




# Relationships between chemical composition, antioxidant activity and genetic analysis with ISSR markers in flower buds of caper plants (*Capparis spinosa* L.) of two subspecies *spinosa* and *rupestris* of Spanish cultivars

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**Abstract** Genetic diversity and variability between populations is essential for the long-term survival of plant species as well as their adaptation to different habitats. The *Capparis spinosa* L. has two subspecies in Spain, *spinosa* with stipules thorny and *rupestris* without them. In Spain, the subspecies used for its cultivation is *spinosa*, which is difficult to manipulate due to its stipules thorny. The capers, unripe fruits and tender shoots are used as food. The caper plant is a rich source of phenolic compounds, due to that many flavonoids have been found in different parts of caper plant and in high quantities, which indicates that it is a good source of functional compounds both as food and for nutraceutical applications. There are no published works on the differences in biochemical and functional compounds of both subspecies, so in this work 32 varieties have been genetically analyzed to know

their subspecies. Afterwards, various biochemical and functional parameters have been analyzed to find out if they present differences between both subspecies. From the results of the biochemical and functional parameters studied, there are no difference between the *spinosa* and *rupestris* subspecies, in all the parameters studied, except chlorophylls. There was more difference between the results of the subspecies *spinosa* among them, than with the subspecies *rupestris*. For all this, it can be concluded that the *rupestris* subspecies that does not present stipules thorniness can be cultivated, instead of the *spinosa* subspecies that does present them, without losing functional or nutritional characteristics of the caper buds.

**Keywords** Antioxidant activity · Flavonoids · Flavonols · Phenols · *Capparis spinosa* · ISSR

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## Introduction

Genetic diversity and variability between populations is essential for the long-term survival of plant species (Wang et al. 2016), as well as their adaptation to different habitats. The family *Capparaceae* consists of 39 genera and 650 species, which are distributed in warm areas around the world. In the genus *Capparis*, about 250 species are known (Gull et al. 2015), they

could be native to the tropics and later spread to the Mediterranean basin and Central Asia (Zohary 1960). The *Capparis spinosa* L. is a common perennial winter-deciduous shrub with a summer cycle, and it has a creeping growth. It grows in North Africa, Europe, West Asia, Afghanistan and Australia (Inocencio et al. 2006; Fici 2014; Grimalt et al. 2019). The caper plant is largely cultivated in the Mediterranean basin. In Spain, the most valuable parts of *Capparis spinosa* used as food are the fresh aerial parts, especially the flower buds (capers), unripe fruits and young shoots. These are pickled or kept in brine and used as an appetizer or as a complement to meat, salads, pasta, and other foods (Argentieri et al. 2012; Grimalt et al. 2019). These plants have been used traditionally to prevent a high number of diseases such as diabetes, hepatitis, obesity and kidney problems (Anwar et al. 2016). In recent years, interest in consuming healthy foods has increased (Wu et al. 2004). The caper plant is a rich source of phenolic compounds, due to that many flavonoids have been found in different parts of capers and in high quantities, which indicates that it is a good source of functional compounds both as food and for nutraceutical applications (Grimalt et al. 2018, 2019; Wodyło et al. 2019).

On the other hand, the caper is a xerophytic shrub with a remarkable adaptability to harsh environments. Mediterranean countries are in a region of the world threatened by global warming and *C. spinosa* is a promising crop for arid or semi-arid regions within the climate change context, since the caper plant is highly tolerant to drought and heat stress (Grimalt et al. 2018).

*C. spinosa* present two different subspecies in wild populations and cultivated forms, *C. spinosa* L. subsp. *spinosa* and *C. spinosa* L. subsp. *rupestris* (Sibth. & Sm.) Nyman in Spain (Mateo and Crespo 1995) and in Italy (Gristina et al. 2014). The subsp. *spinosa* is characterized, in the Mediterranean Region, for having branches spreading or erect, multiramified; stipules conspicuous and thorny, mostly recurved, decurrent at the base. While, the subsp. *rupestris* is characterized, in the Mediterranean Region, for having branches pendulous, unramified or few-ramified; stipules mostly setaceous or caducous, when persisting straight or slightly recurved, not decurrent at the base (Fici et al. 2014). Intermediate phenotypes also appear which increases the genetic diversity of this

species (Gristina et al. 2014). From an agricultural point of view, the cultivation and collection of edible parts of this plant is greatly hampered by these stipules thorny. For this reason, it would be very successful to be able to cultivate the subsp. *rupestris*, which does not show stipules thorny, if this subsp. had a high content of compounds, antioxidant activity and nutritional power with respect to subsp. *spinosa*, which does present stipules thorny, and that is the subsp. more cultivated.

Undersanding the level of genetic diversity and the genetic structure of this species, we wanted to do genetic analysis of the collected samples to ensure that they belong to each of the subsp. mentioned or if they are intermediate phenotypes. For this analysis, we have used the inter-simple sequence repeats, also known as ISSRs. This DNA analysis technique is a quick and simple technique with low running costs and requiring only small quantities of template DNA. The production of large number of fragments and the reproducibility are other advantages of these markers (Reche et al. 2019), they do not require prior knowledge of the DNA sequence and can be universally applied as dominating markers (Liu et al. 2015). The ISSR markers have already been used successfully on *C. spinosa* (Saifi et al. 2011; Al-Safadi et al. 2014; Gristina et al. 2014; Liu et al. 2015; Tamboli et al. 2018; Rhimi et al. 2019; Ahmadi et al. 2020). Other DNA analysis techniques such as RAPD (Özbek and Kara 2013) and AFLP (Inocencio et al. 2005; Aichi-Yousfi et al. 2016) have also been used in this species. However, in all cases these techniques have been used to distinguish between several species of the genus *Capparis* or subspecies of *C. spinosa* with respect to morphological characters of the samples.

The objective of this work is to compare two subspecies of *Capparis spinosa*, the subspecies *spinosa*, which has stipules thorny, and the subspecies *rupestris*, which does not. This study is carried out in order to know if the two subspecies have similar chemical and functional characteristics, since the *spinosa* subspecies, which has stipules thorny, is the only one cultivated in Spain and its spines make agronomic work very difficult. If the subspecies *rupestris*, which does not have stipules thorny, had similar functional characteristics, it could be cultivated instead of the subspecies *spinosa*, which would greatly facilitate agronomic work. To our knowledge,

this is the first time this study has been done. For this, as specific objectives of the work, we would have to:

- (i) to carry out their genetic analysis using ISSR markers of thirty-two Spanish cultivars of *Capparis*, to ensure the subspecies to which each sample belongs
- (ii) to study the functional and nutritional properties of twenty-four Spanish cultivars of *Capparis* and finally
- (iii) to study the relationship between the genetic profile and the functional and nutritional properties, with the purpose of knowing if *C. spinosa* subsp. *rupestris* (without stipules thorny) presents a compositional profile differentiate from *C. spinosa* subsp. *spinosa* (with stipules thorny), to know if subsp. *rupestris* can be cultivated without losing functional and nutritional properties.

## Materials and methods

### Experimental conditions and plant material

During the vegetative growth stage of the caper plant (May) forty leaves from four plant (ten per plant) of thirty-two cultivars were hand-harvested in 2018. The cultivars were collected in five locations in the southeast of Spain of which 9 cultivars belong to the subsp. *rupestris* and 22 to the subsp. *spinosa* (Table 1). The leaves were immediately taken to the laboratory

and frozen at  $-80\text{ }^{\circ}\text{C}$  until they were used for genetic analysis.

During the reproductive growth stage of the caper plant (June and July), forty flower buds (capers) from four caper plant (ten per plant) were hand-harvested. However, there were cultivars that presented pests and did not have the sufficient quality of the capers or were not present, so only 24 cultivars were taken (to study the functional and nutritional properties). Once in the laboratory, forty flower buds of each cultivar in the stage of development called surfines (diameter between 7–8 mm) were selected; since they are the stage most used for culinary purposes. Then, the flower buds were freeze-dried using a freeze dryer (Telstar Technologies LyoQuest-55) for 24 h under reduced pressure, 0.220 mbar. The temperature in the drying chamber was  $-25\text{ }^{\circ}\text{C}$ , while the heating plate reached  $15\text{ }^{\circ}\text{C}$ . After drying, the samples were stored vacuum-packed in a freezer at  $-80\text{ }^{\circ}\text{C}$  until analysis.

### Genetic study

#### DNA extraction

Genomic DNA was extracted from young leaves, following the CTAB method with slight modifications (Doyle and Doyle 1990). The extracted DNA was dissolved in water Milli-Q and the final concentration was adjusted to  $15\text{ ng }\mu\text{L}^{-1}$ , using a Nanodrop spectrophotometer (ThermoFisher Scientific, Waltham, USA).

**Table 1** *Capparis* cultivars and site of collection

Population	Cultivars	Subspecies <sup>a</sup>	Site of collection (town, province, country)	Latitude, longitude, altitude (m)
EPSO	TE1, TE2, TE3, TE4, TE5, TE6, TE7, TE8, TE9, TE10	<i>rupestris</i>	Orihuela, Alicante, Spain	38°5' N, 0°56' W, 24
Serón	TS1, TS2, TS3, TS4, TS5, TS6, TS7, TS8	<i>spinosa</i>	Serón, Almería, Spain	37°21' N, 2°32' W, 822
Águilas	TA1, TA2, TA3, TA4, TA5, TA6, TA7	<i>spinosa</i>	Águilas, Murcia, Spain	37°24' N, 1°34' W, 21
La Alberca	TALB1, TALB2, TALB3, TALB4, TALB5	<i>spinosa</i>	La Alberca, Murcia, Spain	37°56' N, 1°07' W, 88
La Alcayna	TALCY1, TALCY2	<i>spinosa</i>	La Alcayna, Murcia, Spain	38°05' N, 1°09' W, 212

<sup>a</sup>Plant species were identified by an expert botanist from the Department of Applied Biology using the protocol by García-Rollán (1981)

### PCR optimization and ISSR selection

We used 6 markers of the UBC primer set #9 of the University of British Columbia Biotechnology Laboratory (Vancouver, Canada), and 12 markers from the work of Al-Safadi et al. (2014) (Table 2). These markers were the more polymorphic in previous studies (Al-Safadi et al. 2014). Annealing temperature was optimised by running a gradient PCR between 45 and 60 °C. Annealing temperature of 53 °C obtained the best results. Amplification with each arbitrary primer was repeated twice and only those primers that produced reproducible and consistent bands were only selected for data generation.

