Original Research Article

Effect of Climate in Seed Diversity of Wild Tunisian *Rhus tripartita* (Ucria) Grande

ABSTRACT

Rhus tripartita (Ucria) Grande (Anacardiaceae) is a rare and endangered species. Seeds of *Rhus tripartita* were obtained from spontaneous populations in nine different localizations in Tunisia and their shape analyzed by image analysis. Seeds were harvested in 2014 and image analysis was done in *CSIC* laboratories (Salamanca, Spain).

The method for seed shape analysis is based on the comparison of seed images with a cardioid. Five hundred and thirty seeds from nine natural populations in Tunisia were analyzed giving percentages of identity with a cardioid (J index) ranging from 76.2 to 95.3. Variation was higher in the side of the seed containing the micropyle (right side). Seeds are classified in four types: A, B, C, or BC depending on their degree of similarity with the cardioid in both sides (right and left). Type A seeds have high degree of similarity on both sides; type B have high similarity in the right and low in the left; type C present high similarity in the left and low in the right, and finally type BC with low similarity value in both sides. Size and shape were compared for seeds in and among populations. Differences among populations were found both in size as well as in shape (roundness, J index total and partials). The analysis of seed size and shape reveals differences between climatic regions. The largest seeds are found in the lower semi-arid region; the smallest in the upper arid; in the lower arid, seeds are of intermediate size. Four morphological seed types were obtained (A, B, C and BC). Type A represents seeds in the lower semi arid climate whereas type C represents seeds in the arid climates.

Morphological types were characteristic for some of the populations indicating that differences in shape are independent of size Genetic and ecological effects contribute to seed size and shape of *Rhus tripartita.*

Keywords: Biodiversity, cardioid, models, morphology, *Rhus tripartita*, seed, shape, Tunisia

1. INTRODUCTION

Rhus tripartita (syn. *Rhus tripartitum*) or Sumac (Anacardiaceae) is a pluri-annual shrub, up to 2 m high, with short and spiny branches, glossy, of a reddish brown. Leaves are deciduous, alternate and compound with three (rarely five) folioles of a dark green color and with dentate margins (Figure 1). *Rhus tripartita* is a dioecious plant with small clusters of yellowish white flowers. The fruit is a small drupe with a single seed in the interior having spherical shape and a red to black color. Pollination of *R. tripartita* is primarily accomplished by a variety of small Diptera and Hymenoptera [1].

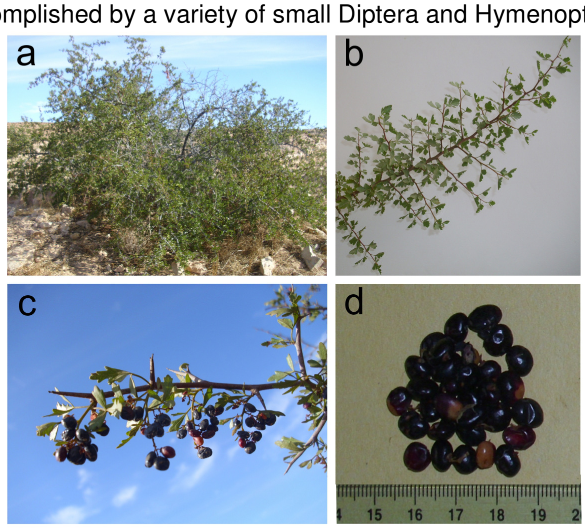

Figure 1. *Rhus tripartita.* (a): Plant, (b) branch with leaves, (c): branch with leaves and fruits and (d) mature fruits. Fruits are single seeded drupes (seeds are shown in figure 3)

