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Genetic Variability of Reproductive Organs of Atlas Pistachio in Tiaret Region, Algeria

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Abstract

Pistacia atlantica Desf., endemic to northern Africa belonging to the family Anacardiceae, is a dioecious species of great ecological and socio-economic importance that exhibits considerable variation between individuals but some cases of monoecis have been discovered within the same family such as *Pistacia atlantica* Desf., *Pistacia therebinthus* and *Pistacia chinensis*. The study was conducted to find for the existence of genetic variability of the Betoum from mid-February to mid-March. The study is made on the morphological parameters of reproductive organs of four sites in the Tiaret region in Algeria. The results showed that this species has a high polymorphism of the morphological, qualitative and quantitative parameters of the reproductive organs. This variation is found in the male and female individuals of the selected stands. Variations in these reproductive organs can in no way be influenced by climatic changes such as leaves and roots and their physiological, biochemical and anatomical parameters. This allowed us to discover the first case of the monoicy in the Rechaiga forest. It is necessary to carry out thorough studies to know all the effects and benefits that this species can bring in our daily life in all the social, ecological and economic medicinal fields with the aim of a better appreciation of the patrimony than presents this species, the maintenance of genetic diversity, its conservation and the prospects for its development.

Keywords: Atlas pistachio, genetic variability, morphological parameters, reproductive organs

1. Introduction

Atlas pistachio (*Pistacia atlantica* Desf.), is called elbetoum, botma, betouma or btoma in Arabic language (Monjauze, 1980). The fruit is a drupe called Ghodim in Algeria. This woody species belonging the Anacardiaceae or Terebinthaceae family (Quezel and Santa, 1963). It is a powerful tree characterized from the ecological point of view by high tolerance to climatic variations. It extends from the wet bioclimate to the central Sahara (Ifticene-Habani, 2020). Among the studies carried out on water stress, the results of research by (Ben Hamed et al., 2016) have shown that *Pistacia atlantica* Desf. is a species that adapts better to water stress followed by rehydration compared to *Pistacia vera* L.

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by physiological, biochemical and gas exchange responses. In an arid environment, *P. atlantica* increases the thickness of leaflets, epidermis, cuticle, and palisadic parenchyma for development while protecting the water inside the internal tissues as well as the rate of terpenes increases with the degree of dryness (Ait Said., 2010). In long dry periods, the Atlas pistachio tree resists by developing long, thick roots at depth (Limane et al., 2014). This species presents natural morphostructural variations. The structure, which is a domain of identification, gives it a special anatomical composition as to variation in shape is visible at the level of organs such as stems, flowers or leaves (Berrichi and Merkati, 2020). The leaves of *Pistacia atlantica* Desf. are composed of finely winged rachis with lanceolate leaflets, obtuse at the apex (Quezel and Santa, 1963). They have 7–11 alternate leaflets and are more than 12 cm long (Monjauze, 1980) and can reach up to 18 leaflets leaf (El Zerey- Belaskri and Benhassaini, 2016) hence it has a high genetic polymorphism between genotypes within the same population and between different populations from different sites (El Zerey-Belaskri et al., 2018). This species is considered a panacea given its medicinal properties. The leaves of *Pistacia atlantica*, rich in essential oils, differ between male and female individuals (Tzakou et al., 2007, Gourine et al., 2009) and in season (Gourine et al., 2009). It also appears that the seeds of this species are rich in nutritional value and their hulls have higher antioxidant properties than those of *P. khinjuk* (Mohammedi et al., 2019). The fruit of *Pistacia atlantica* Desf. in the form of a small walnut and rich in oils, is edible (Monjauze, 1980) and contains fatty acids and sterols (Benhassaini et al., 2007). Moreover, salinity has such an effect on the roots that it reduces their number by inducing a high content of proline (Chelli-Chaabouni et al., 2010) as reported by (Megha and Mummigatti, 2017) that water stress has the same effect on cotton. The flowers are petal less and the inflorescences are axillary (Quezel and Santa, 1963). This species is also used as a pollinator for *Pistacia vera* (Belhadj et al., 2007).

