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Original Research Paper

Leaf Geometric morphometrics among a natural population of Norway maple (*Acer platanoides*L.) in Northern Algeria

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Abstract

Maple (Acer L.) is a diverse tree genera that includes more than a hundred of deciduous and evergreen species in Northern hemisphere, Acer platanoides is a species from the maple's genus with an invasive aptitudes in Europe and North America, this species had never been recorded in North Africa and the main aim of this work is to investigate the shape and size variability within a natural population in Northern Algeria. The study was carried out using a collection of multivariate, bivariate and univariate statistics, 303 A. platanoides leaves were included in the analysis counting 2 taxa from 8 countries. The analyzed data shows some very close results between Algerian and European A. platanoides, One Way ANOVA of size provided a significant p.value <0.001 between the three studied populations, the Bonferroni correction doesn't show any significant p.values between Algerian and European A. platanoides but confirmed the difference of A. platanoides ssp turkestanicum from the others, linear regression of shape and size shows a significant p.value of <0.001 but a low negative coefficient of correlation r= -0.18 and a low coefficient of determination r2= 0.033, Principal component analysis (PCA) shows an inertia of 53.48% between the first two components and revealed three different forms, MANOVA based on shape data revealed a significant p.value <0.001 between groups of taxa, a Pillai trace of 1.108, and a Wilks lambda coefficient of 0.192, the closest squared Mahalanobis distance (d=8.01) was reported between Algerian and European A. platanoides populations while the largest (d=16.74) was scored between Algerian and Iranian populations, clustering using Kmeans was depending on both Elbow and Silhouette methods, the typical number of clusters according to the two methods was k=2, however, clustering doesn't reveal any specific shape or group of leaves, the statistical analysis proved a small phenotypic plasticity between Algerian and European A. platanoides leaves in terms of shape while size remain conserved between both populations, the provided statistical tools confirms the ability of A. platanoides to show an environmental adaptation additionally also approves A. platanoides ssp turkestanicum as distinguished subspecies.

Keywords: North African maples; Acer platanoides L.; PCA; Algeria; Geometric morphometrics

Introduction

Maple (*Acer* L.) is a large and diverse tree genera that includes more than 130 of deciduous and evergreen species(Parsa 2014)it is a part of Sapindales order's and the family of Aceraceae (Siahkolaee et al. 2017),maples are known with their dispersion along the temperate regions of the northern hemisphere from North America, to East Asia, passing by Europe, North Africa and Middle East (Gibbs and Chen 2009).

The European continentis considered the home of many native maples and a number of infraspecific taxa(Blondel 2018), until now, It is not clear how many subspecies and varietiesshould be included into the genius *Acer* as the south-east limits with Asia are not appropriately inspected, however some species are totally well known all over the continent, this includes,*Acer Pseudoplatanus*, *Acerlobelii* and *Acer platanoides*(Turok et al. 1996), in the other hand the rest and the majority of European maples lies across the Euro-Mediterranean area expending toward the West of Asia, actually,there are 7 native maple species reported by (FAO 2013)across the Mediterranean area with North Africa, these maples are reported as spontaneous species, with a very heterogeneousgeographic distribution, (Mediouni & Azira 1992)creating some kind of mixed forest formations with Oak, Numidian fir, Yew and Atlas Cedar (Trabut & Battandier 1890),According to (Quézel & Santa 1963), 4 taxa of maples occursmainlyin Northern Algeria which are*Acermonspessulanum*, *Acercampestre*,*Acer opalus* and *Acer obtusatum*.

Maples taxonomy was always questionable, in fact a lot of investigations are established in order to determine an accurate classification for species within this genus, Early revisions where those of pax1902 and pojarkova 1933 followed by Fang 1966 until the late of the late 20th century where (De Jong 2002) subdivided the *Acer* genus into sixteen sections that are further subdivided into nineteen series, however and with all these efforts North African maples seems to be unbenefited at all and a remarkable lack of data and scientific works regarding the mentioned genius in the statedarea is clearly exposed.

Acer platanoides is a shade intolerant,deciduous maple that could achieve 10 meters high, it is native to Europe but also has a large distribution and found in many parts of the world(Gelderen Van et al. 1994) it belongs to the section platanoidea with *Acer campestre* a well-known species around the Mediterranean rim(Nagy & Ducci 2004), *Acer platanoides* is recognized with two taxa, *Acer platanoides*ssp*platanoides* and *Acer platanoides*ssp*turkestanicum* a subspecies with smaller leaves that is native to west Asia including Afghanistan, Iran andTurkestan(Murray 1969)*Acer platanoides* has a unique capability of high seed survivability, evenunderdifficult conditions, this could achieve several years in freezing temperature(Hong & Ellis 1990).

