Morphological, molecular, and physic-chemical characterization of traditional Moroccan tomato (*Solanum Lycopersicum* L.) genotypes

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This study provides the first explicit use of morphological, molecular, and physic-chemical data to examine distinctness and relationships between 8 traditional Moroccan tomato varieties of the species (*Solanum lycopersicum* L.) and one pure line Saint Pierre (SP) from Vita Morocco Company used as a control. For the morphological traits, significant differences were observed between the traditional genotypes and variation concerns the number of flowers per inflorescence, fruit shape, fruit color, and fruit weight. Heterogeneity was found in fruit shape within the same varieties as Figuig I (FI), Rissani Orange (RO), Rissani white (RB), and Berkane II (BII). It is unavoidable to purify them for the registration as conservation varieties. The Simple Sequence Repeats (SSR) molecular markers, Tom 236-237 and TMS52 that associated with some fruit traits, showed the highest polymorphism degree with five polymorphic amplicons for the local traditional tomatoes, which suggest a putative association among the phenotypic and molecular data. Tomato qualities measured by some physic-chemical parameters such as acidity, pH, conductivity, and total phenolic contents allow to distinguish the studied traditional varieties. The genotypes Hoceima (H) and Figuig II (FII) particularly merit considerable attention because of their phenols richness, high acidity, and low pH. Finally, the observed variations between the studied genotypes could be used by the tomato breeders in different hybridization programs for the crop improvement.

Keywords: *Solanum lycopersicum*; morphological traits; SSR marker; physic-chemical parameters.

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Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most widely consumed vegetable crops in the world. It is a reservoir of diverse phytonutrients. Tomato has an important nutritional value to human diet and plays a big role in human health [1]. In Morocco, tomato production is around 1.7 million tons [2]. The introduction of pure lines, hybrids, and the industrialization of agriculture led to a strong decline in the cultivation of traditional varieties [3, 4]. Now there is a big interest to collect traditional varieties of tomato as sources of variation in breeding programs as well as to conserve them in germplasm banks. Traditional varieties of tomato were used as a base for the development of modern tomato varieties during the nineteen and early twenty centuries [5]. The collected Moroccan traditional tomato varieties selected by farmers in a limited geographic area by using traditional farming system and, in the most cases, the climate of the

cultivation region can be defined as arid inferior. Such traditional tomato genotypes are typically characterized by good stress tolerance and local adaptability [6] and are promising genetic sources to incorporate valuable traits in cultivated varieties [7].

Characterization is an important method for the identification of a genetic diversity between varieties. The genotypes could be easily differentiated by morphological, biochemical, and molecular characterization. Many molecular markers were used to set up genetic variation in tomato cultivar collection. Molecular SSR marker is one of the most suitable markers for variety identification as it has great discrimination power for varieties with limited genetic variation [8]. Although molecular markers are an efficient tool to investigate the genetic basis of agronomic traits between breeding lines, morphological characterization remains essential to define the characteristics of local varieties for their protection and registration as recognized conservation varieties [9, 10]. Additionally, variations found among the genotypes based on morphological and agronomical characteristics could be used by the tomato breeders in different hybridization programs for the crop improvement and could be a tool to link quantitative trait loci (QTLs) responsible of this variation to functional genes. Physic-chemical traits as phenolic contents, titrable acidity, and other quality parameters could be useful in the selection of the cultivated variety either for direct use or breeding purposes [11, 12]. Those parameters are related to genetic features, cultivation conditions, and/or handling and storage methods [13, 14]. Indeed, phenolic compounds have been reported as cultivars and varieties-distinguishing factors in some plant products [15] being dependent on genotype and environmental factors [16].

The present study was aimed to characterize a different set of tomato populations belonging to traditional varieties from different regions of Morocco. Morphological characterization was performed based on highly heritable traits as inflorescence, flower, and fruit. These conventional descriptors have been complemented with a set of 14 nuclear Simple Sequence Repeats (SSR) as molecular markers. We evaluated some physic-chemical parameters as total phenolic contents, titrable acidity, pH, and electrical conductivity, which could be used for increasing nutritional value through germplasm improvement programs.

Materials and methods

Plant materials

Nine lots of tomato were studied in this investigation including one commercial pure line of French origin “Saint Pierre” (SP) from Vita Morocco Company (Casablanca, Morocco) used as a control. The other lots were collected from four different regions of Morocco: (1) Berkane region (north east): two tomato lots noted as Berkane I (BI) and Berkane II (BII); (2) Figuig region (south east): two tomato lots noted as Figuig I (FI) and Figuig II (FII); (3) Rissani region (south west): three batches noted as Rissani white (RB), Rissani orange (RO), and Rissani Black (RN), in which we found a difference in color among the seeds; (4) Al-Hoceima region (north): one tomato lot noted as Al-Hoceima (H).

