

Physiological Behavior of Cork-Oak Acorns (*Quercus suber* L.) and Selection of the Best Ecotypes Adapted to Climate Change

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Abstract – Cork oak forests are extremely variable with regard to their ecological and socio economic conditions. Therefore, the impacts of climate change on cork oak forests are likely to be distributed across different bioclimatic zones. Nevertheless, our understanding of the potential of this species to cope with climate change is necessary. To explore this, we grew Cork oak seedlings from acorns collected at twenty Tunisian populations selected from climatically contrasting localities and covering an aridity gradient (Kroumirie and Relicts areas). Acorn size and moisture content were evaluated as a possible adaptive trait enhancing stress resistance. Seedling morphological traits (height, diameter and number of leaves) were analyzed after three month growing. There were significant correlations among the considered acorn morphology and seedling traits. Relicts populations originating from sites with the driest summers exhibited the highest survival rates. These populations were characterized by bigger germination rate, suggesting selection for this trait across sites experiencing drought. Our findings reveal that Kroumirie populations are not very well adapted to cope with increasing drought. The physiological behavior response was more related to the geographical origin. Seedling traits tolerant to drought might have a high potential for vegetation recovery in afforestation projects and restoration programme particularly under water-limited environments or in degraded areas.

Keywords – Cork Oak, Climatique Change, Kroumirie, Relicts Areas, Acorn Size, Moisture Content, Morphological Traits.

I. INTRODUCTION

Cork oak (*Quercus suber* L.) is one of the most important forest species growing in mediterranean basin, because of its distinctive properties. Ecologically it serves as an environmental protector and stabilising the marginal areas species [1]. In Tunisia, this genus is distributed from north to south in the forests belonging to two different areas (Kroumirie and Relicts areas). In fact, Cork oak forest provide a wide range of environmental activities, including biodiversity conservation, soil conservation, fire prevention resilience to climate change and desertification, carbon fixation [2].

It displays intermediate to tolerance to drought and high temperatures, being considered a transitional

species between the high drought tolerant evergreen oaks [3]. Populations of cork oak become scarce and scattered going northward due to cold winters and eastward due to dry conditions [4]. Models predicting suitable habitat for forest tree species under future climate change scenarios indicate that the range of *Q. Suber* could decrease drastically in southern Tunisia [5]. It could also extend its distribution northwards and to higher altitudes benefiting from a potential increase in winter temperatures [6]. However, those models that predict species redistribution with climate change largely ignore within-species genetic variation [7]. In the short term, the potential of species to respond to these rapid changes will depend on their phenotypic plasticity as much as intra-specific genetic variation for adaptive traits [8].

Seed size is one of the most important traits influencing the early stages of the life cycle of the plant. The importance of seed size in governing the fitness of progeny has been supported by extensive empirical evidence [9]; several studies have found that larger seeds help seedlings to endure drought [10]-[11], and promote germination [12]-[13], growth and survival [14]. Seed size can be affected by the original environment, primarily due to seed sensitivity to resource availability [15]- [16]. In general, growth parameters of seedlings such as height, diameter, and fresh weight increase as seed size increases [17]. A positive relationship among acorn size, seedling height and summer survival has been reported for other Mediterranean oak species [18]. Several studies have reported that bigger acorns lead to increased seedling growth [19] - [22] and higher root/ shoot ratios [23] - [24], which can improve the seedling performance by developing and maintaining deep and extensive root systems accessing water from deep within the soil profile [25]. Owing to large differences in environmental conditions among populations, *Q. suber* presents high inter-population variation in adaptive traits as a consequence of adaptation to the climate at the place of acorns origin and populations from the wettest sites had

the smallest average seed size and the lowest final survival under the dry conditions [26].

In the near future, it will be important to increase long-term studies on natural populations in order to understand plant response to environmental factor fluctuations including climate change. The present study was designed to determine the morphologic response of cork oak acorns (*Quercus Suber L.*) from 2 climatically and genetically different provenances (kroumirie and relicts forests) and selection of the best ecotypes adapted to climate change.

The present study is part of a large programme, which aims to determine the capacity of *Q. suber* to cope with expected climate change and to contribute to the development of a strategy for the extensive production of high quality seedlings of this species for reforestation in the forests of Tunisia. Our specific objectives were (1) to estimate the influence of acorn size on the response of *Q. suber* to local climatic conditions and (2) to identify which populations would be the most susceptible to the climate change expected for the region.

II. MATERIALS AND METHODS

II.1. Study area: We selected twenty populations to cover the main range of climatic conditions of *Q. suber* distribution in Tunisia. Two areas were identified: The Kroumirie and Relicts Areas (Table 1) and (fig.1).

II.2. Acorn collection: Samples of fresh acorns, morphologically mature, collected at the end of November 2012 (200 per locality). The acorns were reaped directly from the selected shrubs (acorns of each shrub per each population were maintained separated) at the time of natural dispersal.

II.3. Acorn Morphology: After cleaning, acorns are dried for one week and a morphological study of these acorns was determined by measuring the size of fruit (Seed length, width) using a slide gauge and expressed in cm and by the shapes (oval, rounded,...), the weight was determined by measuring the average value of the weight of 20 acorns .