Recent studies reveals the importance of morphological characters for identifying maple species and resolving difficulties that occurs into its taxonomical identification(Chikhaoui 2016), for many reasons, the use of both classical and geometric morphometrics with different statistical models became a powerful tool to illustrate variations among groups of taxa (Savriama 2018), weather linear measurement appeared to be quite dealing with quantitative traits, this latter remains very limited in topics, experiments and studies that aims to characterize and distinguish between taxa, a reason could be that the complex shape of an organism cannot be easily summarized by using only linear measurements (Zelditch et al. 2012), conversely, the morpho-geometrics allows to better explore the deformations and graphically display any changes across the anatomical parts of the plants, and this could be a beneficial advantage for species classification and identification (Liu et al. 2018).

Until this moment, we do not have any bibliographic source that confirms the presence of *Acer platanoides* nor its origins in North Africa and this would rise some interesting hypothesis regarding its presence in Chréa forest and its environmental adaptation in Algeria, this work should answer the following questions:

- Could a geometric morphometric method reveal any variations regarding shape and size among the studied *A. platanoides* populations?
- Are there any forms of environmental adaptation that influenced leaves shape and size of Algerian *A. platanoides*?

The exposed work has the objective to highlight some essential informations regarding this species in Algeria and the main aim of this study is to compare collections of Algerian and world wide *A. platanoides* leaves that are very different in terms of ecological and geographical conditions, the presented work also highlight the variability of shape and size of an isolated and naturally grown population of Norway maple using a morpho-geometric method and a collection of statistical tools.

Materials and Methods:

This study took in consideration three populations of Norway maple, the first population is represented by Algerian *A. platanoides*, the second population is represented by European *A. platanoides* and finally the third population is corresponding to Iranian *A. platanoides*ssp*turkestanicum*,303landmarked leaves participates in the analysis matching to8localities (Figure 1), including Algeria, Germany, England, Switzerland, Netherlands, Sweden, Norway and Iran, Algerian leaf samples were collected from the forest of Chréa an area of the national park in northern Algeria at an elevation of 1250m to 1350m, 14 mature and healthy Norway maple trees were selected, naturally grown and dispersed along the forest, mainly with species of *Cedrus*, *Juniperus* and *Quercus*, leaves were scanned on their fresh form using a combined printer (HP all in one 123) to 300 DPI, European *A. platanoides* and *A. platanoides*ssp*turkestanicum* samples were downloaded from virtual herbariums registered at the Global Biodiversity Information Facility data base (GBIF 2021),specimens were chosen from different countries in order to cover a wide geographical area (Table 1), damaged leaves were initially excluded from digitizing, a scale factor was adjusted in order to remove the effect of using pictures in various resolutions

