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Full Length Research Paper

Morphology and chemical composition of Tunisian caper seeds: variability and population profiling

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Caper, as a spontaneous plant, has a large natural distribution in the Mediterranean Sea basin. It is an interesting crop with an economic importance; especially the species *Capparis spinosa*. The morphology of seeds and their composition in lipid and protein were studied in 15 wild Tunisian caper populations: 9 populations represented the subspecies *C. spinosa* subsp. *spinosa* (thorny caper) and 6 populations represented the subspecies *C. spinosa* subsp. *rupestris* (inerm caper). Results show that seeds of *C. spinosa* are especially attractive because they can be grown to produce oil (ca. 30%) and protein (26%). Principal component analysis (PCA) did not show a geographic separation. A subspecies segregation between *C. spinosa* subsp. *rupestris* (as a homogeneous group) and *C. spinosa* subsp. *spinosa* (as a heterogeneous group) was clearly detected.

Key words: Caper (*Cappains spinosa*), morphology; lipids, proteins, principal component analysis (PCA).

INTRODUCTION

Caper is the common name of the genus *Capparis*, family of Capparaceae (Jacobs, 1965). Its origin is the tropical regions (Fici, 2001) and includes more than 250 species in the entire world (Barbera, 1991; Jacobs, 1965). *Capparis spinosa* is the most important species. It is one of the most commonly found aromatics in the Mediterranean Sea basin (Germano et al., 2002). Before its commercialization, the fresh aerial parts, including the fruit and the flower buds (capers) are stored in vinegar or brined and eaten pickled. Previous chemical studies on *C. spinosa* have brought attention to the richness of different parts of this plant with beneficial compounds (Germano et al., 2002; Matthaus et al., 2005; Tlili et al., 2009, 2010).

As a spontaneous plant, caper has a large natural distribution in the Mediterranean Sea basin; it grows from the Atlantic coasts of the Canary Islands and Morocco to the Black Sea and to the Crimea and Armenia, and eastward to the Caspian Sea and into Iran (Romeo et al., 2006). This reflects the adaptation of this plant with varied soil and climatic conditions as drought, high temperature and salinity (Levizou, 2004; Rhizopoulou and Psaras, 2003). This species is an interesting crop with an economic importance in the Mediterranean region over the last years (Romeo et al., 2006). Spain, Morocco and Italy were the main producer countries (Levizon et al., 2004; Özcan and Akgül, 1998).

In Tunisia, caper is characterized by a wide geographical distribution (Barbera, 1991; Jacobs, 1965; Levizou, 2004; Saadaoui, 2007). Indeed, a morphological study of vegetative and reproductive apparatus, show that in Tunisia there is only one species with two different subspecies: *C. spinosa* subsp. *spinosa* (thorny caper) and *C. spinosa* subsp. *rupestris* (inerm caper) (Pottier-Alapetite, 1979; Saadaoui et al., 2007).

The aim of this work is to study and compare the morphologic and storage content (lipid and protein) of seeds of 15 Tunisian *C. spinosa* populations to investigate whether or not *C. spinosa* contains important

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amounts of lipid and protein and to estimate the level of variability within this species.

MATERIALS AND METHODS

Chemicals

All solvents used in the experiments were purchased from Fluka (Ridel-de Han, Switzerland).

Plant material

Sampling was performed from 15 Tunisian regions (Figure 1). From each location seeds were collected from 5 to 10 plants, mixed and then a representative sample was taken for further analysis. The length and the width of the seed were studied. The weight of 1000 seeds was measured by a precision balance. Samples were analyzed in triplicate.

Morphological parameters

The length and the width of the seed were studied. The weight of 1000 seeds was measured by a precision balance.

Lipid content

The oil content was determined according to ISO method 659:1998. *C. spinosa* seeds were ground in a mortar and extracted with petroleum ether in a Soxhlet apparatus for 6 h. The solvent was concentrated using a rotary evaporator under reduced pressure at 60℃. The oil was dried by using a stream of nitrogen.