II.3. Moisture Content: The temperature for moisture measurement was maintained at $130^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for two hours. The moisture content is expressed as follows [26].

$$H\% = 100 \frac{(PF - PS)}{PF}$$

II.4. Acorn sowing and germination: 20 acorns per locality were sown in a plastic pot (60 × 20 cm). Acorns were sown at approximately 2-cm depth in the topsoil, and covered with the respective litters [27]. The pots were kept outdoors under natural conditions, and watered regularly to the field capacity. The germination process was carried out without pretreatments. The germination time was monitored daily, and the beginning of germination was considered when the percentage of germination for each pot was 20% [28]. After germination of 20 seedlings per each locality were selected for measurements.

Germination rate is appreciated by the average germination time (TMG), is expressed is as follows:

$$TMG = n_i \cdot t_i / N$$

ni = number of seeds germinated at time ti,

N = total number of germinated seeds

II.5. Mortality rate (%): is measure of the number of deaths in a particular population, per the total number of emerged plants, per unit of time.

II.6. Growth rate of seedlings: The aerial growth parameters: the length and diameter of the collar of the rod and the average number of leaves were observed every week.

II.7. DATA ANALYSIS: The study results were processed statistically by the analysis of variance using the program SYSTAT (Version 10,2). Pearson's correlation coefficient was determined for different structural parameters.

III. RESULTS

III.1. Acorns Sizes: The morphological study of acorns indicates the presence of significant differences. There was a very high correlation between length and acorn weight ($r^2 = 0.78$). There were no significant intra-population differences among length and diameter ($p \leq 0.05$) (Table 2).

For the acorn morphology of the considered populations; the length ranged from $2.13 \pm 0.47\text{cm}$ to $3.53 \pm 0.66\text{cm}$. Diameter ranged from $0.21 \pm 0.05\text{mm}$ to $0.82 \pm 0.05\text{mm}$. Weight varied from $2.5 \pm 0.97\text{g}$ to $10.28 \pm 2.25\text{g}$ (Fig.2). The statistical comparison of values between different populations showed that acorns differ in terms of their weight which shown the highest variability (Table 2).

Within each region; there was significant variability in the acorn morphology. The highest values were observed in the provenances of JZ (3.2 cm, i.e. 0.82 mm, 10.2g) where the smallest values in both length ($2.91 \pm 0.26\text{cm}$) and diameter ($0.21 \pm 0.063\text{mm}$) are present in provenance of Siliana (JS).

In Kroumirie a significant variation between regions was observed with a highest value in Tbarka (Population of Asn) (3.53cm, 0.64 mm, 6.24g) and Ain Drahem (population of MS) [3.53cm; 0.61mm and 6.33g]. The average minimum value is in Provenances Aslt (Area of Fernana) (2.13 cm, 0.59 cm, 3.53g). Area of Ain Drahem et Nefza was in an intermediate position (Fig.2) and (Table 2).

In addition, the variations in size and weight, there is also a variation in the shapes of acorns. For the provenance of JA, acorns are usually oval. BL DF, BM and Bab are generally rounded or elongated shape. The population JA, acorns are ovoid. Whereas those of MS, AZ, JD, JB and Jk are smaller and rounded. F and Js is stated to have large elongate acorns. (Fig.3). Our results show that acorns have different sizes and shapes ranging from spherical rounded to sub-cylindrical aspects.

III.2. Moisture Content: The moisture content presented significant differences within each provenances. Three different groups can be distinguished: A first group highly hydrated (51.3%) with five provenances of relict area, a second intermediate (47.1%) with seven provenances, and a third population weakly hydrated (44.1%) corresponding to the rest of the population (Tab. 3). The moisture content of acorns collected in the same locality is different.

III.3. Seed Germination: The seed germination rate of Cork oak is shown in Table 4. Our results indicated a significant difference in germination rate ($P \geq 0.05$) among the different provenances. The germination rate is between 40% and 76%, except for provenances Jbel sarj (3%) and Ghorgalia (0%). Relict areas present the best germination rate such as Population of JA (76,66%) and JZ (73%).

III.4. Mortality rate: There were no significant intra-population differences among germination rate and rate of mortality ($p \leq 0.05$) (table 4). The mortality rate was high in population of JB (26%) and low in JZ (2,27%) and Bab (2,63%) where it was 0 % in BL, BLr, SS, JS and JA.

III.5. Seedling growth: Significant increase in diameter, length and total number of leaves (Fig.4.5.and 6). Significant differences were found in the stem growth, diameter and number of leaves in seedlings which developed from acorns (Tab .2). The values of Seedling growth show a significant variation between different populations. Throughout the observation period, the population JS (relict area) (Fig.4A.5A.and 6A) and Tb (kroumirie population) (Fig.4C.5C.and 6C) remarkably distinguished from the other populations by significantly lower height growth, diameter and number of leaves (125.2 ± 6.29 mm; 0.85 ± 0.31 mm; 8.5 ± 1.27) (165.2 ± 40.6 mm; 1 ± 0.23 mm; 13.66 ± 3.88) respectively.

Population JA, JB and JZ (Relict populations) seedlings had the highest diameter (320 ± 21.67 mm) (314 ± 16.74 mm) (294 ± 15.06 mm) respectively, and distinguished by the significantly highest number of leaves JZ(25.33 ± 2.21), JB(22 ± 1.7) and JA (21.83 ± 2.5), while provenances of Fernana have the lowest number of leaves (15.11 ± 1.23 mm), diameter (1.32 ± 0.03 mm) during the growth period (Fig. 4D, 6D) (Tab 5). The average diameters was recorded in Ain Drahem (1.24 ± 0.007 mm), Tabarka 1.24 ± 1.33 mm and Nefza (1.24 ± 0.07 mm) (Tab 5).