A few case of monoicy are found in Turkey (Kafkas et al., 2000; Kafkas et al., 2001; Kafkas et al., 2005; Isfendiyaroglu, 2009) where there are two different genotypes, the first contains a mixture of male and female inflorescences and the second has branches containing only male flowers and other branches carry only female inflorescences (Kafkas et al., 2005). In northern China, the case of monoicy found in *P. chinensis* Bunge has provided research to elucidate the phenomenon of sex differentiation (Chen et al., 2019). In the species of the genus Pistacia, the sexual expression shows a great diversity and the origin of the monoecism remains an enigma (Bai et al., 2019). This species presents a great genetic variability within and between species (Mehdeb-Hireche et al., 2021).

2. Materials and Methods

We had choosen four natural populations of the Atlas pistachio tree (*Pistacia atlantica* subp. Atlantica), Tagdempt, Guertoufa, Rechaiga and Rosfa, in the Tiaret region, located in the northwest of Algeria and tree and flower sampling was random during the period from mid-February to mid-March 2018. This choice was made to find the existence of intra and inter-population variability as long as there are isolated feet in other places in the city of Tiaret.

The experiment was carried out in late winter and early spring at the laboratory of the Mostaganem higher school of Agronomy, when the flower buds of this species broke and at different times when there is a lag in the maturation of male and female flowers. We used dissection clamps to separate the pieces that make up the flower in order to count them. In the first place, we dissociated the flowers that are intertwined against each other, then bracts and sepals to finally arrive at the reproductive parts of the flowers.

To estimate differences in morphological characteristics of the reproductive organs, 300 flowers were collected from 15 male and 15 female trees at each station, for a total of 2400 flowers. Using a binocular magnifying glass, we were able to observe and count the different parts constituting the flowers.

The data from the samples were processed by factorial correspondence analysis (FCA) using the software "XLSTAT 2017" which allows to search the affinities existing between the male and female individuals of the different stations.

3. Results and Discussion

First of all, it was difficult to recognize the male trees of the female trees but because of the repeated exits, we had the opportunity to distinguish between the two trees and it is because of the colour of the flowers in the first place and their shape in the second place. But (Sun et al., 2014) were able to develop a female-specific SCAR marker to distinguish sex in *Pistacia chinensis* and this technique could be used in the sex determination of all individuals of the genus Pistacia.

Based on the different outputs made at the selected sites, we found that in Atlas pistachio trees, the flowering of female individuals is delayed by an interval of 7 to 10 days from that of male individuals. Indeed, it starts in mid-January at the stations of Rechaiga and Rosfa while it is done in mid-February at the level of Guertoufa and Tagdempt where the cold lasts longer.

The reproductive organs of Bethum are in compact axillary clusters of inflorescences at the beginning of their formation and when pollen grains are released, for male flowers and after fertilization, for female flowers, start to grow longer and bigger (a, b and c figures 1 and 2). After the pollen grains are released, the anthers lose their vigour and turn black as shown in Figure 2b.

3.1. The male flowers

They are compact, close together, separated by bracts. Each flower is protected by one or two pale green bracts at the same tree or in the trees selected from the selected sites. The mean of the bracts of all flowers is (1.042±0.00) and that of

each station is mentioned in Table 1. There is also another light green or brown glabrous bract that surrounds a number of flowers (Figure 1).

The flowers of the *Atlas pistachio* tree, like all species of the Anacardiaceae family, are aperitif. They are protected by bracts and sepals. The number of sepals that have different sizes is variable and the mean of all flowers is (4.50±0.02) and Table 1 shows that at each station.

Figure 1: The reproductive organs of the *Atlas pistachio* tree *Pistacia atlantica* Desf.; a: Male flowers; b: The female flower with small and large bracts

Table 1: Mean of the quantitative endpoints studied for male flowers

	No. of stamens	No. of pollination boxes	No. of dehiscence slots	No. of bracts	No. of sepals
Rechaiga	5,680	2,000	2,000	1,100	3,650
Rosfa	6,600	2,000	2,000	1,030	4,590
Tagdempt	5,940	2,000	2,000	1,030	4,940
Guertoufa	6,170	2,000	2,000	1,010	4,840

The number of stamens varies within the flowers of the same tree, at the level of the trees of the same station and also between stations. Based on the results obtained, the average of the stamens for the 1200 flowers treated is (6.10±0.04) and that of each station is mentioned in Table 1. We found flowers containing a high number of stamens. In this case, 14 and 15 stamens at a single tree at Rosfa Station (a, b: Figure 2).

All the stamens recorded have an invariable number of pollen lodges and dehiscence slots per anther. Each anther contains two cells and two slots of longitudinal dehiscence (Table 1).

