of onion bulb were blended and mixed with 50 ml of 2% metaphosphoric acid and then filtered. The extracts' solutions were excited for 15 min and filtered through a 0.45 µm filter syringe for HPLC analysis. The extract was studied using an LC-MS-2020 mass spectrometer (Shimadzu, Kyoto, Japan).

Sugars Sugar Analysis by HPLC

Sugars were studied according to Sharma et al. [19] with little bit modifications. Approximately 5 g of dry samples were mixed with 50 ml of 80% ethanol and refluxed for 1h. The mixture was then filtered through a Whatman No. 4 filter and readjusted to 50 ml with 80% ethanol. The resulting extracts were evaporated at 50ºC. These concentrated extracts were immediately diluted with 10 ml of water and then stored at -20ºC until used for analysis.

Carbohydrates proportion of the dry weight of onion bulbs (mg g^{-1} DW) were determined by HPLC (Shimadzu – Japan). The mobile phase was acetonitrile-water (85:15; v/v) at 0.4 mL /min flow rate. The column temperature AQUASIL C18-HL column (150 mm × 3 mm, 3 µm particle sizes) was maintained at 40ºC. The analysis was done by using a Shimadzu UFLC XR series chromatograph with an LC-20 AD XR manual injector. Chromatograms were integrated using the Shimadzu Lab solutions software. Assays were performed in triplicate. The retention times were compared to external standards solution of glucose, fructose and sucrose (Sigma Chemical Co., St Louis, MO, USA).

Statistical Analysis

For each onion genotype, the mean and standard deviation of each evaluated parameter (n = 3) was calculated. For the statistical analysis, the mean values of each parameter were used for the analysis of variance. Means were separated using the Tukey's b test ($p<0.05$). SPSS for Windows (Version 20) was used to perform data analysis. Multivariate relationships among genotypes were revealed by Xlstat 2019 software, via a principal component analysis (PCA) by using a correlation matrix derived from the significant traits after the analysis of variance. Agglomerative Hierarchical Cluster analysis (HCA) was used to determine differences and similarities among onion genotypes, and the distance measure used was Euclidean distance computed between each population by the Ward method.

Results and Discussion

Total Phenolic and Flavonoid Contents

High variation was observed within and among the 19 Tunisian onion genotypes for the TPC and the TFC of bulb extracts (Table 2). The TPC ranged from 2.64 to 9.23 mg GAE g^{-1} DW for local landraces and 4.83 to 12.52 mg GAE g^{-1} DW for local breeding lines. Among the onion genotypes, the highest TPC was found in the local breeding line OP3-W (white onion, 12.52 mg GAE g^{-1} DW). It seems that selection strategies carried out by breeders of CRRHAB aiming at high yield and good ability for storage enhanced the phytochemical composition and quality of onion.

For the local landraces, the highest TPC was found in LL1-r (red onion, 9.23 mg GAE g^{-1} DW). The lowest value in LL10-w (white onion, 2.64 mg GAE g^{-1} DW). For local landraces, the red onion had a higher TPC than the violet, yellow and white onions. The results were compatible with a previous studies of varieties of onion with different colors (red, violet, white and green) [20, 21]. Besides, Lachman et al. [22] found that red onion had the highest amount of total polyphenol content, the white onion had the lowest amount while yellow onion had an average content of total polyphenol content. Results from TPC values revealed substantial variation among onion bulbs. According to Bibi et al. [23], various environmental factors (air, water, soil, temperature, precipitation, altitude), genetic variation between different genotypes or within individuals of the

Table 2. Bioactive compounds and antioxidant activity evaluated in onion genotypes.

