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Ecological acclimation on the pheno-characters of *Ginkgo biloba* L. outside its native range: Perspective to pollen grains, Egypt

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Key words: Acclimation, *Ginkgo biloba,* Gymnosperm*,* Male cone, Phenology, Pollen grains

Abstract

A fertile branch at three meters high was chosen from a 25-year-old Ginkgo biloba male tree for this investigation. The tree was examined daily to record the suitable time of mature yellow catkins (male cone), which was in April 2023. Pollen grains from the mid catkin from the nine fertile nodes beside those collected from the four Earths directions have been examined carefully by both the light and Scanning electron microscopes. Element contents have been investigated within the different pollen stages. The data obtained revealed that most of the investigated pollen pheno-characters showed highly significant differences between the catkin developmental stages and in between the different positions in each catkin stage, except within few characters. Despite the state of differences recorded within the different positions of the catkins there were highly significant differences between the different nine stages of the catkin position. This investigation showed that *G. biloba* trees growing in Alexandria city are adapted to the city climate and release their pollen grains gradually to ensure successful pollination. The microsporangia mature gradually; accordingly, their pollen grains are in different developmental and hydrolytic states. The obtained results proved the high adaptation processes which are shown in both the phenol-morphological states beside their mineral contents of the *G. biloba* pollen grains.

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Introduction

Ginkgo biloba is a mysterious tree; it is the oldest living gymnosperm tree which overcomes much generic extinction, and it is the only species still adapt with all the environmental disorders from the Ginkgophyta (Zhou & Zheng, 2003). It is considered as a unique tree in many aspects, it occupies its unique taxonomic division, class, order, family and genus with great genetic distances with its relatives beside its important food content, and medicinal values. This species is considered a living fossil as it overcomes many global catastrophes and adapts with great environmental and climate changes. This species has many forms of adaptation which made it modulate with the environmental disturbance meanwhile it has its specific way of fertilization. It produces huge amounts of light pollen grains to succeed its anemophilous mode of pollination. *G. biloba* pollens carried inside microsporangia in subsequent degrees of development to insure long fertile time (Lu *et al.*, 2016). After pollen dispersal, the pollen grain releases water and becomes typically folded inwards in its aperture or leptoma to keep the exposed aperture area in hydrated state (Hesse *et al.*, 2009). Worth noticing that the shape of dry pollen grains after dispersal is bilaterally symmetrical with monosulcate wide aperture and nearly smooth exine ornamentation (Lu *et al.* 2011a). After hydration with the pollination drop, the pollen converts into a round shape (Tekleva *et al.*, 2007). This pollen shape change is considered as a mode of adaptation to keep more pollen grains in fertile and hydrated form.

Trees of *G. biloba* are native to China and introduced to Japan, Europe, and North America (Del Tredici 1991 & 2000; Tsumura *et al.*, 1992). Its trees are commonly planted in the parks as ornamentals for their woody trunk and characteristic leaves. The trees are dioecious i.e., the female megasporangia carried in trees separate from those who carried the microsporangia. The microsporangia are carried in an inflorescence called catkin. The catkins originated from the axils of the leaves on short shoots and their numbers differ from node to another. Each sporangiophore consists of a stalk and sterile extension which bears two pendant pollen sacs and release their pollens by the aid of longitudinal slit (Klimko *et al.*, 2016).

Pollen morphological characteristics have been studied in detail as they are essential for successful fertilization and reproduction in *G. biloba*. This study presents information on aspects of pollen development of *G. biloba,* which are relevant to understanding its ecological characteristics as an introduced species in Egypt. Meanwhile to find out how much the catkin-like developmental stages affect the pollen morphological characters and its hydration state which in turn affect its fertility to help in the process of the species conservation and reproduction. In addition, to highlighting the special strategy of *G. biloba* to acclimate with environmental factors outside its native range with respect to pollen grain characters.

Materials and methods

Study Species

Male reproductive structures were studied for about 25-yr-old male trees of *G. biloba*, grown in the Botanic Garden of Alexandria University in Alexandria city, Egypt. Catkin-like inflorescences (male cone) were collected by the authors. Trees were investigated daily to record the suitable time with yellow male cones indicating mature micro-sporangia, which were in April (2023). A fertile branch at elevation of about three meters high was chosen for this study. The mid catkin from the nine fertile nodes beside those collected from the four directions (North, East, South and West) have been dealt with in this study. Three positions from each catkin have been chosen; near the peduncle (L), mid the catkin (M) and near the tip (U) (Fig. 1 A & B); and carefully smeared onto glass slide with drop of glycerol jelly for light microscope examination. Counting the total number of pollen grains, number of hydrated (Spherical) and dehydrated (Prolate or Oval) in each position from each node, pollen measurements and photographed using OPTICA (B-150D) light microscope fitted with USB digital-Video Camera and Computer Software at 10X40 lenses. The polar, equatorial axis or radius as well as the exine thickness and aperture measurements were recorded in at least 30 pollen grains to obtain the minimum, maximum and calculate the mean and standard deviation of each item.

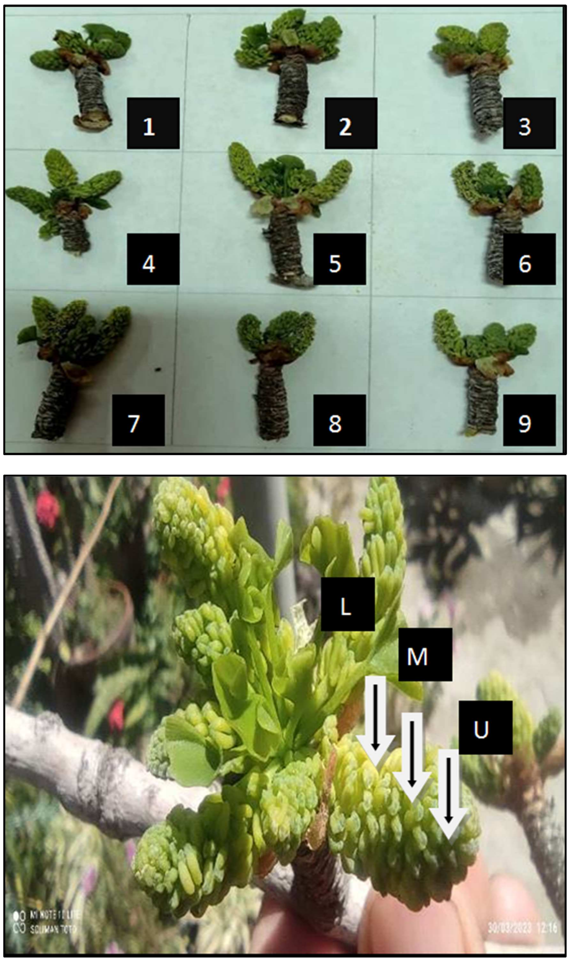

Fig. 1. A-The nine studied stages B- The three Position in each catkin (L, M. U). [Photos taken by Heneidy S. and Toto S.]