### PCR amplifications

Reactions were carried out in 25- $\mu$ L volume containing 30 ng template DNA, 0.5 U TaqDNA polymerase, 10 mM dNTP, 10  $\mu$ M primer in 1  $\times$  reaction buffer that contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl and 2.5 mM MgCl<sub>2</sub>. The temperature profile used was: a denaturation for 2 min at 94 °C, then 35 cycles consisting each of a denaturation step for 30 s at 94 °C;

an annealing step for 30 s at 53 °C; an extension step for 1 min at 72 °C and the final extension for 5 min at 72 °C, using a Eppendorf Mastercycler Gradient (Hamburg, Germany).

### Electrophoresis conditions

Amplified products were loaded on 1.5% agarose gel and separated in 1  $\times$  TAE buffer at 100 V. The gels were visualized under UV after staining with ethidium bromide and documented using a gel documentation and image analysis system (Vilber Lourmat, Collégien, France).

### Data analysis

The banding patterns were scored as present (1) or absent (0). Only clear and repeatable fragments were considered in the genetic analysis. Band size determination was carried out using the molecular weight marker GeneRuler100 bp Plus DNA Ladder (ThermoFisher Scientific, Waltham, USA).

Three indexes were calculated: MR (Multiplex Ratio), PIC and RP (Resolving Power). The MR is defined as the number of polymorphic loci found in a reaction (Powell et al. 1996). For dominant (presence/absence) markers the PIC is defined as  $1 - F_{aa}^2 - F_{an}^2$ , where  $F_{aa}^2$  is the frequency of the amplified allele and  $F_{an}^2$  is the frequency of the non-amplified allele. The RP is defined as  $\sum I_b$ , being  $I_b = 1 - (2|0.5 - p|)$ , where  $p$  is the frequency of the genotypes that contain the band. It represents the ability of a marker to discriminate against the different studied accessions.

Phylogenetic relationships among accessions were estimated from the molecular characterization data, using the package NTSYSpc 2.0 (Adams et al., 1998). Dendrogram was constructed using the Unweighted Pair Group Method with arithmetic averaging (UPGMA) clustering analysis based on the genetic similarity coefficient matrices (Nei and Li 1979). Statistical stability of the branches in the cluster was estimated by bootstrap analysis with 1000 replicates, using the Winboot software program (Yap and Nelson 1996). Population structure was estimated using a model based Bayesian procedure implemented in the software Structure v. 2.3.4 (Pritchard et al. 2000). An admixture model with correlated allele frequencies without prior population information was used. The most informative number of subpopulations was

**Table 2** ISSR markers and primer sequence

Marker	Primer sequence
UBC807	AGAGAGAGAGAGAGAGT
UBC814	CTCTCTCTCTCTCTA
UBC817	CACACACACACACAA
UBC820	GTGTGTGTGTGTGTGTC
UBC825	ACACACACACACACT
UBC829	TGTGTGTGTGTGTGTGC
ISSR1	CACCACCACCACCACCACT
ISSR2	GAGAGAGAGAGAGAGAC
ISSR7	CACACACACACACACACAG
ISSR8	CTCTCTCTCTCTCTCTG
ISSR10	TCCTCCTCCTCCTCC
ISSR13	AGAGAGAGAGAGAGAGG
ISSR14	GAGAGAGAGAGAGAGAT
ISSR15	GAGAGAGAGAGAGAGAC
ISSR16	GAGAGAGAGAGAGAGAA
ISSR19	CTCTCTCTCTCTCTTT
ISSR22	GTGTGTGTGGTGTGTA
ISSR43	ACACACACACACACCTA

identified using the K method (Evanno et al. 2005) with the aid of Structure Harvester (Earl and Vonholdt 2012). The estimated cluster membership coefficient matrices of the 20 runs were permuted so that all replicates have the closest match possible and then averaged across replicates, using the Greedy algorithm of the software CLUMMP (Jakobsson and Rosenberg 2007). To validate the predefined or the estimated population structure, we calculated pairwise *F<sub>st</sub>* and Nei's standard genetic distance between populations (Nei and Li 1979). The reference distribution for *P* value calculation of the *F<sub>st</sub>* analysis was calculated using 10,000 permutations. These analyses were performed with the Genalex 6.5 software (Peakall and Smouse 2012).

#### Biochemical, nutritional and functional parameters

In the capers the following parameters were measured in triplicate:

Chlorophylls *a* and *b* were extracted for each sample using 85% acetone according to Official Method AOAC (1990). The absorbance was read at 664 nm, using Helios Gamma spectrophotometer (model, UVG 1002E; Helios, Cambridge, UK). The results were expressed in mg 100 g<sup>-1</sup> dry weight (dw).

Total carotenoids were extracted according to Valero et al. (2011), with acetone and diethyl ether to promote phase separation. The lipophilic phase was used to estimate the total carotenoid content and the absorbance was measured at 450 nm using the same Helios Gamma spectrophotometer named above. The results were expressed as mg of carotenoids 100 g<sup>-1</sup> dw.

To determine the protein content, the Bradford method (1976) was used, using the Bio-Rad reagent. For the quantification, a standard curve of pure bovine serum albumin (BSA) was used, according to Grimalt et al. (2019). The absorbance was measured at 595 nm using the same Helios Gamma spectrophotometer named above. The results were expressed as mg g<sup>-1</sup> dw.

To estimate the total flavonoids and flavonols, extracts of methanolic caper buds were used using 80% methanol and a weight-to-volume ratio of 1/50, stirring for 24 h (Argentieri et al. 2012). The total phenols were quantified according to Singleton et al. (1999), with slight modifications, using the Folin-

Ciocalteu reagent and the calibration curve was performed with gallic acid. The absorbance was measured at 760 nm using the same Helios Gamma spectrophotometer named above. The results were expressed as mg GAE 100 g<sup>-1</sup> dw.

Total flavonoids were quantified using the method of Chang et al. (2002) with some modifications. The reaction was kept at room temperature for 30 min and the absorbance was measured at 415 nm, using the same Helios Gamma spectrophotometer named above. Total flavonols were quantified using the Kumaran et al. (2007) method with some modifications, and absorbance was measured at 440 nm. The results of flavonoids and flavonols were expressed as mg rutin Eq. 100 g<sup>-1</sup> dw.

The activity of hydrophilic-total antioxidant activity (H-TAA) and lipophilic antioxidant activity (L-TAA) of caper buds were determined in the aqueous and organic phases, respectively. The reaction mixture contained 10 mM ABTS, 1 mM hydrogen peroxide, and 10 mM peroxidase in a total volume of 1 mL of 50 mM glycine-HCl buffer (pH 4.5) for H-TAA, or ethyl acetate for L-TAA. The reaction was monitored at 730 nm until a stable absorbance was obtained using the same spectrophotometer named above. After that, a suitable amount of caper buds extract was added and the observed decrease in absorbance was determined. A calibration curve was performed with Trolox as antioxidant standard for both H-TAA and L-TAA (Arnao et al. 2001). The results were expressed as mg Trolox equivalent 100 g<sup>-1</sup> dw.

The determination of the total antioxidant activity made by three methods FRAP, ABTS<sup>+</sup> and DPPH<sup>•</sup>. The ferric reducing ability of plasma (FRAP) and (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) (ABTS<sup>+</sup>) antioxidant assays were determined following Benzie and Strain (1996) and Re et al. (1999), respectively, using the same Helios Gamma spectrophotometer named above. The radical scavenging activity was evaluated using the DPPH<sup>•</sup> radical (2,2-diphenyl-1-picrylhydrazyl) method, as described by Brand-Williams et al. (1995). The results were expressed in mM Trolox per 100 g of dw.

#### Statistical analysis

For physical, chemical and biochemical parameters, a basic descriptive statistical analysis was followed by one-way analysis of variance test (ANOVA) for mean



comparisons. Three analyzes have been carried out: grouping the cultivars by subspecies, grouping cultivars by populations and without grouping. The method used to discriminate among the means (multiple range test) was Fisher's LSD (Least Significant Difference) procedure at a 95.0% confidence level. These analyses were performed using the software package SPSS 18.0 for Windows (SPSS Science, Chicago, USA).

Correlation between the different determined parameters was calculated in R using the package 'reshape2' v. 1.4.3 (Wickham 2007). A Principal Component Analysis (PCA) was performed to determine which combination of attributes are contributing to phenotypic diversity in our populations. PCA was conducted in R using the package 'FactoMineR' v. 1.41 (Lê et al. 2008). Three different samples from each population were used to perform the PCA, and the mean from values of weight and diameter/length parameters was included in each population in the data matrix.

## Results and discussion

### Genetic diversity and hierarchical classification

The 32 *Capparis* cultivars were amplified consistently with 8 of the 18 primers (Table 2 and Fig. 1). The number of products generated per primer was found to range from 5 to 15 of different sizes in the range of 0.23–2.20 kb (Table 3). The primer ISSR15 exhibited the maximum (15) product whereas primer ISSR10 gave the least (5) number of products. A total of 83 amplified products were produced with an average of 10.37 products per primer, of which 81 (97.6%) were polymorphic and 2 (2.4%) products were monomorphic (Table 3). The percentage of polymorphic bands ranged from 80% for primer ISSR10 to 100% for the other primers. These ISSR primers gave a high PIC value of 0.446 for primer ISSR16 and low PIC value of 0.305 for primer UBC825, with an average PIC value of 0.356 per primer. An average RP of 5.56 per primer was obtained with the highest RP value of 7.59 for primer ISSR16, and the lowest value of 2.40 for primer ISSR10 (Table 3). These results are similar with obtained through ISSR by Gristina et al. (2014) in Italy and Al-Safadi et al. (2014) in Syria, lower than those obtained by Tamboli et al. (2018) in India and Rhimi et al. (2019) in Tunisia, while they are clearly superior

to those obtained with AFLP by Inocencio et al. (2005) in Spain. All works included several *Capparis* species and/or subspecies.