		TPC (mg GAE/ g DW)	TFC (mg of RE/g DW)	ascorbic acid (mg/100g FW)	ABTS (µmol TE/g)
Local landraces	LL1-r	9.23±0.05b	37.58±1.23b	6.84±0.14b	13.25±0.36a
	LL2-r	2.97±0.18j	9.54±0.34j	1.90±0.05k	10.80±0.59b
	LL3-r	5.13±0.02e	21.35±0.81f	3.18±0.07g	8.74±0.77c
	LL4-p	4.31±0.06g	13.92±0.19i	2.46±0.06i	9.95±0.64b
	LL5-p	3.27±0.15i	12.26±0.52i	3.46±0.02g	6.44±0.19d
	LL6-p	4.07±0.04h	10.53±0.23j	1.84±0.05k	14.19±0.23a
	LL7-p	4.71±0.15g	19.90±0.92g	2.04±0.08k	10.43±0.98b
	LL8-y	3.72±0.13h	14.42±0.08h	8.50±0.11a	13.28±0.16a
	LL9-w	6.08±0.26d	19.66±0,87g	2.89±0.05h	9.79±1,50b
	LL10-w	2.64±0.09j	10.30±0.29j	3.18±0.07g	10.70±0.08b
	LL11-w	3.50±0.13i	10.08±0.25j	3.45±0.07g	13.28±0.15a
	Mean	4.51	16.32	3.61	10.99
Local Breeding lines	BL1-r	4.88±0.04f	22.81±0.17f	5.15±0.04d	12.29±0.65a
	BL2-y	5.55±0.23e	12.58±0.26h	2.58±0.01i	6.02±0.94d
	BL3-y	6.02±0.23d	28.44±0.62d	5.84±0.14c	7.17±0.19c
	BL4-w	8.33±0.04c	31.55±0.50c	4.15±0.05f	5.42±0.12d
	BL5-w	6.06±0.02d	26.55±0.06e	4.71±0.26e	12.82±1.19a
	OP1-y	5.25±0.1e	20.48±1.32g	2.23±0.05j	4.45±0.18d
	OP2-w	4.83±0.14f	13.45±0.16h	3.46±0.02g	10.33±1.78b
	OP3-w	12.52±0.19a	48.28±0.10a	5.84±0.05c	12.63±0.88a
	Mean	6.68	25.52	4.25	8.89

For each column, means followed by different letters are significantly different (Tukey b test, $p<0.05$).

same genotype are leading to a great influence on plant secondary metabolites contents and their synthetic bioactive potential.

The TFC in local landraces and local breeding lines varied significantly among genotypes ranging from 9.54 to 37.58 mg of RE g^{-1} DW and from 12.58 to 48.28 mg RE g^{-1} DW, respectively (Table 2). These results were in accordance with the findings of Benitez et al. [24] who reported that TF content fluctuated between 7-43.1 mg GAE g^{-1} DW. For the local breeding lines, the highest level of TF content (48.28 mg of RE g^{-1} DW) was observed for the OP3-w while the lowest (12.58 mg of RE g^{-1} DW) was in the BL2-y.

For the local landraces, TFC oscillated between 9.54 mg of RE g^{-1} DW in LL2-r (red onion collected from the arid inferior climate stage) to 37.58 mg of RE g^{-1} DW in LL1-r (red onion collected from the Saharian superior stage). In our study, colored onion (red and violet) had higher amounts than yellow and white onions. Pérez-Gregorio et al. [14] reported that red onion cultivars have higher flavonoid content than white onions. Many studies have reported that flavonoid content depends widely on the genotype, bulb size, agricultural technique and day length sensitivity [14, 25, 26]. Onion is one of the most important sources of flavonoids that provide a protective effect on human health.

Antioxidant Activity

Onion genotypes showed significant antioxidant activities ($p<0.05$). Local landraces had higher antioxidant potential than the local breeding lines, by the ABTS method (Table 2). For the local breeding lines, the ABTS content varied from a maximum of 12.82 µmol TE g^{-1} in BL5-w to a minimum of 4.45 µmol TE g^{-1} in OP1-y.