The Pearson's correlation analysis underlines a significant correlation among Acorn size, Moisture Content and growth parameters between the different studied cork oaks populations (Tab.2).

IV. DISCUSSION

The significant differences in the seed germination and size of the considered *Quercus* acorns seem to be in relationship with the local conditions [29]. The results of our research indicate that *Q. suber* seedlings from the two areas: Kroumirie and relicts are characterised by different morphological (morphological include the size) and physiological traits. This morphological study of acorn should produce guidelines for the selection of genotypes that yield fruits with desirable characteristics. A direct correlation between acorn size and young seedling survival and a positive correlation of seedling size and acorn size are reported [30]. Our results confirm these observations with significant differences in physiological behaviour of cork-oak acorns in the different populations.

For relict population; the large acorns size (may be justified by the drier climatic conditions of their original localities [31, who underlines that large seed size

accelerates germination time and increases germination rate, seedling survival, and seedling growth. This is what has been observed in Relict populations JZ, JB and JA who presented some of these adaptation characteristics, the highest acorn size and weight with highly water content, the high germination capability, the lowest mortality rate and the highest growth in length, weight and number of primordial leaves. Relicts populations are considered the most fertile population that could be considered as an ecotype of reforestation in the future [32].

On the contrary, Js (Area and Siliana) and Tb (Area of Nefza) presented lowest parameters of acorn size, weight and water content with the highest mortality rate and lowest growth parameters. These results indicate that morphological seedling traits probably driven by the climate of the localities from which they were originated. These areas have unfavorable climatic conditions in areas that are in the limits of Cork oak distribution [32].

In Kroumirie: The small acorns size Asn (Area of Fernana), can be related to the cold stress, because of the lowest photosynthetic rates of the cold stressed *Quercus* parent plants [33]. In fact, the cold stress inhibits the activity of sucrose synthesizing enzymes like sucrose phosphate synthase which has a high sensitivity to low temperatures [34]. Several authors underline that Acorns from this provenance had a lower size and volume during the harvest and so thereafter shorter growth and less robust over time [35], they have acquired over the years a great capacity to mobilize soil water reserves stored during the winter and to regulate their evapotranspiration [36]-[37].

This new generation is a direct descendant of cork oak trees located on the south side of the Kroumirie at low altitude and subjected to continental influences with very hot and very dry summers. These trees are small and have low circumference; regarding the altitude of oaks [35].

For Ain Drahem (population of Bab, AZ and Ms), Tabarka (population of ASn) and Nefza (Blr) present intermediate acorns size which are justified by the favorable climatic [38]. This suggests that provenances from bioclimate subhumide, presents a weather conditions which are favorable for the growth of Cork oak [32].

In general, growth parameters of seedlings such as height, diameter, and fresh weight increase as seed size increases [39]. Large seed size has often been linked to enhanced seedling survival and regeneration success [40] - [41]. The effect of seed size on seedling performance is highly variable and depends on environmental conditions such as drought [42]. Our results indicate that large seeds of *Q. suber* from populations can render restoration programs in the Tunisia forests more successful [43]. In fact, seed size variation is thought to be important for the establishment of seedlings under field condition [44], and larger seeds can improve the establishment of seedlings under competitive and resource-limiting conditions [45]. For example, several research found that seedlings from smaller seeds were better under moderate levels of water stress [46]; however, under severe water stress seedlings from large seeds were more tolerant and also found that larger seeds of *Quercus suber* from drier populations

exhibited the highest survival rates under dry conditions [47]-[48].

The discriminate Analysis confirms the obtained results identifying on the basis of the considered acorn and seedling traits, eight groups and underlining a complete separation between the region of Silana and Fernana, which explains 50% of the total variance, and an intermediate position of Zaghouana included with nabeul, Ain Drahem and Tbarka. Nevertheless, Nefza and Tbarka are closer to each other, by their similar climatic and soil conditions (Fig.7).

Overall our results underline that *Q. suber* is characterised by a large variability of acorn and seedling traits which could be considered for afforestation projects and restoration programmes. The knowledge of seed germination and seedling growth capability in response to the intensity and duration of water stress in the Mediterranean Basin is important because water availability is a limiting factor of prime importance in the oak regeneration [49] (Trubat et al. 2010).

The early stages of oak population's seedlings development are affected differently by changes in soil water reserves and temperature [35]. In general, forest trees such as oak cork show a phenological variability as well as morphology, as shown by several provenance tests [50]-[51]. The variability observed between populations can depend on two factors: the intra-population variability and the inter-population variability related both to the effect of the environment and to the genetic structure of the population [52].

In particular, the large acorns may have a high potential in resource-limited or degraded areas by their better germination rate and growth seedlings capability in the establishment phase, and by the highest capability to maintain a more stable water-use efficiency which may be advantageous during the early life stages, particularly during the first summer drought following planting in afforestation projects. Physiological knowledge concerning populations of *Q. suber* seedlings from different climate are important to forecast the potential productivity to increasing drought stress.

V. CONCLUSION

The purpose of this study of acorn morphological and physiological characteristics was to establish the nature and range of variation within and between populations. The results from this study should serve as guidelines for the selection of the best ecotypes of cork-oak acorns (*Quercus suber* L.) adapted to climate change throughout its distribution range. In this study, The relicts zones are the best compared to the Kroumirie area. Ecophysiological behaviour of relict population represents a population model of type 'resistance model' or type 'stabilisation model' (Ramírez et.2010). In the near future, it will be important to increase long-term studies on natural populations in order to understand plant response to environmental factor fluctuations including climate change.