SEM investigation and mineral contents

Non-acetolysed pollen grains have been sputtered onto cleaned, Aluminum labeled stubs, coated with 20 nm Gold in a Polaron JFC-1100 coating unit, examined and photographed using JEOL-JSM.I T200 Series Scanning Electron Microscope allocated in the electron microscope unit, Faculty of Science, Alexandria University, Egypt. Pollen mineral contents were measured in pollen grain pellets and subjected to X-ray analysis under 20 kv using the same SEM. The pollen terminology was adopted from Faegri and Iversen (1989), while the shape of the pollen grains followed that of Erdtman (1952).

Study area

The studied trees of *G. biloba* are grown the Botanic Garden of Alexandria University in Alexandria city in Northern Egypt. Alexandria is the second largest city in Egypt. It is located in the northern Mediterranean coastal region of Egypt. In general, Egypt has arid climate, but Alexandria has its characteristic climate. It has hot, humid weather during the summer, with the hottest months are July and August and cool rainy weather during the winter (Fig. 2)

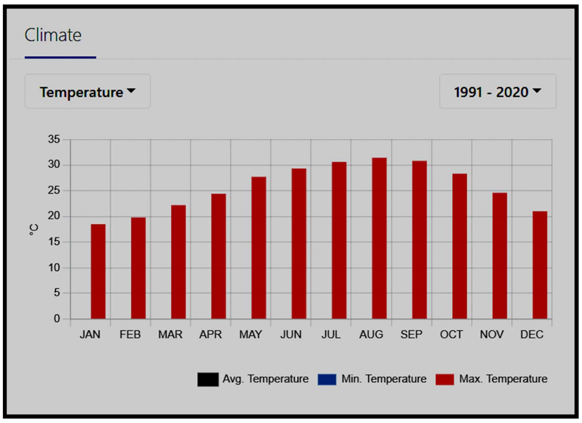

Fig. 2. The mean temperatures from 1991 till 2020 in Alexandria, Egypt. Show the average temperatures in Alexandria in Celsius.

Data analyses

Quantitative data were expressed as mean and standard deviation for normally distributed quantitative variables. One way ANOVA test was used for comparing the different studied groups and followed by Post Hoc test (Tukey) for pairwise comparison. The significance of the obtained results was judged at the 5% level. Five replicas for each group and the data were expressed using Mean ± SD.

Results

The chosen branch had nine fertile nodes with one or two sterile nodes in between. Each fertile node has from three to five catkin-like inflorescences (Fig.1 A). The micro-sporophylls are in spiral arrangement and bear two fertile micro-sporangia. Each micro-sporangium contains different total numbers of pollen grains and different ratios between the hydrated (Spherical) to the dehydrated (Oval or Prolate) (Table 1).

These variations recorded in between the position of the micro-sporangia of the same catkin. The ratio between the spherical pollen grains to the oval ones (S/P) was greater in the low micro-sporangia; near the peduncle; than the middle and upper ones. Catkins from the ninth node and those from the four main directions; mid and upper positions had S/P pollens greater than the lower position (Fig. 3, 4, 5).

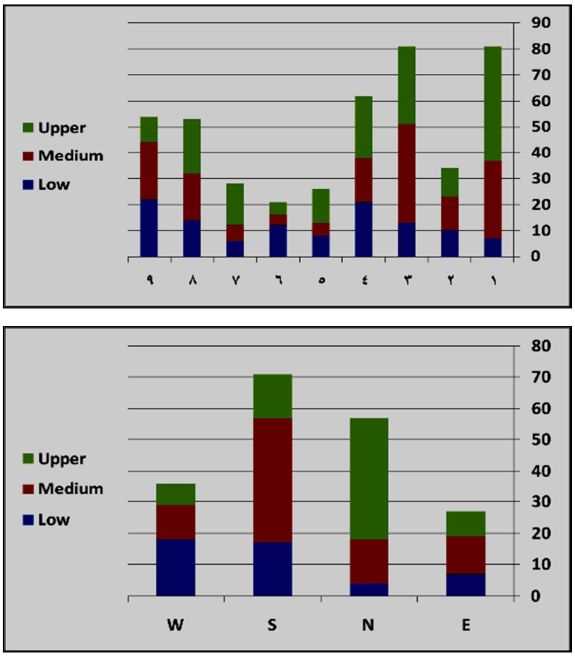

Fig. 3. Total number of pollen grains A- nine studied stages, B-four directions

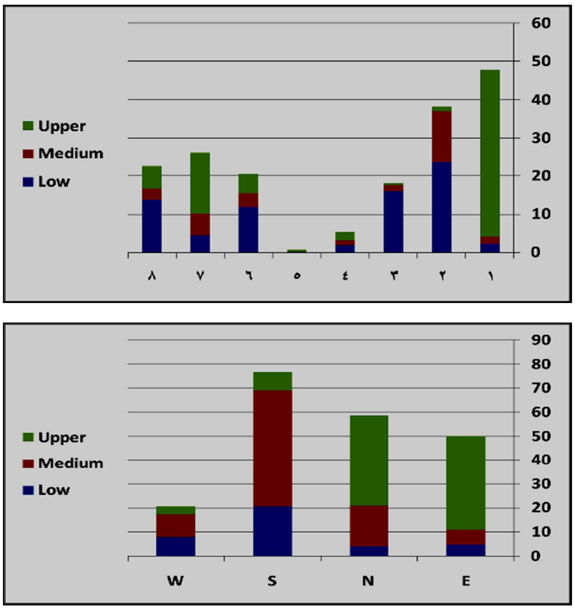

Fig. 4. Ratio between spherical and prolate A-studied stages, B-four directions.