For the local landraces, the red genotype LL6-r had significantly higher ABTS content 14.19 µmol TE g^{-1}, whereas LL5-p showed the lowest ABTS content (6.44 µmol TE g^{-1}). Previous studies [6, 27] reported that red onion bulbs have a higher antioxidant capacity than yellow and white onion, which is in accordance with our findings. Antioxidant activity may be affected

by the presence of other compounds such as sulfur compounds and gallic acid, among others, which were not included in this research. Rodrigues et al. [28] in Portugal reported that levels of antioxidant activity in local red and white onions varied significantly between seasons. The content of flavonoids will directly affect the TPC and TAC values of samples. The method of extracting polyphenols from plant materials is also an important factor for the determination of antioxidant activity [15]. Tan et al. [29] showed that the antioxidant capacity in many plants can be attributed to their levels of phenolic compounds.

Based on the results obtained, we can deduce that local landraces are a good source of phenolics with high antioxidant capacities, and that strategies should be developed to conserve and valorize these local landraces.

HPLC Analysis of Phenolic Acids and Flavonoids

Significant differences for the polyphenol compounds among the local breeding lines and local onion landraces were found (Table 3, Fig. S1). Five free phenolic acids (quinic, protocatechuic, ρ-coumaric, trans-ferulic, and salviolinic acid) and three flavonoids (quercetin, luteolin, and cirsiliol) were quantified by the LC-ESI-MS method (Table 3). For all genotypes, the most abundant phenols were quercetin, quinic acid and protocatechuic acid, whereas luteolin was the least abundant phenolic compound with low concentrations (0.05 to 3.73 mg 100 g^{-1} of extract).

As illustrated in Table 3, quercetin is the major flavonoid in local landraces and local breeding lines with a concentration of about 73.51 to 687.14 mg 100 g^{-1} DW and 12.51 to 1142.19 mg 100 g^{-1} DW, respectively. These findings are higher than the results reported by Liguori et al. [30] for onions of the Mediterranean area. The highest values of this parameter were obtained by Kwak et al. [31] who studied quercetin content in three red onion cvs which varied from 32.21 to 127.92 mg g^{-1} DW. The quercetin content variability may be influenced by the genetic background [32], bulb size and bulb weight [33]. Also, this variability can be related to environmental factors and geographical origin.

Other flavonoids were detected in our study, such cirsiliol and luteolin but at minor concentrations (Fig. S1), fluctuating between 0.04 and 15.91 mg 100 g^{-1} DW. Quinic acid, an intermediate in plant phenolics biosynthesis, was the second important phenol with concentrations which ranged from 72.19 to 282.39 mg 100 g^{-1} DW in the local landraces LL3-r and LL9-w, respectively. Simin et al. [34] found that this compound varied from 551.1 to 1132 μg g^{-1} DW in bulb and aerial part extracts of *A. flavum*. Among hydroxybenzoic acids, we found that protocatechuic acid accounted for 7.2-136.66 mg 100 g^{-1} DW. These findings were similar to those found by Prakash et al. [20] (3.1-138 μg g^{-1}) in India. Salivonic acid was also present but in lower amounts.

We also found that the dominant hydroxycinnamic acid was ρ-coumaric acid, with concentrations ranging between 3.18-29.5 mg 100 g^{-1} DW followed by -transferulic acid, ranging between 0.82-23.18 mg 100 g^{-1} DW. For comparison, Liguori et al. [30] found that the concentration of ρ-coumaric acid accounted for 17.3-33 mg 100 g^{-1} DW. These metabolites play an important role in health benefits and can replace synthetic antioxidants, so that consuming onion would be beneficial [35].

For the quantified ascorbic acid content (Table 2), the local landrace of yellow color LL8-y showed the highest value (8.5 mg 100 g^{-1} FW) whereas the purple genotype LL6-p showed the lowest values (1.84 mg 100 g^{-1} FW). For the local breeding lines, the highest vitamin C concentration was found in BL3-y (5.84 mg 100 g^{-1} FW) followed by LL1-r (6.84 mg 100 g^{-1} FW), while OP1-y showed the lowest value (2.23 mg 100 g^{-1} FW). The results presented in this study fell well within the published range for ascorbic acid content in onion bulbs, which was reported to vary from 1.68 mg 100 g^{-1} [36] to 8.4 mg 100 g^{-1} FW [7]. Lester [37] reported that ascorbic acid contributes significantly to the onion size. In our study, yellow onions showed the highest ascorbic acid content. However, Gorinstein et al. [38] showed that ascorbic acid content in red onion is higher than yellow and white onion bulbs. The variability in ascorbic acid among onion bulbs can be attributed to climatic conditions or soil type, which can influence the genetic information of the onion genotypes.