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Table 1: Cork oak populations and some geographical and climatic characteristics of the study localities.

	Subdivision	Pop	Altitude	Geographic Characteristics		Climatic Characteristics		
				Latitude	Longitude	Annual mean Temperature °C	Annual mean precipitation (mm)	Bioclimatic floor
kroumirie area	Ghardimaou	Ast	836	36°52'52'' N	8°33'05''E	17,7	580	Semiarid
	Fernana	F	793	36°50'21'' N	8°30'68''E	16	370	Semiarid
	Fernana	SS	375	36°64'76'' N	8°66'34''E	22	351	Semiarid
	Sejnane	BL	158	37°07'74'' N	9°16'46''E	18	741	Humid
	Tbarka	W	151	36°09'57'' N	8°93'62''E	18,1	865	Humid
	Tbarka	Asn	868	36°81'43'' N	8°74'12''E	19	816	Humid
	Tbarka	JK	375	36°93'93'' N	8°95'01''E	18	829	Humid
	Nefza	BLr	270	37°02'01'' N	9°08'07''E	16	724	Humid
	Nefza	GH	166	37°07'28'' N	9,05'13''E	18	657	Humid
	Nefza	JD	477	36°68'84'' N	8°62'21''E	16,11	732	Humid
	Nefza	Tb	353	36°89'52'' N	9°06'73''E	19	551	Humid
	AinDrahem	AZ	720	36°78'77'' N	8°88'54''E	15	835	Humid
	Ain Drahem	BM	498	36°74'98'' N	8°73'13''E	16	1020	Humid
	Ain Drahem	Bab	660	36°78'26'' N	8°65'75''E	16,2	1093	Humid
	AinDrahem	DF	862	36°78'86'' N	8°74'19''E	15,5	1200	Humid
Ain Drahem	MS	552	36°76'31'' N	8°79'02''E	15,9	963	Humid	
Relicts areas	Sliman	JA	466	36°85'16'' N	10°78'18''E	17,8	444	Semiarid
	Grombalia	JB	364	36°50'78'' N	10°44'71''E	17,7	459	Semiarid
	Zaghouan	JZ	939	35°92'45'' N	9°51'37''E	16,1	483	Semiarid
	Siliana	JS	709	36°47'70'' N	10°31'68''E	17,9	432	Semiarid

Table 2: Results of the Pearson's correlation analysis among the considered seed variables. [Bold type indicate significant correlation (P < 0.05)].

	Width	Weight	Moisture Content	Seed Germination	Growth In Length	Growth In Diameter	Number of Leaves
Length	0,04204601	0,78194235	0,72090648	0,16790066	0,20466704	0,12804738	0,18663463
width		0,17891461	0,04491937	0,19659097	0,20155254	0,07714053	0,22219995
Weight			0,94670071	0,31949801	0,26059292	0,29248391	0,29803442
Moisture Content				0,34823252	0,33832199	0,32503486	0,37009385
Seed Germination					0,72885231	0,62452456	0,68123401
Growth in length						0,84973257	0,85064085
Growth in diameter							0,86283713

Table 3: Moisture content variation.

Groups	Populations	Moisture Content (%)
I	Jzid (a)	53,78±6,68
	Blr (a)	52,62±4,99
	JA (a)	51,92±7,38
	AZ (ab)	50,35± 7,87
	JB (ab)	49,63±6,63
	Moyenne	51,66±1,68
II	JK (b)	48,77±7,56
	Wech(b)	47,77±4,35
	MS (c)	46,45±8,98
	Asn (c)	46,91±10,35
	F (c)	46,65±10,63
	JS (c)	46,11±8,61
	SS (cd)	45,78±11,03
	Moyenne	46,92±1,03
III	GH (e)	44,94±7,56
	Jdis (e)	44,36± 8,31
	Tb (e)	43,75±11,88
	DF (e)	43,69±6,22
	BM (ef)	42,60±10,63
	Aslt(ef)	42,52±6,42
	BL (ef)	41,72±12,03
	Bab(ef)	42,52 ±6,42
	Moyenne	43,22±1,22

For populations, a significant difference (P < 0.05) is observed when the letters are different.

Table 4: Germination rates (%) and rate of mortality % of Quercus Cork acorns.

Provenances	Germination rates (%)	Rates of mortality (%)
Ast	63,33	7,89
F	60	5,55
SS	40	0
BL	13,33	0
W	25	6,66
Asn	45	3,70
JK	43,33	3,84
BLr	51,66	0
GH	0	-
JD	43,33	7,69
Tb	40	8,33
AZ	38,33	13,04
BM	60	5,55
Bab	63,66	2,63
DF	28,33	17,64
MS	70	4,76
JA	76,66	0
JB	70	26,19
JZ	73,33	2,27
JS	5	0

Table 5: Evolution of Seedling Growth by region.

