

Investigation of Altitudinal and Longitudinal Variations of Pollen Grains of the Oak Species (The Genus *Quercus* L.) in Duhok Province Iraq¹

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ABSTRACT

Pollen grains of 3 Iraqi oak (*Quercus*) species belonging to *section Quercus* and *section cerris* growing naturally in Duhok province Iraq. Three altitudinal zones (500-1000 m, 1000-1500 m, 1500-2000 m) were examined by light microscope (LM) and scanning electron microscope (SEM). The main issue focused on in this study is to determine whether oak pollens have changed dimensionally and structurally according to both different latitudes and longitudes. Based on the averages obtained from seven variables, it has been demonstrated comparative data. The mean value, range (min.-max.), and standard deviation (SD) were calculated for polar axis (P), equatorial diameter (E), polar/equatorial ratio (P/E), apocolpium (AP), mesocolpiuin (ME), colpus length (CL) and exine (wall) thickness (ET). Mean values were assessed by the statistical tests to compare the mean values for each variable.

Pollen grains of all species are in monad, isopolar, radially symmetrical, their apertures are mostly tricolpate. Rarely 4-colporate as in *Q.brantii* and *Q.libani*. Overall mean polar length of *Q.brantii*, *Q.infectoria* subsp. *Veneri* and *Q.libani* were 24.34, 24.29, and 27.92 μm respectively. Pollen shape classes range from prolate-spheroidal to sub-prolate at varying proportions for the species; *Quercus brantii* (P/E; 1.07 μm), *Quercus infectoria* (P/E; 1.06 μm) and *Quercus libani* (P/E; 1.14 μm) According to pollen size classes, pollen grains of *Q. brantii* and *Q. infectoria* were 67% minute and 33% mediate, while in *Q.libani* are 100% mediate. Exine sculpturing pattern is gemmate-verrucate for all taxa.

Keywords: Pollen; *Quercus*; LM; SEM; Altitude; Duhok Iraq

1. INTRODUCTION

The Fagaceae is a large plant family comprising more than 900 species belonging to 8-10 genera (Kremer *et al.* 2012). Among these, *Quercus* is the largest genus containing over 400 species in the world. As stated in many classifications, oaks can be divided into three groups based on the inner surface of the acorn shell (glabrous vs tomentose), leaf lobes (bristle tipped vs not), maturation time (one vs two seasons) and the presence of tylosis in the latewood vessels (available vs absent): (i) White Oak Group (**Section *Quercus***), (ii) Red Oak Group (**Section *Labatae***) and (iii) Intermediate (=Evergreen) Oak Group (**Section *Protobalanus***).

The addition of pollen morphology, a character that was ignored in previous classifications, to the morphological classification characterizing phylogenetic lineages resulted in a new classification that would require new studies in biogeography, ecological and evolutionary ways (Denk *et al.*, 2017). The members of the genus have a wide geography spreading in the forests and steppe areas in the Americas, Europe, and Asia continent areas of the Northern Hemisphere. (Tiffney and Steven, 2001; Tucker, 1974). The Genus *Quercus* has a complicated taxonomy because of the enormous

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population size and wide range distribution. Furthermore, hybridization and morphological changes due to heterophylly are very common among the species and the infrageneric taxa (Burger, 1975; Colombo, *et al* 1983; Johnson;1986)

Forest areas in Iraq are mostly located in the north and north-eastern mountainous areas of the country. There are 2,000 hectares of forestland in Iraq and a large part of this area consists of oak forests. Forests constitute a limited territory covering an area of about 140.000 ha, As already it is known, more than half of the country has a desert ecosystem. Iraqi forests account for 2% of the country's total surface area (Aref; 210) and oaks are the main tree species in these areas.