Fructose, glucose and sucrose were found in all the genotypes with significant differences (Table 4). Fructose was the predominant sugar in almost all of the samples, followed by sucrose and glucose. The local landrace LL4-p had the highest total sugar content 267.04 mg g^{-1} DW (103.11 mg g^{-1} DW fructose, 77.96 mg g^{-1} DW glucose and 85.97 mg g^{-1} DW sucrose) followed by the local breeding line BL3-w with 244.36 mg g^{-1} DW (156.33 mg g^{-1} DW fructose, 69.42 mg g^{-1} DW glucose and 18.61 mg g^{-1} DW sucrose). These values were in accordance with the results mentioned by Benítez et al. [39]. In a study on a local Greek cv of onion, Petropoulos et al. [25] reported that sugar composition may be mainly due to the genotype. Besides, the onions sugars composition plays an important role, to determine their intended use. In this context, Randle and Lancaster [40] proved that onions with high DMC (>15%) and low levels of reducing sugars are appropriate conditions to long storage. Petropoulos et al. [25] proved that onion sweetness is closely related to TSS and sugars composition. Sugars are responsible for the sweet sensation of foods.

Table 3. Phenolic compounds in the onion genotypes (mg/100g DW) identified by LC-ESI-MS analysis.

		Quinic acid	Protocatechuic acid	ρ -coumaric acid	Trans ferulic acid	Salviolinic acid	Quercetin	Cirsiliol	Luteolin
Local Landraces	LL1-r	235.61 ± 9.09b	44.65±0.59e	6.66±0.04e	2.76 ±0.12g	16.59±0.89b	178.66±8.44h	6.57±0.02h	0.10±0.05h
	LL2-r	76.01±7.24f	12.71±1.76g	4.77±0.26f	1.01±0.02i	1.06±0.15f	109.48±2.00i	8.56±0.02f	3.73±0.17a
	LL3-r	72.19±2.15f	18.09±1.64f	3.96±0.26 g	0.82±0.01i	13.21±0.07c	90.71±0.72i	7.4±0.02g	0.63±0.23f
	LL4-p	218.7 ±9.13b	77.22±3.94d	16.33±1.18c	6.35±0.40e	ND	687.14±43.83c	13.15±0.53b	0.07±0.01h
	LL5-p	182.27±4.10c	14.99±0.28g	6.41±0.24e	0.96±0.05i	11.4±0.91d	73.51±4.68i	15.85±0.38a	0.87±0.06e
	LL6-p	116.07±11.29e	29.72±4.39f	11.61±0.27d	10.23±0.22d	12.33±0.40c	523.62±12.75d	13.35±0.02b	0.18±0.09h
	LL7-p	168.24±5.38c	25.75±0.25f	5.34±0.10f	5.65±0.30e	9.87±0.15e	281.27±3.20g	0.04±0.01j	0.31±004g
	LL8-y	232.83±9.08b	92.63±4.80c	10.11±0.31d	4.35±0.12f	14.96±0.18b	560.33±2.22d	11.06±0.09d	2.23±0.01b
	LL9-w	282.39±7.88a	ND	6.09±0.08e	2.81±0.19g	21.94±0.19a	588.01±0.37c	13.53±0.11b	0.81±0.02e
	LL10-w	74.25±3.55f	24.88±3.48f	3.49±0.05g	1.87±0.13h	10.74±0.37d	161.79±1.76h	4.44±0.11i	0.12±0.01h
	LL11-w	112.38±2.15e	16.34±0.14f	6.72±0.22e	6.27±0.16e	10.27±0.11d	501.39±3.47e	10.51±0.05e	0.45±0.01f
	Mean	160.99	35.70	7.41	3.92	12.24	341.45	9.50	0.86
Local Breeding lines	BL1-r	153.92±1.34d	5.10±0.12g	7.51±0.53e	10.28±0.33d	17.61±4.07b	20.76±0.57j	15.91±0.16a	0.08±0.02h
	BL2-y	266.65±4.71a	93.73±5.90c	7.44±0.47e	4.17±0.10f	ND	431.39±10.07f	10.04±0.05e	0.42±0.01f
	BL3-y	179.51±3.09c	7.21±0.24g	3.18±0.19g	2.76±0.07g	ND	31.99±0.92j	6.49±0.04h	0.05±0.01h
	BL4-w	153.78±14.54d	22.51±3.51f	6.17±1.72e	1.30±0.18h	ND	259.64±15.02g	7.76±0.08g	1.91±0.02c
	BL5-w	270.61±3.59a	136.66±1.65a	29.50±0.28a	19.05±0.28b	ND	1002.01±20.07b	6.69±0.03g	1.32±0.01d
	OP1-y	180.41±0.90c	ND	6.84±0.31e	6.17±0.16e	ND	12.51±0.43j	10.11±0.06e	0.05±0.01h
	OP2-w	230.92±9.94b	134.81±17.01a	23.10±0.76b	23.18±0.67a	1.31±0.04f	1142.19±52.74a	11.96±0.39d	1.30±0.01d
	OP3-w	223.73±3.75b	120.85±3.04b	10.44±0.36d	12.56±0.43c	1.28±0.09f	971.68±34.09b	10.23±0.06e	1.34±0.01d
	Mean	207.44	74.41	11.77	9.93	6.73	484.02	9.90	0.81