	Region	Length(mm)	Diameter (mm)	Number of leaves
Kroumirie Area	Ain Drahem	240,8±4,79	1,24±1,33	16,70±2,1
	Nefza	258,33±3,21	1,24±0,07	17,88± 2,23
	Bizerte	280±0,57	1,4±0,08	19,66±1,6
	Tbarka	264±0,30	1,63±1,82	18,27±3,3
	Fernana	274,66±0,29	1,32±0,03	15,11±1,23
Relicts Areas	Nabeul	317±0,09	1,49±0,15	21,99±2,11
	Zaghouana	294±0,22	1,78±0, 64	25,33±2,09
	Siliana	125,2±0,26	0,85±0,063	8,5±1,27

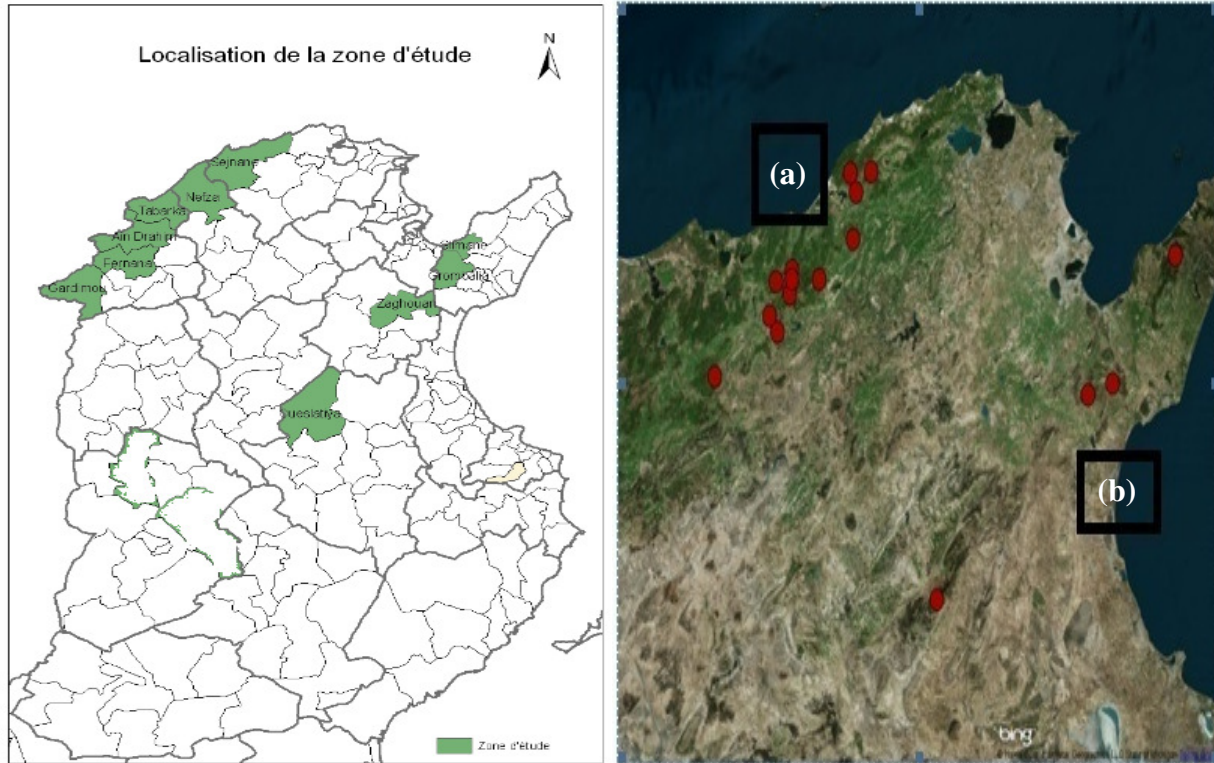


Fig.1. Map showing the locations of acorn collection sites in Tunisia : Kroumirie (a) and Relict (b) populations.

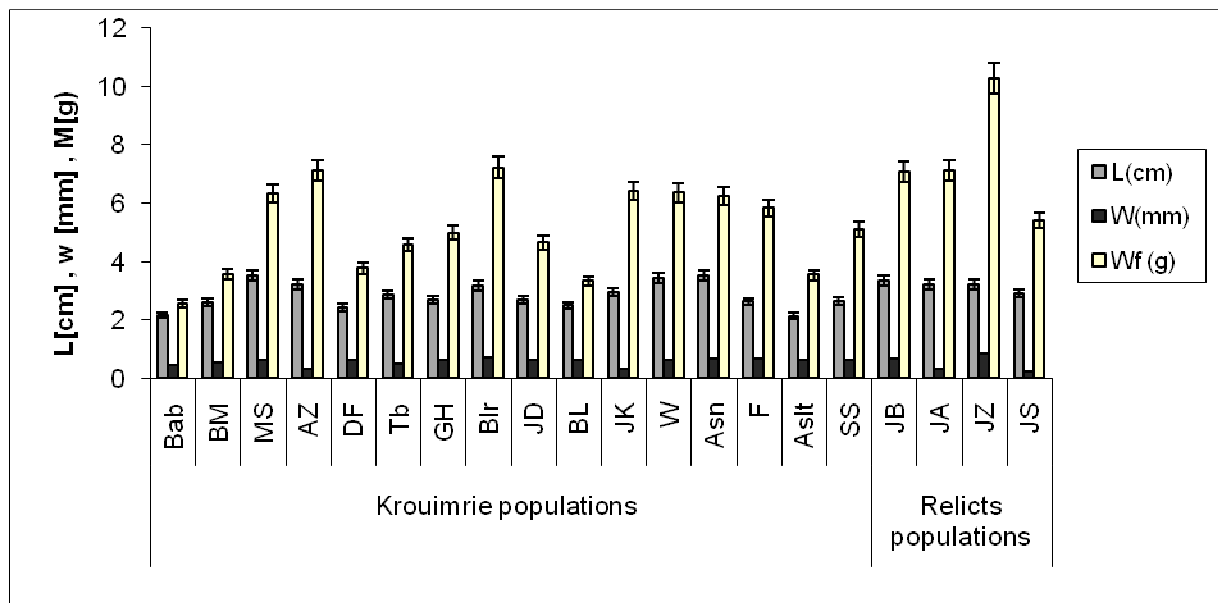


Fig.2. Acorn length (L), acorn width (W) and acorn fresh weight (W_f) from the considered populations.