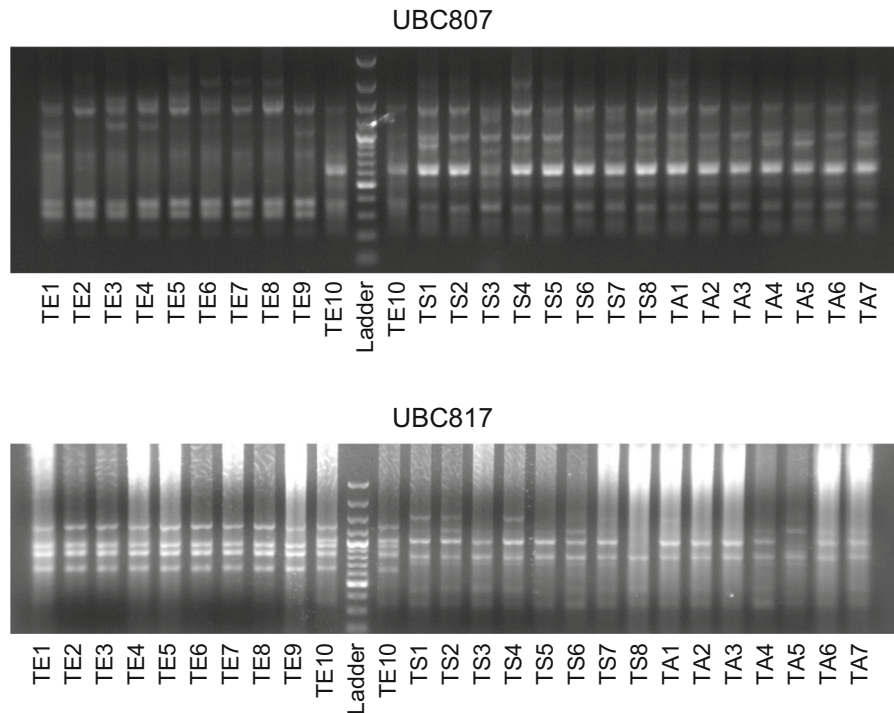
The dendrogram obtained by the ISSR data appears in Fig. 2. Nei's similarity coefficient ranged from 0.20 to 0.96. In the dendrogram, obtained only with 8 ISSR markers, two thirds of the nodes were supported by bootstrap values high than 25%, which indicates the robustness of the result obtained. All cultivars could be distinguished with the ISSR markers used. Intra-population variability was obtained in all the populations. Subspecie-specific or unique fragments were detected in all the markers (Tables 2 and 3), as in previous works with ISSR (Al-Safadi et al. 2014; Gristina et al. 2014) and AFLP markers (Inocencio et al. 2005).

In the dendrogram (Fig. 2) the cultivars are grouped in two main clusters. Cluster I contains all the TE cultivars with the TE10 cultivars as the most different. Cluster II contains all the rest cultivars, with a good grouping obtained for the others groups: all TS cultivars, except TS7, all TA cultivars, all TALB cultivars except TALB4, and all TALCY cultivars. The clustering of cultivars by subspecies was also found by Gristina et al. (2014) in Italy, as well as by species by Al-Safadi et al. (2014) in Syria.

Figure 3 shows the PCA obtained with the ISSR results. The first two main principal components (PC1 and PC2) explained the 58.9 and the 7.1% of the variability, respectively. The cultivar distribution was very similar to that of the dendrogram, with two large groups, and the TE10 cultivar in an intermediate position. No cultivar TE has stipules thorny except TE10, which does. Due to these stipules thorny and the results obtained with PCA, it can be thought that TE10 is a hybrid between the *spinosa* and *rupestris* subspecies.

### Genetic structure analysis

The identification of genetically similar groups of plants was performed using an admixture model-based clustering analysis implemented in the software Structure. The Evanno's test indicated that the most informative number of subpopulations (K) is 2, suggesting the existence of two major clusters in present *Capparis* cultivars. The inferred population structure is presented in Fig. 4. The groups defined by the Structure's analysis represent statistically different



**Fig. 1** Banding profiles obtained with the markers UBC807 and UBC817 in part of the studied plants. In each gel, the ladder is GeneRuler100 bp Plus (ThermoFisher)

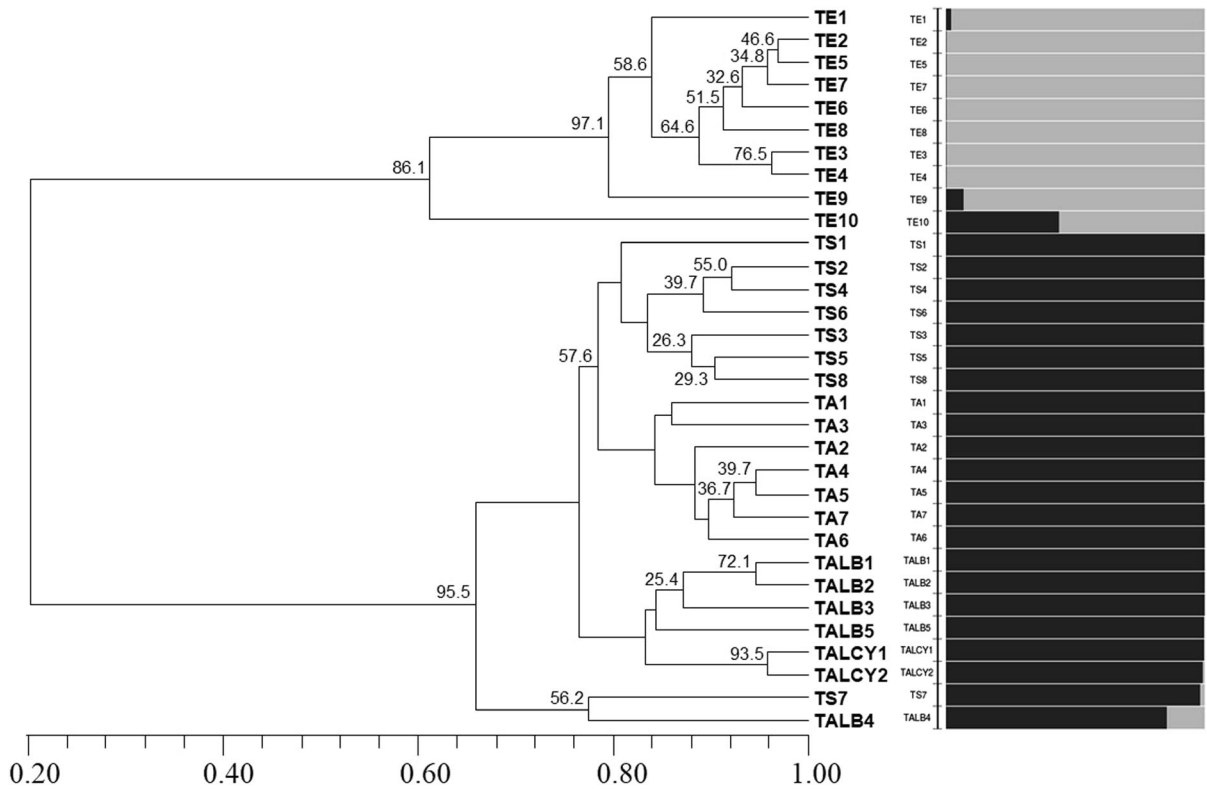
**Table 3** Genetic variation obtained with ISSR markers

Marker	Number of bands	Size range (pb)	MR	PIC	RP	Unique bands (Number, population)
UBC807	12	320–2200	11	0.323	6.00	3, TE; 4, rest
UBC817	11	350–1650	11	0.417	6.69	4, TE; 7, rest
UBC820	7	1000–2200	7	0.349	3.83	2, TE; 1, rest
UBC825	10	460–1750	10	0.305	4.44	1, TE
ISSR10	5	800–1800	4	0.336	2.40	2, TE; 2, rest
ISSR14	12	250–1500	12	0.364	6.57	2, rest
ISSR15	15	230–1750	15	0.312	6.94	4, TE; 3, rest
ISSR16	11	300–1350	11	0.446	7.59	3, TE; 2, rest
Total	83	ND	81	ND	ND	18, TE; 21 rest
Average	10.37	ND	10.12	0.356	5.56	

MR multiplex ratio, RP resolving power, PIC polymorphic information content, ND not determined

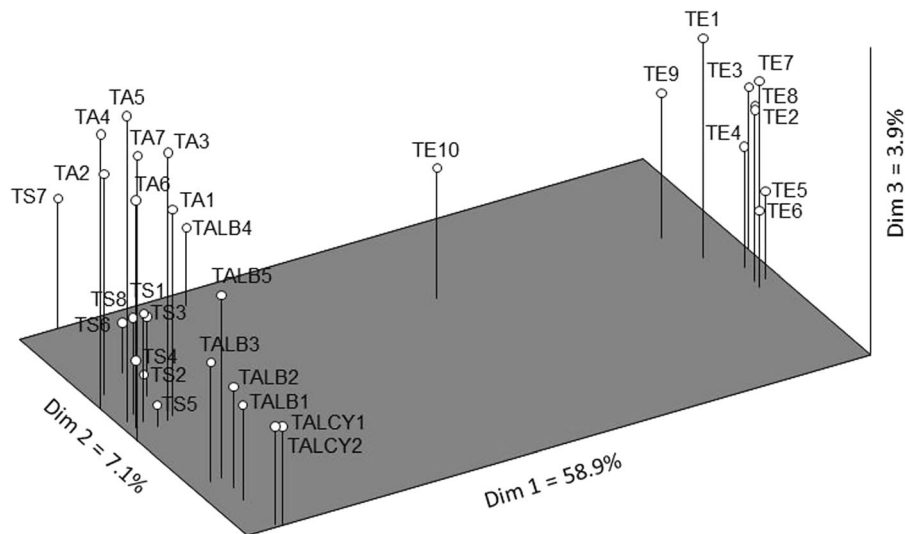
subpopulations, as indicated by the evaluation of genetic differentiation. All the cultivars formed two subpopulations: subpopulation 1 consisting of all the TE cultivars (*C. spinosa* subsp. *rupestris*) and

subpopulation 2 containing the rest of the cultivars of *C. spinosa* subsp. *spinosa* (TS, TA, TALB and TALCY). Bayesian analysis performed in Structure agrees the results obtained with the UPGMA