The seeds of each lot of tomato were sown in the greenhouse for germination and growth. After two weeks of sowing, the seedlings were transplanted into the experimental plot with a spacing of 90 x 40 cm^2 per standard cultural recommendations for the area in a randomized complete block design and grown in a greenhouse at the nursery of the park Lala Aïcha with regular watering. The climate in this region can be defined as arid inferior. Tomatoes were harvested at the optimum ripeness stage and immediately used for analysis.

Traits evaluated

Phenotypic data were evaluated for growth, yield, and fruit quality traits during 2016 - 2017. Morphological characters were studied in selected tomato accessions by already set

standards for morphological characters by International Plant Genetic Resources Institute (IPGRI) tomato descriptor [17]. These characterizations include the plant growth type, number of flowers per inflorescence, fruit weight, fruit morphology i.e. fruit shape and color, and numbers of fruit locules.

Genomic DNA isolation

A group of 8 commercial hybrids tomatoes named 620, 621, 622, ABR 620 Global,1-G-48-6032, Emperador RZ, BrigeorE, RTM105 were added to evaluate the SSR markers and to determine the relationship with the local varieties.

For total genomic DNA extraction, fresh leave of each lot of tomato were grounded in liquid nitrogen and stored at -80°C. Genomic DNA isolation was performed following the procedure of the DNeasy Qiagen Plant mini kit (QIAGEN, Germantown, MD, USA).

SSR-PCR amplification

For SSR analysis, among the relatively high number of SSR loci already reported in tomato, 14 simple sequence repeat (SSR) markers were selected from the published data [18, 19] or on the website of Solanaceae Genomics Network (http://solgenomics.net) to obtain a good coverage of the tomato genome. Some SSRs were selected to include a group of loci in regions harboring reported QTLs that affect several fruit features like Tom 236-237, TM 52, and TM 63, whereas the remaining SSRs do not have a known linkage with genes of interest. These molecular markers are used to search for possible associations between molecular markers and morphological traits.

PCR amplification was performed in a 20 µl total volume, containing 20 ng of genomic DNA, 0.25 mM of each primer, 200 µM dNTPs, 1.5 mM $MgCl_2$, 1 mg/ml BSA, 1X PCR buffer, and 1 U Taq DNA polymerase (Promega, Madison, WI, USA).

The amplifications were conducted with Thermal Cycler (Applied Biosystems, Grand Island, NY, USA) with an initial 5 min at 94°C followed by 35 cycles of 30s at 94°C, 45s at X°C, and 1min 30s at 72°C, ended by 7min final extension cycle at 72°C. The amplification products were separated and analyzed on a LI-COR sequencer (Westburg, Leusden, The Netherlands) using a 6.5% acrylamide gel. The length of the alleles was determined by comparison with marker loaded on adjacent gel tracks. The raw data were collected and analyzed by the analysis software "Gene ImageIR" (Westburg, Leusden, The Netherlands).

Cluster analysis

All tested varieties (Traditional varieties, hybrids commercial, and the pure line SP) were clustered based on the estimated genetic distance. The positions of an SSR bands were scored and transformed into a binary character matrix. Genetic similarities between genotypes were calculated according to the method of Nei and Li [20]. The similarity matrix was subjected to cluster analysis by the unweighted pair group arithmetic method (UPGMA) [21]; and phylogenetic tree was created using the output data and the graphical module of the MVSP 3.1 software.

Titrable acidity, pH, electrical conductivity and dry weight

Physic-chemical traits as total solid percentage dry weight (DW), titrable acidity (TA), pH, and electrical conductivity (EC) were recorded for traditional genotypes and the control SP.

Tomatoes were harvested at the optimum ripeness stage and immediately used for analysis. Five to six tomatoes per sample were weighed and washed with sterile water. To obtain juice, fruit pieces were homogenized in a conventional blender for 1 min. Two hundred grams of homogenate were centrifuged at 4°C, 12,400 g for 10 minutes. The juice was filtered (Whatman #4 filter paper) and used for the analytical determinations. The tomato homogenate was dried at 65°C for 72h to determine the dry weight (DW).