Female flowers: Female flowers also have no petals. They have one to two bracts (b: figure 1) and sepals of different shapes and have the general averages of these two parameters that are variables are (1.04±0.00) and (5.68±0.05) respectively and that of each station are mentioned in Table 2.

The results obtained for the number of ovaries show that the average for the 1200 flowers treated is (1.00±0.00). Most flowers have only one ovary per flower. With the exception of a few flowers that have only two flowers at the rate of one flower per station (Rechaiga and Tagdempt) except for the Guertoufa station where we were able to find two flowers with two ovaries with two opposing styles (Figure 3).

Table 2: Mean of the quantitative endpoints studied for female flowers per station

	Ovary	Stigma	No. of bracts	No. of sepals
Rechaiga	1,000	1,980	1,060	8,250
Rosfa	1,000	2,360	1,040	4,950
Tagdempt	1,000	1,990	1,010	4,720
Guertoufa	1,000	1,930	1,030	4,820

The pistil has three distinct parts: the ovary, the style and the stigma. The results obtained indicate that the number of

Figure 2: Male flowers of the Atlas pistachio tree; a and b: Panicles of young male flowers; c: Male flower panicles after release of pollen grains; d: Male flowers with 12; e: Male flowers with 14; f: Female flowers with 15 stamens

Figure 3: *Pistacia atlantica* Desf. flower with two ovaries

styles and stigma varies from 1 to 3 for all flowers. Indeed, the redundancy of the number of 3 is (12.67%), the number 2 is (82.58%) and finally, the number 1 is (4.75%) (Figure 4) as well as the average of the stigmas of the flowers is (2.06±0.01) of the stigmas and that of each station is indicated in Table 2.

For all the flowers studied, we found that they have only one box per ovary (1.00±0.00). Therefore, this parameter is invariable and unchangeable.

Concerning the qualitative characteristics; in all the treated flowers, we observed that each flower has short, medium and long sepals. For the male flowers, the colour of the kittens differs from one tree to another, we observe the predominance of the «green» colour in all the male flowers representing a rate of 79.75% while the red colour represents only 20.25%. The stamens showed a degree of hair growth. Indeed, in the locality of Rosfa, the stamens have a high hair density compared to those of the other stations and this, almost throughout the length of the stamen especially at the level of the summit.