As Iraq's neighbours, Turkey and Iran are rich in oak taxa and most of which have been studied morphologically but pollen studies in the Middle East region are still lacking (Panahi *et al.*,2012). While morphological information provides definite evidence for differentiating species, they also reflect habitat characteristics in vating regions. The ranges of variation of the pollen characteristics of the oak (*Quercus*) taxa in the Middle East region have not yet been established. Since 1971. Only taxonomic collecting and a few local studies have been done about the oaks in Iraq. There were no comprehensive studies, so in the present study, the 7 most useful morphological pollen characters belonging three oak taxa and the statistical analysis have been provided as comparative analysis according to three altitudinal zones and three provinces located at different latitude/longitude degrees in Duhok province Iraq.

MATERIAL AND METHODS

Study Area and Field Works

Pollen grains of three taxa of *Quercus*. were collected from living plants throughout their geographic range in Iraq. Most of the Iranian *Quercus* taxa are also distributed in the neighboring countries, mainly in Turkey, Iraq and Caucasian area. The voucher specimens are placed in the Herbarium of Kahramanmaraş Sutcu Imam University, Faculty of Forestry (KSU) in Turkey. At least 3 voucher specimens from Duhok province and three altitudinal zones (500-1000 m, 1000-1500 m, 1500-2000 m) were studied in order to ensure constancy of pollen characters among different populations of each particular species. Materials and collecting data of examined taxa in the present study are listed in Table. 1.

Species	N O	Altitudu de	Locality	Altitude	Cordinate
<i>Quercus brantii</i>	1	LA	Duhok, Qarqarava,near Galley dum is located 9km of duhok city	733 m	36.907306 ;43.03840166
	3	MA	Duhok, Rosting village near sarsing district is located33 km from duhok city	1130 m	37.01520543; 43.52438774
	5	HA	Duhok, Maten mountain near,A medya district is located,78kmof duhok city	1559 m	37.11764766 ;43.52438774
<i>Quercus infectoria</i>	2	LA	Duhok, Qarqarava,near Galley dum is located 9km of duhok city	733 m	36.907306 ;43.03840166
	4	MA	Duhok, Rosting village near sarsing district is located 33 km of duhok city	1130 m	37.01520543; 43.52438774

	6	HA	Duhok, Maten mountain near, Amedya district is located, 78 km of duhok city	1559 m	37.11764766 ;43.52438774
<i>Quercus libani</i>	7	HA	Duhok, Maten mountain near, Amedya district is located, 78 km of duhok city	1559 m	37.11764766 ;43.52438774

Table 1. List of species and the collection data

Duhok Province

Duhok province is situated (43.20-44.10 longitude; 36.40-37.20 latitude) in the Kurdistan of Iraq. The province is very close to both the Syrian and Turkish borders. Elevation ranges from 300 to 2300 in the province of MAM Mount and ranges are given as follows; Spi Mount to the north, Mam Seen Mount to the east, and Zawa Mount to the south. The west side of the province expresses the plain structure which is called the Seimeil agriculture plain. (Rajab, 2014).

Laboratory Works

Pollen grains were prepared by the standard method of (Radford *et al.*, 1974), after which fresh material of pollen grain was mounted in methyl green-glycerine jelly (1 gm methyl green dissolved in 100 ml absolute alcohol, 95%) and then the slides were observed by using a light microscope and photographed. Pollen grains were studied by MOTIC LM / 40x-100x lenses for LM studies. Measurements were made on 30 pollen grains which were chosen randomly in each slide for each taxa. The mean value range (min-max), and standard deviation (SD) were calculated for polar axis (P), equatorial diameter (E), polar/equatorial ratio (P/E), apocolpium (AP), mesocolpium (ME), colpus length (CL) and exine (wall) thickness (ET) which were measured from the altitudinal zones (Table 2). The ratio of the polar axis to equatorial diameter (P/E) was provided as an index of pollen shape (Erdtman, 1971) while pollen sizes were provided according to the length of the longest axis (Erdtman, 1945). Also, exine ornamentation was determined.