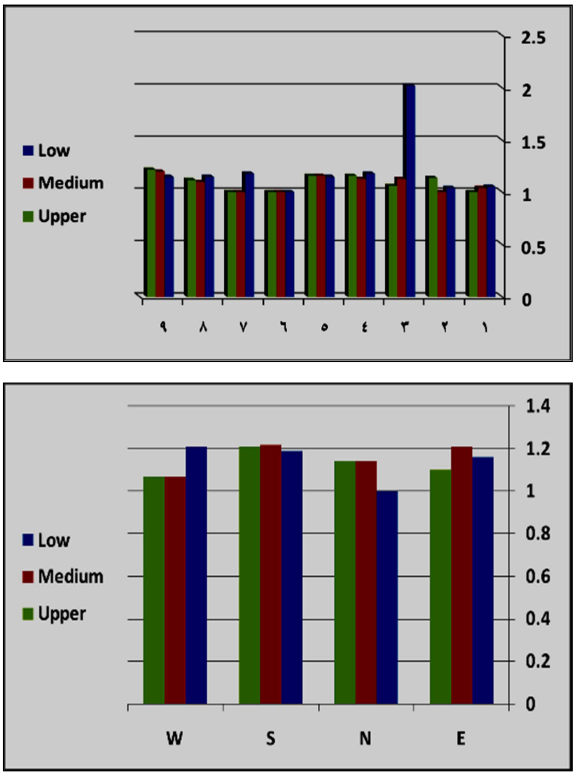

Fig. 5. Shape of pollen grains according to P/E ratio A- nine studied stages B-four directions.

Pollen grains morphology

Light microscope investigation

The pollen grains by the first investigation; under the light microscope (Fig.6-17 and Table 1); appear either apolar or isopolar, bilateral symmetric, spherical (P/E=1), oval (P/E>1) or subprolate (P/E±1.2). The radius of the spherical pollen grains varied between 21.3µm in the lowest micro-sporangia on the 1st. node near the peduncle, while it reaches over 26 µm in the upper nodes (8 & 9) as well as in the East (M) and North (Up) directions (Fig. 15).

Apertures are either mono-aperturate (Fig. 6, 8) or anaporate (Fig. 12, 15, 16) in the spherical pollens. They are mono-sulcate in the oval or subprolate pollen grains (Fig. 7, 9, 10, 11, 14). Under the light microscope, the apertures appear as bi-colpate and this is due to the wide opening (Fig. 13, 17).

The exine is considerably thin it ranges from 0.5 to 0.8 µm thick which appears as punctuate or ornamented with small granules under the light microscope (Fig.7,9,11,14,15,16).

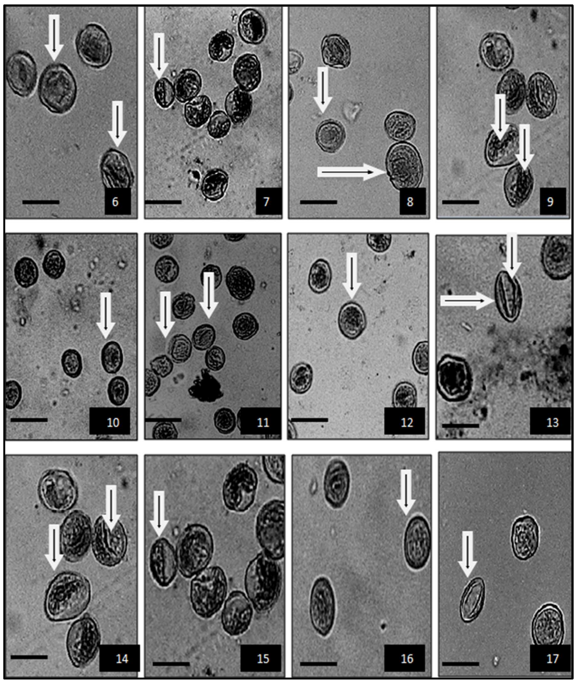

Fig. 6-17, LM photographs. Bar = 20 µm. Fig. 6 stage 1L spherical and subprolate pollen grains, Fig. 7 stage 1M spherical and subprolate pollen grains with wide sulcus, Fig.8 stage 2L spherical pollen grains with anaporate aperture, Fig.9 stage 5M prolate pollen grains with wide sulcus, Fig.10 stage 5U spherical and prolate pollen grains with narrow sulcus, Fig.11 stage 7L spherical and prolate pollen grains with narrow sulcus, Fig.12 stage 6U spherical pollen grain with anaperturate aperture, Fig.13 stage 8M prolate pollen grains with two slit like apertiures, Fig.14 stage 9U subprolate pollen grains with wide and slit-like apertures, Fig. 15 stage EM spherical and subprolate pollen grains with wide sulcus, Fig.16 stage NL spherical pollen grains with anaporate aperture, Fig.17 stage WL prolate pollen grains with two slit-like apertures.

Scanning Electron Microscope investigation (SEM)

Under the SEM investigation the detailed features of both the apertures and exine ornamentation have been clarified (Fig. 18-30 and Table 1). The apertures are mono-sulcate in the oval pollen grains with unidentified margo (Fig. 18, 25). The sulcus is almost ellipsoidal and extends till near the poles (Fig. 18, 23, 25). The spherical pollen grains have very wide nearly rounded aperture called anaporate (Fig. 28, 29). The sulcus always extends from distal to proximal poles and its apex is either round (Fig. 18) or sharp (Fig. 23, 25). The sulcus membrane is psilate (Fig. 19, 22). The exine ornamentation differs between the studied pollen grains, from the psilate or faintly rugate (Fig. 20, 21) to ulcerate (Fig. 27, 30) or even tectate perforate ornamented with small granules (Fig. 26). In a few pollen grains, whatever the developmental stage or directions, the tectum ornamented with rod-shaped extensions (Fig. 23, 24).