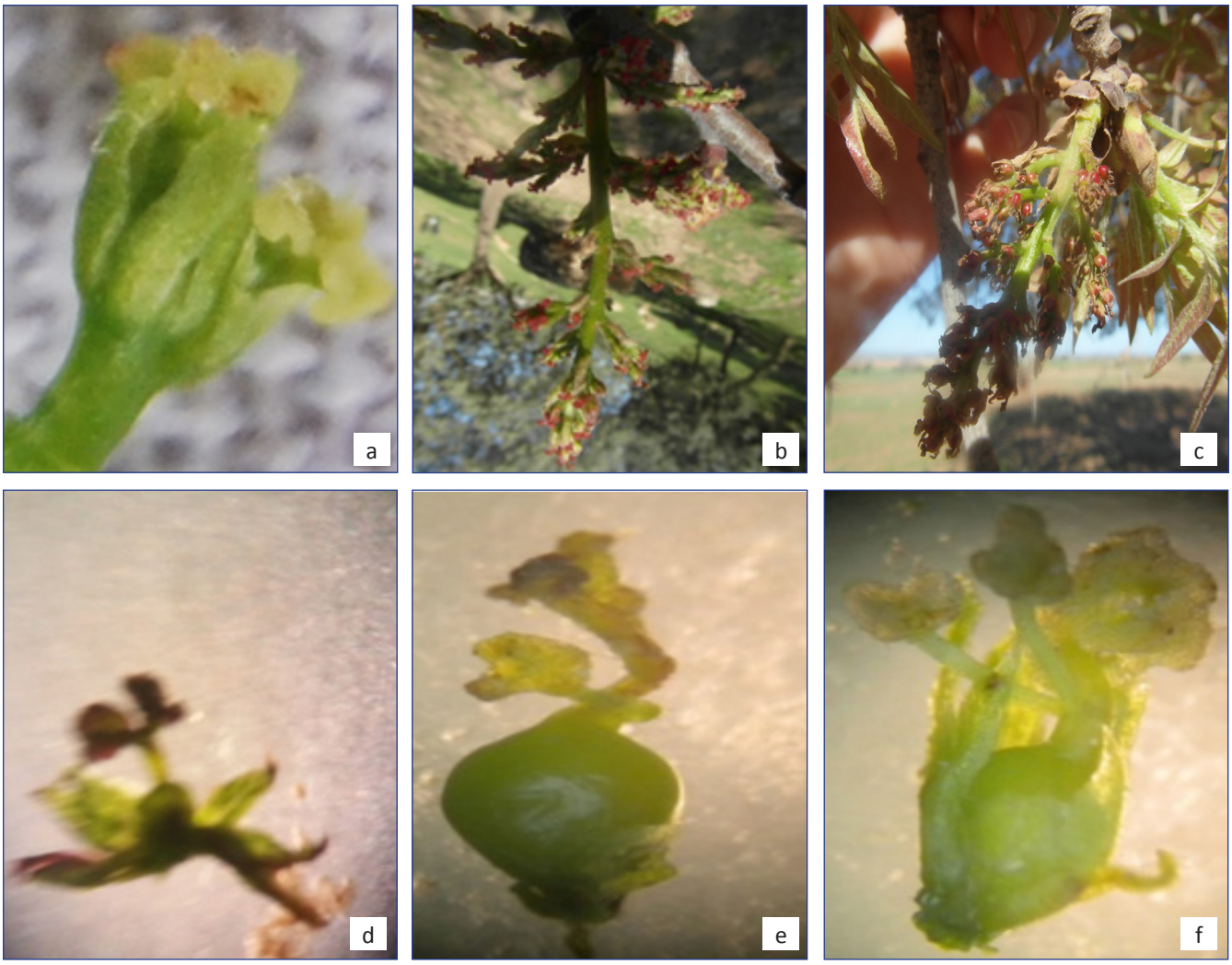

Figure 4: Female flowers and different forms of ovaries of the *Atlas pistachio* tree; a, b and c: Clusters of female flowers; d: Ovary with 1 style and 2 stigmas, e: Ovary with 2 styles and 2 stigmas, f: Ovary with 3 styles and 3 stigmas

During this work, we noticed that the insertion of the ovary on the floral receptacle differs from one flower to another. However, we noted that there are two modes of insertion: horizontal and oblique. (52.67%) of flowers presented the horizontal mode and (47.83%) presented the oblique mode.

The Atlas pistachio tree is a dioecious species belonging to the family Anacardiaceae. One case of monoicy was discovered in Algeria and others were found in Turkey (Kafkas et al., 2001). In contrast, other cases belonging to the genus Pistacia have been discovered in Bulgaria and in China.

Based on the results obtained, we find that there is a fairly pronounced variability within the tree and between individuals in the populations. For the study of the characteristics of flowers, they are well protected against climate variations and cannot be affected by these variations.

First of all, it was difficult to recognize the male trees of the female trees from afar but because of the repeated exits, we had the opportunity to distinguish between the two trees and it is because of the colour of the flowers in the first place and their forms in the second place.

A Correspondence Factor Analysis was conducted to reveal the possible commonalities of the parameters and their interactions with the selected stations.

During our study, we were able to observe that there is intra- and interspecific flower variability.

The floral pieces in the Perigord pieces play a great role in their intra and interspecific number.

Flowers are without petals which make them bare flowers (Mlika, 1987). The number of dehiscence slits and the number of pollen lodges are stable. At their level, the stamens have pubescence while those of Rosfa are very hairy. The flowers have a high number of stamens but those in the centre of the flower have a reduced size and short nets.

The histogram in the following figure indicates the existence of two very distinct heterogeneous groups which allowed us to neglect the 3rd axis.

With an inertia of 82.57%, the F_1 axis is positively defined by the number of stamens by individuals in the Rechaiga region (Figure 5). As a result, this axis highlighted in the positive direction, the station of Rechaiga being characterized by the existence of a reduced number of stamens varying from 4 to 9 while the average of the bracts is equal to 1.1 and that of the sepals is 3.65 varying between 1 and 5.