Elongated acorns



Acorns sub-rounded



Large Acorns



Rounded Acorns

Fig.3. Variation of shapes acorns of cork oak (*Quercus Suber. L.*)

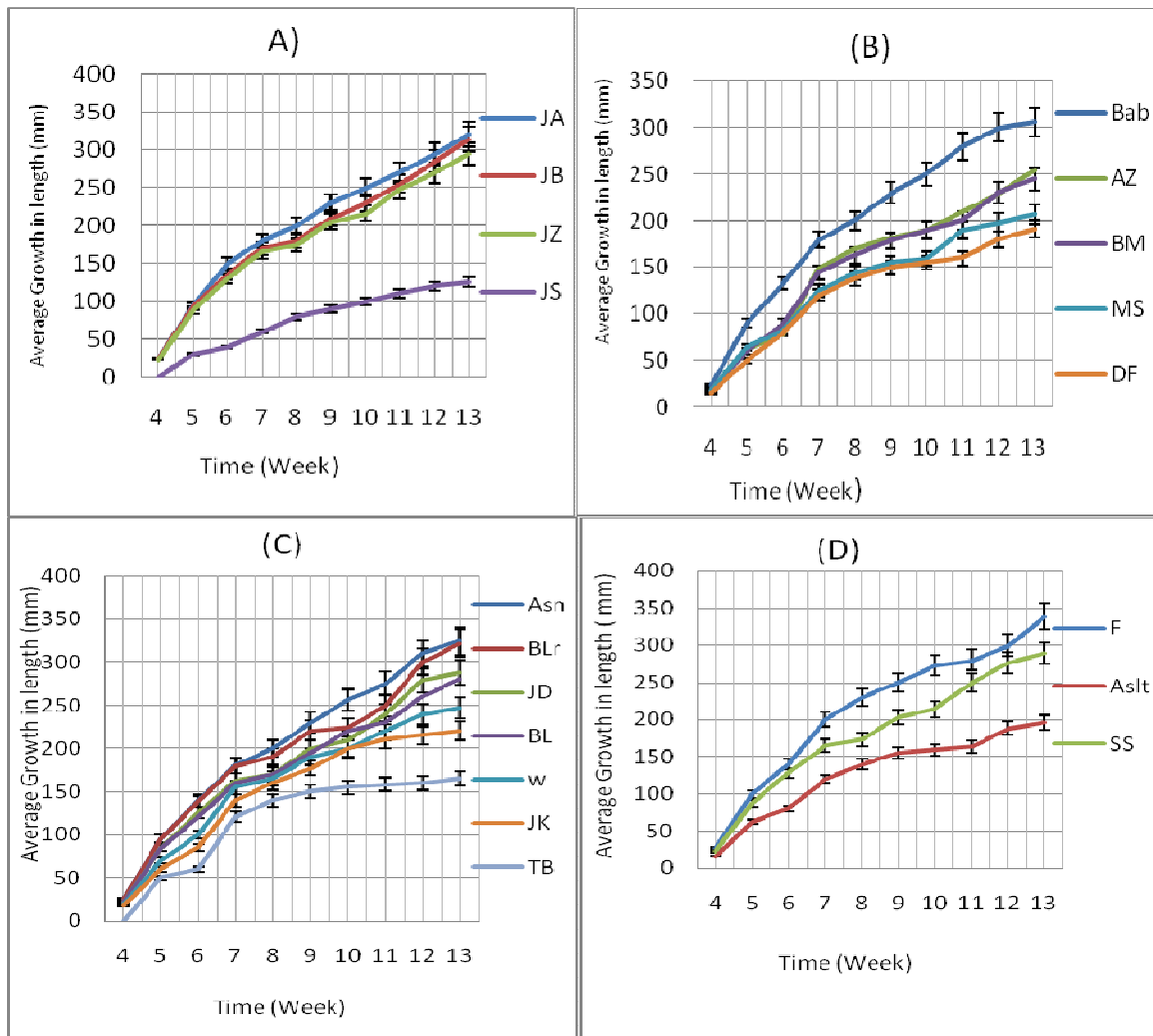


Fig.4. Growth in length of cork oak populations :
(A)= Relicts areas, kroumirie area; [(B) Ain Drahem , (C) Tbaraka and Nefza , (D) Fernana].