The EC and pH were directly determined in the juice using a conductivity meter and a pH meter, respectively. Titrable acidity (TA) was determined according to the Association of Official Analytical Chemists (AOAC) Official Method 942.15 [22]. The pH of the tomato juice (100 mL) was raised to pH 8.1 with 0.1 N NaOH.

Determination of total phenolic contents

Dried tomato slices (10 g) were stirred with 100 mL MeOH at 30°C for overnight. The extract was filtered through Whatman #1 filter paper for removal of seed particles. The residue was re-extracted with 60 mL methanol. The obtained extracts were filtered again, pooled and concentrated under vacuum at 40°C. These methanolic extracts were used for phenolic and antioxidant analyses.

The Folin-Ciocalteu method was used to measure the total phenolic compounds [23]. For the analysis, from each sample, 0.5 mL of methanolic extract solution was added to 0.5 mL of Folin- Ciocalteu reagent (Prolabo, Paris France) followed by 4 mL of 1 M sodium carbonate. The test tubes were incubated at 45°C for 5 min and cooled in cold water. Absorbance was measured at 765 nm using a Shimadzu 1600-UV spectrophotometer (Shimadzu, Kyoto, Japan). The results were compared to a gallic acid calibration curve.

Statistical analysis

Statistical analysis was performed using SPSS program (version 20). Analysis of variance (ANOVA) and Duncan's multiple range test ($P \leq 0.05$) were used to establish possible significant variation among the traditional genotypes.

Results and discussion

Morphological analysis

In this study, 9 local tomato accessions were used including one pure line variety (SP) from Vita Morocco Company used as a control. The traditional genotypes were collected from farmers in a limited area and were issued from natural and farmers' selection and adapted to an arid climate. The evaluation of the diversity of these accessions was based on some phenotypic fruit traits, which indicated that fruit size, shape, and colour were the most important characters of the tomato [24] and they were essential for the definition of a variety [5]. Furthermore, other study noticed in the morphological characterization of Greek tomato landraces that the majority of genotypic variation was found especially in fruit traits [25]. The 9 tomato accessions were characterized morphologically by comparing the plant growth type, inflorescence characters, number of flowers per inflorescence, fruit weigh, fruit morphology i.e. fruit shape, colour, and numbers of fruit locules. Those characters were evaluated in each plant and fruit characterization was performed using a pool of at least 25 fruits per accession. The fruits were harvested at the mature stage.

In growth type, all the traditional cultivars produced plants with semi-undetermined growth habit except for the control SP, FI, and H accessions which were undetermined.

For the other morphological and yield traits, significant differences were shown between the traditional genotypes with certain morphology for each genotype. Flowering was an important parameter directly related to yield and productivity of plants. This trait was different across the different tested genotypes. Indeed, the number of days to flowering after sowing ranged from 71 days for FI to 88 days for RB. Inflorescence type varied from uniparous to multiparous and the mean value of flowers per inflorescence varied from 3.91 for FII to 6.07 for RO genotypes (Table 1). Exterior colour of mature fruit varied from red for H and BI to pink for FI including orange for the rest genotypes as the control. In a similar way the largest mean fruit weight (174 g) was recorded by FI (Table 1) while RB had the smallest fruit weight (95 g). Weight difference was found between populations of the studied traditional genotypes and was in general higher than that obtained in the SP control. On a collection of 94 commercial

Table 1. Morphological differences among the selected tomato accessions for some fruit traits.

	fruit weight (g)	fruit width (cm)	fruit lenght (cm)	fruit lenght/ fruit width ratio	number of locules	number of flowers per inflorescence	external ripe fruit color
FI	174.45±69.74^{b}	6,98±1,18^{d}	6,23±1,07^{c}	0,92±0,18^{ab}	6,5±1,12^{e}	4,64±0,79^{ab}	pink
RO	146,64 ± 53,60^{ab}	6,9±0,8^{cd}	5,45±1,17^{bc}	0,79±0,15^{a}	4,66±1,26^{b}	6,07±1,13^{c}	orange
BI	129,60± 61,10^{ab}	6,25±1,11^{bcd}	5,59±1,2^{bc}	0,9±0,13^{ab}	5,75±0,44^{cde}	4±0,67^{a}	red
FII	147,65± 57,67 ^{ab}	6,36±0,88^{bcd}	6,03±1,34^{c}	0,94±0,14^{b}	5,83±1,18^{de}	3,91±0,74^{a}	orange
RB	95,11± 90,62^{a}	4,44±1,37^{a}	3,54±1,19^{a}	0,79±0,09^{a}	3,66±0,68^{a}	5,23±0,73^{b}	orange
SP	140,91±46,38^{ab}	6,11±0,26^{bc}	5,71±0,68^{bc}	0,93±0,08^{b}	5±1,17^{bc}	5±0,87^{b}	orange
BII	135,18±60,65^{ab}	5,82±0,91^{b}	4,81±0,36^{b}	0,85±0,18^{ab}	6±0de	5,17±1,04^{b}	orange
RN	149,50±41,32^{ab}	5,71±0,61^{b}	4,87±0,39^{b}	0,86±0,08^{ab}	5,32±0,7^{bcd}	4,48±1,29^{b}	orange
H	147,60±80,11^{ab}	6,45±0,70 ^{bcd}	5,32±1,47^{bc}	0,84±0,26^{ab}	5,5±0,88^{cd}	4,94±0,98^{b}	red