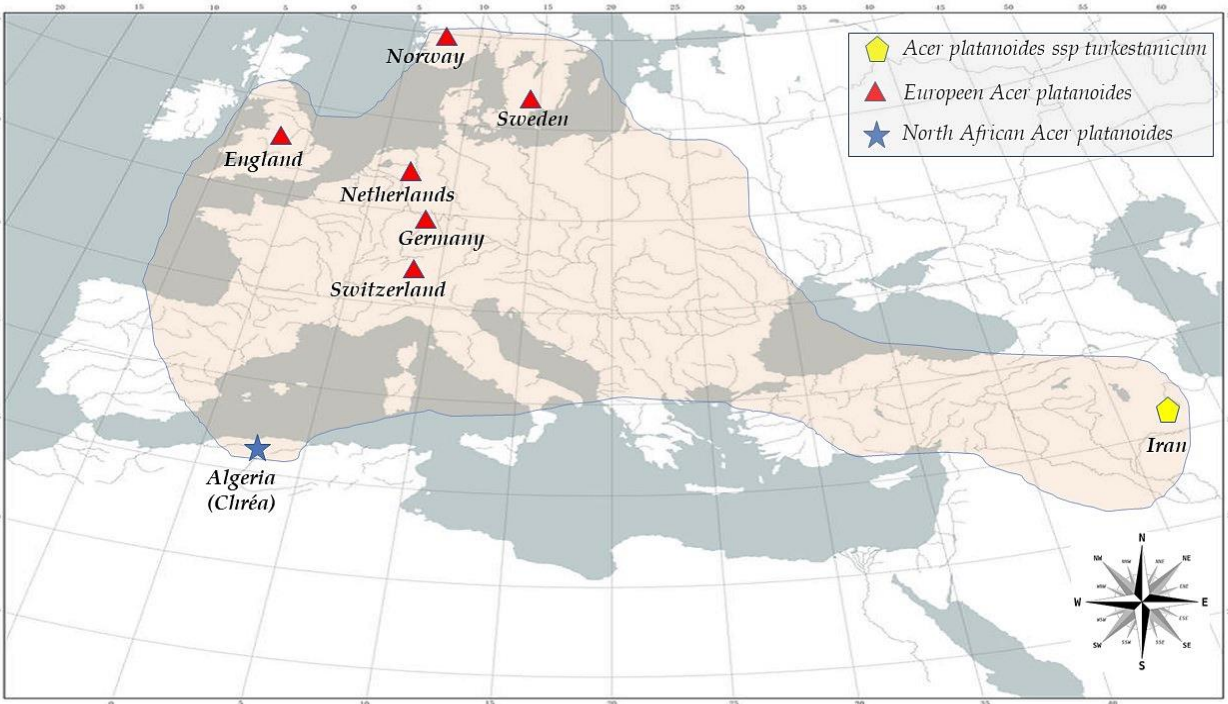

Figure 1. Theoretical distribution map of Acer platanoides species in Eurasia, Algerian Acer platanoides appeared in blue, Eropean Acer platanoides appeared in red while Acer platanoidesssspturkestanicum appeared in yellow.

A configuration of 14 landmarks (LM's) was used in leaf digitizing (Figure 2), this procedure is done through TPSdig32 ver2.31, a software from Rholf's geometric morphometrics packages, during the analysis LM 1 represents leaf base, 2 and 14 are apex of the lower teeth's , 3 and 13 are the inner sinus between the lower teeth's and the lower lateral lobes, 4 and 12 apex of lower lateral lobes, 5 and 11 are the inner sinus between upper lateral lobes and lower lateral lobes, 6 and 10 are the apex of upper lateral lobes, 7 and 9 are the inner sinus between the upper lateral lobes and central lobe, 8 is the apex of central lobe, this configuration is very similar to this of (Wahlsteen 2021), petioles had been excluded from the analysis, in general, petioles are highly unstable while scanning leaves and it is really difficult to estimate their correct length due to its curvature and deviation from a straight line hence this could indicates errors while digitizing.

The used statistical methods varied between multivariate, bivariate and univariate statistics including principal component analysis (PCA) a very common method in geometric morphometrics used for exploring the shape trends and variability among specimens, leaves Centroid Size (CZ) was calculated according to Procrustes shape distances, the scale factor plays and important role in this operation(Hammer & Harper 2005), size was tested using descriptive statistics and One way analysis of variance (ANOVA) in addition to a supplementary post-hoc test using Bonferroni coefficients for identifying similar groups, according to (Ghasemi & Zahediasl 2012)normality should be ignored due to the large number of observations 303, the relationship between shape and size was tested using linear regression, shape data were represented by the scores of "PC1" the leading component in PCA function with the highest inertia and variations, discrimination between groups of taxa was tested using multivariate analysis of variance(MANOVA) at a confidence level equal to 95%.