means followed by different letters are significantly different (Tukey b test, $p<0.05$).

Table 4. Sugars content of the studied onion genotypes determined by HPLC analysis.

		Fructose (mg /g DW)	Glucose (mg /g DW)	Sucrose (mg /g DW)	Total
Local landraces	LL1-r	85.92±0.20h	55.02±0.18d	100.42±0.58a	241.36
	LL2-r	42.90±0.08o	16.33±0.05l	58.98±0.26f	118.21
	LL3-r	36.26±0.83p	15.97±0.10l	61.83±0.18e	114.06
	LL4-p	103.11±0.62c	77.96±0.28b	85.97±0.37b	267.04
	LL5-p	62.21±0.42l	18.26±0.58k	73.52±0.61d	153.98
	LL6-p	44.24±0.10o	11.06±0.05m	32.36±0.07j	87.66
	LL7-p	74.94±0.15j	37.16±0.47g	26.45±0.08k	138.55
	LL8-y	89.02±0.39g	46.35±1.17e	57.63±0.95f	193.00
	LL9-w	70.71±0.4k	29.07±0.38i	53.30±0.51g	153.08
	LL10-w	79.82±0.17i	70.35±0.18c	79.72±0.15c	229.89
	LL11-w	48.72±0.47n	16.12±0.08l	30.14±0.50j	94.98
	Mean	67.08	35.79	60.03	162.89
Local Breeding lines	BL1-r	112.30±0.25b	46.76±0.31e	41.03±0.17h	200.09
	BL2-y	92.73±0.21f	84.39±0.04a	53.61±1.71g	230.73
	BL3-y	55.53±0.55m	19.77±0.10k	24.35±0.06k	99.65
	BL4-w	56.75±0.14m	24.90±0.05j	63.48±0.39e	145.13
	BL5-w	156.33±0.53a	69.42±0.09c	18.61±0.25l	244.36
	OP1-y	97.72±0.35e	34.09±0.19h	36.91±0.71i	168.72
	OP2-w	100.06±0.15d	42.31±0.02f	35.89±0.49i	178.26
	OP3-w	110.61±0.02 b	76.46±0.62b	21.65±0.42l	208.72
	Mean	97.75	49.76	36.94	184.46

For each column, means followed by different letters are significantly different (*Tukey* b test, $p<0.05$).