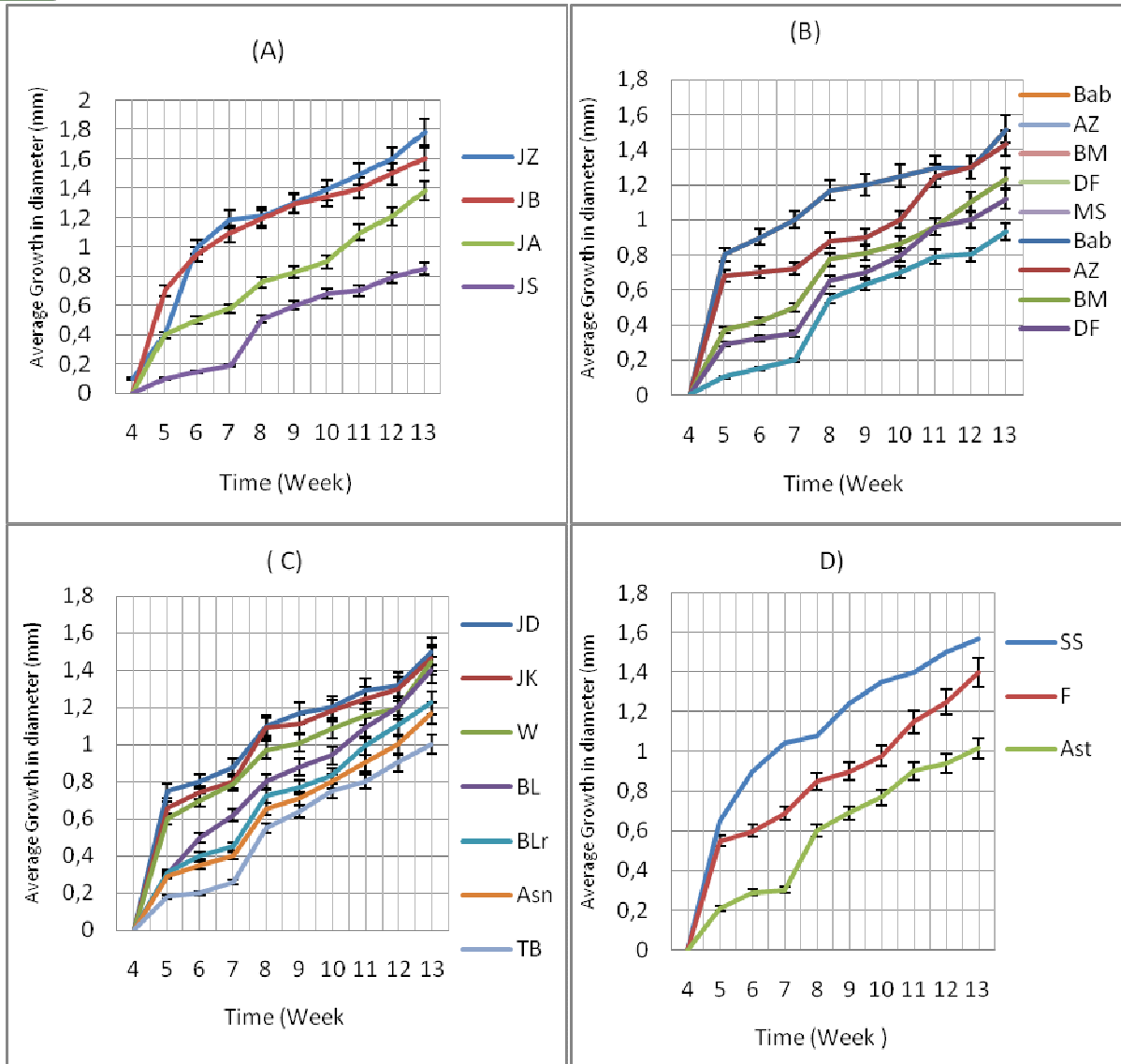
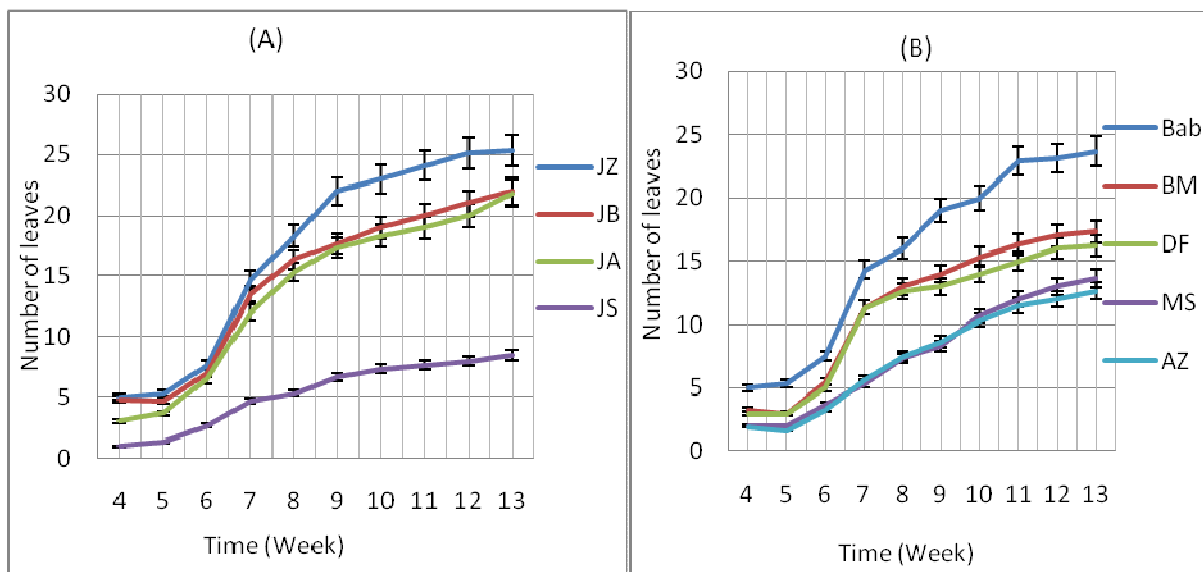


Fig.5. Growth in diameter of cork oak populations: (A)= Relicts areas, kroumirie area: [(B) Ain Drahem , (C) Tbarka and Nefza , (D) Fernana] .