Rhus tripartita is indigenous to arid and semiarid regions in the North of Africa where it is a rare and endangered species due to human pressure and climate change [2]. It may have emigrated there from the Irano-turanian region in Asia, and its geographical distribution goes across North Africa into the Middle East including Canary Islands, Morocco, Algeria (incl. Hoggar), Tunisia, Sicily, Libya, Egypt, N. Sudan (Nubia), Palestine, Lebanon, and Syria [3]. According to Furth [1], there are two basic environmental types of *R. tripartita* populations. First, most common and widespread, are the populations that live in desert environments under harsh xeric conditions across the North African Sahara into the Middle Eastern deserts. These populations are usually quite small and greatly isolated from the nearest populations. Second, are those populations found in the Mediterranean environment usually near the coast (SW Morocco, Algeria, Canary Islands, Sicily, Egypt, Israel and Lebanon). In Tunisia, plants grow in the semi-arid, arid and Saharan regions including the rocky valleys in the south and mountains in the center. Although *R. tripartita* requires calcareous soil, it can grow in a variety of edaphic situations, from rather deep clay-textured soil to fissures in hard limestone, dolomite rock, or granite, where some soil has accumulated [1]. This plant adapts well to some difficult environmental conditions such as high salinity [4], but stomatal conductance, photosynthesis rate, transpiration rate and isotopic discrimination of *R. tripartita* young plants are affected by water deficit [5]. *R. tripartita* is a plant frequently used in traditional medicine and pharmacology [6], also its Bark is used for tanning in desertic regions.

Morphological seed characters, such as shape and size can be used to distinguish species, ecotypes and varieties [7, 8, 9, 10, 11]. Object morphometry may be achieved with five parameter categories: size, shape, harmonic analysis, fractal dimension and topology [12, 13, 14, 15]. In addition, the germination of *R. tripartita* is low; our work in nursery (3/4 sand and ¼ manure) shows 4.25% (0 to 6.6% depending on the population). In this case, the analysis of seed diversity will be a necessity to improve the germinative bahavior of the species and aims at the rehabilitation of the natural populations

Seeds of nine different locations of Tunisian *Rhus tripartita* are compared in size and shape; the sites are characterized by different bioclimatic conditions. The method of analysis is based in the comparison of the outline of the seed image with a cardioid curve. Similar analysis have been done for *Arabidopsis thaliana* [16], and the model legumes *Lotus japonicus* and *Medicago truncatula* [17], detecting differences in seed shape between mutant genotypes. In *Capparis spinosa*, the analysis of ten spontaneous populations by this method allowed to find differences in seed shape between sub-species *rupestris* and *spinosa* [9]. The objectives of this work are to characterize the seed for any individual / population and to analyze the effect of genetic factors and climate on seed shape and size variation.

2. MATERIAL AND METHODS

2.1. Plant Material

Mature fruits were collected in the field from 27 plants belonging to 9 populations corresponding to geographical locations in Ain Jelloula (AJ), Haffouz (HA), Bou Hedma (BH), Beni Kdech (BK), Dkhilet Toujene (DK), Ksar Hdada (KH), Jbel Omrane (JO), Toujene (TJ), and Tounine (TN; Table 1). AJ and HA are in the lower semi-arid climate; BH, in the upper arid climate, and the rest (six populations) belong to the lower arid climate (Figure 2). The comparison was done between three groups: Group 1 comprising plants from lower semi-arid climate (AJ and HA); Group 2 of plants in the upper arid climate (BH), and Group 3 in the lower arid climate (BK, DK, KH, JO, TJ, and TN).