Cluster and Principal Component Analyses

Multicriteria analysis via PCA coupled with hierarchical cluster analysis was used in several studies as a performing tool of classification regarding the different phenotypic, biochemical and molecular assets to fix the most discriminant parameters and to identify the similarities existing among the studied genotypes [41, 42].

In this study, both multicriteria analysis were computed *via* Pearson correlations approach between the assessed traits. Overall, significant positive correlations were obtained between fructose *Vs* quinic acid (r = 0.652), protocatechuic acid (r = 0.668), p-coumaric acid (r = 0.698), transfrulic acid (0.649), quercetin (r = 0.518), and glucose (r = 0,769); between glucose *Vs* quinic acid (r = 0.517) and protocatechuic acid (r = 0.657) and between ascorbic acid *Vs* flavonoids content (r = 0532). Otherwise, the sole significant negative correlation was registered between sucrose *Vs* transfrulic acid (r = -0.556).

Concerning the PCA analysis performed on the biochemical data, the first two main components (PC) comprised about a half of the variability existing in the analyzed genotypes (Table 5, Fig. 1a). PC-1 explains 36.05% of the total variability. The most important traits related to this axis were: quercetin, quinic acid, protocatechiuc acid, p-coumaric acid and trans-ferulic acid, representing the phenolic compounds. The most important traits of PC-2, which explains 15.33% of the total variation, were TPC, TFC and ascorbic acid, representing the bioactive compounds. Fructose, glucose and sucrose were the most important traits in PC-3, representing the sugars content.

The dendrogram (Fig. 1b) clustered onion genotypes into three main groups based on quinic acid, protocatechuic acid, p-coumaric acid, trans-ferulic acid, quercetin, TFC, TPC, ascorbic acid, fructose, glucose and sucrose. The 1st cluster containing 10 genotypes, namely LL2-r, LL3-r, LL10-w, BL1-r, OP1-y, LL5-p, BL3-y, LL7-p, BL4-w and LL1-r), this cluster was characterized by the highest concentrations of sucrose and low concentrations of polyphenol compounds. The 2nd cluster comprised three white breeding lines,

Table 5. Principle component analysis of onion traits studied

	PC-1	PC-2	PC-3
Explained proportion of variation (%)	36.05	15.33	11.33
Cumulative proportion of variation (%)	36.05	51.38	**62.71**
Trait		Eigenvectors	
Quinic acid	**0.702**	0.215	0.338
Protocatechuic acid	**0.909**	-0.116	0.024
ρ - coumaric acid	**0.838**	-0.422	0.019
Trans ferulic acid	**0.815**	-0.389	-0.185
Salionilic acid	-0.346	0.138	0.006
Quercetin	**0.850**	-0.294	-0.130
Cirsiliol	0.066	-0.289	0.247
Luteolin	0.069	-0.167	-0.458
TFC	0.399	**0.819**	-0.284
TPC	0.473	**0.745**	-0.224
Fructose	0.840	0.061	**0.311**
Glucose	0.663	0.221	**0.525**
Sucrose	-0.338	0.256	**0.571**
ABTS	0.330	-0.122	-0.395
Ascorbic Acid	0.349	**0.579**	-0.186