Protein content

Protein contents were determined according to the AOAC (1984) official method using a micro-Kjeldhal apparatus. Each sample (100 mg) was digested for about an hour with 100 mg of digestion mixture (8 g K_2SO_4 + 20 g $CaSO_4$ + 2 g selenium) and 6 ml of concentrated H_2SO_4. When digestion was completed, the solution became clear. The solution was then made up to 30.0 ml in a volumetric flask with distilled water. For the nitrogen determination, 10 ml of 2% boric acid solution was first taken in a beaker with a few drops of methyl red as indicator. Then 10 ml of the digested mixture, 30 ml of 40% NaOH solution and 10 ml of distilled water were transferred to the distillation chamber. Ammonia was liberated, and it combined with NaOH to form NH_4OH, which was then received into the boric acid solution to form ammonium borate (pink color to yellow). Distillate (ammonium borate) was then titrated with 0.1 N H_2SO_4. The volume of acid that had been added at the point when the color of the distillate changed from yellow to pink was recorded. Protein was calculated according to the following formula:

%protein = %N × 6.25

Statistical analysis

Principal component analysis (PCA) is a chemometric method to visualize information contained in experimental data and to find the true dimensions of a dataset (Miloun et al., 1992). The v-parameters (variables) measured for each sample describes each sample (object) in a v-dimensional space. PCA generates a set of new orthogonal variables (axes) and linear combinations of the original ones, so that the maximal amount of variance contained in the dataset (information) is concentrated in the first principal components. The loadings are the coefficients of the original variables defining each principal component. The scores are the coordinates of the objects on the new axes (Armanino et al., 2002; Nasri et al. 2005). PCA was conducted with SAS statistical software.

RESULTS AND DISCUSSION

Seeds description

Table 1 gives descriptive results for the analysed seeds of *C. spinosa* (seed length, seed width and 1000 seeds weight). The average values are 3.45 ± 0.30 mm, 2.86 ± 0.27 mm and 10.14 ± 2.46 g for length, width and weight, respectively. The statistical analyses show a significant difference between populations. It is interesting to report that the high values for the three used parameter are detected with Bellaregia (B) (3.95 mm; 3.37 mm and 12.88 g length, width and weight, respectively). Tataouine, show low level for length, width (2.92 and 2.46 mm, respectively), while the low 1000 seeds weight was detected with Ksar Hdada KH (7.13 g). It is also interesting to know that *C. spinosa* subsp. *spinosa* has a high value of seed weight, since seed weight is generally considered the least plastic of plant traits (Harper, 1970). In our study, the seed weight varied the most, which was probably influenced by the environment of the maternal plants, and together with internal variables affects seed weight (Krannitz, 1997).

Lipid content

The present study show the contents of seed oil from different *C. spinosa* populations from different regions in Tunisia. The oil content of seeds (Table 2) varied from 27.64% in Ghar El Melh (GM) to 37.93% in Joumine (J) on a dry weight basis (dw%) with an average of 30.38 ± 3.16% (dw%). These results are in agreement with other authors (Akgül and Özcan, 1999; Matthaus and Özcan, 2005), who reported that the values are between 27.3 and 37.6% (dw%).

Oil content is not affected by location and the difference is not significant between the two subspecies (*C. spinosa* subsp. *spinosa* and *C. spinosa* subsp. *rupestris*), whereas it is significant between samples. Theses results are is in agreement with other authors (Berganza et al., 2005).

Due to a rapid increase in the world population, seed oils have attracted much attention because of their biological properties and possibly use as food or in industry. Research has increased to investigate new plant sources of oil, especially from underexploited seeds. These results support the use of caper seeds as a new vegetable source of oil, which is in agreement with Daulatabad et al. (1991) who reported that this plant represents a new and