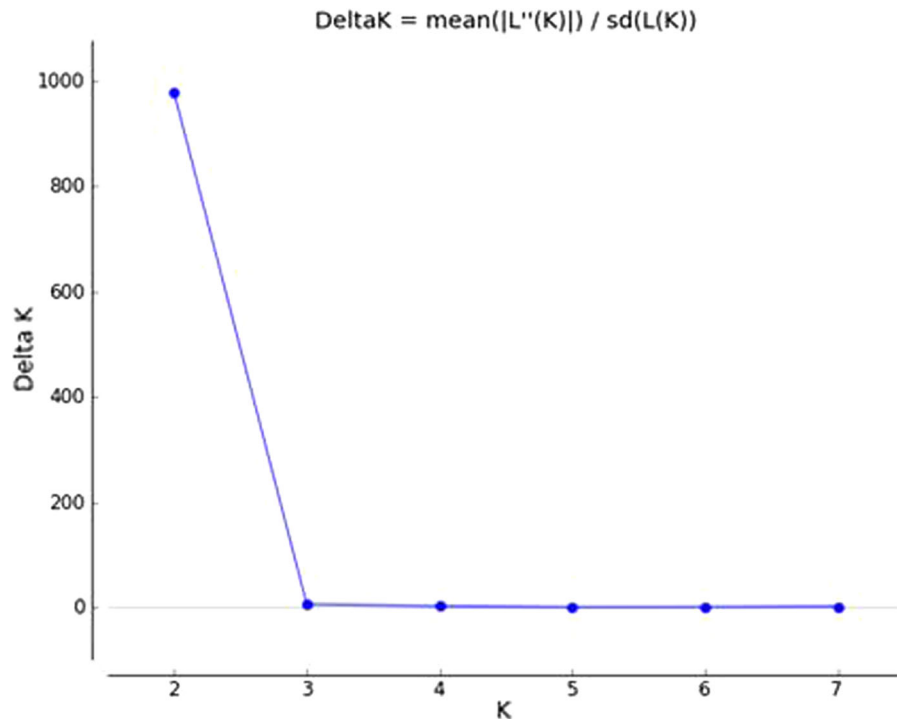
**Fig. 2** Genetic relationship among accessions. Left: Dendrogram (UPGMA) of the *Capparis* plants based on genetic distances calculated with the genetic similarity coefficient matrices. The numbers near nodes represent the percentage of time when the node occurred among 1000 bootstraps (only for

nodes with bootstrap values  $> 25\%$  are given). Right: estimated population structure of the pea genotypes for  $K = 2$ . Each genotype is represented by a horizontal line, which is partitioned into colored segments that represent the estimated membership fractions in the  $K$  clusters



**Fig. 3** Principal component analysis (PCA) using the ISSR results





**Fig. 4** Estimation of the optimum number of clusters for the pea genotypes according to the Evanno's method. The graph displays the Delta K [ $\text{mean}(|L''(K)|)/\text{SD}(L(K))$ ] for each K value

dendrogram and the PCA, indicating the robustness of the results obtained.

Chlorophyll, carotenoid and protein content in flower buds

The content of chlorophyll *a*, chlorophyll *b* and total chlorophylls is shown in Table 4. Chlorophyll *a* presented values from 9.07 to 21.65 mg 100 g<sup>-1</sup> dw in cultivars 'TA5' and 'TE9', respectively. Chlorophyll *b* content presented its highest value in cultivar 'TE1' with a total of 8.48 mg 100 g<sup>-1</sup> dw, followed by cultivar 'TE4' with a total of 8.44 mg 100 g<sup>-1</sup> dw, being cultivar 'TALCY2' the one with the lowest value, with a total of 4.25 mg 100 g<sup>-1</sup> dw. Regarding the total chlorophylls, the values oscillated from 14.09 to 29.44 mg 100 g<sup>-1</sup> dw, in cultivars 'TA5' and 'TE9', respectively. As observed in Fig. 5, there was a positive correlation between chlorophylls *a*, chlorophylls *b* and total chlorophylls.

The total content of chlorophylls was influenced by the subsp and population (Table 4). Thus, the *C. spinosa* subsp. *rupestris* showed the highest content of total chlorophylls compared to *C. spinosa* subsp.

*spinosa*. Regarding the populations, the highest mean value was observed for the TE, TS and TALB (> 23.3 mg 100<sup>-1</sup> dw) and the lowest for TA and TALCY (< 17.47 mg 100<sup>-1</sup> dw).

Therefore, *spinosa* cultivars show a low content of chlorophylls with respect to *rupestris*, although there are some populations of *spinosa* that have not presented significant differences with *rupestris*. Therefore, there are more differences between the *spinosa* populations than the average between *spinosa* and *rupestris*.

The carotenoid content ranged from 20.88 to 59.73 mg β-carotene 100 g<sup>-1</sup> dw, being the cultivar 'TS2' the one with the highest value, followed by cultivar 'TS1' (56.90 mg β-carotene 100 g<sup>-1</sup> dw). On the contrary, the cultivar 'TA4' has presented a lower value in carotenoid content. If we relate the results by geographical areas, the TS population presented the highest values of carotenoid content with a mean value of 49.62 mg β-carotene 100 g<sup>-1</sup> dw, followed by 'TALB', 'TALCY' and 'TE' with similar values without significant differences between them, with means values of 45.91, 44.92 and 42.20 mg β-carotene 100 g<sup>-1</sup> dw, respectively.

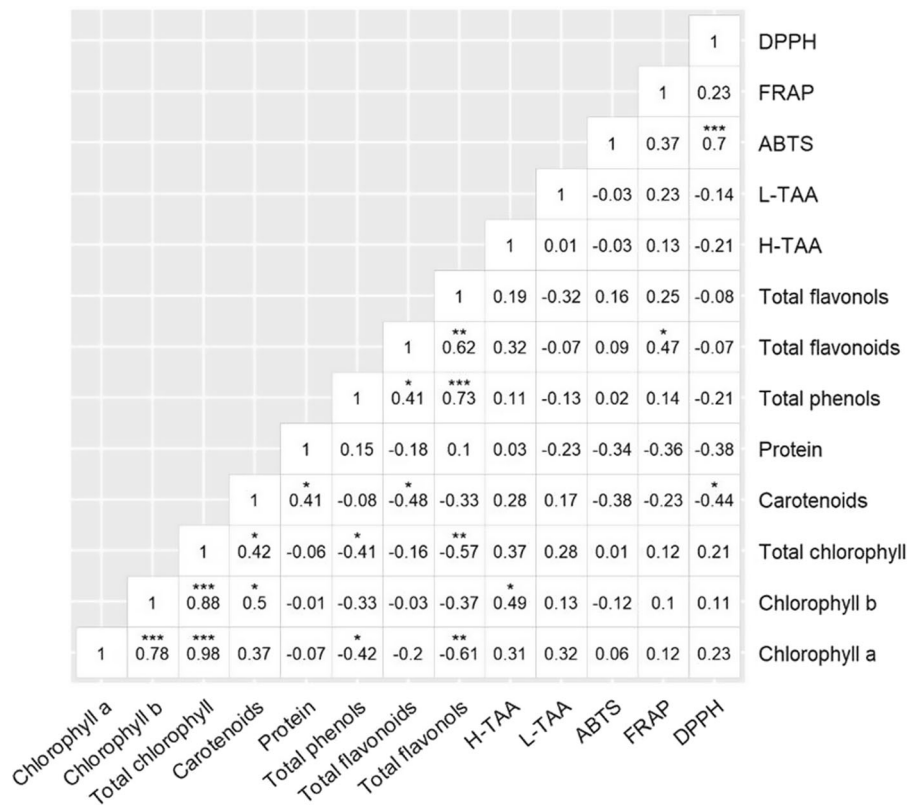
**Table 4** Photosynthetic pigments (mg 100<sup>-1</sup> g dw), carotenoids (mg β-carotene 100 g<sup>-1</sup> dw) and proteins content (mg g<sup>-1</sup> dw) of flowers buds