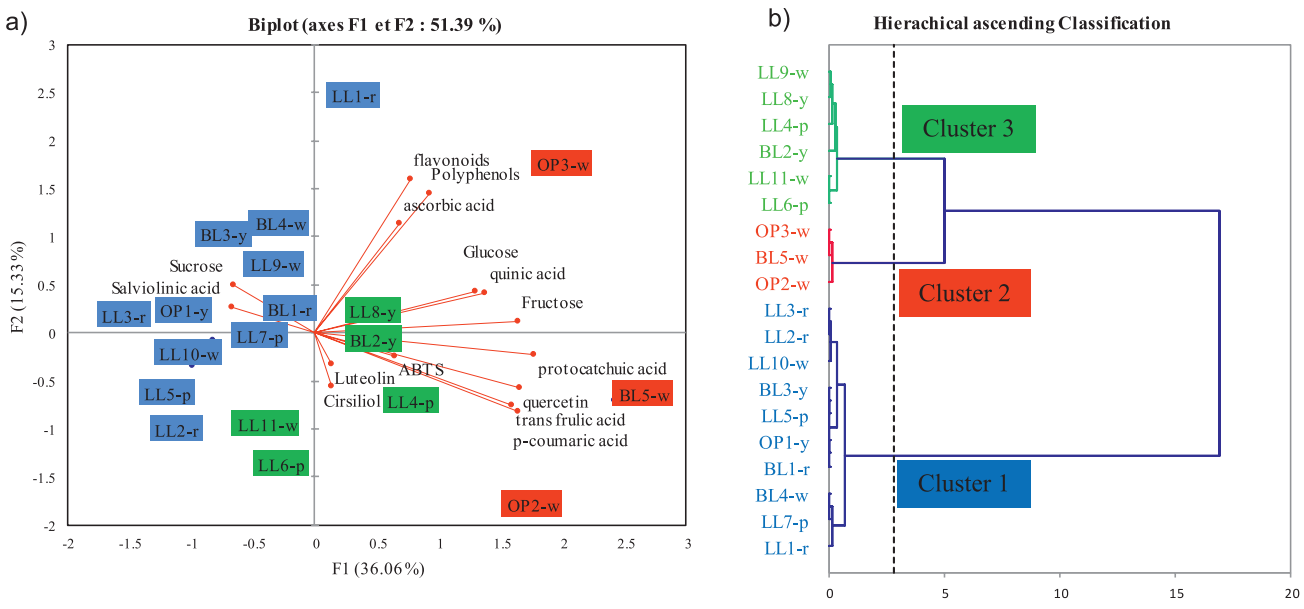

Fig. 1. PCA biplot a) and Hierarchical ascending classification b) of onion genotypes. Onion genotypes were clustered into three main groups based on Quinic acid, Protocatchuic acid, *p*-coumaric acid, *Trans* ferulic acid, Quercetin, TFC, TPC, Ascorbic acid, Fructose, Glucose and Sucrose. The 1st cluster containing 10 genotypes (LL2-r, LL3-r, LL10-w, BL1-r, OP1-y, LL5-p, BL3-y, LL7-p, BL4-w and LL1-r). The 2nd cluster comprised of three genotypes BL5-w, OP3-w and OP2-w, the white local breeding lines. The 3rd cluster comprised of genotypes LL6-p, LL11-w, LL8-y, LL9-w, LL4-p and BL2-y.

BL5-w, OP3-w and OP2-w, which are characterized by the richest content of quinic, protocatechuic and ρ-coumaric acids, quercetin, total flavonoid and phenolic content. This cluster represents a good source of bioactive compounds and a nutritional quality that can enhance health and well-being. The 3rd cluster

comprised of six genotypes (LL6-p, LL11-w, LL8-y, LL9-w, LL4-p and BL2-y) which are characterized by low concentrations of ascorbic acid, total phenolic and flavonoid content. This cluster is also characterized by intermediate concentrations of quercetin, quinic acid, protocatechiuc acid, p-coumaric acid and trans-ferulic acid. So, the breeding lines BL5-w, OP3-w and OP2-w could be selected as the best genotypes for further onion breeding program to obtain hybrids.

The diversity in chemical and nutritional composition found here highlights the great value of this onion germplasm. This diversity, especially from the three main onion clusters, shows that some genotypes are featured with several phytochemical characteristics and seem to have a high potential for further exploitation as promising materials in cultivation and/or in breeding for the creation of new varieties/hybrids.

Conclusions

This study has shown the great phenotypic diversity of onion genotypes from Tunisia based on biochemical traits and the bioactive component content. This onion collection is very rich in sugars, ascorbic acid, natural antioxidants and phenolic compounds. The obtained results can be used for the sustainable conservation and management of onion genetic resources. Indeed, some local landraces showed a great potential and are of great interest given their nutraceutical characteristics and their bioactive components. That's why, strategies must be developed to conserve these valuable local landraces. The interesting local breeding lines are going to be registered in the catalogue of the new varieties. According to our results the breeding lines BL5-w, OP3-w and OP2-w can be selected as the best genotypes.