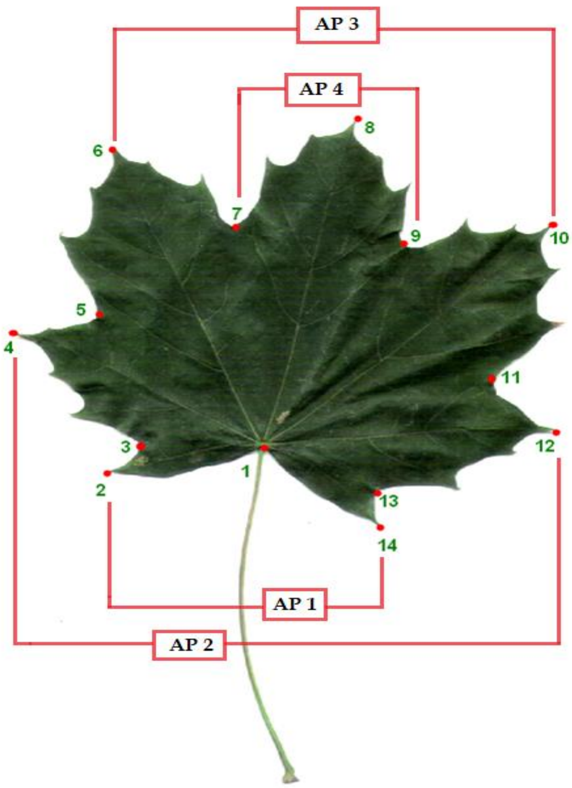

Fig 2. The 14 land marks (LM's) configuration applied on Algerian Acer platanoidesleaf with the analyzed anatomical parts (AP's) here AP1: shows the left and right lower teeth's, AP2 shows left and right lower lateral lobes, AP3: shows the upper lateral lobes and finally AP4: reveals the central lobe

Finally the clustering was done using Kmeans method, thetypical number of clusters for this method was estimated using both Silhouette and Elbow methods, both are depending on the visual selection (Kodinariya & Makwana 2013)

Table 1. Number of digitized Acer platanoides leaves from each country and continent according to their taxa, here Algeria is represented by 206 leaves in total, European Acer platanoides by 68 including England, Germany, Netherlands, Sweden, Norway and Switzerland, finally Acer platanoidesssspturkestanicum is represented by 29 from Iran.

Country	**Number of leaves**	**Continent**	**Taxon**
Algeria	206	North Africa	*Acer platanoides*subsp. *platanoides* L.
England	11	Europe	*Acer platanoides*subsp. *platanoides* L.
Germany	11	Europe	*Acer platanoides*subsp. *platanoides* L.
Norway	30	Europe	*Acer platanoides*subsp. *platanoides* L.
Sweden	3	Europe	*Acer platanoides*subsp. *platanoides* L.
Switzerland	6	Europe	*Acer platanoides*subsp. *platanoides* L.
Netherlands	7	Europe	*Acer platanoides*subsp. *platanoides* L.
Iran	29	Asia	*Acer platanoides*subsp. *turkestanicum* (pax) P.C.de Jong
8 countries in Total	303 leaves in Total	3 continents in Total	2 taxa in Total

The statistical analysis was purified from some outliers and carried out using a collection of softwares and packages, Initial Shape data were storedin files of TPS format created by TPSutilver1.78,leaf landmarking(digitizing) was done using TPSdig32ver2.31 (Rohlf 2015), before running the statistical analysis all the shape data were transferred to a two dimensional Procrustes fit by Past software.

(R Core Team 2020)version 3.6.3 was used to calculate ANOVA, MANOVA, Descriptive statistics and also to estimate the optimal number of clusters, the package fact extra version 1.0.7 in R (Kassambara & Mundt 2020)was an R extension used to plot the K means function, the PCA was released using Morpho J version 1.06d (Klingenberg 2011), Past software version 4.03 (Hammer et al. 2001) was used for testing linear regression

Figure 3. Algerian Acer Platanoides located in the forest of Chréa, Original picture from the Author Mediouni Mohammed Rida

Results:

Principal Component Analysis and global shape trends:

Most of the variance in the PCA is explained by the first two components, the function is dealing with *A. platanoides* leaves that are originally recorded from different regions (Figure 6), PC1 leads the highest value of inertia by 44.56% followed by PC2 explaining an inertia of 8.92% (53.48% in total).

The scatter plot revealed three distinguished populations in terms of shape, European *A. platanpoides* appeared on the positive score of the first component while *A. platanpoides*ssp*turkestanicum* dominated the negative score of the same component, Algerian *A. platanpoides* appeared mainly in the positive side of the second principal component, leaf shapes could be also clearly distinguished, European *A. platanpoides* appeared with five palmately lobes, a wide middle lobe, lateral lobes were radially positioned with an open angle however and conversely, a very narrow angle of approximately 60° at the level of lower theeth's while Algerian *A. platanpoides* appeared with contracted lateral lobes leveled at the same direction of middle lobe also with an open angle at the level of lower theeth's, in the other hand, *A. platanpoides*ssp*turkestanicum* appeared different to the previous taxa with smaller and narrow middle and lateral lobes compared to those of European *A. platanpoides* however the main difference occurs in the open angle at the level of lower theeth's that almost reached 180°.