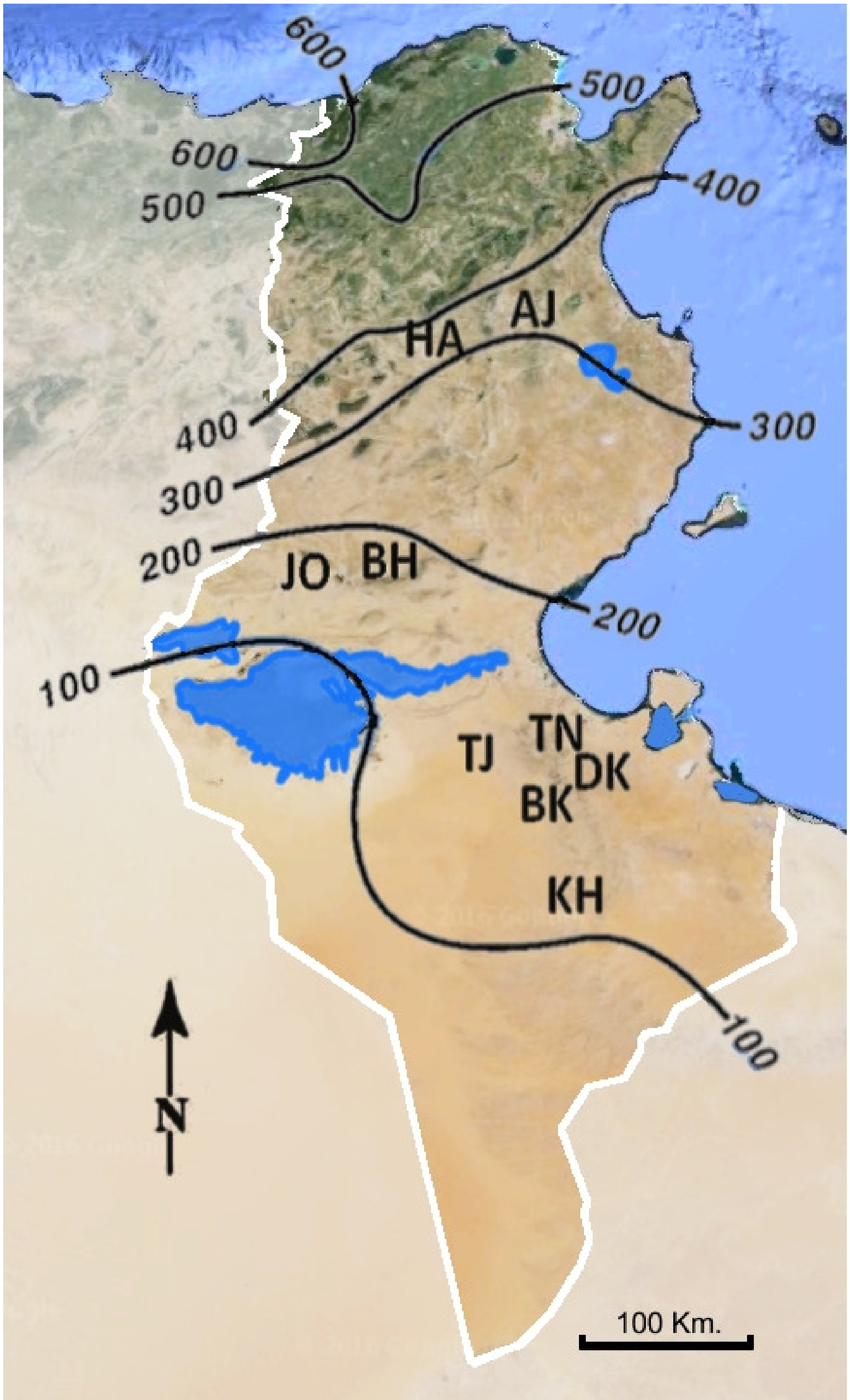

Figure 2. Map of Tunisia showing nine geographic localizations where *Rhus tripartita* seeds were collected. Coordinates are shown in table 1

Table 1. Characteristics of each population

Populations	Latitude (N)	Longitude (E)	Altitude	Climatic region*	Average annual rainfall** (mm)	Average annual temperature** (°C)
Ain Jelloula (AJ)	35° 47' 58.09''	9° 48' 46.49''	209	lower semi-arid	393	17.2
Bou Hedma (BH)	34° 28' 08.38''	9° 31' 35.65''	342	upper arid	200	19.5
Beni Kdech (BK)	33° 14' 57.74''	10° 12' 01.74''	488	lower arid	145	19.8
Dkhilet Toujene (DK)	33° 25' 59.70''	10° 11' 29.65''	291	lower arid	183	19.8
Haffouz (HA)	35° 38' 31.30''	9° 39' 17.93''	262	lower semi-arid	364	17.8
Jbel Omrane (JO)	34° 21' 29.09''	9° 05' 59.21''	458	lower arid	160	19.2
Ksar Hdada (KH)	33° 06' 25.97''	10° 18' 15.89''	329	lower arid	118	20.2
Toujene (TJ)	33° 28' 13.70''	10° 07' 37.11''	536	lower arid	183	19.8
Tounine (TN)	33° 30' 32.76''	10° 08' 15.68''	206	lower arid	152	19.3

*Climates were obtained according to the classification of Emberger [17]
** Data of National Institute of Meteorology. Tunis, Tunisia.