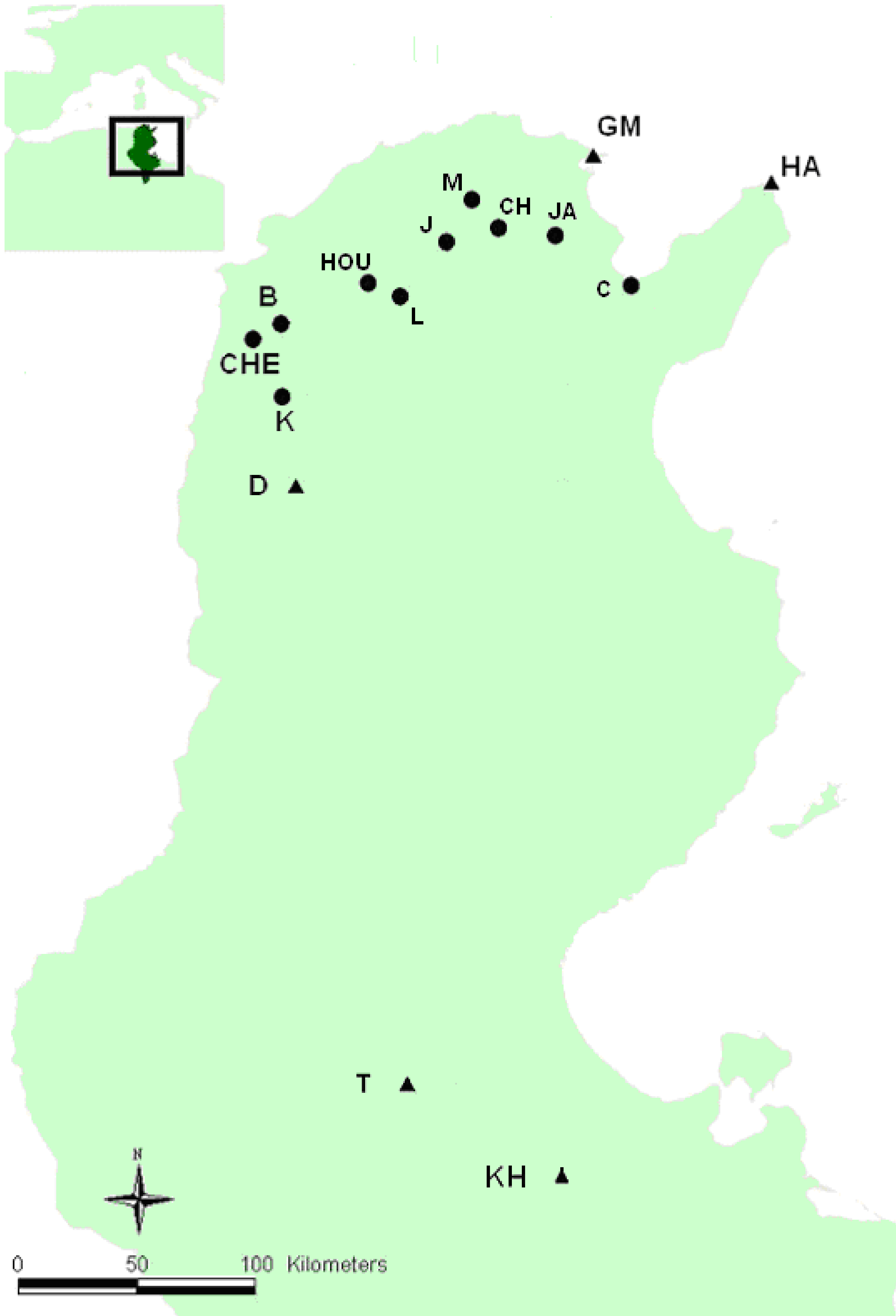

Figure 1. Geographic location of the 15 harvested samples of *C. spinosa*. M: Mateur, C: Campus, L: Lafareg, CH: Chouigui, B: Bullaregia, CHE: Chemtou, JA: Jebel Ammar, J: Joumine, K: Kef, HOU: Houmana, GM: Ghar El Melh, HA: Haouaria, D: Dahmani, T: Tataouine, KH: Ksar Hdada. ▲ *C. spinosa* subsp. *rupestris* (inerm caper); ● *C. spinosa* subsp. *spinosa* (thorny caper).