Values with different letters are different at α= 0.05

cultivars, the calculated mean, minimum, and maximum values for the fruit weight were 58.2 g, 6.8 g, and 161.4 g respectively [26], which was lower than the results of our collection. The study on 142 Ramellet varieties from Balearic Islands and 29 other local varieties showed a variation for the fruit weight quite lower than the present work [27]. Tomato germplasm showing high variance in some traits indicated that these characteristics had more chance to be improved [28]. Variation in fruit morphology is a prevalent characteristic among cultivated tomato [29]. Fruit shape was heterogeneous within the local cultivars. According to this trait, analysis showed that the collection could be subdivided into four genetic groups. Indeed, obovoid fruit shape was a dominant one for FI, FII, and H genotypes with a moderate admixture level of round and flat shape. The control SP and RN genotype had only round shape. RO, RB, and BII landraces showed flat fruit morphology with round/obovoid mixture. BI genotype displayed round fruit shape with some mixture of flat and obovoid shape. Heterogeneity in fruit shapes (round and flattened) within the same variety was found in the Greek variety “Santorini” [25], as well as in the Italian traditional variety “A pera Abruzzese” in which predominantly round fruit, but also flattened and obovoid fruit in a considerable number of cases were observed [30]. This variation is probably originated by spontaneous crossings during multiplication and by possible seed mixing since seed exchange and mixing is quite usual among variety [5]. Regarding fruit locules, the mean value scaled between 3.66 for RB to 6.5 for FI genotype. The width and length of fruit means (Table 1) varied between 4.44 to 6.98 cm and 3.54 to 6.23 cm respectively, which indicated that RB genotype presented the lowest values and FI genotype presented the highest one. In those genotypes FI and RB, the mean fruit weight was strongly and positively related to flowering date, fruit size (fruit length and width), and the number of locules (NL).

Morphological analysis showed that the local RB genotype clearly differentiated from the other traditional genotypes and showed weak fruit traits. Considering these data, variation among genotypes was particularly evident for some traits such as fruit weight and number of locules. The rest descriptors did not show a wide range of variations. However, the studied genotypes were quite distinct. Other studies on phenotypic diversity on tomato landraces from different countries had shown similar or lower values of diversity [25, 31]. The traits observed in this collection were found during the two years of testing indicating that this diversity was maintained through environmental changes and these traditional varieties represented an interesting model to use them as a source of useful genes for future breeding programs.

Molecular analysis

In this investigation fourteen microsatellite markers were selected from the published data

Table 2. Set of simple sequence repeats (SSR) primers used in this investigation.