Each population is represented by one to six plants, and each plant is represented by twenty seeds in the morphological analysis. Only one plant was used as the source of all seeds in three populations (HA, TJ, TN); two plants in DK and JO; three plants in BK; five in KH and six plants in AJ and BH. All seeds were collected in summer 2013, except for five plants in AJ that were collected in summer 2014. Individual plants were treated independently in each location, thus AJ1 to AJ6 refer to six independent plants from location AJ. Seeds were collected, allowed to dry at room temperature, and conserved in the laboratory conditions.

2.2. Photography and image analysis

Individual seeds were placed over a flat surface, and observed with an 'SMZ-2T' stereo-microscope. Photographs of orthogonal views of the seeds oriented with their micropyle to the right were taken with a digital camera Nikon 'Coolpix 950'.

Groups of seeds, containing between 10 and 50 units, where also photographed with a Canon Ixus 135, for the semi-automated calculation of area, perimeter and roundness.

2.3. Quantitative morphology

Roundness [19] is given by

$$\mathrm{I} = 4\frac{\mathrm{area}}{\pi \times A^2} \ ,$$

Where *A* is the length of the major axis. Roundness is a measure of the similarity between a plane figure and a circle. It ranges from zero to one giving the value of 1 for circles and it is a useful magnitude as a first approximation to seed shape. It is preferred here to circularity index [20], because the latter is very sensitive to alterations in the margin of the figure.

For the quantification of area, perimeter and roundness the profile of the seeds needs to be taken manually to avoid the irregularities. These magnitudes are obtained automatically with the program Image J.

2.4. Seed shape analysis

Cardioid figures were superimposed to the seed images (Figure 3a). The cardioid is the trajectory described by a point of a circle that rolls around another fixed circle with the same radius. Quantification of the adjustment was done in each seed by the comparison of the areas in two regions: the common region in the cardioid and the seed images, and the regions not shared between both images (Figure 3b). The index of adjustment (J index; [16]) is defined by:

$$\mathrm{J} = \frac{(\mathrm{area\ I})}{(\mathrm{area\ I} + \mathrm{area\ D})} x100$$

Where area I represents the common region and area D the regions not shared. Note that J ranges between 0 and 100, and decreases when the size of the non-shared region grows. It equals 100 when cardioid and seed image areas coincide, i.e., area D is zero.

J Index was calculated for a total of 530 seeds (20 seeds randomly selected per each plant, except in one plant from AJ that contained only 10 seeds). The images of the seeds with the superimposed cardioid curve were divided in two halves and percent of identity with the cardioid was calculated for each half of the seed, left or right. This gave four seed types (A, B, C, or BC) depending on their similarities with the cardioid (Figure 3c). Type A is defined for seeds whose values of similarity with the cardioid are superior to 92 in the left region and superior to 80 in the right. These values were chosen arbitrarily to have an equilibrated distribution of the seed among the four types. Type B is defined for seeds whose values of similarity with the cardioid curve are lower than 92 per cent in the left region and superior to 80 in the right of the seed. Type C is defined for seeds whose values of similarity with the cardioid curve are superior to 92 in the left part of the seed and lower than 80 per cent in the right. Finally, type BC corresponds to seeds whose values of similarity with the cardioid curve are lower than 92 per cent in the left part of the seed and lower than 80 per cent in the right.

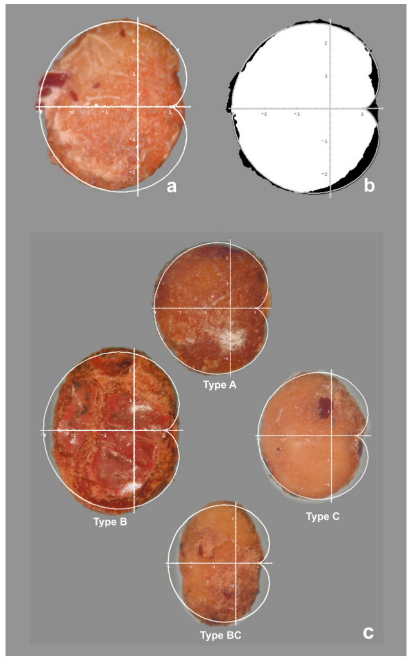

Figure 3. 3a: Adjustment of *Rhus tripartita* seed images to a cardioids. The index of adjustment (J index) is defined by: J= (area I)/(area I + area D) x100, where area I represents the common region (white in 3b) and area D the regions not shared (black in 3b). 3c: Four morphological types described in this work. Seed types are defined in the text (section 2.4).