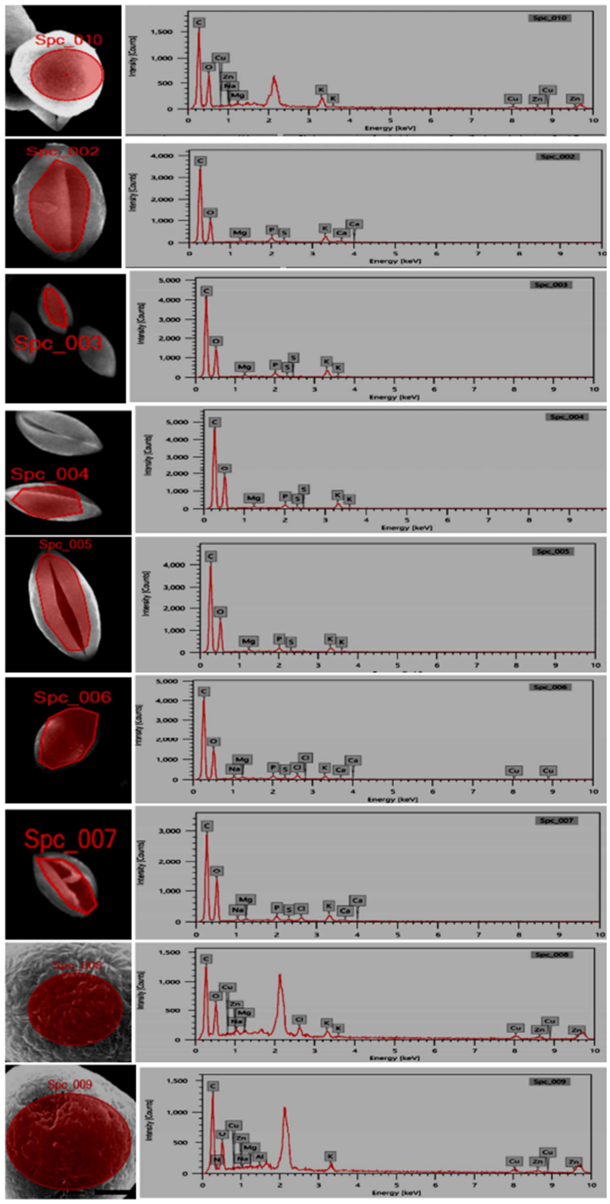

Fig.31. Elements in the different pollen shapes and positions in the nine studied stages

Pollen grains mineral contents

The mineral contents in representative developmental stages show that all the pollen stages shared the elements C, O, mg & K in abundant contents.

The two elements; P & S; present in trace amounts in all the stages except the 1st, 8th and 9th stages (P 0.77 in stage 6 to 1.2 in stage 2 and S 0.15 in stage 4 to 0.3 in stage 2). Traces of Na (0.19) recorded in the first stage only and Cu & Zn recorded in the 1st, 8th and 9th stages only with small amounts (2.93 & 1.65; 4.12 & 2.31; 5.53 & 2.97 respectively).

Traces of Ca recorded in the 2nd, 6th and 7th stages only (0.42, 0.29, 0.21 respectively), while Al recorded in trace amount (0.95) in stage 9 (Table 2 and Fig.31).

Table 1. Number of mature catkins, total pollen grains count in five microscopic fields, ratio between spherical/prolate pollens, radius, or polar and equatorial axis lengths in µm: min-max (mean ±SD) and ratio between polar and equatorial axis.

St	No of Ca	TPG	SphPG		PrPG				Exine	
			Radius	Pore width	PAL	EAL	Sulcus L	Sulcus W	Thickness	Orn
1 L		7-10 7.2±2.95	21.3-24.6 23.5±1.98	18.3-18.6 18.5±0.08	0.0-24.5 19.4±5.82	0.0-23.5 18.4±4.82	22.2-24.5 21.4±3.62	2.2-4.1 3.2±1.08	0.5-0.6 0.58±0.05	
1 M	3	15-48 29.6±16.01	24.0-25.2 24.7±0.08	15.2-15.8 15.5±0.18	0.0-26.8 14.97±13.68	0.0-25.0 14.4±13.17	22.8-26.8 24.7±2.68	1.4-2.9 2.2±0.63	0.5-0.6 0.58±0.05	P or FR
1 U		16-52 43.6±9.5	24.7-25.2 24.94±0.18	20.7-21.2 20.84±0.38	0.0	0.0	0.0	0.0	0.06	
2 L		5-24 19.8±6.11	24.8-25.1 24.9±0.32	20.8-22.1 21.9±0.22	0.0-24.5 14.0±10.66	0.0-23.6 14.0±9.55	22.3-24.5 22.9±1.96	1.8-3.9 2.5±1.38	0.5-0.6 0.58±0.05	
2 M	5	5-26 15.4±10.21	24.2-24.5 24.4±0.13	19.2-21.5 20.6±1.13	0.0	0.0	0.0	0.0	0.6-0.8 0.68±0.11	R
2 U		9-13 11.2±1.48	22.9-24.5 23.9±0.75	19.9-22.5 20.5±1.75	23.1-26.6 24.8±1.22	19.6-24.4 21.7±1.90	23.1-26.6 24.8±1.22	1.6-4.2 3.5±0.67	05-06 0.58±0.05	
3 L		6-25 15.8±8.08	24.5-25.3 24.9±0.31	20.2-22.9 21.6±1.31	0.0-26.4 25.8±1.02	0.0-22.2 12.8±11.67	24.6-26.4 25.8±3.28	2.0-4.8 3.7±1.08	0.6	
3 M	4	18-58 (39.8±17.70)	24.0-25.3 24.7±0.61	20.0-22.3 21.0±1.31	24.6-26.5 25.6±0.72	21.5-24.4 22.7±1.28	24.6-26.5 25.6±0.72	2.8-4.8 3.9±0.73	0.6-0.8 0.68±0.11	FR
3 U		19-42 33.4±8.74	24.0-24.7 24.4±0.26	19.8-21.7 20.2±1.36	25.2-26.0 25.6±0.33	23.8-25.2 24.2±0.62	25.2-26.0 25.6±0.33	2.0-3.8 3.2±0.62	0.6	
4 L		14-33 24.8±7.40	25.4-27.6 26.1 ±0.96	22.2-23.6 23.1 ±0.42	26.0-28.0 26.9±0.84	22.9-23.7 23.2±0.50	26.0-28.0 26.9±0.84	1.8-2.6 2.0±0.58	0.5-0.6 0.56±0.06	
4 M	4	14-26 19.4±6.31	24.8-25.6 25.2±0.36	20.2-21.9 21.2±0.66	23.2-26.6 24.9±1.33	21.0-22.9 22.1±0.97	23.2-26.6 24.9±1.30	2.0-3.2 2.4±0.77	0.6	FR or TP wRSsT
4 U		18-28 23.8±4.03	24.6-25.3 24.9±0.27	20.6-21.3 20.7±0.77	24.4-27.5 25.6±1.2)	22.4-23.2 22.8±0.33	24.4-27.0 25.5±1.22	2.0-4.6 3.2±1.38	0.6-0.8 0.68±0.11	
5 L	4	4-14 9.8±4.15	24.4-25.4 24.9±0.48	20.2-21.8 (20.2±1.48)	24.9-25.8 (25.2±0.46)	21.1-22.4 (21.9±0.38	24.9-25.8 (25.2±0.46)	2.4-4.8 3.6±1.17	0.6-0.8 0.68±0.11	P or TP wRSsT
5 M		3-9 6.8±2.39	0.0	0.0	25.4-26.2 25.8±0.30	21.9-23.4 23.0±0.4	25.4-26.2 25.2±0.30	2.2-3.2 2.5±0.7	0.6-0.8 0.68±0.11	