For SEM, the pollen grains were put in absolute alcohol. a few drops of which were placed on previously numbered aluminum stubs, air dried at room temperature, and sputter-coated with gold-palladium for 5 minutes in a Humer II Sputtering Device before observation with a Zeiss/Eivo LS10 SEM. The micromorphological features and quantitative characters of pollen grains were studied and photographed at a voltage of 20 KV and under the magnification of 500X, 200X and 10X. The terminology follows mainly that of the following references (Erdtman, 1952; Solomon, 1983; Colombo *et al.*, 1983; Nixon, 1993; Hesse, 2009; Naryshkina, 2010; Panahi *et al.*, 2012). Pollen surface terminology followed (Punt *et al.*, 2007).

RESULTS

The palynological characteristics of the taxa investigated in this study are illustrated in Table 2 and Figures 2 and 3. Pollen size classes are based on (Erdtman, 1945) and pollen shape classes are based on (Erdtman, 1971).

Quercus brantii

Pollen grains are in monad, isopolar, mostly 3-porate and rarely 4-porate. According to pollen size classes, based on the size expressed as length of the longest axis, the results refer to minute (56%) and mediate (44%) pollen grains. According to pollen shape classes using P/E mean values, the results refer to overall P/E mean values is (1.08 μm) and the shape classes are prolate-spheroidal (78%) and sub-prolate (22%). Exin sculpturing pattern is gemmate-verrucate. The measurements of pollen characters were calculated as the mean value, such as; polar length (24.69 μm), equatorial length (22.98 μm), apocolpium (7.55 μm), mesocolpium (8.91 μm), colpus length (14.51 μm) and exine thickness (1.37 μm).

Quercus infectoria

Pollen grains are monad, isopolar, radially symmetrical. Their pores are sometimes indistinct and their apertures are tricolpate, tricolporoidate or tricolporate with colpi of medium length. According to pollen size classes, based on the size expressed as the length of the longest axis, the results refer to minute (67%) and mediate (33%) pollen grains. According to pollen shape classes using P/E mean values, the results refer to overall P/E mean values is (1.05 μm) and the shape classes are prolate-spheroidal (100%). Exin sculpturing pattern is gemmate-verrucate. The measurements of pollen characters were calculated as the mean value, such as; polar length (24.29 μm), equatorial length (23.15 μm), apocolpium (8.14 μm), mesocolpium (8.24 μm), colpus length (13.40 μm) and exine thickness (1.38 μm).

Quercus libani

Pollen grains are in a monad, isopolar, radially symmetrical. Their pores are sometimes indistinct and their apertures are tricolpate, tricolporoidate or tricolporate with colpi of medium length. According to pollen size classes, based on the size expressed as length of the longest axis, the results refer to mediate (100%) pollen grains. According to pollen shape classes using P/E mean values, the results refer to overall P/E mean values is (1.11 μm) and the shape classes are prolate-spheroidal (67%) and subprolate (33%). Exin sculpturing pattern is gemmate-verrucate. The measurements of pollen characters were calculated as the mean value, such as; polar length (27.34 μm), equatorial length (25.42 μm), apocolpium (8.73 μm), mesocolpium (7.74 μm), colpus length (17.74 μm) and exine thickness (1.45 μm).