2.5. Statistical analysis

Normal distribution was checked by Kolmogorov-Smirnov test. Comparison between different groups was done with ANOVA. Kruskal-Wallis method, which is the method of choice for multiple comparisons in non-normal distributions, has also been applied finding similar differences between populations. Post-hoc analysis was carried out using Scheffé test (comparison of samples of different sizes). In general, statistical analysis was done with software IBM SPSS v.21. For the analysis of principal component analysis (PCA), the software Statistica Kernel Version 5.5, Stat Soft. Inc. (Johannesburg, SA) was used for the analysis of six variables: area, perimeter, circularity index, J index total, J index left and J index right.

3. RESULTS

3.1. Seed size

Seed image areas range between 10.6 and 40.6 mm^2. Coefficient of variation in a single plant oscillates between 6.3 (KH3) and 29.4 (AJ3). Thus a large amount of the diversity in size is observed in some individual plants, but not in others. Differences between plants were found in AJ, DK and KH (not shown). Seeds were larger in lower semi-arid climate and smaller in upper arid climate (Table 2).Mean seed weight per population were (mg): AJ (45), BH (37) , BK (36) , DK (37) , HA (33), JO (40), KH (53) , TN (29) and TJ (34).

3.2. Variation in seed shape

3.2.1. Roundness

Roundness values ranged between 0.64 and 0.97 (Table 2). Coefficient of variation in a single plant oscillated between 2.9 (KH2) and 9.0 (AJ3). Differences between plants were found for the populations BK, and KH (not shown).

3.2.2. J Index

Total J Index values range between 76.2 and 95.3 (Table 2). Coefficient of variation in a single plant oscillates between 1.4 (KH5) and 4.3 (DK1). Differences between plants were observed in populations BH, BK, and KH (not shown**)**. In populations, the lowest J index was found in BK, and the highest in AJ

Variation is smaller in the left side of the seed and larger in the right. Mean values of J index in the left side of the seed oscillated between 83.2 and 98.6 (Table 2). In populations, the lowest J index in the left part of the seed was found in KH; whereas in AJ, BH, DK, HA and TN, values were higher than in KH (not shown).

Mean values of J index in the right side of the seed oscillated between 40.8 and 93.6 (Table 2). Among populations, the lowest J index in the right part of the seed was found in BK; whereas values in AJ, BH, KH, HA, JO, TN and TJ (all except DK) were higher than BK (not shown).

The lowest J index observed in BK is due to the lower values in the right side of the seeds. KH has lowest values of J index in the left side, associated with lower roundness, but not in total J index.

3.3. Climatic effects in seed size and shape

The average value of seed area varies significantly between climates ($P = 0.05$). It is 25.3; 18.4 and 16.5 mm² respectively for lower semi-arid, upper arid and the lower arid (Table 2). Also, roundness varies between bioclimatic zones, but only between the lower semi-arid (0.82) and arid. In the populations of arid region, we have not seen significant difference between upper arid (0.77) and lower arid (0.76) (Table 2).

The same result was obtained for J index, J index left and J index right; these parameters vary significantly between semi-arid and arid (upper arid and lower arid). Indeed, for the last two climates, the difference was not significant (Table 2).

Different seed types are predominant in three climatic regions. The mean seed type is different in each of the three climatic regions. Type A corresponds to lower semi arid with 55.4%, whereas type C corresponds to upper and lower arid regions with 37 and 32.1% respectively. Table 3 shows the number and percentage of each type in each of the three climatic regions.