St	No of Ca	TPG	SphPG Radius	SphPG Pore width	PrPG PAL	PrPG EAL	PrPG Sulcus L	PrPG Sulcus W	Exine Thickness	Orn
						7		2		
5 U		10-18 14.0±3.39	24.2-24.8 24.4±0.38	21.8-22.6 22.0±0.88	23.7-25.7 24.7±0.86	20.8-21.5 21.1±0.33	23.7-25.7 24.8±0.86	3.6-5.2 4.4±0.77	0.6-0.8 0.68±0.11	
6 L		5-19 13.8±5.07	24.2-25.3 24.7±0.43	20.2-22.3 21.2±1.03	0.0	0.0	0.0	0.0	0.6	
6 M	3	3-6 4.6±1.34	23.9-24.9 24.6±0.39	20.6-22.5 21.2±1.39	0.0	0.0	0.0	0.0	0.5-0.6 0.54±0.05	Ulcerate
6 U		3-9 6.6±2.35	23.5-24.5 24.1±0.36	20.2-22.6 21.2±1.36	0.0	0.0	0.0	0.0	0.5-0.6 0.54±0.05	
7 L		3-8 5.6±1.95	24.9-25.3 25.1±0.15	20.4-22.3 21.1±1.22	0.0-26.8 12.4±14.06	0.0-22.8 9.1±12.52	23.6-25.8 22.6±3.50	3.4-5.2 4.2±0.98	0.5-0.8 0.66±1.34	
7 M	4	4-8 5.8±1.68	25.3-26.0 25.5±0.39	20.3-21.2 20.2±1.02	0.0	0.0	0.0	0.0	0.6	Ulcerate
7 U		9-25 19±6.05	25.1-25.5 25.3±0.15	21.6-22.5 21.9±0.37	0.0	0.0	0.0	0.0	0.5-0.8 (0.62±0.11)	
8 L		9-17 13.8±3.63	25.1-26.0 25.6±0.34	21.2-24.2 23.6±0.54	0.0	0.0	0.0	0.0	0.6-0.8 (0.68±0.11)	
8 M	3	14-25 18.2±6.96	24.9-26.4 25.6±0.58	20.9-22.6 21.2±0.88	23.7-26.6 25.1±1.07	20.6-22.9 21.9±1.03	23.7-26.6 25.1±1.07	3.2-5.2 4.4±0.77	0.6	FR
8 U		3-32 21.8±10.8	25.1-26.3 25.8±0.45	20.1-22.3 21.2±1.07	22.6-26.1 24.6±1.45	20.0-22.6 21.4±1.11	22.6-26.1 24.1±1.45	2.6-3.5 2.8±0.67	0.5-0.5 0.58±0.05	
9 L		11-39 27.4±10.57	23.9-25.4 (24.7±0.55)	19.8-22.4 (20.4±2.05)	0.0-27.1 21.1±6.81	0.0-23.4 18.4±5.30	24.2-27.1 25.1±1.81	2.8-4.2 3.5±0.67	0.5-0.8 0.62±0.11	
9 M	4	6-30 21.6±8.61	24.9-26.4 25.5±0.65	20.9-22.6 21.6±0.95	0.0-24.0 14.2±10.73	0.0-20.01 11.0±8.92	21.2-24.0 22.8±1.76	2.8-4.4 3.6±0.82	0.5-0.8 0.6±1.22	P or FR
9 U		7-13 9.8±2.89	25.5-26.2 25.7±0.35	21.4-22.8 21.7±1.12	0.0-24.6 14.02±10.00	0.0-20.2 11.04±9.03	20.2-24.6 21.4±3.17	2.4-4.2 3.6±0.48	0.5-0.8 0.6±1.34	
E L		5-13 8.8±3.49	24.7-25.4 24.9±0.28	20.6-22.8 21.5±1.32	0.0-24.5 10.7±13.34	0.0-21.2 10.8±10.34	21.2-24.5 22.7±1.34	2.0-3.4 2.8±0.54	0.6-0.8 0.64±0.09	
E M		8-17 11.8±5.13	24.7-26.5 25.4±0.73	20.6-22.2 21.0±1.23	0.0-25.1 14.4±10.88	0.0-20.8 11.4±9.14	21.2-24.6 22.2±1.88	2.4-4.2 3.2±0.98	0.6	FR or TP
E U	3	5-13 9.2±3.46	24.5-25.8 25.2±0.46	20.5-22.8 22.0±2.66	0.0-24.5 14.2±10.96	0.0-22.2 12.4±9.93	21.2-23.5 21.9±1.66	2.4-4.4 3.4±0.97	0.6	
N L		3-5 4.0±1.00	23.4-24.7 23.9±0.46	20.4-21.5 20.8±0.72	0.0	0.0	0.0	0.0	0.6	
N M		11-25 18.2±6.09	23.5-24.2 24.0±0.22	20.5-21.2 20.8±0.44	0.0-23.2 10.2±12.60	0.0-20.4 9.1±11.12	20.4-23.0 21.2±2.02	2.0-4.8 3.5±1.18	0.5-0.6 0.58±0.04	Ulcerate
N U		35-42 38.8±3.27	25.1-26.9 25.9±0.69	20.1-21.9 20.9±0.89	0.0-23.8 11.1±12.46	0.0-20.9 9.1±11.14	20.2-23.6 22.1±1.46	1.8-4.8 3.6±1.17	0.6	