	Chlorophyll <i>a</i>	Chlorophyll <i>b</i>	Total chlorophyll	Carotenoids	Protein
<i>Subsp.</i>					
<i>rupestris</i>	18.86 b	7.42 b	26.27 b	42.20	10.47
<i>spinosa</i>	14.79 a	6.28 a	21.07 a	42.50	11.96
<i>Population</i>					
TE	18.86 d	7.42 b	26.27 b	42.20 ab	10.47 a
TS	16.05 bc	7.27 b	23.31 b	49.62 b	11.79 ab
TA	12.36 a	5.11 a	17.47 a	32.86 a	11.84 ab
TALB	17.97 cd	7.25 b	25.21 b	45.91 ab	13.53 b
TALCY	11.13 a	4.25 a	15.38 a	44.92 ab	8.63 a
<i>Cultivar</i>					
'TE 1'	20.90 ± 1.31 <sup>lmn</sup>	8.48 ± 1.17 <sup>g</sup>	29.36 ± 1.07 <sup>j</sup>	52.04 ± 5.89 <sup>ijklm</sup>	9.84 ± 0.64 <sup>bc</sup>
'TE 2'	18.97 ± 1.76 <sup>ijklmn</sup>	8.24 ± 0.59 <sup>g</sup>	27.21 ± 2.28 <sup>ij</sup>	38.40 ± 2.73 <sup>defg</sup>	9.82 ± 0.40 <sup>bcd</sup>
'TE 3'	19.77 ± 1.50 <sup>ijklmn</sup>	8.23 ± 0.62 <sup>g</sup>	28.00 ± 1.90 <sup>ij</sup>	33.45 ± 1.47 <sup>bcd</sup>	9.01 ± 0.06 <sup>abcd</sup>
'TE 4'	19.08 ± 0.55 <sup>ijklmn</sup>	8.44 ± 0.49 <sup>g</sup>	27.51 ± 1.00 <sup>ij</sup>	46.83 ± 0.57 <sup>ghijk</sup>	9.47 ± 0.38 <sup>bc</sup>
'TE 5'	19.85 ± 1.20 <sup>ijklmn</sup>	6.82 ± 0.63 <sup>defg</sup>	26.67 ± 1.03 <sup>ij</sup>	34.66 ± 4.91 <sup>defgh</sup>	9.39 ± 0.62 <sup>abcd</sup>
'TE 6'	20.12 ± 0.99 <sup>klmn</sup>	6.21 ± 0.83 <sup>cde</sup>	26.33 ± 0.20 <sup>hij</sup>	49.65 ± 2.73 <sup>hijkl</sup>	11.12 ± 1.09 <sup>bcdef</sup>
'TE 7'	17.24 ± 0.96 <sup>fghij</sup>	7.70 ± 0.45 <sup>efg</sup>	24.93 ± 1.19 <sup>ghi</sup>	46.49 ± 2.09 <sup>ghijk</sup>	11.11 ± 0.57 <sup>bcdef</sup>
'TE 8'	18.50 ± 0.25 <sup>hijklm</sup>	6.63 ± 0.36 <sup>def</sup>	25.12 ± 0.50 <sup>ghi</sup>	50.34 ± 1.05 <sup>hijklm</sup>	11.90 ± 0.32 <sup>cdef</sup>
'TE 9'	21.65 ± 0.97 <sup>n</sup>	7.80 ± 0.46 <sup>fg</sup>	29.44 ± 1.18 <sup>j</sup>	45.42 ± 2.94 <sup>ghij</sup>	11.62 ± 1.04 <sup>bcdef</sup>
'TE 10'	12.48 ± 0.45 <sup>bcd</sup>	5.67 ± 0.51 <sup>abcd</sup>	18.14 ± 0.87 <sup>bcd</sup>	24.72 ± 0.32 <sup>ab</sup>	11.46 ± 0.20 <sup>bcdef</sup>
'TS 1'	14.94 ± 1.20 <sup>cdef</sup>	7.80 ± 0.55 <sup>fg</sup>	22.73 ± 0.65 <sup>efg</sup>	56.90 ± 3.38 <sup>lm</sup>	10.47 ± 0.68 <sup>bcde</sup>
'TS 2'	18.40 ± 1.02 <sup>hijkl</sup>	8.42 ± 0.36 <sup>g</sup>	26.81 ± 0.72 <sup>ij</sup>	59.73 ± 1.64 <sup>m</sup>	13.03 ± 1.48 <sup>ef</sup>
'TS 3'	17.65 ± 0.86 <sup>ghijk</sup>	7.46 ± 0.43 <sup>efg</sup>	25.10 ± 1.12 <sup>ghi</sup>	43.55 ± 1.75 <sup>efghij</sup>	11.58 ± 0.47 <sup>bcdef</sup>
'TS 4'	14.18 ± 0.09 <sup>cde</sup>	5.82 ± 0.43 <sup>bcd</sup>	19.99 ± 0.45 <sup>cdef</sup>	42.93 ± 4.73 <sup>efghi</sup>	12.24 ± 1.54 <sup>def</sup>
'TS 5'	15.06 ± 0.44 <sup>bcdefgh</sup>	6.86 ± 0.04 <sup>cdefg</sup>	21.91 ± 0.41 <sup>efg</sup>	45.02 ± 3.67 <sup>ghij</sup>	11.67 ± 0.95 <sup>cdef</sup>
'TA 1'	12.59 ± 0.77 <sup>bcd</sup>	4.40 ± 0.33 <sup>ab</sup>	16.99 ± 0.46 <sup>abc</sup>	27.17 ± 2.71 <sup>abc</sup>	9.02 ± 1.04 <sup>ab</sup>
'TA 2'	13.30 ± 0.28 <sup>bcd</sup>	5.44 ± 0.26 <sup>abcd</sup>	18.73 ± 0.14 <sup>cde</sup>	32.35 ± 3.12 <sup>bcd</sup>	11.29 ± 1.36 <sup>bcdef</sup>
'TA 3'	11.69 ± 0.20 <sup>abc</sup>	5.79 ± 0.22 <sup>bcd</sup>	17.48 ± 0.35 <sup>bcd</sup>	47.65 ± 5.45 <sup>ghijk</sup>	16.66 ± 1.26 <sup>g</sup>
'TA 4'	15.16 ± 0.40 <sup>defg</sup>	4.92 ± 0.32 <sup>abc</sup>	20.08 ± 0.54 <sup>cdef</sup>	20.88 ± 2.20 <sup>a</sup>	10.79 ± 1.55 <sup>bcdef</sup>
'TA 5'	9.07 ± 0.21 <sup>a</sup>	5.02 ± 0.09 <sup>abc</sup>	14.09 ± 0.29 <sup>a</sup>	36.23 ± 2.18 <sup>cdef</sup>	11.44 ± 0.41 <sup>bcde</sup>
'TALB 1'	16.58 ± 0.49 <sup>efghi</sup>	6.43 ± 0.46 <sup>cdef</sup>	23.01 ± 0.39 <sup>fgh</sup>	53.96 ± 1.90 <sup>klm</sup>	15.71 ± 0.49 <sup>g</sup>
'TALB 2'	21.35 ± 1.53 <sup>mn</sup>	7.93 ± 0.78 <sup>fg</sup>	29.27 ± 2.28 <sup>j</sup>	52.66 ± 0.28 <sup>ijklm</sup>	14.42 ± 0.35 <sup>fg</sup>
'TALB 4'	15.97 ± 0.21 <sup>defghij</sup>	7.39 ± 0.51 <sup>defg</sup>	23.36 ± 0.75 <sup>efghi</sup>	31.11 ± 1.61 <sup>bcde</sup>	10.46 ± 1.80 <sup>bcdef</sup>
'TALCY 2'	11.13 ± 2.25 <sup>ab</sup>	4.25 ± 0.47 <sup>a</sup>	15.38 ± 2.72 <sup>ab</sup>	44.92 ± 3.75 <sup>fghijkl</sup>	8.63 ± 1.59 <sup>a</sup>

Values (means ± standard error) followed by the same letter, within the same column, were not significantly different according to Fisher's least significant difference (LSD) procedure at 95% confidence level (n = 30). TE: cultivar EPSO; TS: cultivar Serón; TA: cultivar Aguilas; TALB: cultivar La Alberca; TALCY: cultivar La Alcayna

On the other hand, the protein content was not affected by subsp. but population affected it. The 'TALB' population presented the highest values (13.53 mg 100 g<sup>-1</sup> dw), followed by the 'TS' and

'TA' population that presented mean values of 11.79 and 11.84 mg 100 g<sup>-1</sup> dw, respectively, without significant differences between them. The lowest mean values were observed for 'TE' and 'ALCY'



**Fig. 5** Pearson coefficient of the correlations between chemical and biochemical parameters. Positive correlations are represented by positive values, and negative correlations by negative

values. The significance level of correlations is represented by \* ( $p$  value < 0.5), \*\* ( $p$  value < 0.01) and \*\*\* ( $p$  value < 0.001)

(10.47 and 8.63 mg 100 g<sup>-1</sup> dw, respectively). The protein content was in a range of values between 8.63 and 16.66 mg 100 g<sup>-1</sup> dw, being the cultivar 'ALCY2' the one that has presented the lowest content and the cultivar 'TA3' the one that has presented the highest value. The protein content is affected by the genetic differences of the cultivars.

However, the protein content did not show significant differences between the *spinosa* and *rupestris* subspecies, finding more differences between the *spinosa* populations than between the average *rupestris* and *spinosa*.

In Fig. 5, a positive correlation is observed between chlorophylls *b* and carotenoids, for this reason it is observed that the cultivar 'TE1' presents the highest value in chlorophylls *b* and carotenoids. In the case of cultivar 'TA4' the same happens it has presented low values in both chlorophylls *b* and carotenoids.

There are not many previous studies on the biochemical properties of *C. spinosa* in Spain. These results did not agree those reported by Grimalt et al. (2019), who observed higher content of total chlorophylls and proteins and lower content of carotenoids compared to ours. This may be due to the fact that the collection dates were different and possibly the climatic effects have generated these differences. Ulukapi et al. (2016) obtained a carotenoid content of 21.24 mg kg<sup>-1</sup> in capers from Turkey, values lower than those of the present study. Tlili et al. (2009) obtained a carotenoid content in a range between 411.3 and 3452.5 µg g<sup>-1</sup> fw, so our values are in the same interval. Grimalt et al. (2019), Özcan and Akgül (1998) and Ulukapi et al. (2016) have been obtained higher results in the content of protein that in this study. This is possibly due that they are found in different geographical areas and different cultivars.