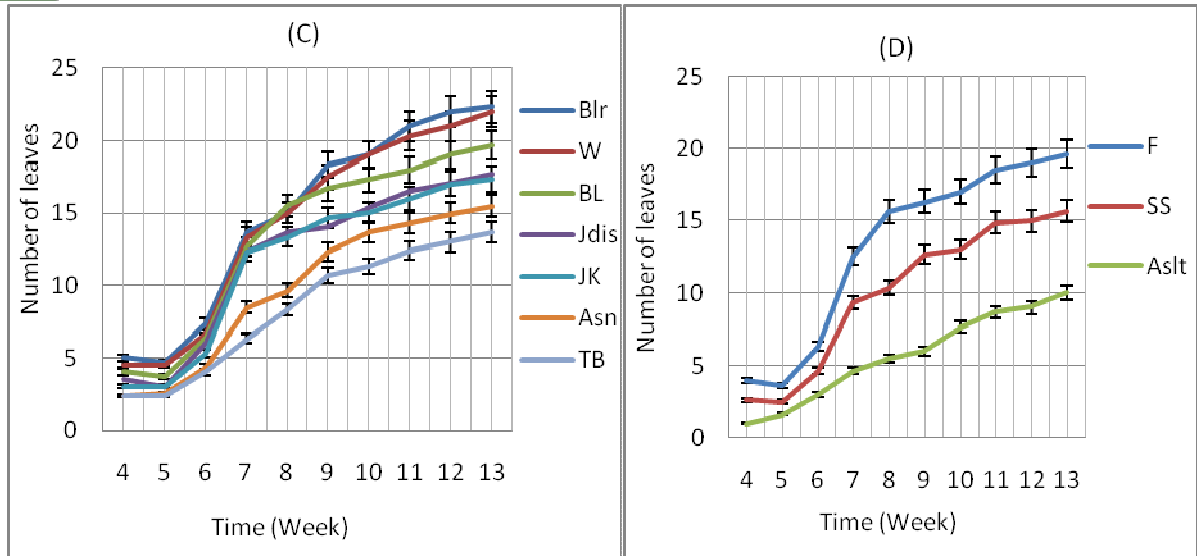


Fig.6. Growth in number of leaves of cork oak populations: (A)= Relicts areas, kroumirie area: [(B) Ain Drahem , (C) Tbaraka and Nefza , (D) Fernana] .

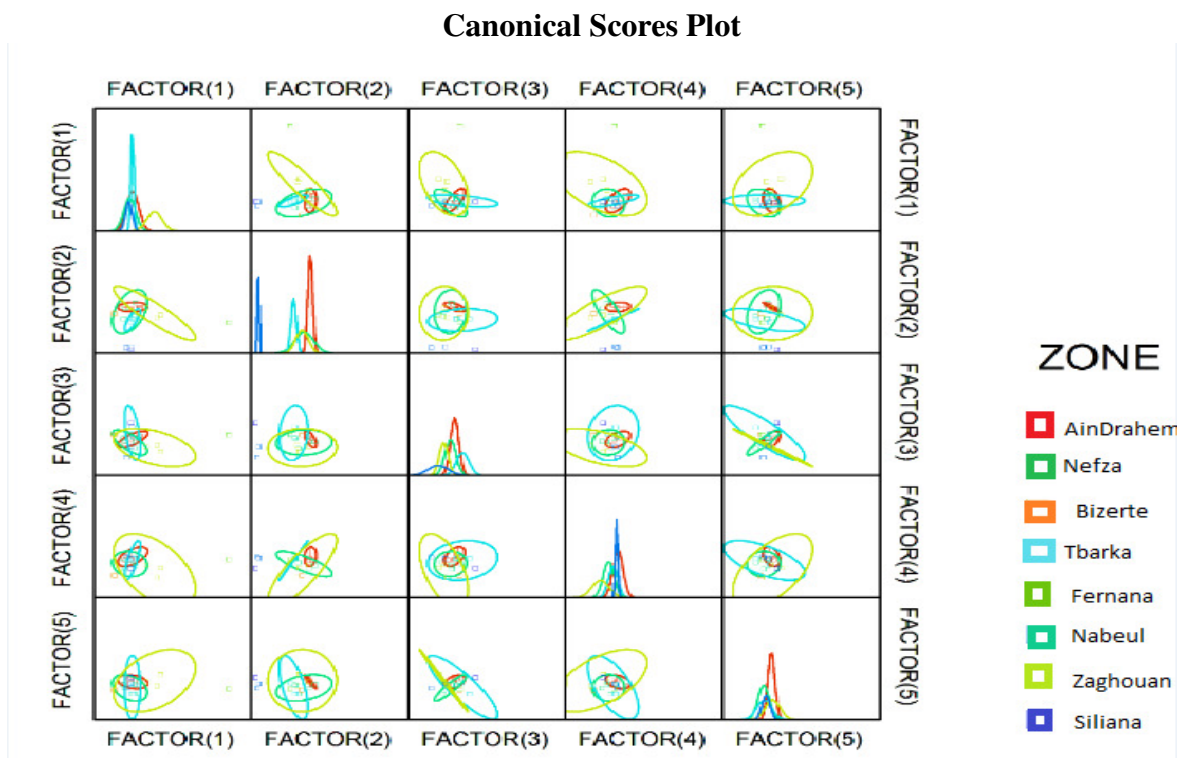


Fig.7. Analysis of total base composition of Cork Oak L. Results of the linear discriminant analysis along the first and second discriminant axes [Factor (1) and Factor(2)]