St	No of Ca	TPG	SphPG		PrPG				Exine	
			Radius	Pore width	PAL	EAL	Sulcus L	Sulcus W	Thickness	Orn
S L		8-21 17.2±4.18	24.5-25.4 24.9±0.38	20.5-22.4 21.9±0.58	0.0-24.8 10.8±13.45	0.0-20.6 11.1±9.07	21.6-24.8 22.8±1.45	2.2-4.2 3.8±0.42	0.6	
S M	5	38-42 39.6±1.82	24.2-25.2 24.5±0.41	20.2-22.0 21.5±0.41	0.0-26.8 13.6±12.29	0.0-22.2 12.8±8.57	23.2-24.8 23.8±0.39	2.0-3.6 2.8±0.78	0.6	Ulcerate
S U		6-22 15.2±6.16	24.9-25.2 25.0±0.13	20.9-23.2 22.6±1.13	0.0-26.7 20.2±5.45	0.0-21.5 16.7±4.36	22.6-25.7 24.2±1.35	1.8-3.6 2.8±0.77	0.6	
W L		14-25 18.8±5.54	24.7-25.4 25.1±0.26	21.6-22.6 21.9±1.06	0.0-22.8 18.0±3.57	0.0-19.2 14.9±5.37	19.8-21.8 21.0±0.17	3.2-5.2 4.2±0.98	0.6	
W M	3	10-13 11.4±1.52	24.2-24.6 24.4±0.15	20.4-22.6 21.4±1.15	0.0-22.4 17.6±4.86	0.0-21.0 16.5±4.24	19.6-21.6 20.4±0.86	3.6-5.4 4.4±0.96	0.5-0.6 (0.58±0.04)	Ulcerate
W U		5-9 7.0±1.58	24.4-25.0 24.7±0.26	20.2-22.4 21.2±1.06	0.0-22.4 14.5±7.02	0.0-21.0 14.2±6.39	19.8-21.6 20.4±1.52	3.2-5.2 4.4±0.77	0.6	

Abbreviations: Anap.=Anaporate, Aper=Aperture, Ca=Catkin, EAL=Equatorial axis length, FR=Faintly rugate, L=Length, No=Number, Orn=Ornamentation, P=Psilate, PAL=Polar axis length, P/E= Polar/Equatorial axis, PrPG=Prolate pollen grain, Sph=Spherical pollen grain, St=Stages, TP= Tectate perforate, TPG=Total pollen grains, RS/P= Ratio between Spherical /Prolate , W=Width, wRSsT=with Rod shape supra tectum.

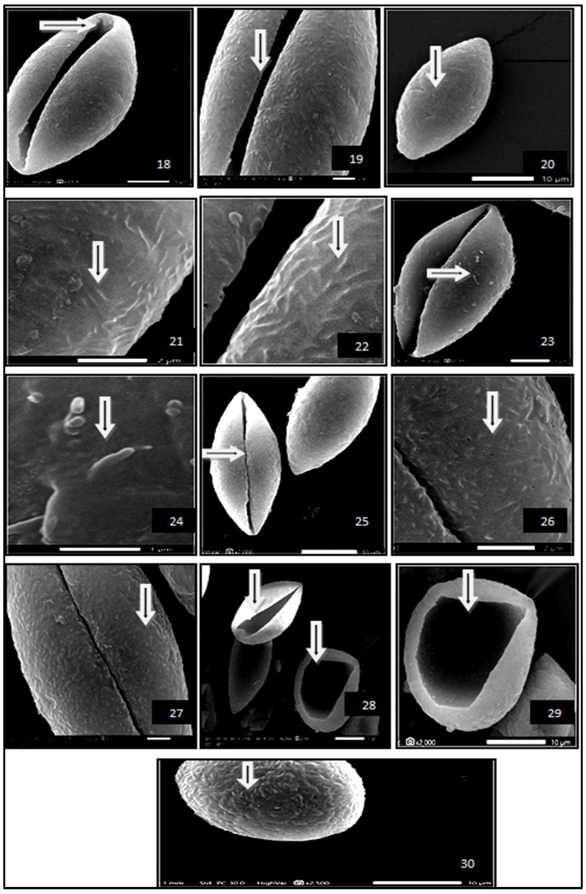

Fig. 18-30. SEM photographs showing the main different characters within the studied stages. Bar=2µm in 19, 21, 22, 24, 26, 27; Bar=5µm in 18, 23, 25, 28; Bar=10µm in20, 29,30. Fig.18 rounded end sulcus, Fig.19 narrow sulcus and rugate exine, Figs.20& 21 faintly rugate exine, Fig.22 rugate exine, Figs.23& 24 rod shapes exinous protrusions, Fig.25 narrow very long sulcus, Fig.26 tectate perforate exine, Figs.27& 30 ulcerate exine, Figs 28 &29 anapore and wide sulcus.

Data analyses

Most of the investigated pollen pheno-characters showed highly significant differences between the catkin developmental stages (F2 & P2) and in between the different positions (L, M, U) in each catkin stage (from 1-9 and the four directions F1 & P1), except within few characters (Tables 3). Despite the state of differences recorded within the different positions of the catkins (L, M, U) there were highly significant differences ($p \leq 0.001$) between the different nine stages of the catkin position.

Table 2. Element contents in both spherical and prolate pollen grains at K line, 20 Kv, and 30 second exposure rate (EDX as X-ray exposure by SEM).