<i>Quercus Brantii</i>									
No	A	ST	P	E	P/E	APO	MES	COL	EXT
1	LA	Mean ± St.d.	22.30 ± 2.08	21.05 ± 1.82	1.07 ± 0.15	6.30 ± 1.40	7.42 ± 2.14	15.03 ± 1.74	1.27 ± 0.28
		Min-Max	20-30	18-24		3-10	4-11	11-19	1-2
3	MA	Mean ± St.d.	24.23 ± 2.94	21.74 ± 3.28	1.15 ± 0.29	6.85 ± 1.19	6.13 ± 1.42	13.34 ± 2.62	1.42 ± 0.33
		Min-Max	16-30	12-28		5-11	4-10	9-20	1-2
5	HA	Mean ± St.d.	25.48 ± 3.09	24.20 ± 2.90	1.06 ± 0.17	6.32 ± 1.71	12.36 ± 2.39	15.39 ± 2.40	1.39 ± 0.22
		Min-Max	21-30	20-28		3-10	9-18	12-22	1-2
\bar{x}			24.69	22.98	1.08	7.55	8.91	14.51	1.37
<i>Quercus infectoria</i>									
No	A	ST	P	E	P/E	APO	MES	COL	EXT
2	LA	Mean ± St.d.	21.99 ± 2.29	19.97 ± 2.33	1.12 ± 0.17	7.80 ± 1.09	7.14 ± 1.46	14.94 ± 2.51	1.50 ± 0.35
		Min-Max	18-27	15-24		6-10	5-10	9-20	1-2
4	MA	Mean ± St.d.	22.94 ± 1.47	22.52 ± 1.88	1.03 ± 0.13	10.30 ± 2.44	7.30 ± 1.42	13.18 ± 2.66	1.13 ± 0.17
		Min-Max	17-28	19-26		6-14	5-10	9-21	1-1
6	HA	Mean ± St.d.	25.58 ± 2.97	23.52 ± 2.56	1.10 ± 0.17	7.84 ± 1.18	6.27 ± 1.49	16.08 ± 2.35	1.32 ± 0.26
		Min-Max	20-31	18-29		6-10	3-9	9-21	1-2
\bar{x}			24.29	23.15	1.05	8.14	8.24	13.40	1.38
<i>Quercus libani</i>									
No	A	ST	P	E	P/E	APO	MES	COL	EXT
7	HA	Mean ± St.d.	25.58 ± 2.97	23.52 ± 2.56	1.10 ± 0.17	7.84 ± 1.18	6.27 ± 1.49	16.08 ± 2.35	1.32 ± 0.26
		Min-Max	20-31	18-29		6-10	3-9	9-21	1-2
\bar{x}			27.37	25.42	1.11	8.73	7.74	17.74	1.45

Table 2. Pollen grain characters of *Quercus* taxa, polar axis (P), equatorial diameter (E), exine (wall) thickness (EXT), apocolpium (APO), mesocolpium (MES) and colpus length (COL)