Table 1. Seed length, width and weight of *C. spinosa* seeds[a]

Sub-species	Sample	Seed length (mm)	Seed width (mm)	1000 seeds weight (g)
C. spionosa* subsp. *spinosa	M	3.55	2.86	12.23
	C	3.53	2.74	9.98
	L	3.81	3.17	14.85*
	CH	3.74	2.98	11.48
	B	3.95	3.37*	12.88
	CHE	3.43	3.10	12.41
	JA	3.41	2.79	9.39
	J	3.60	2.91	12.22
	K	3.78	3.01	9.46
	HOU	3.50	3.13	10.19
	Mean ± SD	3.63 ± 0.18	3.01 ± 0.19	11.51 ± 1.75
C. spionosa* subsp. *rupestris	GM	3.13	2.64	7.15
	HA	3.21	2.70	8.45
	D	3.01	2.54	7.15
	T	2.92	2.46	7.16
	KH	3.15	2.53	7.13
	Mean ± SD	3.08 ± 0.11	2.57 ± 0.09	7.41 ± 0.58
	Total mean ± SD	3.45 ± 0.30	2.86 ± 0.27	10.14 ± 2.46

M: Mateur, C: Campus, L: Lafareg, CH: Chouigui, B: Bullaregia, CHE: Chemtou, JA: Jebel Ammar, J: Joumine, K: Kef, HOU: Houmana, GM: Ghar El Melh, HA: Haouaria, D: Dahmani, T: Tataouine, KH: Ksar Hdada. [a] Values are mean of three repetitions; * values are significantly higher than the mean at p = 0.05.

Table 2. Total lipid and proteins (%DW) of Tuisian *C. spinosa* seeds[a].

Subspecies	Samples	Oil	Protein
C. spionosa* subsp. *spinosa	M	30.38	29.55
	C	27.92	27.62
	L	36.9*	27.58
	CH	30.29	27.58
	B	30.42	27.01
	CHE	27.83	25.68
	JA	30.10	25.28
	J	37.93	24.79
	K	28.24	21.37
	HOU	27.93	27.37
	Mean ± SD	30.79 ± 3.67	26.38 ± 2.24
C. spionosa* subsp. *rupestris	GM	27.64	27.30
	HA	29.49	26.39
	D	30.89	25.97
	T	27.91	24.93
	KH	31.85	22.74
	Mean ± SD	29.56 ± 1.83	25.47 ± 1.75
	Total mean ± SD	30.38 ± 3.16	26.08 ± 2.07

M: Mateur, C: Campus, L: Lafareg, CH: Chouigui, B: Bullaregia, CHE: Chemtou, JA: Jebel Ammar, J: Joumine, K: Kef, HOU: Houmana, GM: Ghar El Melh, HA: Haouaria, D: Dahmani, T: Tataouine, KH: Ksar Hdada. [a] Values are mean of three repetitions; * values are significantly higher than the mean at p= 0.05.

Table 3. Contribution of different characters to the definition of PCA axes on *C. spinosa*.

Component	Axe		
	1	2	3
Inertia %	66.59	27.17	5.76
Cumulative %	66.59	93.77	99.53
Characters defining axes	(-) 1000 seeds weight	(+) protein content (-) oil content	(-) seeds length (-) seeds width

rich source of vegetable oil.

Protein content

The Kjeldahl method gives approximately the amount of total protein present in *C. spinosa* seeds from different Tunisian location (Table 2). *C. spinosa* seeds are rich in protein. The content ranged from 21.74 (dw%) in Ksar Hdada (KH) to 29.55 (dw%) in Mateur (M), with an average 26.08 ± 2.07% on a dry weight basis (dw%). These results are in agreement with those published by Akgul and Ozcan (1999) who reported that the seed protein content is ca. 22% for Turkish caper. The difference between populations is significant. This difference might be due to geographic distribution, which is in agreement with other studies (Breene et al., 2007; Nasri and Triki, 2007). It is interesting to know that difference between *C. spinosa* subsp. *spinosa* and *C. spinosa* subsp. *rupestris* is not significant.

Proteins play significant roles in human health. Especially in developing countries where the average protein intake is less than required, it is essential to find new sources of edible protein and other nutrients to overcome the population problem (Samanta and Laskar, 2009). Plant proteins remain a very important source of food and feedstock, for human and animal consumption, respecttively (deMan, 1999). Legume seeds, say colza (15 to 30%), lentils (23 to 32%) and lupain (30 to 50%) (Dulau and Thebaudin, 1998) are the most important sources of protein for human and animal nutrition, whereas protein content of cereals is between 10 and 15% (Guéguen and Lemarié, 1996).