However, the two remaining stations Tagdempt and Guertoufa are positioned on the negative side of the F_1 axis. Their male individuals have a number varying between 4

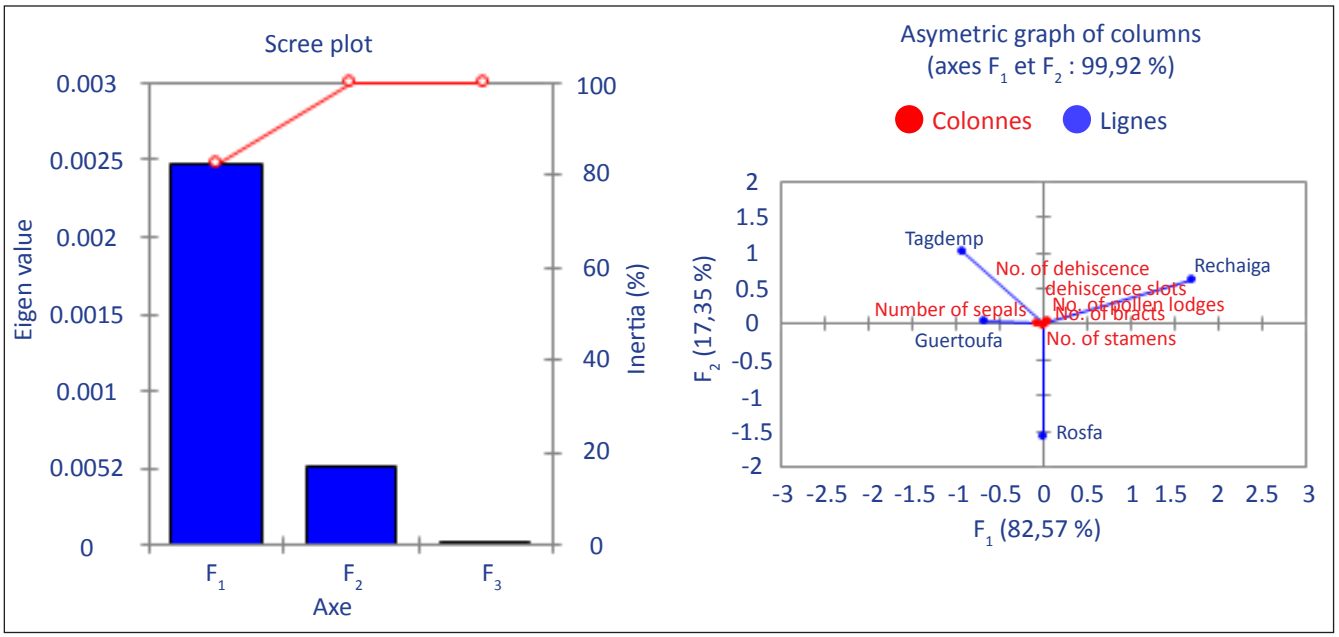

Figure 5: Histogram of eigenvalues and percentages of inertia (FCA1) and factorial plane of stations according to quantitative parameters of male flowers

and 12 stamens per flower while the average of the bracts is equal to 1.03 with a number of sepals varying in an interval of 3 to 7 with an average of 4.59 for the region of Guertoufa and 4.94 for Tagdempt.

The F_2 axis has an inertia value equal to 99.92%. The station of Rosfa is positioned on the negative side of this axis with male flowers and is characterized by the existence of a high number of stamens where we could find a tree containing a number of stamens equal to 15 while the number of sepals oscillates between 2 and 7 with an average of 4.84 and that of bracts is equal to 1.01.

As in many dioecious species, we observed in the pistachio tree of the Atlas that female flowers bloom before male flowers as mentioned by this author (Mlika, 1987).

The cumulative total inertia rate of the FCA2 for all observations is 100% explained by the 3rd axis with the first two axes F_1 and F_2 having the highest contribution with 96.36% and 99.97% respectively. We then deduce two distinct heterogeneous groups as shown in Figure 6.

According to the F_1 axis, with an inertia rate of 96.36%, the Rechaiga station is on the positive side of the axis which is in opposition to the other stations. Indeed, it was said that this station is characterized by a high number of sepals because it varies between 0 and 11 sepals per flower with an average of 8.25 including 67% of the flowers containing 8 sepals and an average of 1.06 for the number of bracts. It turns out that at this station we were able to find a flower with two ovaries and therefore four stigmas.

The remaining stations are positioned on the negative side of the F_1 axis. And according to axis F_2 which has an inertia rate equal to 99.97%, the Rosfa station is opposed to the two remaining stations Guertoufa and Tagdempt. Indeed, thanks to the recorded data, the average stigma is equal to 2.36. As for the number of sepals, it varies between 2 and 11 with an average of 4.95 whereas the average of the bracts is 1.04 of which 35.67% of the flowers containing 4 sepals and 35% contain 5.