SSR names	Reference	Motif	Forward	Reverse	chromosome	T° hybridation
SSR14	http://solgenomics.net/	(ATA)9	TCTGCATCTGGTGAAGCAAG	CTGGATTGCCTGGTTGATTT	3	55°C
SSR22	http://solgenomics.net/	(AT)11	GATCGGCAGTAGGTGCTCTC	CAAGAAACACCCATATCCGC	3	50°C
SSR26	http://solgenomics.net/	(CGG)7	CGCCTATCGATACCACCACT	ATTGATCCGTTTGGTTCTGC	2	50°C
SSR63	http://solgenomics.net/	(AT)39	CCACAAACAATTCCATCTCA	GCTTCCGCCATACTGATACG	8	55°C
SSR248	http://solgenomics.net/	(TA)21	GCATTCGCTGTAGCTCGTTT	GGGAGCTTCATCATAGTAACG	10	55°C
SSR578	http://solgenomics.net/	(AAC)6(ATC)5	ATTCCCAGCACAACCAGACT	GTTGGTGGATGAAATTTGTG	6	55°C
TOM236-237	Suliman-Pollatschek *et al.* 2002	(AT)16	GTTTTTTCAACATCAAAGAGCT	GGATAGGTTTCGTTAGTGAACT	9	47°C
TOM184	Suliman-Pollatschek *et al.* 2002	(ATTT)3 (ATT)7	CAACCCCTCTCCTATTCT	CTGCTTTGTCGAGTTTGAA	4	45°C
TOM196-197	Suliman-Pollatschek *et al.* 2002	(GA)14	CCTCCAAATCCCAAAACTCT	TGTTTCATCCACTATCACGA	11	45°C
TOM210-211	Suliman-Pollatschek *et al.* 2002	(ATA)15	CGTTGGATTACTGAGAGGTTTA	ACAAAAATTCACCCACATCG	4	45°C
TMS52	Areshchenkova and Ganal 2002	(AC)14 (AT)18	TTCTATCTCATTTGGCTTCTTC	TTACCTTGAGAATGGCCTTG	12	55°C
TMS56	Areshchenkova and Ganal 2003	(CT)19	GATCTCAAAGGATGAACAATAC	TCATTAGGAGATTCTTTGTATCA	1	55°C
TMS63	Areshchenkova and Ganal 2002	(AT)4(GT)18(AT)9	GCAGGTACGCACGCATATAT	GCTCCGTCAGGAATTCTCTC	1	60°C
TMS65	Areshchenkova and Ganal 2002	(TA)25 (GA)20	AGCTTCATCCATTACGCCAC	GTGCATCTGGCGTACCTACC	12	60°C

or on the website of Solanaceae Genomics Network (Table 2) and were used to screen the traditional tomato varieties and some other commercial tomato hybrids. A group of 8 commercial hybrids tomatoes named 620, 621, 622, ABR 620 Global, 1-G-48-6032, Emperador RZ, BrigeorE, and RTM105 were added to evaluate the SSR markers and to trace the genetic relationship among all tested tomato cultivars.

Almost all the 14 SSR markers used for the genetic analysis, a polymorphic profile was generated with a total of 83 alleles observed (Table 3). High level of polymorphism was observed within the commercial varieties with 46 alleles while a slightly lower allele number was found within the traditional cultivars (37 alleles). The number of alleles amplified for the traditional tomato cultivars assessed from 2 to 5 alleles while this number varies between 2 and 7 for commercial varieties. In self-pollinated crops, such as tomato, genetic variability is low, which results in low polymorphism [32]. Limited allelic variation was observed in other tomato studies by using high number of SSR markers [33]. In the present study it has been shown that phenotypic and genetic analysis confirmed that these traditional cultivars had maintained a wealth of genetic variation likely mediated by human migration, seed exchange, and natural selection. This type of distinctiveness and relevance materials could represent a good chance to add diversity to analyse the structure of the variation in tomato.

The association between molecular markers and morphological traits, mostly those associated with fruit traits, were investigated in several studies [34, 35] showing a significant association between molecular markers linked with known QTLs and morphological descriptors. The loci Tom 236-237 located on chromosome 9 was significantly associated with the number of flowers per inflorescence and the loci TMS52, located on chromosome 12 was linked among other traits with fruit weight. In the present study, Tom 236-237 and TMS52 markers showed the high polymorphism degree for the local traditional tomatoes with five polymorphic amplicons (Table 3) and seemed to be related to the morphological traits observed in those

Table 3. Total number of polymorphic bands and their seize range for 14 SSR markers used on 8 traditional Moroccan tomato genotypes and 9 commercial tomato varieties.

		Polymorphic bands	
SSR names	Seize (pb)	Local Moroccan Tomato genotypes	Commercial varieties
SSR14	166-235	-	3
SSR22	208-214	2	2
SSR26	172-178	-	3
SSR63	206-248	4	-
SSR248	220-251	3	5
SSR578	290-299	-	2
TOM184	163-206	2	4
TOM196-197	206-214	3	4
TOM210-211	216-222	3	2
TOM236-237	154-210	5	7
TMS52	148-178	5	3
TMS56	102-126	3	4
TMS63	154-181	3	3
TMS65	288-298	4	4

genotypes. According to the tested traditional batches, the number of flowers per inflorescence scaled between 3.66 to 6.5, and the fruit weight ranged from 95 to 174 g. The loci TMS 63 located on chromosome 1 was associated with fruit shape obovoid [35], which is a dominant trait in our traditional varieties. This SSR marker gave a polymorphic pattern within the studied tomato Moroccan lots. The correlation with the other markers without a known linkage with QTLs of interest such as SSR 63 and TMS 65 who gave polymorphic amplicons requires further studies.