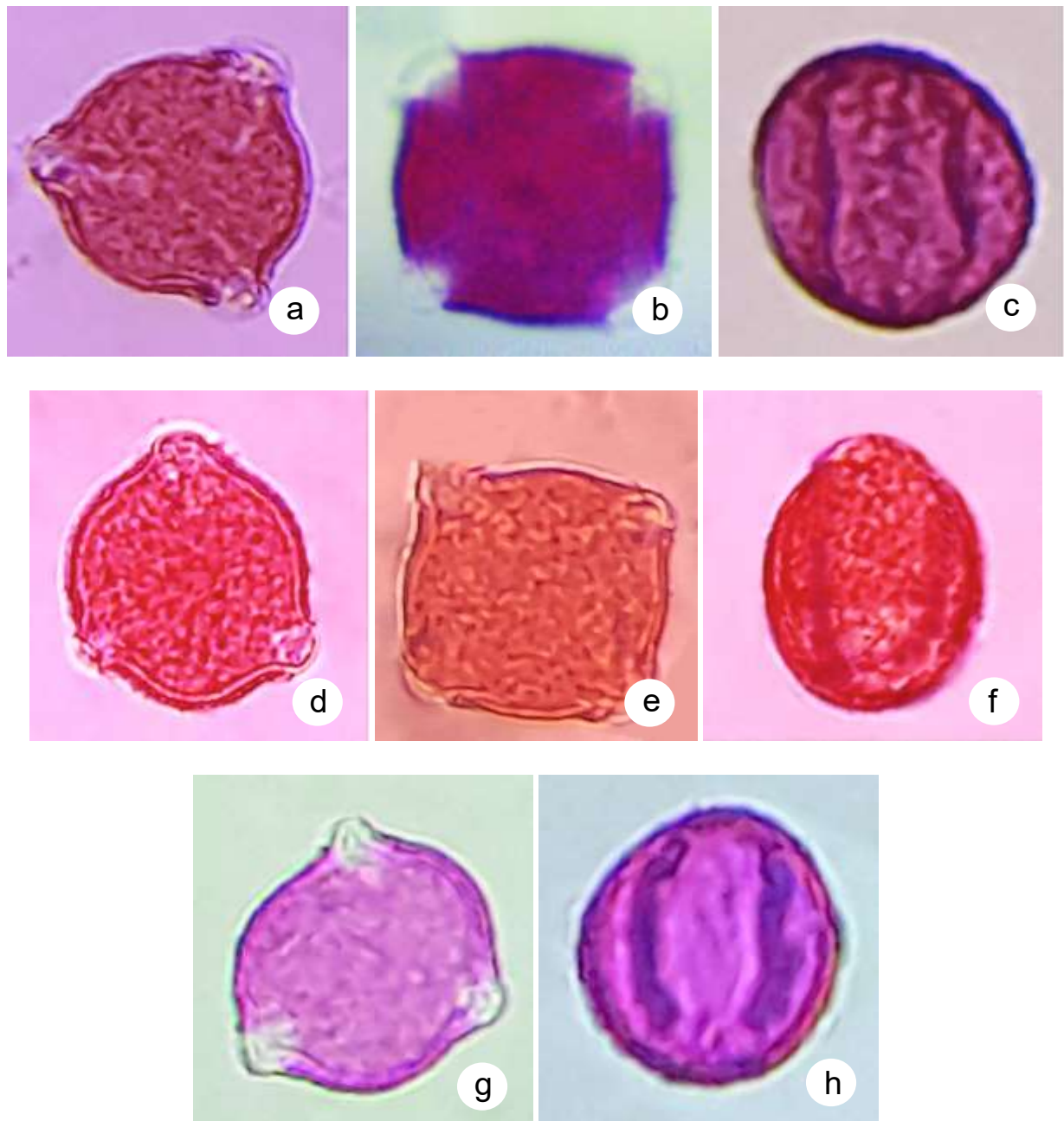
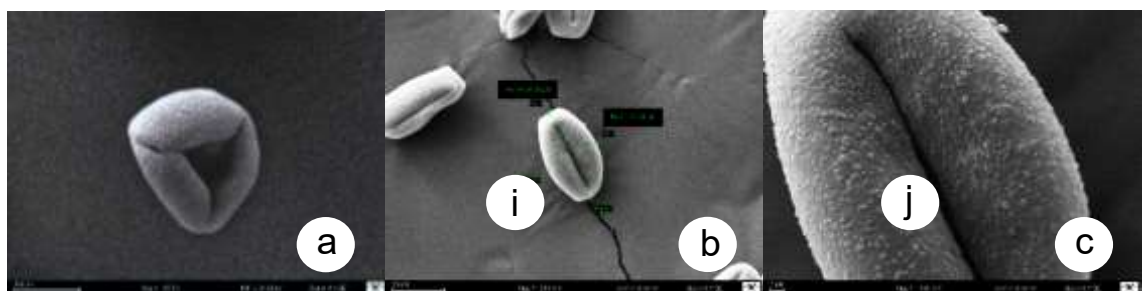


Figure 1. LM views of pollen grains; from left to right: polar view of tricolporate pollen, polar view of tetracolporate pollen, equatorial view. *Quercus brantii* (a, b, c), *Quercus libani* (d, e, f), *Quercus infectoria* (g, h).