Therefore, the Tagdempt individuals are characterized by the presence of a single flower on a set of 300 while two flowers have this particularity of having two ovaries registered at the level of Guertoufa. The mean of the stigmas is 1.99, that of the sepals is 4.72 and their number varies between 2 and 57.67% of flowers with 5 sepals with an average bract count of 1.01 for the Tagdempt population.

On the other hand, the population of Guertoufa has an average stigma of 1.93 and that of the sepals is 4,82 and their number varies from 2 to 7 of which 66% of the whole possesses 5 and 94.33% of the flowers which possess 2 bracts whose average is 1,03.

We found that the lowest number of sepals is 0, recorded

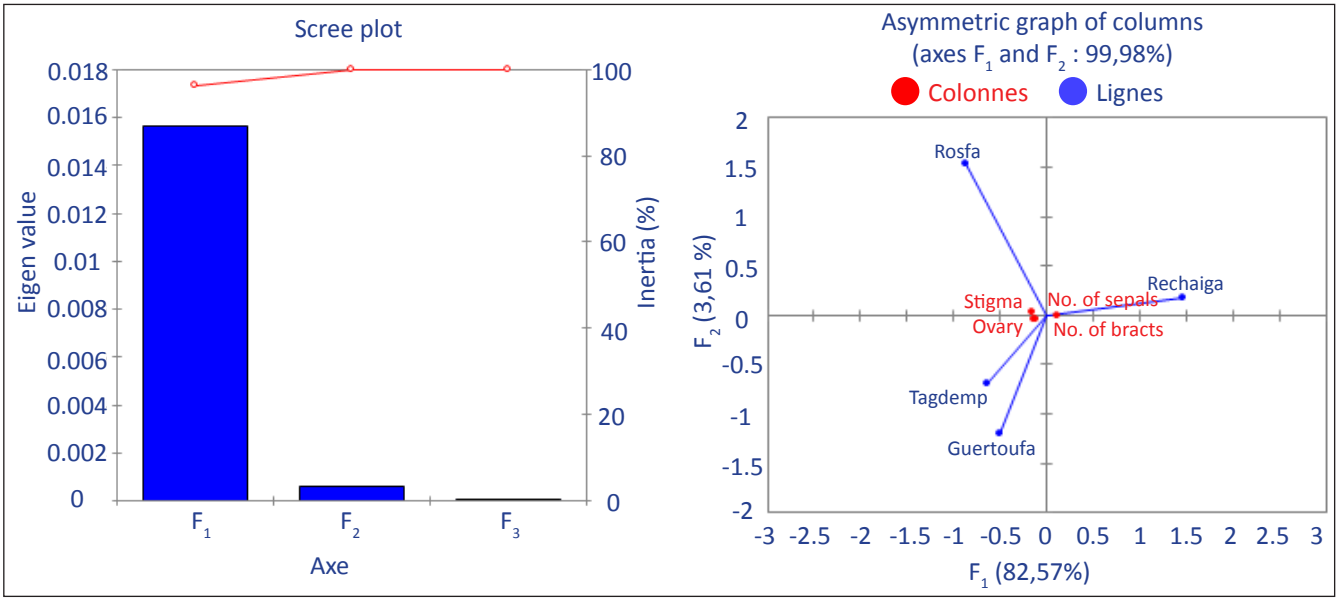

Figure 6: Histogram of eigenvalues and percentages of inertia (FCA2) and Factorial plan of stations according to quantitative parameters of females' flowers

at the Rechaiga station, while the highest number is equal to 11 sepals of variable size (Rechaiga and Rosfa). The number of stigmata varies from 1 to 3 stigmata per flower, as quoted in the literature; these results are consistent with the work cited by (Quezel and Santa, 1963). Only one flower had a peculiarity: the possession of two ovaries in Rechaiga, Tagdempt and Rosfa and two flowers in Guertoufa. The double ovarian formation has not been reported for Pistacia species to date what was confirmed by (Bachelier and Endress, 2009; Isfendiyaroglu, 2009).

4. Conclusion

The results of this study will add value to the estimate of this species. The latter is endowed with great plasticity allowing it to live in severe conditions of the surrounding environment where it is necessary to value and protect it. Its show a very pronounced divergence intra- and interspecies reproductive organ divergence and found that there is a great deal of genetic diversity between species, both qualitative and quantitative, that differs between males and females at the stations studied.

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