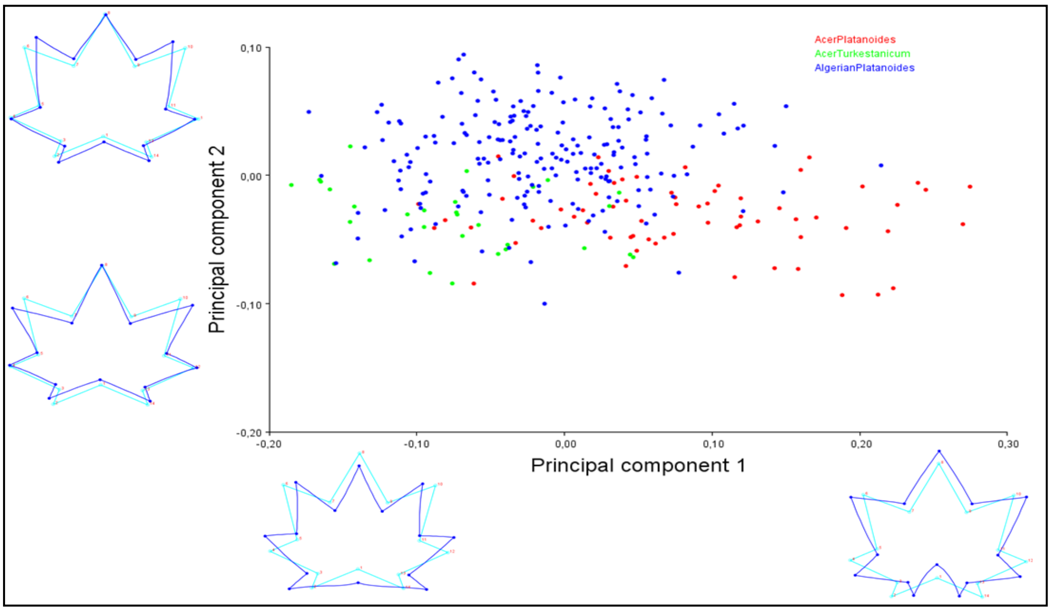

Figure 6. Principal Component Analysis of 303 Acer platanoides leaves, here Algerian Acer platanoides appears in (Blue), European Acer platanoides appears in (Red) and Iranian Acer platanoidesssspturkestanicum appears in (Green)

The scatter plot revealed three distinguished populations in terms of shape, European *A. platanpoides* appeared on the positive score of the first component while *A. platanpoidesssspturkestanicum* dominated the negative score of the same component, Algerian *A. platanpoides* appeared mainly in the positive side of the second principal component, leaf shapes could be also clearly distinguished, European *A. platanpoides* appeared with five palmately lobes, a wide middle lobe, lateral lobes were radially positioned with an open angle however and conversely, a very narrow angle of approximately 60° at the level of lower theeth's while Algerian *A. platanpoides* appeared with contracted lateral lobes leveled at the same direction of middle lobe also with an open angle at the level of lower theeth's, in the other hand, *A. platanpoidesssspturkestanicum* appeared different to the previous taxa with smaller and narrow middle and lateral lobes compared to those of European *A. platanpoides* however the main difference occurs in the open angle at the level of lower theeth's that almost reached 180°.

Regression Analysis of Shape versus Size:

The relationship between shape and size was tested by linear regression (Figure 5), this latter shows a significant <0.001 but negative correlation r= (-0.18) between the two studied parameters, also shows a low determination coefficient r^2=(0.033), the reported results indicates some slight changes in shape over the diminution of size therefore the correlation coefficients remains low and doesn't allow us to confirm a strong relationship between the shape and size.