By using the data scored from the 14 SSR primers, it was possible to subdivide the investigated accessions into two main groups (Figure 1). One group includes 8 tomatoes commercial hybrids named 620, 621, 622, ABR 620 Global,1-G-48-6032, Emperador RZ, BrigeorE, RTM105, and the other contains the traditional varieties which can be in its turn divided into four main clusters. The first separate and more distant cluster holds FII genotype. The second cluster contains two cultivars with each one presented in one branch H and BI. The third one includes the pure line SP, RO, and RN. The fourth cluster is divided into two sub-clusters. One is branched BII and the second one contains RB and FI. Traditional tomato cultivars RO, RN on the one hand, RB and FI on the other hand are closely related to each other and seem to have common origin. This could be explained by the geographical situation of these regions, Rissani and Figuig are both situated in the south of Morocco. It is probably that the farmers exchange the same seeds with more or less an admixture level.

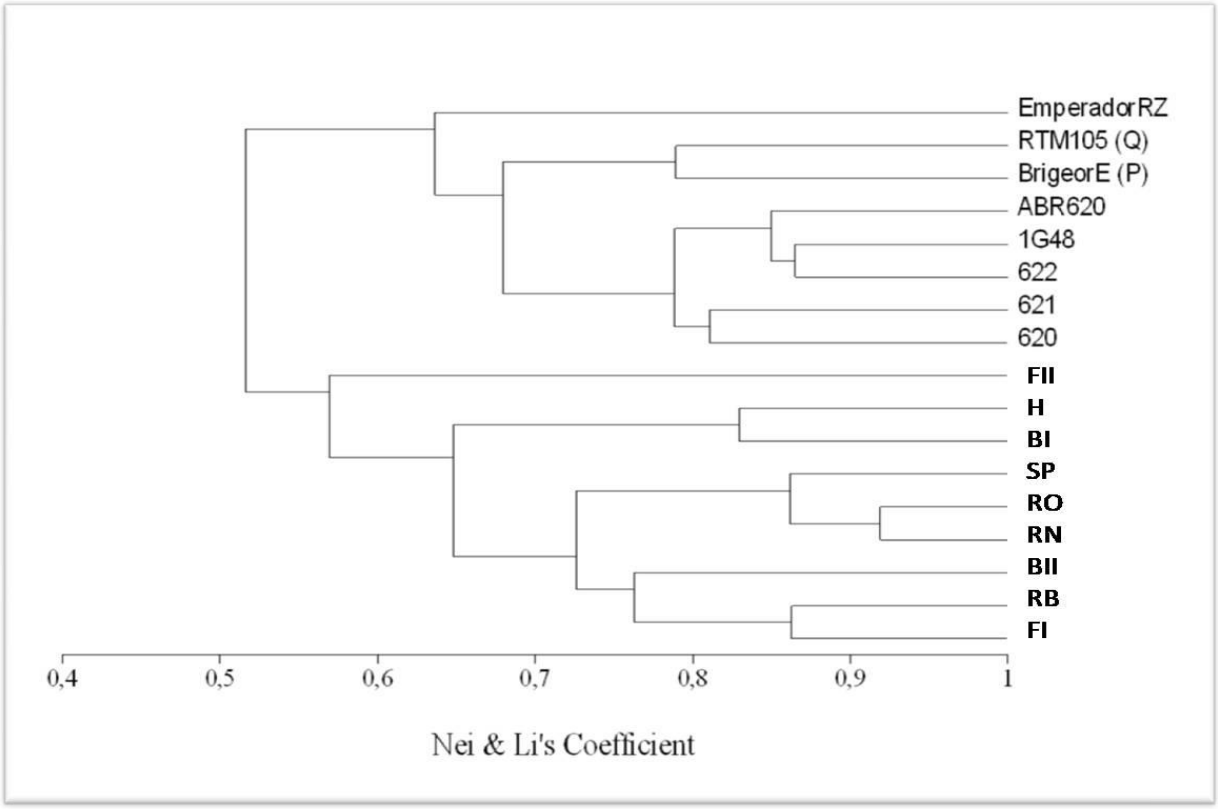

Figure 1. Dendrogram constructed from SSRs data showing relationship among the local Moroccan tomato genotypes and commercial tomato varieties based on Nei and Li (1979) distance and the unweighted pair group arithmetic method (UPGMA).