**Table 5** Total phenols (mg GAE 100 g<sup>-1</sup> dw), flavonoids and flavonols (mg rutin Eq. 100 g<sup>-1</sup> dw) of flowers buds

	Total phenols	Total flavonoids	Total flavonols
<i>Subsp.</i>			
<i>rupestris</i>	1571.45 a	2166.70	1426.43 a
<i>spinosa</i>	2183.54 b	2187.79	1810.32 b
<i>Population</i>			
TE	1571.45 a	2166.70 ab	1426.43 a
TS	2445.69 b	2197.61 ab	1854.39 b
TA	2440.54 b	2305.08 b	1993.95 b
TALB	1547.84 a	2046.02 a	1551.37 a
TALCY	1494.86 a	1977.69 a	1448.62 a
<i>Cultivar</i>			
'TE 1'	1694.14 ± 32.87 <sup>bc</sup>	2334.27 ± 55.01 <sup>defgh</sup>	1353.31 ± 49.93 <sup>ab</sup>
'TE 2'	1676.79 ± 43.12 <sup>bc</sup>	2404.08 ± 79.22 <sup>fgh</sup>	1642.30 ± 116.25 <sup>bcdefgh</sup>
'TE 3'	896.25 ± 4 9.26 <sup>a</sup>	2157.95 ± 156.90 <sup>abcdefgh</sup>	1428.35 ± 230.24 <sup>abcd</sup>
'TE 4'	1396.44 ± 97.72 <sup>ab</sup>	2140.19 ± 65.93 <sup>abcdefgh</sup>	1410.55 ± 26.73 <sup>abc</sup>
'TE 5'	2030.31 ± 2.17 <sup>cde</sup>	1954.20 ± 84.26 <sup>abc</sup>	1378.24 ± 50.27 <sup>abc</sup>
'TE 6'	1679.32 ± 28.96 <sup>bc</sup>	2054.93 ± 75.14 <sup>abcde</sup>	1363.02 ± 66.71 <sup>ab</sup>
'TE 7'	1724.51 ± 22.59 <sup>bc</sup>	2043.17 ± 91.89 <sup>abcde</sup>	1449.51 ± 85.33 <sup>abc</sup>
'TE 8'	1491.06 ± 129.54 <sup>b</sup>	2032.83 ± 166.00 <sup>abcd</sup>	1299.80 ± 72.48 <sup>a</sup>
'TE 9'	1633.26 ± 59.60 <sup>bc</sup>	2215.48 ± 81.54 <sup>bcdefgh</sup>	1421.08 ± 54.86 <sup>abcd</sup>
'TE 10'	1492.40 ± 162.13 <sup>b</sup>	2329.86 ± 195.99 <sup>defgh</sup>	1518.16 ± 122.96 <sup>abcde</sup>
'TS 1'	2899.88 ± 66.53 <sup>g</sup>	2274.37 ± 82.88 <sup>bcdefgh</sup>	2107.02 ± 82.02 <sup>jk</sup>
'TS 2'	1784.84 ± 289.65 <sup>bcd</sup>	2063.61 ± 60.18 <sup>abcdef</sup>	1669.10 ± 30.01 <sup>cdefg</sup>
'TS 3'	2416.73 ± 249.64 <sup>ef</sup>	2293.41 ± 125.07 <sup>cdefgh</sup>	1897.33 ± 121.84 <sup>fghij</sup>
'TS 4'	2432.23 ± 361.11 <sup>ef</sup>	2099.84 ± 21.05 <sup>abcdefg</sup>	1758.88 ± 146.34 <sup>defghij</sup>
'TS 5'	2694.75 ± 168.28 <sup>fg</sup>	2256.83 ± 93.01 <sup>bcdefgh</sup>	1839.64 ± 16437 <sup>fghij</sup>
'TA 1'	2771.26 ± 150.92 <sup>fg</sup>	2248.99 ± 155.73 <sup>bcdefgh</sup>	1869.77 ± 170.12 <sup>fghij</sup>
'TA 2'	2772.67 ± 240.67 <sup>fg</sup>	2276.86 ± 8.25 <sup>bcdefgh</sup>	1930.43 ± 39.08 <sup>ghijk</sup>
'TA 3'	2787.94 ± 244.86 <sup>fg</sup>	2349.45 ± 141.45 <sup>efgh</sup>	1951.59 ± 80.72 <sup>hijk</sup>
'TA 4'	2308.72 ± 43.12 <sup>def</sup>	2495.46 ± 153.91 <sup>h</sup>	2227.77 ± 225.85 <sup>k</sup>
'TA 5'	1562.10 ± 46.65 <sup>bc</sup>	2154.63 ± 58.86 <sup>abcdefgh</sup>	1990.21 ± 68.06 <sup>ijk</sup>
'TALB 1'	1506.16 ± 181.30 <sup>b</sup>	1849.55 ± 43.59 <sup>a</sup>	1425.30 ± 50.85 <sup>abc</sup>
'TALB 2'	1473.36 ± 73.12 <sup>b</sup>	1995.32 ± 190.12 <sup>abcd</sup>	1453.98 ± 109.27 <sup>abcde</sup>
'TALB 4'	1664.00 ± 69.45 <sup>bc</sup>	2293.18 ± 158.43 <sup>bcdefgh</sup>	1774.82 ± 211.57 <sup>efghi</sup>
'TALCY 2'	1494.86 ± 103.08 <sup>bc</sup>	1977.59 ± 71.00 <sup>ab</sup>	1448.62 ± 57.21 <sup>abc</sup>

Values (means ± standard error) followed by the same letter, within the same column, were not significantly different according to Fisher's least significant difference (LSD) procedure at 95% confidence level (n = 30). TE: cultivar EPSO; TS: cultivar Serón; TA: cultivar Aguilas; TALB: cultivar La Alberca; TALCY: cultivar La Alcayna

Total phenols content (TPC), flavonoids and flavonols in the flower buds

Table 5 shows the results of total phenols content (TPC), flavonoids and flavonols analyzed in flower buds. The total phenols content and total flavonols were affected by subsp and population, while the total flavonoids was only affected by population.

Regarding the cultivars, higher value of total phenols content was observed in the cultivar 'TS1'

with a total of 2899.88 mg GAE 100 g<sup>-1</sup> dw, followed by the cultivar 'TA2', being on the contrary the cultivars 'TE3' the one who presented the lowest values (896.25 mg GAE 100 g<sup>-1</sup> dw). On the other hand, significant differences were detected between populations and also among subsp. Flowers buds of 'TS' and 'TA' populations showed the highest total phenols content (> 2.440 mg GAE 100 g<sup>-1</sup> dw) in opposition to populations 'TE', 'TALB' and 'TALCY'; while between subsp. the flower buds of

subsp. *spinosa* showed the higher total phenols content in comparison with subsp. *rupestris*. The results obtained showed that the total phenols content was influenced by the genotype and environment conditions.

In reference to the total flavonoid content, this was not affected by the subsp.; however, significant differences were reported for populations and among cultivars. Thus, the maximum values were obtained for the flower buds of 'TA', 'TS' and 'TE' populations ( $> 2160$  mg rutin Eq.  $100\text{ g}^{-1}$  dw). Regarding among cultivars the total flavonoid content ranged from 1849.55 to 2495.46 mg rutin Eq.  $100\text{ g}^{-1}$  dw ('TALB1' and 'TA4', respectively). Finally, the total flavonols content, was also significantly affected by subsp., populations and cultivars. Thus, the flower buds with higher value was observed for subsp. *spinosa* and among populations for 'TS' and 'TA' ( $> 1854.39$  mg rutin Eq.  $100\text{ g}^{-1}$  dw); while among cultivars the higher values were observed for cultivar 'TA4' (2227.77 mg GAE  $100\text{ g}^{-1}$  dw), while the cultivar 'TE8' presented the lower content compared to the other cultivars.

There is a negative correlation between chlorophylls *a* and total chlorophylls with the total content of total phenols and flavonols, and this is reflected in the results obtained (Fig. 5). Thus, the cultivar 'TE9' has presented a high content of chlorophylls *a* and total with respect to the other cultivars, while in the content of total phenols and flavonols it has presented one of the lowest values.

Aliyazicioglu et al. (2013) obtained a total phenol value of 37.01 mg GAE  $100\text{ g}^{-1}$  DW, which is much lower than those obtained in this study. In another study carried out in different regions of Tunisian (Tlili et al. 2010), low values were obtained compared to those obtained in the present work, with a total of 2.74 mg  $100\text{ g}^{-1}$  fw. However, Mansour et al. (2016) obtained higher values in *C. spinosa* than those of the present work, with a total of 427.27 mg GAE  $\text{g}^{-1}$  dw in total phenols and 57.93 mg QE  $\text{g}^{-1}$  dw in flavonoids. Tesoriere et al. (2007) obtained 48.75 mg GAE  $100\text{ g}^{-1}$ . Maldini et al. (2016) obtained values of total phenols between 98 and 149 mg  $100\text{ g}^{-1}$  FW, among which flavonoids were the majority with 82–117 mg RE  $100\text{ g}^{-1}$  FW. Inocencio et al. (2000) observed a wide variation in the flavonoid contents of capers from different regions and they proposed that environmental and physiological factors could have

important effects. Compared with total phenols of other crops such as mango (208 mg  $100\text{ g}^{-1}$ ), bilberry (525 mg  $100\text{ g}^{-1}$ ) or raspberry (517 mg  $100\text{ g}^{-1}$ ), the results in *Capparis spinosa* showed that it is an excellent natural source of these compounds (Tlili et al. 2010).

#### Total content of antioxidant activity (TAA) in the flower buds

The total antioxidant activity has been quantified by the ABTS<sup>+</sup> method in the hydro-soluble (H-TAA) and lipidic-soluble (L-TAA) fractions in the caper flower buds of *C. spinosa* (Table 6). Results from TAA (both H-TAA and L-TAA) showed that genotype plays an important role in determining the antioxidant capacity of each cultivar. H-TAA did not show differences between subspecies, although it did between populations, being the cultivar 'TS' the one that presents the highest values, with an average of 1403.36 mg Trolox Eq.  $100\text{ g}^{-1}$  dw, followed by 'TE' cultivars that presented an average of 1036.93 mg Trolox Eq.  $100\text{ g}^{-1}$  dw. 'TA', 'TALB' and 'TALCY' presented the lowest values and without significant differences between them. Regarding cultivars, the H-TAA content showed values in a range between 384.99 and 1883.13 mg Trolox Eq.  $100\text{ g}^{-1}$  dw in cultivars 'TALB4' and 'TS1', respectively. In the case of the L-TAA content, there were differences between subspecies and population, being the *rupestris* subspecies and the 'TE' population the ones that presented the highest L-TAA. Regarding the cultivars, 'TE6' presented the highest value with 460.00 mg Trolox Eq.  $100\text{ g}^{-1}$  dw and the lowest was presented by 'TALB2' with a total of 158.49 mg Trolox Eq.  $100\text{ g}^{-1}$  dw. Although the differences were not significant in the mean of the subsp. *rupestris* and *spinosa* for the H-TAA parameter and they have been for the L-TAA parameter, in both cases more differences were found in the contents of both parameters between the populations of *spinosa* than between the means of *spinosa* and *rupestris*.