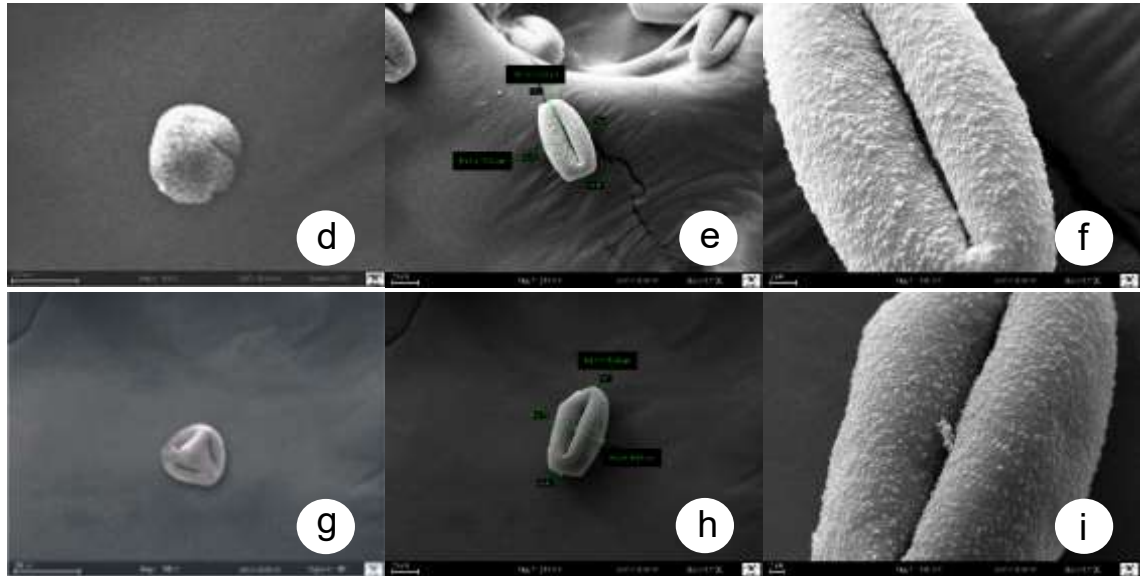


Figure (X). SEM views of pollen grains from left to right: polar view, equatorial view, pollen exine patterns. *Quercus brantii* (a, b, c); *Quercus infectoria* (d, e, f), *Quercus libani* (g, h, i),

DISCUSSION

In this study, important data to determine the variation boundaries of pollen morphological characteristics of oak taxa were obtained. In addition, comparable measurements have been made as a result of LM and SEM studies. Pollen sizes and shapes always provide valuable visual and dimensional data for future studied. From this point of view, it has been shown for the first time how the pollen dimensions changes according to the altitudinal zones (low to high) in different latitudes and longitudes.

Oak pollens are invariably present individually (in monad type). Also, exine ornamentation reflects an invariant character (gemmate-verrucate) among the oak species as stressed by Naryshkina and Evstigneeva (2010). This is generally a conservative character within the genus. P/E is the most commonly used value that determines the pollen shape. If the polar axis (P) is larger than the equatorial axis (E), the result is prolate. if the polar axis is smaller than the equatorial axis, the result is oblate. Data from an Iranian study (Panahi *et al.*, 2012) show that P/E ratios are lower than 1 in most oak species they studied. Oblate-spheroidal (P/E=0.88-1.00 in *Q. ceforum*, *Q. infectoria* subsp. *boissieri* var. *boissieri*, *Q. infectoria* subsp. *boissieri* var. *tenuicarpa*, *Q. komarovii*, *Q. longipes*, *Q. robur* subsp. *pedunculiflora*), prolate-spheroidal (P/E-1.00-1.14 in *Q. petraea* subsp. *iberica*). These results do not coincide with our study. It is concluded that for the same taxa present in both Iranian and present works (*Quercus infectoria* subsp. *veneris*), different results have been achieved. Considering the mean values in the present study, prolate- spheroidal and sub-prolate results were obtained in varying percentages in certain species *Quercus brantii* prolate-spheroidal (78%, P/E =1.01-1.14) and sub-prolate (22%, P/E =1.15- 1.33); *Quercus infectotria* subsp. *veneris* prolate-spheroidal (100%, P/E =1.01-1.14); *Quercus libani* prolate-Spheroidal (67%, P/E = **1.01-1.14**) and sub-prolate (33%, P/E **1.15-1.33**).

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