Table 2. Area, roundness and J index values in three climatic regions

Climatic region (number of plants)	Area (mm²) Mean ± SD	Roundness Mean ± SD	J index total Mean ± SD	J index left Mean ± SD	J index right Mean ± SD
lower semi-arid (7)	$25.3^{c} \pm 6.2$	$0.82^{b} \pm 0.053$	$89.8^{b} \pm 2.2$	$94.4^{b} \pm 2.3$	$82.3^{b} \pm 5.5$
upper arid (6)	$16.5^{a} \pm 2.8$	$0.77^{a} \pm 0.043$	$87.2^{a} \pm 3.1$	$92.4^{a} \pm 2.3$	$76.1^{a} \pm 8.5$
lower arid (14)	$19.4^{b} \pm 4.9$	$0.76^{a} \pm 0.062$	$87.4^{a} \pm 4.5$	$92.5^{a} \pm 3.0$	$76.7^{a} \pm 9.6$

Scheffé test was used as the post-hoc test grouping in ANOVA

SD: standard deviation

Table 3. Number and percentage of the four morphological types in three climate regions of *Rhus tripartita.*

Type	Lower semi-arid	Upper arid	Lower arid	total
A	72 ± 55.4	24 ± 20.2	77 ± 27.5	173 ± 32.7
B	16 ± 12.3	19 ± 16.0	50 ± 17.9	85 ± 16.1
C	30 ± 23.1	44 ± 37.0	90 ± 32.1	164 ± 31.0
B+C	12 ± 9.2	32 ± 26.9	63 ± 22.5	107 ± 20.2

3.4. Multivariate analysis: Principal Component Analysis

The plot of principal component analysis (PCA) for individuals identified two principal components that explained 99.51% of the total variance: 86,51% for axis 1 and 13% for axis 2. The principal component analysis (PCA) for individuals shows the presence of two groups, the first includes individuals from both populations (KH and AJ) and they are characterized by developed seeds. The second group comprises individuals belonging to the remaining populations and few individuals in the population AJ (AJ2 and AJ3) and KH (KH1 and KH2) (Figure 4). For populations, the two principal components plot of PCA explained 98.47% of the total variance. The PCA of population shows the distinction of populations into two groups too. A first group is characterized by large seeds and three populations AJ, HA and KH. The second group comprises the six remaining populations, characterized by reduced seed size (Figure 5).

The correlation matrix did not show a significant correlation (<0.3) between parameters related to size (area and perimeter) and the parameters related to seed shape (circularity index, J index total, J index left and J index right).

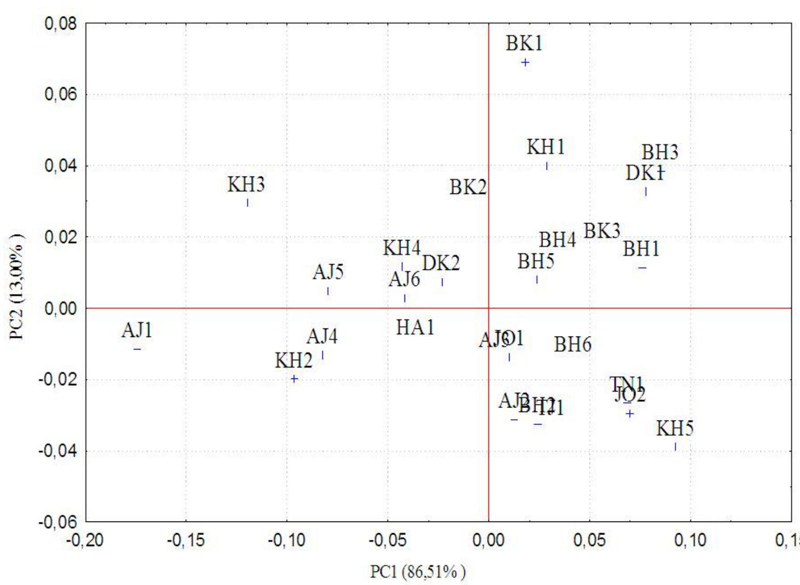

Figure 4. Distribution of the populations after principal components analysis (PCA) for all studied parameters (axis 1-2)