Antioxidant activity was not significantly different among the subsp. and populations, but yes among cultivars (Table 6). In all cases of TAA, there were more differences between the *spinosa* populations than between *spinosa* and *rupestris* populations. The antioxidant activity by the ABTS<sup>+</sup> method presented the highest values in the cultivar 'TE5' with a total of

**Table 6** H-TAA and L-TAA and total antioxidant activity by different methods: ABTS<sup>+</sup>, DPPH<sup>•</sup> and FRAP (mg Trolox Eq. 100 g<sup>-1</sup> dw) of flower buds

	H-TAA	L-TAA	ABTS <sup>+</sup>	FRAP	DPPH <sup>•</sup>
<i>Subsp.</i>					
<i>rupestris</i>	1036.93	305.85 b	547.00	1799.91	804.46
<i>spinosa</i>	967.56	214.54 a	550.46	1653.09	723.12
<i>Population</i>					
TE	1036.93 ab	305.81 b	547.7	1799.91	804.46
TS	1403.36 b	215.88 a	535.92	1787.52	578.87
TA	821.18 a	246.74 ab	585.28	1720.64	686.29
TALB	654.44 a	168.67 a	481.81	1389.95	986.39
TALCY	459.84 a	184.46 a	655.01	1432.66	838.72
<i>Cultivar</i>					
'TE 1'	1126.15 ± 72.48 <sup>fgh</sup>	279.11 ± 33.75 <sup>fg</sup>	497.07 ± 37.54 <sup>cdef</sup>	2191.79 ± 221.01 <sup>gh</sup>	462.79 ± 22.53 <sup>abcd</sup>
'TE 2'	1223.37 ± 32.55 <sup>fghij</sup>	276.41 ± 83.45 <sup>hi</sup>	578.92 ± 20.02 <sup>fg</sup>	2033.86 ± 114.38 <sup>fgh</sup>	582.17 ± 28.28 <sup>cdefgh</sup>
'TE 3'	1133.69 ± 98.56 <sup>efghi</sup>	279.34 ± 100.66 <sup>hi</sup>	661.02 ± 186.22 <sup>gh</sup>	1259.21 ± 64.07 <sup>bc</sup>	1392.11 ± 93.36 <sup>lm</sup>
'TE 4'	1046.38 ± 107.14 <sup>efgh</sup>	359.80 ± 26.30 <sup>hi</sup>	544.13 ± 27.03 <sup>ef</sup>	1733.26 ± 14.52 <sup>def</sup>	961.86 ± 15.77 <sup>ijk</sup>
'TE 5'	562.26 ± 235.40 <sup>ab</sup>	264.66 ± 49.14 <sup>def</sup>	801.68 ± 10.26 <sup>k</sup>	1751.53 ± 31.79 <sup>bcddef</sup>	1939.25 ± 164.69 <sup>n</sup>
'TE 6'	619.55 ± 4.53 <sup>abcd</sup>	460.00 ± 14.15 <sup>j</sup>	460.03 ± 30.03 <sup>bcd</sup>	1857.65 ± 73.84 <sup>fg</sup>	439.51 ± 10.26 <sup>abc</sup>
'TE 7'	1033.70 ± 139.08 <sup>efg</sup>	324.63 ± 13.74 <sup>gh</sup>	515.10 ± 43.05 <sup>def</sup>	1964.28 ± 97.11 <sup>fgh</sup>	645.75 ± 92.86 <sup>efgh</sup>
'TE 8'	929.89 ± 98.90 <sup>def</sup>	361.32 ± 5.71 <sup>hi</sup>	514.35 ± 31.54 <sup>def</sup>	2009.33 ± 43.55 <sup>fgh</sup>	571.41 ± 85.85 <sup>cdefg</sup>
'TE 9'	1548.77 ± 57.88 <sup>ijkl</sup>	267.81 ± 21.53 <sup>f</sup>	552.64 ± 63.07 <sup>ef</sup>	1922.23 ± 286.58 <sup>fgh</sup>	533.12 ± 42.55 <sup>abcde</sup>
'TE 10'	1145.59 ± 34.37 <sup>fghi</sup>	185.01 ± 48.29 <sup>a</sup>	345.15 ± 14.02 <sup>a</sup>	1275.98 ± 174.20 <sup>bc</sup>	516.60 ± 65.33 <sup>abcde</sup>
'TS 1'	1883.13 ± 243.83 <sup>l</sup>	277.90 ± 21.87 <sup>fg</sup>	431.25 ± 31.54 <sup>abcd</sup>	1876.67 ± 38.54 <sup>fg</sup>	503.58 ± 81.34 <sup>abcde</sup>
'TS 2'	1696.57 ± 422.64 <sup>ghijk</sup>	189.55 ± 37.05 <sup>ab</sup>	490.82 ± 12.26 <sup>cdef</sup>	1819.61 ± 121.14 <sup>f</sup>	544.38 ± 48.31 <sup>bcd</sup>
'TS 3'	1445.03 ± 160.89 <sup>hijk</sup>	171.49 ± 10.12 <sup>abc</sup>	688.30 ± 29.78 <sup>hi</sup>	1797.08 ± 86.85 <sup>efg</sup>	765.89 ± 36.04 <sup>hij</sup>
'TS 4'	1237.76 ± 136.07 <sup>fghij</sup>	235.17 ± 11.6 <sup>def</sup>	653.51 ± 46.30 <sup>gh</sup>	1797.08 ± 116.64 <sup>efg</sup>	698.81 ± 42.30 <sup>fghi</sup>
'TS 5'	754.29 ± 101.82 <sup>bcd</sup>	205.31 ± 5.85 <sup>bcd</sup>	415.73 ± 10.51 <sup>abc</sup>	1647.16 ± 196.23 <sup>cdef</sup>	381.69 ± 39.55 <sup>ab</sup>
'TA 1'	787.52 ± 164.32 <sup>cdef</sup>	241.19 ± 16.70 <sup>ef</sup>	483.06 ± 21.78 <sup>cdef</sup>	1117.54 ± 224.01 <sup>b</sup>	511.84 ± 111.88 <sup>abcde</sup>
'TA 2'	470.78 ± 176.44 <sup>a</sup>	235.85 ± 12.97 <sup>cdef</sup>	541.63 ± 404.72 <sup>ef</sup>	1910.46 ± 315.87 <sup>fgh</sup>	752.37 ± 131.15 <sup>ghi</sup>
'TA 3'	889.61 ± 148.29 <sup>def</sup>	265.23 ± 5.59 <sup>f</sup>	560.40 ± 287.33 <sup>f</sup>	1259.96 ± 192.47 <sup>b</sup>	459.28 ± 44.80 <sup>abcd</sup>
'TA 4'	1312.24 ± 347.10 <sup>ijkl</sup>	248.72 ± 33.89 <sup>def</sup>	774.40 ± 178.46 <sup>ij</sup>	2974.45 ± 111.63 <sup>h</sup>	1225.92 ± 95.61 <sup>l</sup>
'TA 5'	645.74 ± 101.01 <sup>abcd</sup>	242.69 ± 23.94 <sup>ef</sup>	566.91 ± 193.72 <sup>fg</sup>	1340.80 ± 15.52 <sup>bcd</sup>	482.06 ± 48.81 <sup>abcde</sup>
'TALB 1'	448.45 ± 21.93 <sup>ab</sup>	186.48 ± 9.80 <sup>abcd</sup>	384.95 ± 4.51 <sup>ab</sup>	1069.49 ± 61.07 <sup>b</sup>	371.18 ± 9.76 <sup>a</sup>
'TALB 2'	1129.87 ± 114.29 <sup>efghi</sup>	158.49 ± 27.95 <sup>a</sup>	480.56 ± 52.26 <sup>bcd</sup>	393.21 ± 10.26 <sup>a</sup>	992.65 ± 78.34 <sup>k</sup>
'TALB 4'	384.99 ± 32.96 <sup>ab</sup>	161.05 ± 18.18 <sup>abcd</sup>	579.92 ± 159.43 <sup>fg</sup>	2707.14 ± 115.88 <sup>h</sup>	1595.35 ± 39.05 <sup>m</sup>
'TALCY 2'	459.84 ± 29.11 <sup>abc</sup>	184.46 ± 6.49 <sup>abcde</sup>	655.01 ± 183.46 <sup>gh</sup>	1432.66 ± 108.88 <sup>bcd</sup>	838.72 ± 28.28 <sup>ijk</sup>

<sup>a</sup>Values (means ± standard error) followed by the same letter, within the same column, were not significantly different according to Fisher's least significant difference (LSD) procedure at 95% confidence level (n = 30). TE: cultivar EPSO; TS: cultivar Serón; TA: cultivar Aguilas; TALB: cultivar La Alberca; TALCY: cultivar La Alcayna

801.68 mg Trolox Eq. 100 g<sup>-1</sup> dw, followed by the cultivar 'TA4' with a total of 774.4 mg Trolox Eq. 100 g<sup>-1</sup> dw, being on the contrary the cultivar 'TE10' the one that presents a low value with a total of

345.15 mg Trolox Eq. 100 g<sup>-1</sup> dw. On the other hand, the antioxidant activity by the FRAP method presented a range between 393.21 and 2974.45 mg Trolox Eq. 100 g<sup>-1</sup> dw, in cultivars 'TALB2' and



'TA4' respectively. The cultivar 'TE5' presented the highest value in DPPH method (1939.25 mg Trolox Eq. 100 g<sup>-1</sup> dw), followed by the cultivar 'TALB4' (1595.35 mg Trolox Eq. 100 g<sup>-1</sup> dw). On the contrary, the cultivar 'TALB1' was the one that presented the lowest value, with a total of 371.18 mg Trolox Eq. 100 g<sup>-1</sup> dw.