Electrical conductivity and dry weight

Electrical conductivity (EC) gives an idea on the nutritional quality of a fruit as well as total dissolved solids and organic acids which contribute significantly to fruit's flavour. The EC was directly determined in the juice using a conductivity meter. Tomatoes of the different lots have higher EC. There is a significant difference ($P \leq 0.05$) between varieties tested. EC values ranged from 13.41 ns for RN to 16.48 ns for BII (Table 4). Other study showed that environmental conditions had greater influence on EC than the genetic component [36].

Tomato fruit dry weights (DW) are reported on Table 4. Dry matter corresponds mainly to 30% of soluble sugars (fructose and glucose) and about 25% to fibers [41]. DW values of all tested varieties were between 8.49 and 12.89 %. These values are well above those reported by other authors (4–7%) [37, 38, 39]. In Mediterranean conditions, the values of DW are high at the end of June [37], which was the date of our harvest. Some authors [40] have suggested that the increase in fruit DW observed in summer, which might be the result of a slight water stress that induced a decrease in fruit water content. The highest DW contents were found in RO with 12.89%.

Titrable acidity

Tomato acidity is due to organic and inorganic acids. In fact, organic acids such as citric and malic acids constitute more than 10% of the dry matter. Acids and sugars contribute little to the nutritional value of tomatoes but play an important role in the flavour (acidity-sweetness). During maturation, organic acids can be converted into sugars resulting in a decrease in acidity and an increase in pH. Organic acids (weak acids) have a strong influence on the acidity but not on the pH value [42].

Table 4. Mean value of physic-chemical parameters evaluated in tomato fruits of traditional genotypes and one commercial variety (SP) used as a control.

Genotypes	Electrical conductivity (ns)	Dry weight DW%	Titrable acidity (g/100g citric acid)	pH	Total phenolic contents (mg GAE/100g dry matter)
RB	13,64±0,5a	9.62±0.17a	0.22±0,09a	4,51±0,08bc	361,27±4,28b
FI	14,57±1,00ab	10.64±0.18a	0.46±0,42cde	4,56±0,20cde	421,82±6,45c
FII	13,49±0,51a	9.32±0.13a	0.516±0,11e	4,37±0,05a	669,71±34,14e
H	14,82±0,94abc	10.4±0.15a	0.490±0,70de	4,4±0,02ab	764,40±37,56f
BI	15,21±0,43bcd	8.49±0.25c	0.4±0,11bc	4,67±0,02de	276,34±30,68a
BII	16,48±0,86d	10.51±0.08a	0.517±0,09e	4,53±0,09bcd	430,36±2,44c
SP	16,37±0,64d	9.41±0.07a	0.432±0,10cd	4,78±0,02e	435,23±32,46c
RN	13,41±0,62a	9.46±0.10a	0.35±0,35b	4,53±0,03bcd	465,71±15,99c
RO	15,28±1,15cd	12.89±0.15b	0.516±0,11a	4,64±0,08cde	526,67±34,14d

Values with different letters are different at $\alpha = 0.05$

A significance variation was shown in the titrable acidity (TA) among the tested traditional tomato varieties. The TA ranged from 0.221 for RB to 0.517 g/100 g citric acid for BII genotype (Table 4). Acidity was also evaluated in fruit of 12 tomato genotypes and was reported that fruit acidity varied from 0.256 to 0.704 g/100 g [11]. In similar way, another study reported a variation in TA of fruits of the three processing and six fresh market tomato varieties. The TA values ranged from 0.748 to 0.889 g/100 g [43]. Citric acid contents were not location dependent but were variety dependent [44]. It was reported that higher fruit acidity was an advantage as it caused a lower incidence for fungal infection [45]. In this regard, FII, RO, BII, and H traditional genotypes that have a higher TA value around 0.490 - 0.517 g/100 g are suitable genotypes.

pH values

In this study, tomato fruits of the traditional lots and the control were harvested at the same ripening stage of development. The pH of ripe tomatoes may exceed 4.5 [46]. In this investigation, pH of tomato fruits was significantly different among the tested varieties ($P \leq 0.05$) and varied from 4.37 to 4.78 (Table 4). The pH value of FII was found to be the lowest compared to the pH values of all other traditional tomatoes. The control SP has the highest pH value. This variation seems to be genotypic dependent. The pH below 4.5 is a desirable trait because it halts proliferation of microorganisms and indicates a well quality maintenance [43, 45]. Moreover, high acid value is required for the best flavor.