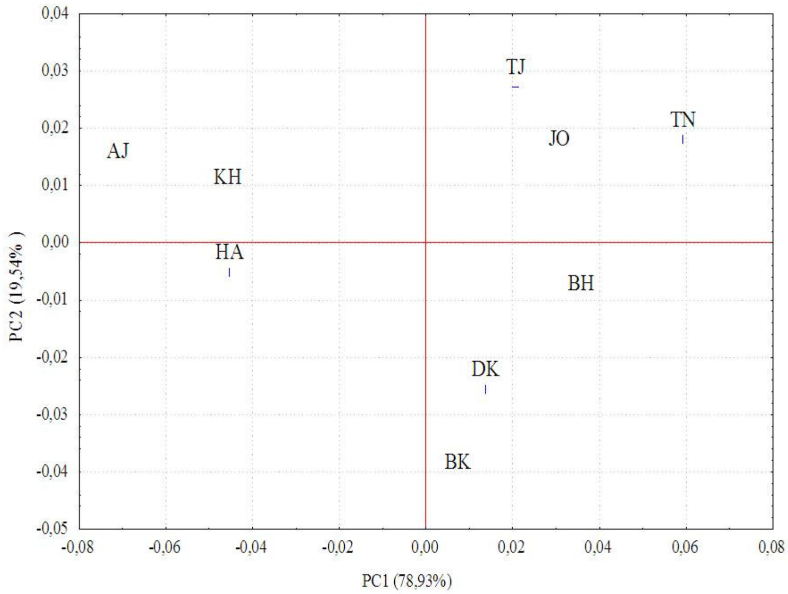

Figure 5. Distribution of the populations after principal components analysis (PCA) for all studied parameters (axis 1-2)

4. DISCUSION

The comparison between individuals and populations show an interesting variation in size and shape indices (roundness and J index); Zouaoui *et al*. [20] investigated morphological parameters and compared the size of *Rhus tripartita* seeds between four Tunisian populations and showed high diversity. The values of J index are close to 90 and mean population values range between 84.9 (BK) and 90.4 (AJ and TN); this result confirms the high variability in seed shape. Similar results were obtained for *Lotus japonicus* and *Capparis spinosa* seeds that adjust well to cardioids [9, 17]. Zouaoui *et al.* [21] explained phenotypic variability of *Rhus tripartita* by genetic effects.

Probably, size difference between populations is a consequence of ecological conditions of habitats; populations in the lower semi-arid region (AJ, HA with 393 and 364 mm for annual rainfall) have larger seeds, as a result of growth in favorable environmental conditions. But, populations growing in more severe conditions of arid climates had smaller seeds. However, the population of Ksar Hadada (KH) which occupies the lower arid region, showed relatively large seeds because these individuals grows in a valley where accumulates the water of the rain. Already, we have in this region developed plants.

Conversely, seed shape allows the segregation of all populations into two groups, the first is dominated by the seeds of the type "A" and contains the two most northern populations: AJ and HA and the second group is dominated by the seeds of the type "C", it includes the rest of the populations. This geographical separation of studied populations in two groups according to geographical distribution North/South suggests the genetic effects on seed shape of *R. tripartita*. Indeed, the population KH is different from other populations for the size, but it approaches these populations in the arid region for shape index. Our results raise the possibility that the two basic environmental types of *R. tripartita* populations described by Furth [1], i.e. the populations that live in desert and those populations living in the Mediterranean environment.

Finally, seed characteristics (size and shape) of *R. tripartita* appear to be influenced by genetic and environment interaction, this is confirmed by Brittaine and Lutaladio [22], after studying the morphological traits of *Jatropha curcas* seeds. In addition, seed size affects plant germination, growth and physiology [23, 24, 25]; the large seed size can contribute to a better survival and growth of its seedlings [26]. Mtambalika *et al*. [27] studied *Afzelia quanzensis* and registered the highest seedlings height and largest root collar diameter with large seeds; in fact, seed size is a component of seed quality which has impact on the performance of crop [28]. Generally, large seed has better field performance than small seed [29].

In this study, we have developed a method for seed shape analysis in *Rhus tripartita* seeds and compared size and shape between seeds obtained from nine different locations from central and southern Tunisia, characterized by different bioclimatic conditions. Application of this method may give the basis for the study of genetic variation, not only in the genus *Rhus*, but in the complex Anacardiaceae family.

5. CONCLUSION

The study shows that the variation in seed shape in Tunisian *Rhus tripartita* is essentially related to genetic factors. However, the size of the seed is related to the climatic factors of each population. Differences between populations were found both in size as well as in shape (roundness, J index total and partials). Morphological types were characteristic for some of the populations indicating that differences in shape are independent of size. Future studies with a larger number of seeds may contribute to define the relationship between seed shape and the influence of genetic or environmental conditions for this endangered plant, to improve plant multiplication and rehabilitation of natural populations of *Rhus tripartita*.

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