Proteins from seeds of non-conventional plants which are found in abundance should be explored to overcome the population problem. Results showed that seeds of *C. spinosa* (ca. 26 %) can be used as a new source for dietary proteins and it may contain good quality proteins that can be exploited to meet the people's increasing demand.

Principal component analysis

Morphological and biochemical parameters of *C. spinosa* seeds have been submitted to principal component analysis (PCA) to confirm the presence of two subspecies of *C. spinosa* in Tunisia. From this analysis, three axes were retained because they express 99.53% of the total variation as seen in Table 3. Axis 1 explains 66.59% of the total variation and associates negatively to 1000 seeds weight. Axis 2 (27.17%) is characterized positively by protein content and negatively by oil content. Most discriminate variables along axis 3, explaining 16.392% of the variation, which are associated negatively to length and width.

Data projections on planes as defined by inertia axes of PCA (Figures 2 and 3) did not show significant structuring in Tunisian populations; samples are not structured according to the geographical origin, which are in agreement with other authors (Saadaoui et al., 2007; RSF, 2001). The presence of two groups could be distinguished: GI group that represent the inerm samples (*C. spinosa* subsp. *rupestris*) and has a wide geograp-hical distribution, and IM group that represent the thorny samples (*C. spinosa* subsp. *spinosa*). Figures 2 and 3 clearly reflect that each provenance in IM group appears to be individual without any particular geographical structuring, while provenances in GI group are associated. These results are in agreement with other authors (Fici, 2001, 2004; Inocencio et al., 2005; Saadaoui et al., 2007), who reported that inerm caper, as an ancestral subspecies, is characterized by low morphological variability, while the level of the variability within thorny caper is very important.

It is concluded that seeds of *C. spinosa* could be an alternative source of edible oil (ca. 30%) and proteins (ca. 26%). Seeds of *C. spinosa* are especially attractive because they can be grown to produce compounds for both food and industry. Morphologic parameters show a significant difference between the two subspecies: *C. spinosa* subsp. *spinosa* and *C. spinosa* subsp. *rupestris*.

Statistical analysis (PCA) did not show geographic segregation. The subspecies segregation can be confirmed. *C. spinosa* subsp. *rupestris* (inerm caper) form one group, while *C. spinosa* subsp. *spinosa* (thorny caper) present an individual repartition. It seems that inerm caper, with a wide geographic distribution, is more adapted to different climates than the thorny one.

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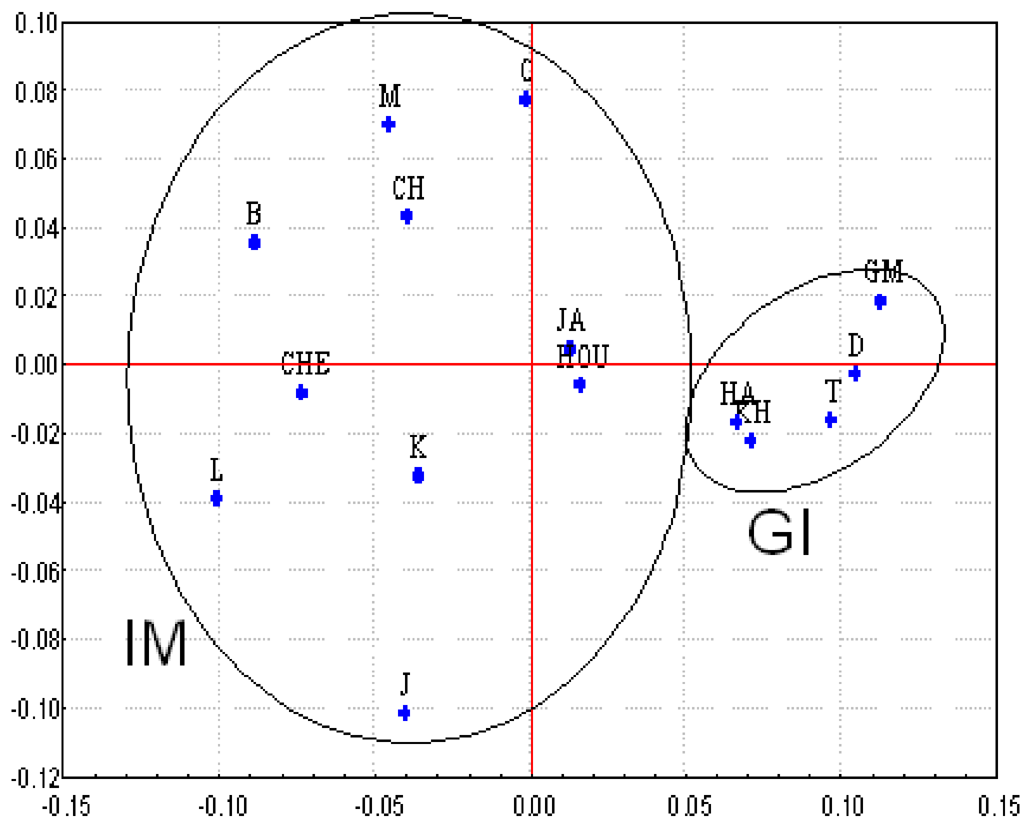