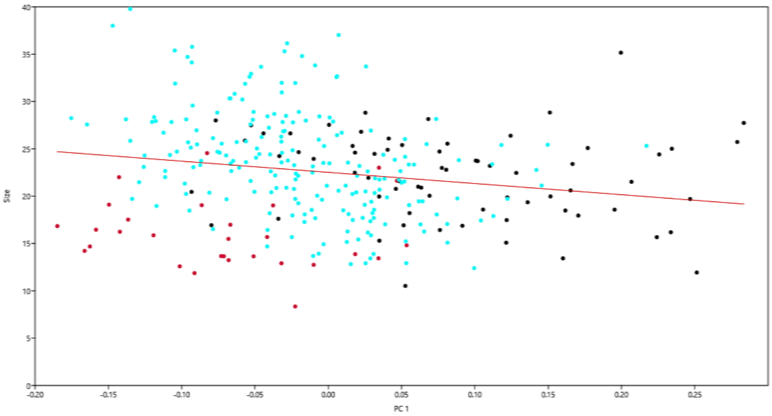

Figure 5. Linear regression of shape and size databased on three Acer platanoides leaf collections, here Algerian Acer platanoides appeared in (Blue), European Acer platanoides in (Black) and Asian Acer platanoidesssspturkestanicum in (Red

According to the regression, *Acer platanoidesssspturketanicum* appeared with a considerably smaller size compared with Algerian *Acer platanoides* and European *Acer platanoides*, the two laters appeared with very heterogeneous forms and shapes however, it is noticeable that Algerian *Acer platanoides* gain a slight amount of size against European*Acer platanoides*.

Size Analysis using ANOVA and descriptive statistics:

Descriptive statistics shows that Algerian *A. platanpoides* and European *A. platanpoides* are very close in terms of size (Table 2) since the two populations achieved a mean of 23.57 and 22.09, a median of 23.33 and 22.63 and a standard deviation of 5.53 and 4.57 respectively, while *A. platanpoidesss*sp*turkestanicum* appeared very different from these populations with considerably lower valuescompared to continental *Acer Platanoides*, the Analysis of variance showed no significant difference between Algerian *A. platanoides* and European *A. platanpoides* but a very significant difference *A. platanpoides*ssp. *turkestanicum* and continental *A. platanpoides*.

Table 2. Descriptive statistics and One-Way ANOVA p.values based on leaves centroide size (cz)

Population	N*	Min	Max	Mean	Median	Variance	Std Deviation	Std Error	Overall P.value	Post Hoc Significance**
Algeria	206	12.40	39.78	23.57	23.33	30.66	5.53	0.38		Non Significant
Europe***	68	10.51	35.16	22.09	22.63	20.96	4.57	055	< 0.0001	Non Significant
Asia	29	8.35	24.54	15.97	15.48	13.26	3.64	0.67		Significant

**: Number of leaves according to each population.*
***: Post Hoc Significance was calculated according to Bonferroni coefficients.*
****: Participating countries from Europe were England, Sweden, Germany, Netherlands, Norway & Switzerland*

Shape discrimination using MANOVA:

The multivariate analysis of variance based on shape data provided a high significant overall probability p <0.001, a Pillai trace of 1.108, which indicates the good contribution of the applied landmark data to the test (Pillai 1955), additionally, a Wilks lambda value of 0.19, which indicates some important statistical variations among the groups of taxa (Shi 2019), the Bonferroni correction also provided high significant probabilities between the tested groups and indicates a clear discrimination between the studied populations.

MANOVA also scored a matrix of squared Mahalanobis distances, the highest values were reported between Algerian *A. platanoides* and *A. platanoides*ssp *turkestanicum* giving a value of 16.74 followed by 13.92 between European *A. platanoides*and *A. platanoides*ssp turkestanicum while the lowest distance was of 8.01 between Algerian *A. platanoides*and European *A. platanoides*, the results shows the clear separation of *A. platanoides*ssp *terkestanicum* from the other taxa, while Algerian and European *A. platanoides*remains very close in terms of shape.

Table 3. Results of MANOVA according to the three studied populations

Compared Taxa	Squared Mahalanobis distances	Bonferroni Correction	Wilk's lambda	Pillai trace	Overall P value
platanoides ssp turkestanicum *Algerian A. platanoides*	16.74	<0.0001			
platanoides ssp turkestanicum *European A. platanoides*	13.92	<0.0001	0.192	1.108	<0.0001
Algerian A. platanoides *European A. platanoides*	8.01	<0.0001			

K means Clusterring:

During the analysis both Elbow and Silhouette methods proposed an optimal number of clusters equal to k=2 (Figure 7), the scatter plot of the Kmeans revealed two main groups with a very heterogenous leaf composition, the first dimension provided and inertia of 33.7% While the seconddimension provided and inertia of 13.5% (47.2% in total)

Kmeans was depending on 1000 permutations, additionally, 2 initial random centroids were set at the beginning of the analysis, the function does not reveal any specific or distinct group of leaves (Figure 8) however the first cluster

appears dominated with 136 Algerian *Acer platanoides* leaves followed by 24 Iranian *Acer platanoidesssspturkestanicum*and finally come European *Acer platanoides* with only 12 leaves, in the other hand the second cluster appears to be balanced with Algerian *Acer platanoides* and European *Acer platanoides* with 70 to 56 recorded leaves respectively and only 5 leaves of *Acer platanoidesssspturkestanicum*.