Spc.	Mag.	Count Rate	Element	Mass%	Atom%
010 Fitting ratio 0.2058	700X	2141.00	C	53.22±0.30	63.27±0.36
			O	38.23±0.60	34.12±0.54
			Na	0.19±0.09	0.12±0.05
			Mg	0.39±0.07	0.23±0.04
			K	3.40±0.11	1.24±0.04
			Cu	2.93±0.21	0.66±0.05
			Zn	1.65±0.20	0.36±0.04
			Total	100.00	100.00
020 Fitting ratio 0.0314	2500X	1851.00	C	60.59±0.22	68.62±0.25
			O	34.78±0.42	29.57±0.36
			Mg	0.31±0.03	0.17±0.02
			P	1.20±0.04	0.53±0.02
			S	0.30±0.02	0.13±0.01
			K	2.40±0.06	0.83±0.02
			Ca	0.42±0.03	0.14±0.01
			Total	100.00	100.00
030 Fitting ratio 0.0291	700X	2141.00	C	57.66±0.19	65.64±0.22
			O	38.38±0.39	32.80±0.33
			Mg	0.35±0.03	0.20±0.01
			P	0.93±0.03	0.41±0.02
			S	0.18±0.02	0.08±0.01
			K	2.5±0.06	0.88±0.02
			Total	100.00	100.00
040 Fitting ratio 0.0250	1100X	2369.00	C	55.96±018	63.75±0.20
			O	40.92±0.36	35.00±0.31
			Mg	0.28±0.02	0.16±0.01
			P	0.93±0.03	0.41±0.01
			S	0.15±0.01	0.07±0.01
			K	1.76±0.05	0.61±0.02
			Total	100.00	100.00
050 Fitting ratio 0.0342	2000X	2081.00	C	58.1±0.20	65.98±0.22
			O	38.79±0.39	32.89±0.33
			Mg	0.28±0.03	0.16±0.01
			P	0.92±0.03	0.40±0.02
			S	0.17±0.02	0.07±0.01
			K	1.42±0.05	0.49±0.02
			Total	100.00	100.00
060 Fitting ratio 0.0266	1300X	2178.00	C	59.25±0.20	67.20±0.22
			O	36.46±36	31.05±0.31
			Na	0.51±0.04	0.30±0.02
			Mg	0.29±0.02	0.16±0.01
			P	0.77±0.03	0.34±0.01
			S	0.20±0.02	0.09±0.01
			Cl	0.95±0.03	0.36±0.01
			K	0.93±0.04	0.32±0.01
			Ca	0.29±0.02	0.10±0.01
			Cu	0.35±0.04	0.07±0.01
			Total	100.00	100.00
070 Fitting ratio 0.0343	650X	1815.00	C	54.93±0.22	63.14±0.25
			O	40.47±0.42	34.92±036
			Na	0.56±0.04	0.34±0.03
			Mg	0.25±0.03	0.14±0.02
			P	0.94±0.04	0.42±0.02
			S	0.23±0.02	0.10±0.01
			Cl	0.74±0.03	0.29±0.01
			K	1.66±0.05	0.59±0.02
			Ca	0.21±0.03	0.07±0.01
			Total	100.00	100.00
080 Fitting ratio 0.3520	5000X	2858.00	C	54.77±0.34	66.22±0.41
			O	32.21±0.54	29.24±0.49
			Na	1.54±0.11	0.98±0.07
			Mg	0.62±0.07	0.37±0.04
			Cl	2.44±0.08	1.00±0.03
			K	1.99±0.09	0.74±0.03
			Cu	4.12±0.23	0.94±0.05
			Zn	2.31±0.22	0.51±0.05
			Total	100.00	100.00
090 Fitting ratio 0.4064	4300X	2644.00	C	54.06±0.35	65.52±0.42
			N	0.77±0.50	0.80±0.52
			O	33.14±0.61	30.15±0.56
			Na	0.20±0.12	0.13±0.07
			Mg	0.43±0.08	0.26±0.05
			Al	0.95±0.09	0.46±0.05
			K	2.05±0.10	0.76±0.04
			Cu	5.53±0.29	1.27±0.07
			Zn	2.97±0.28	0.66±0.06
			Total	100.00	100.00

Table 3. Data analyses (F1, P1, F2, P2) of the different catkin stages (1-9 & E, N, S, N) and microsporangium position (L, M, Up) according to the means and standard deviation in the studied parameters. F: F for One way ANOVA test, pairwise comparison between each 2 groups were done using Post Hoc Test (Tukey), p_1: p value for comparing between the three studied locations in each stage, p_2: p value for comparing between the different studied stages.

Param →	TPG (F1, P1)			No SpPG (F1, P1)			R SpPG (F1, P1)			No PrPG (F1, P1)		
Stages↓	L	M	Up	L	M	Up	L	M	Up	L	M	Up
1	F1 10.008*, P1 0.003*			F1 11.105*, P1 0.002*			F1 2.748, P1 0.104			F1 4.850*, P1 0.029*		
2	F1 0.425, P1 0.663			F1 1.596, P1 0.243			F1 4.851*, P1 0.029			F1 106.400*, P1<0.001*		
3	F1 5.441*, P1 0.021*			F1 2.957, P1 0.090			F1 2.140, P1 0.160			F1 8.561*, P1 0.005*		
4	F1 1.389, P1 0.287			F1 2.352, P1 0.137			F1 5.260, P1 0.023*			F1 0.030, P1 0.971		
5	F1 7.506*, P1 0.008*			F1 6.000*, P1 0.016*			F1 1.043, P1 0.345			F1 2.106, P1 0.164		
6	F1 8.745*, P1 0.005*			F1 8.745*, P1 0.005*			F1 3.933*, P1 0.049*			F1 --, P1 --		
7	F1 8.039*, P1 0.006*			F1 9.112*, P1 0.004*			F1 5.083*, P1 0.025*			F1 2.500, P1 0.124		
8	F1 1.032, P1 0.386			F1 0.483, P1 0.629			F1 0.003, P1 0.997			F1 5.346*, P1 0.022*		
9	F1 3.639, P1 0.058			F1 0.483, P1 0.629			F1 4.892*, P1 0.028*			F1 5.871*, P1 0.017*		
F2	4.976*	7.033*	12.023*	5.464*	5.415*	15.614*	3.119*	6.171*	13.188*	14.771*	6.033*	30.082*
P2	<0.001*			<0.001*			0.009*	<0.001		<0.001*		
E	F1 1.853, P1 0.199			F12.135, P1 0.161			F1 1.096,P1 0.465			F1 3.573, P1 0.168		
N	F1 99.186*, P1<0.001*			F1 75.461*, P1<0.001*			F1 24.603*, P1<0.001*			F1 2.533, P1 0.282		
S	F1 19.853*,P1 <0.001*			F1 24.286*,P1 <0.001*			F1 3.559, P1 0.061			F1 1.439, P1 0.487		
W	F1 12.462*, P1 0.001*			F1 7.175, P1 0.009*			F1 11.945*,P1 0.001*			F1 6.264*, P1 0.044*		
F2	6.707*	54.398*	70.172*	6.317*	58.096*	71.653*	11.346*	9.096*	6.567*	5.927	2.151	5.301
P2	0.004*	<0.001*		0.005*	<0.001*		<0.001*	0.001*	0.004*	0.115	0.542	0.151