Figure 2. Principal component analysis and dispersion of *C. spinosa* populations in planes formed by axes PC1 and PC2 of the PCA.

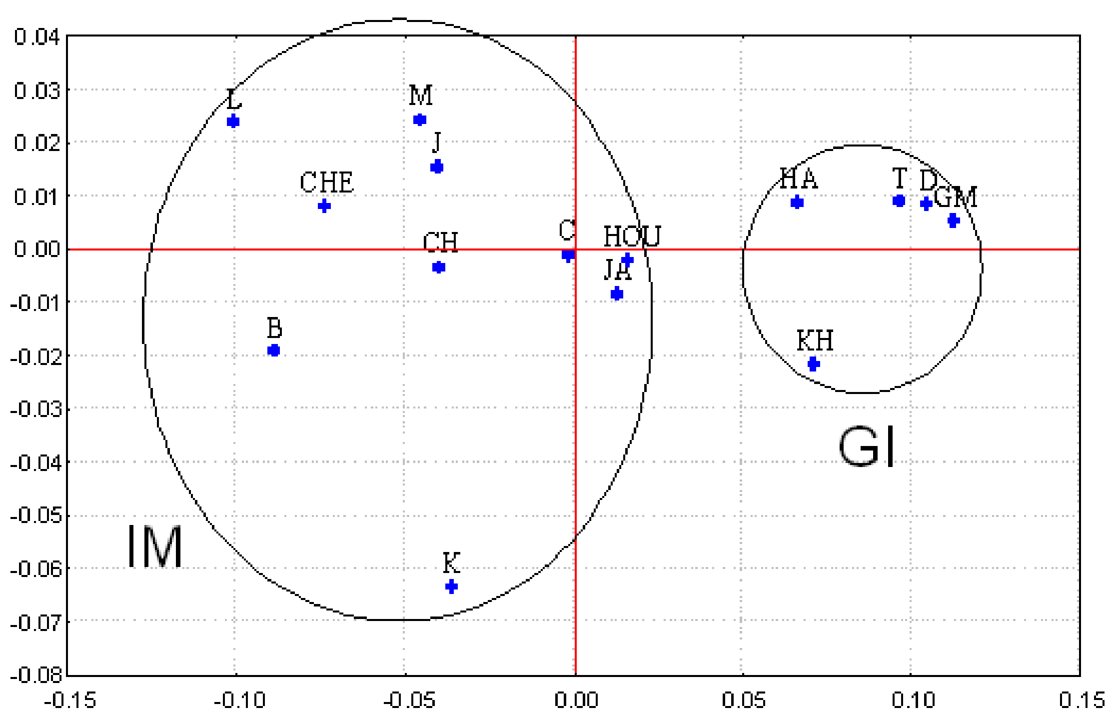

Figure 3. Principal component analysis and dispersion of *C. spinosa* populations in planes formed by axes PC1 and PC3 of the PCA.

collection.

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