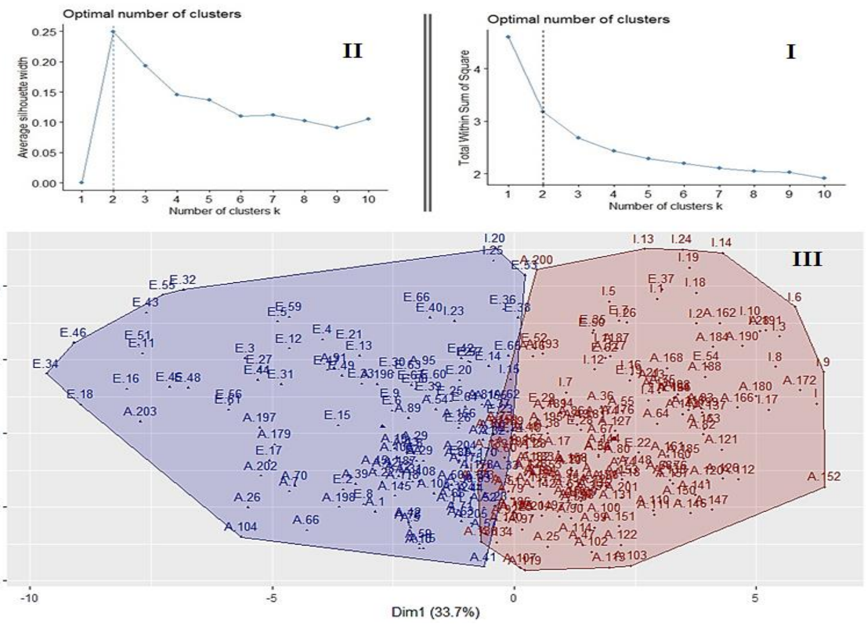

Figure 7. Kmeans clustering revealing 2 clusters according to both Elbow "(I)" and silhouette "(II)" methods, in the scatterplot "(III)",Algerian Acer platanoides appears with abbreviation"A",EuropeanAcer platanoides appears with abbreviation"E" while "I" means Iranian platanoidesssspturkestanicum.

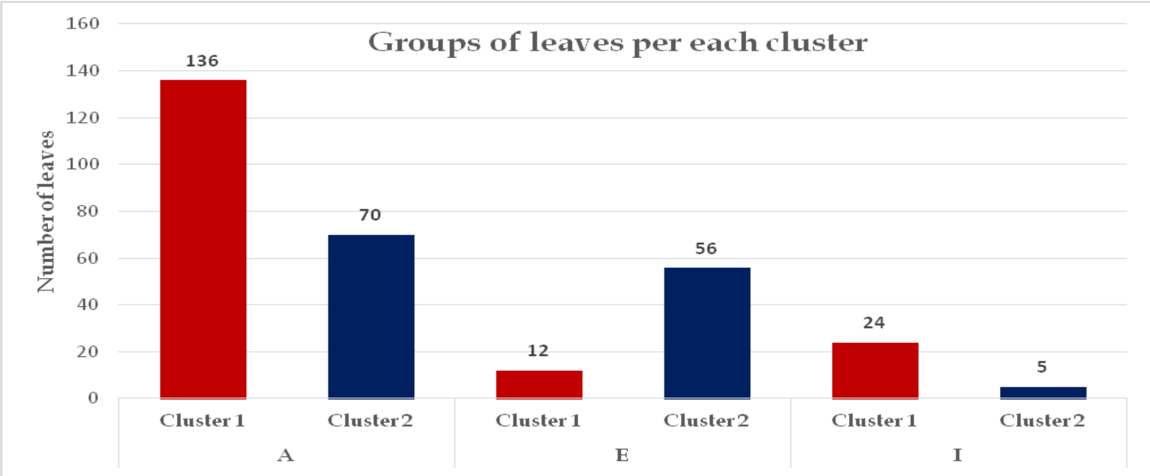

Figure 8. leaves distribution according to each cluster, Algerian Acer platanoides appears with abbreviation“A”,EuropeanAcer platanoides appears with abbreviation “E” while “I” means Iranian platanoidesssspturkestanicum, Cluster1 (Red) is represented by 172 leaves while Cluster2 (Blue) is represented by 131 leaves.