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Conflict of Interest

The authors declare no conflict of interest.

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Supplementary Data

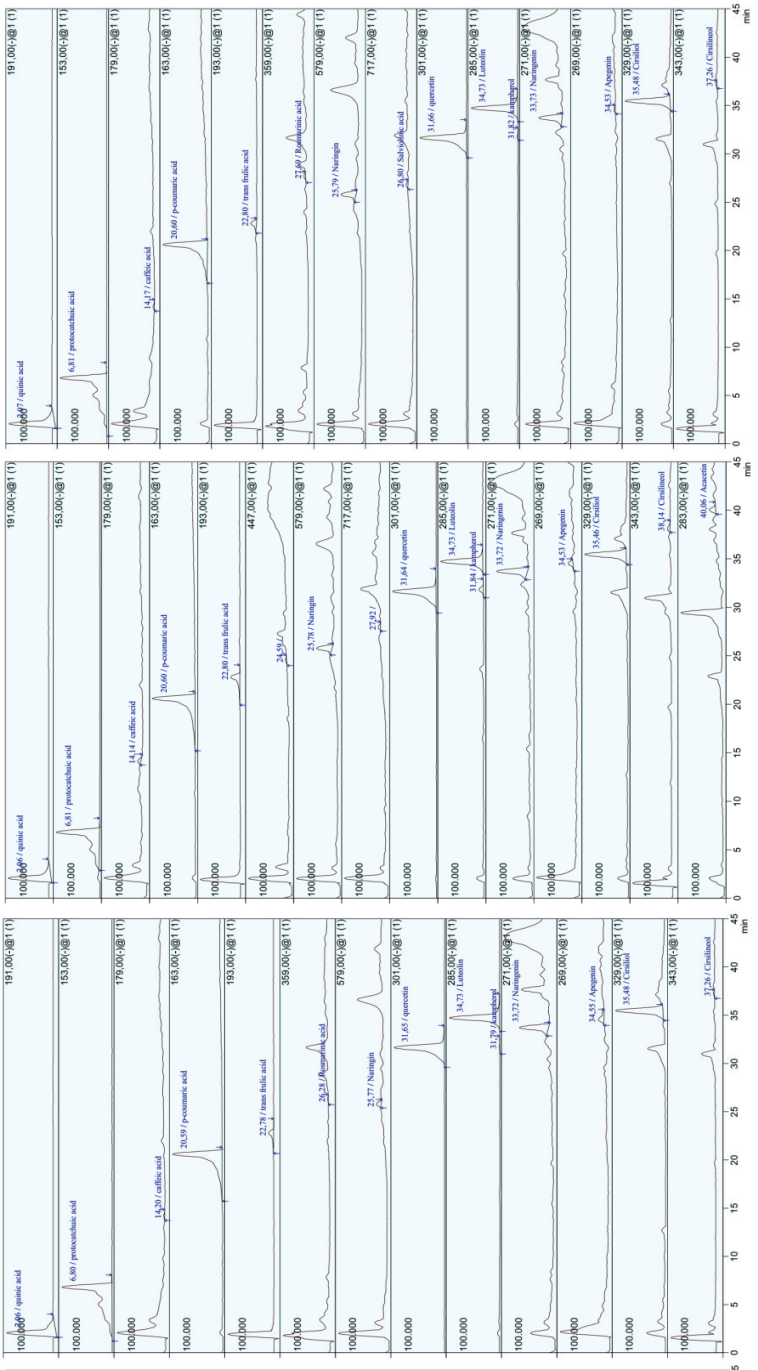

Fig. S1. Chromatograms of phenolic compounds identified by LC-ESI-MS analysis in the onion genotypes LL4-p (A), BL5-w (B), OP2-w (C), and OP3-w (D).