Determination of total phenolic contents

Phenolic compounds are natural antioxidants and are used like antibiotics and natural pesticides. They are found in plant tissues and act as pollinator attractants. In addition, they have a potential role as a defence mechanism against pathogens and injuries by insects [47].

Tomatoes are known as the most important suppliers of phenolic compounds with other nutrients in human diet [48]. Moreover, genetic factors and growing conditions may play an important role in the formation of phenolic compounds [49]. In this investigation, significantly higher levels of total phenolics were detected in H and FII local tomato genotypes with 764.40 and 669.71 mg GAE/ 100 g DW respectively ($p < 0.05$). BI had lower phenolic contents (276.34 GAE/100 g DW). For the other genotypes, the total extractable phenolic concentrations were found within this range (Table 4). In a study reported on five tomato varieties showed that these values were much lower and ranged from 263.0 to 306.9 mg GAE/100 g DW [50]. In a comparative study, the values of phenolic varied as a function of genotype from 188 to 465 mg GAE/ 100 g DW [11]. The total phenol content has shown to be a very good parameter to distinguish traditional

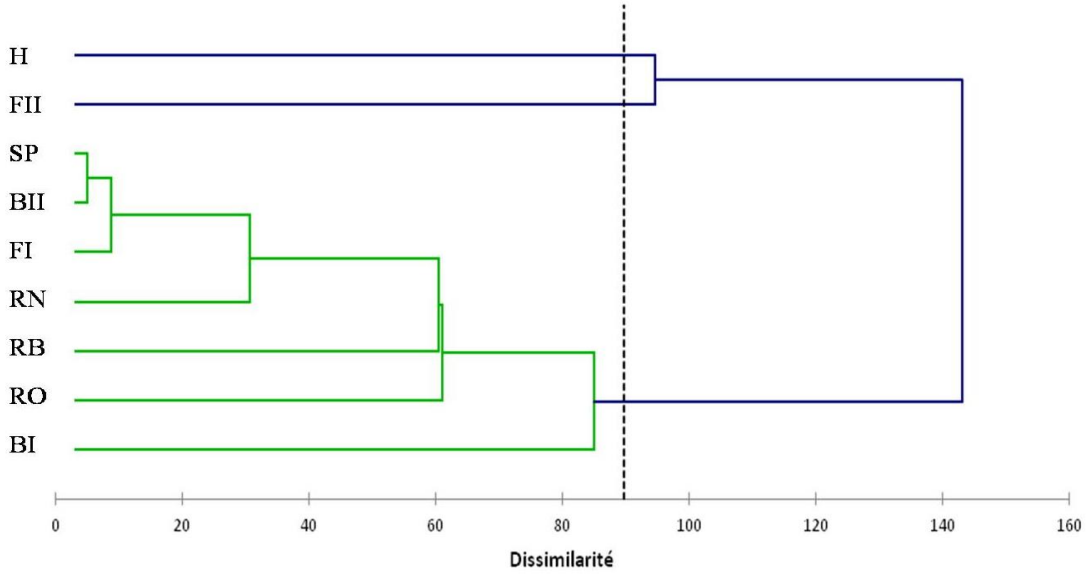

Figure 2. Dendrogram based on physic-chemical analysis of 8 traditional Moroccan tomato genotypes and one commercial variety Saint Pierre (SP).

varieties. It is interesting to note that these local Moroccan tomato genotypes have a high level of phenolic contents which could be useful for germplasm improvement programs.

To evaluate relationships within the traditional tomato varieties, the data scored from physic-chemical composition were combined and used to construct a dendrogram (Figure 2). Two major groups were recognized within the traditional varieties. In the first group, we found that the genotypes H and FII formed a separate cluster and were concordant with the molecular marker data where FII genotype presented a separate and distant cluster. In the second group, six genotypes were clustered together including the commercial variety SP. In this group the genotypes were related to each other (Figure 2) despite their geographical situation, which suggested seed admixture since seed exchange and mixing was quite usual among the farmers.

Conclusions

The current study describes for the first time a thoroughly characterization of Moroccan traditional tomato genotypes based on morphological, molecular, and physic-chemical traits. The results showed that traditional genotypes had several specific traits that could be relevant for their use in local markets as well as for crop improvement and breeding programs. The genotypes (H) and (FII) particularly merit considerable attention because of their phenols richness, high acidity, and low pH. However, for some traditional genotypes, a depuration is necessary to acquire a representative sample in order to increase the level of uniformity and to register these materials as genetic resources conservation in germplasm banks.

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