Grimalt et al. (2019) obtained slightly higher results on the content of H-TAA in flower buds and in L-TAA lower than those of the present work. Tesoriere et al (2007) found lower values of H-TAA and L-TAA in flower buds of Italian cultivars. Aliyazicioglu et al. (2013) obtained lower FRAP values in Turkish cultivars. Mansour et al. (2016) also obtained lower ABTS values in Tunisian cultivars. These differences may be because genotypic and environmental differences.

#### Correlation between traits and principal component analysis

Those traits that measure chlorophylls content have a very close positive correlation among them. Chlorophyll *a* and total chlorophyll are correlated negatively with total phenols and total flavonols, whereas that chlorophyll *b* is positively correlated with carotenoids and H-TAA. Carotenoids are positively correlated with total chlorophyll, chlorophyll *b* and protein, and negatively with total flavonoids and DPPH. Phenols, flavonoids and flavonols have a positive correlation among them. FRAP and total flavonoids are correlated positively, as well as DPPH and ABTS (Fig. 5).

In order to gain a better understanding of the results and trends of the variables studied (13 traits and 24 *Capparis* cultivars), the main components analysis (PCA) was applied and results are showed in Table 7 and Figs. 6 and 7.

The first three main components accounted for 68.44% of the total variation for the results obtained in the caper buds, and the 52.01% of the variability of the data studied were explained by the first two components. PC1 and PC2 from the PCA explained the 31.35 and the 20.66% of the variability, respectively (Table 7). The 'TE' group (except 'TE10') is mainly defined by the amount of chlorophylls, while the 'TA' and 'TS' groups (except 'TS2'), 'TYALC' and 'TE10' are principally correlated with phenols, flavonols and flavonoids. 'TS2', 'TALB1' and 'TALB2' are defined by the amount of carotenoids, but not 'TALB4'

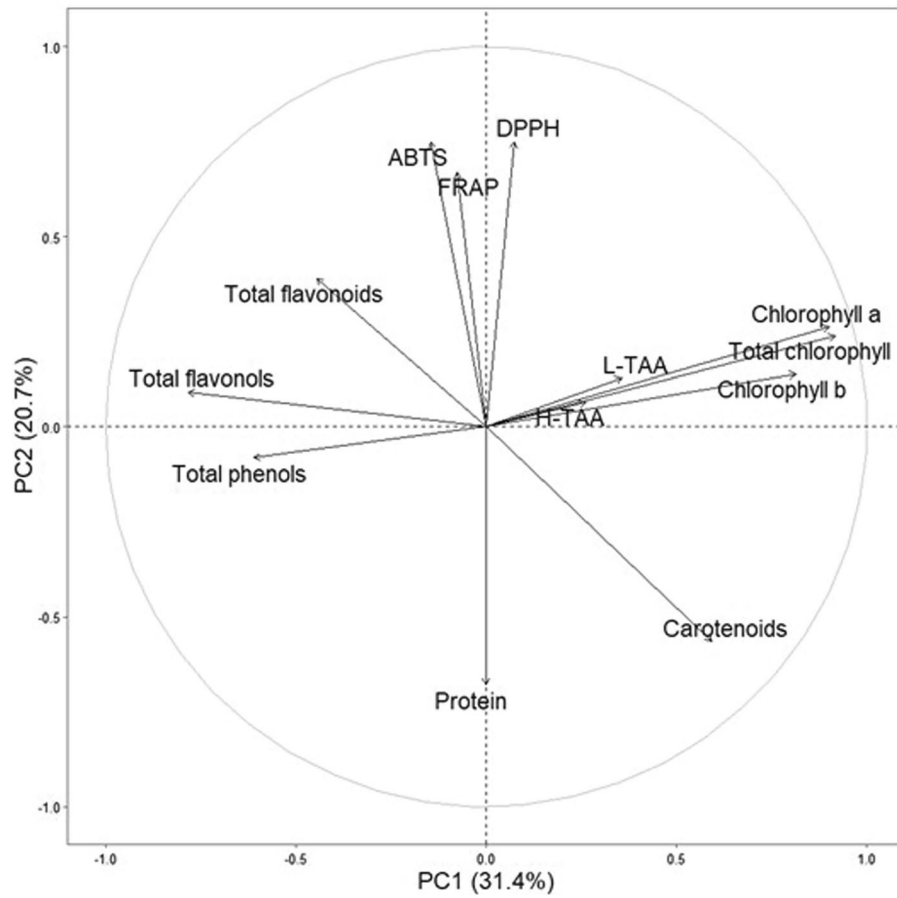
**Table 7** Eigenvectors of each variable in the three first Principal Components obtained with chemical and biochemical parameters

	PC1	PC2	PC3
Eigenvalues	4.08	2.69	2.14
Cumulative variation (%)	31.35	52.01	68.44
Parameters	Eigenvectors		
Chlorophyll a	0.90	0.26	0.13
Chlorophyll b	0.81	0.14	0.40
Total chlorophylls	0.92	0.24	0.22
Carotenoids	0.59	- 0.56	0.25
Protein	0.00	- 0.68	0.17
Phenols	- 0.61	- 0.08	0.50
Flavonoids	- 0.44	0.39	0.63
Flavonols	- 0.78	0.09	0.50
H-TAA	0.26	0.07	0.78
L-TAA	0.36	0.13	0.04
ABTS <sup>+</sup>	- 0.14	0.75	- 0.18
DPPH*	0.07	0.75	- 0.40
FRAP	- 0.08	0.67	0.36

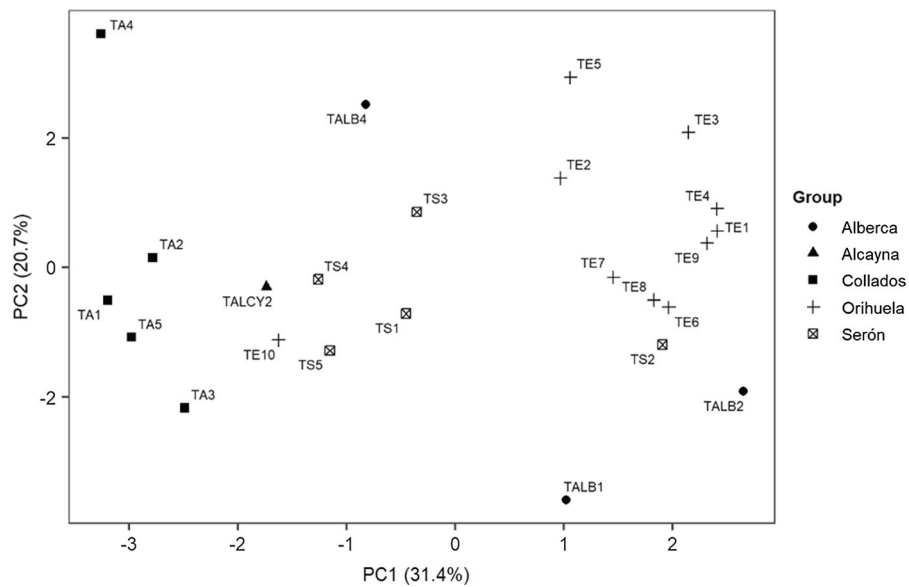
(Figs. 6 and 7). The first component (PC1) was positively related to chlorophylls, H-TAA and L-TAA, and negatively related to total phenols, flavonoids and flavonols. The PC2 was positively correlated with ABTS, FRAP and DPPH, and was negatively correlated with proteins (Fig. 6). PCA clearly distinguishes the 'TE' except 'TE10' population on the right of Fig. 3 along with TS2, in positive PC1. The rest of the cultivars are PC1 negative including 'TE10', but not including 'TALB1' and 'TALB2' (Fig. 7).

#### ISSR markers and chemical and bioactive compounds comparison

Comparing the PCA obtained with chemical and functional compounds and ISSR results (Figs. 3 and 7, respectively), we shall observe that although the cultivar distribution is not identical, figures were very similar. In both cases, 'TE' cultivars were grouped, with the exception of 'TE10' cultivar. In the PCA obtained with chemical and functional compounds the 'TE10' cultivar was grouped with the rest of the cultivars no with the other 'TE' cultivar. In the same



**Fig. 6** Variables factor map from principal component analysis using chemical and biochemical parameters



**Fig. 7** Principal component analysis using chemical and biochemical parameters

way, 'TALB1', 'TALB2' and 'TS2' cultivars were grouped with 'TE' cultivars.

## Conclusions

The PCAs carried out seem to indicate that the 'TE' cultivars, except 'TE10', belong to a subspecies, which, since they do not have stipules thorny, must be the *rupestris* subspecies, while the rest of the populations belong to the *spinosa* subspecies since they do have stipules thorny. According to the genetic PCA, the cultivar 'TE10' seems to be a hybrid between both subspecies, since it is in an intermediate position, or it belongs to the *spinosa* subspecies according to the results of the PCA using biochemical parameters.

In any case, from the results of the biochemical and functional parameters studied, it is clear that there is no difference between the *spinosa* and *rupestris* subspecies, but rather that in all the parameters studied, except chlorophylls, there is more difference between the results of the subspecies *spinosa* among them, than with the subspecies *rupestris*. For all this, for the first time, it can be concluded that the *rupestris* subspecies that does not present stipules thorny, can be cultivated instead of the *spinosa* subspecies that does present them, without losing functional or nutritional characteristics of the flower buds, facilitating agronomic work.

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**Data availability** All data generated or analyzed during this study are included in this published article.

## Declarations

**Conflict of interest** The authors have no conflicts of interest to declare that are relevant to the content of this article.

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