Discussion:

The value of this work is not occurring only on its content as a research paper that deals with North African maples however as one of the rare articles that deals with *Acer platanoides* geometric morphometrics, in the mean time we can find many manuscripts dealing with maple’s diversity, classification, and taxonomy, using different methods from geometric morphometrics counting (Jensen et al. 2002)on *Acer rubrum* and *Acersaccharinum*(Kostic et al. 2017)on *Acer pseudoplatanus*,(Wahlsteen 2020) on *Acer campestre*, to molecularanalysisincluding(Khademi et al. 2016) on *Acer monspessulanum*, (Pandey 2005)and (Grimm et al. 2007)on *Acer pseudoplatanus*

Actually, the data concerning*A. platanpoides*morphogeometricsare so few, and the reasonwhycoulddepend on thetechnical difficulties that appearswhilestudying a rathercomplicatedleafshape(Gavrikova & Ignatyuk 2014), the morphometrical method applied in this manuscript could give us an idea on how Algerian *A. platanpoides*appears in terms of shape and size rather than provide us with a simple leaf configuration based on 14 landmarks for further geometric morphometrics studies.

The statistical analysis in this manuscript tested leaves size using descriptive statistics, ANOVA and linear regression, the mentioned tools confirms the smaller size of *A. platanpoides*ssp*turkestanicum*on the other hand it does not allow us toseparate between the Algerian and the European Norway maple, since this latter remains independent from shape variations according to the regression results but conserved between both populations.

In the next step we managed to use Principal component analysis, a multivariate tool that mainly deal’s with leaves shapes and trends in geometric morphometrics, the PCA distinguished 3 different types of shape conformation where *A. platanpoides*ssp*turkestanicum* was clearly noticed to be different and this is totally expected since it is from a very different ecology that extends from East of Iran to Mid Afghanistan and other neighboring regions, therefore, the main shape differences were reported between Algerian and European *A. platanpoides*principallyat the level of lateral lobes and lower teeths.

The discrimination based on shape data using MANOVA showed a significant difference between all the studied groups of taxa and provided a matrix of squared Mahalanobis distances where Asian and Algerian populations of *A. platanpoides*appears very different, and this would confirm the ability of discrimination tools in geometric morphometrics to distinguish groups of taxa based on their shape data.

The clustering using Kmeans method provided a set of 2 optimal clusters however it failed to identify a specific group of shapes nor a group of taxa, all what we can justify is that cluster 1 was almost dominated with Algerian *A. platanoides* while cluster 2 was a hybrid of the groups of taxa.

According to the provided results, the problematic of differentiation between North African and continental*A. platanoides* appears to be a matter of shape not size since this latter remains conserved between the two studied populations of Europe and Algeria, this would rise some interesting hypothesis regarding leaf phenotypic plasticity of this species and its adaptation capacities to different environments and climates, it should be noted that this is not the first report regarding North African maple's behaviors since a most recent study done by (Mediouni et al. 2021)reveals that both shape and size of three separated populations of Algerian *A. monspessulanum* were influenced by environmental conditions compared to Eurasian groups of *A. monspessulanum*.

An expression of isolation by distance phenomena is not excluded also from the list of hypothesis meanwhile we don't have the accurate information concerning the presence of the studied species in Algeria and for this reason, studies concerning the genetic diversity using molecular markers like SSR's or SNP's are highly important at this stage since SSR's are relatively reliable and does not require much efforts nor costs(Kvesić et al. 2020), Further studies should also include the statistical analysis of samaras taking in consideration that the anatomy of this compartment plays an important and discriminant role in the identification and evaluation of worldwide maples

According to our field prospections, the species does not show any tendency or behaviors of invasiveness contrarily to its influence in Europe and North America(Straigyte & Baliuckas 2015) hence Norway maple in Chréa forest now is considered as a richness to the Algeria and North African flora.

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Conflicts of Interest

The authors declare no conflict of interest.

Plant Identification

Algerian *Acer platanoides* collections were examined and identified by Professor Medjahdi Boumediene from the university of Tlemcen, Departement of Forestry.

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