Table 3. Conted.

Param→ Stages↓	PAL PrPG (F1, P1) L	M	Up	EAL PrPG (F1, P1) L	M	Up	Exine thickness L	M	Up
1	F1 5.080*, P1 0.025*			F1 5.038*, P1 0.026*			F1 0.500, P1 0.619		
2	F1 21.440*, P1 <0.001*			F1 17.056*, P1 <0.001*			F1 3.125, P1 0.081		
3	F1 2.413, P1 0.132			F1 4.195*, P1 0.042*			F1 2.667, P1 0.110		
4	F1 4.291*, P1 0.039			F1 2.109, P1 0.164			F1 3.733, P1 0.055		
5	F1 3.467, P1 0.065			F1 16.623*, P1 <0.001*			F1 0.000, P1 1.000		
6	F1 --, P1 --			F1 --, P1--			F1 4.933*, P1 0.027*		
7	F1 2.666, P1 0.110			F1 2.667, P1 0.110			F1 0.467, P1 0.638		
8	F1 891.344, P1 <0.001*			F1 1023.84*, P1 <0.001*			F1 3.000, P1 0.088		
9	F1 3.521, P1 0.063			F1 3.885*, P1 0.049*			F1 0.311, P1 0.738		
F2	6.122*	21.814*	56.144*	5.917*	20.550*	65.937*	1.479	1.509	1.754
P2	<0.001*			<0.001*			0.199	0.188	0.119
E	F1 3.019, P1 0.221			F1 1.726, P1 0.422			F1 0.684, P1 0.523		
N	F1 2.471, P1 0.291			F1 2.471, P1 0.291			F1 1.000, P1 0.397		
S	F1 2.308, P1 0.315			F1 2.77, P1 0.320			F1 0.000, P1 1.000		
W	F1 4.502, P1 0105			F1 3.575, P1 0.167			F1 1.000, P1 0.391		
F2	4.208	4.117	7.283	4.208	2.970	3.993	6.006*	1.303	1.000
P2	0.240	0.249	0.063	0.240	0.396	0.262	0.006*	0.308	0.418

Summary of table 3.

Characters	Insignificant between positions	Significant between positions	Significant between stages	Significant between dir.
TPG	2, 4, 8, 9, E	1, 3, 5, 6, 7, N, S, W	+	+
No SpPG	2, 3, 4, 8, 9 E	1, 5, 6, 7, N, S, W	+	+
R SpPG	1, 2, 3, 5, 8, E, S	4, 6, 7, 9, N, W	+	+
No PrPG	4, 5, 6, 7, E, N, S	1, 2, 3, 8, 9, W	+	--
PAL	3, 4, 5, 6, 7, 9, E, N, S, W	1, 2, 8	+	--
EAL	4, 6, 7, E, N, S, W	1, 2, 3, 5, 8, 9	+	--
Exine thickness	1, 2, 3, 4, 5, 7, 8, 9, e, N, S, W	6	--	--

The differences in most characters were insignificant according to the four directions of the catkins, except in the total pollen numbers, number of spherical pollen grains and their radius ($p \leq 0.001$).

The exine thickness variation was insignificant in all the positions and directions, except in the catkins collected from stage 6 ($p \leq 0.027$) (Table 3).

Discussion

Ginkgo is the sole living survivor of a genus that has changed little for more than 80 million years, holding the secret to its immeasurable durability. When it became the first plant to bud at the central area of the Hiroshima bomb blast, the *Ginkgo* tree also became a symbol of indomitability and hope (Beuchert 1995). Recently, the world faced great alteration in climatic conditions, especially the fluctuation in the temperature and precipitation. *G. biloba* is a species that challenged great environmental disorders and overcame many world catastrophes. This species attracted the attention of many scientists in different lines of interest. *G. biloba* is a woody tree, it prefers moderate rainfall and hot summer regions such as Mediterranean climates (Dallimore 1967). Accordingly, it was the target of this investigation as most of the previous studies dealt with the ovule development after fertilization. Pollen grains development and dispersal beside their hydrolytic state are important factors to succeed pollination and fertilization process. In fact, the fluctuations in the air temperature and degree of air humidity have resulted either in the extinction or evolution of various species (Dixit *et al.*, 2016). This study was carried out in Alexandria city, which located in the northern coastal Mediterranean region in Egypt. This city has its own characteristic climate with high contents of air humidity all over the year with hot summer. This weather has changed over the last ten years with an increase in temperature and humidity.

The pollen investigation of the nine developmental catkin stages as well as those collected from the four Earth directions showed variation in the number of the catkin in each node from three to five and this agree with Coder (2020) in his description of the *Ginkgo* gender role. The differences in the pollen shape and size between the studied stages and positions can be attributed to developmental stages and cultivar type.

This observation was mentioned by Korszun and Klimlko (2014), as they noticed great variations in *G. biloba* pollen sizes between seven studied cultivars. Differences in pollen aperture types in the same species as well as variations in their shapes have been recorded within angiosperm pollen grains by Taia *et al.* (2023). Meanwhile, Dixit *et al.* (2016) found different pollen shapes in *Pinus roxburghii* due to environmental disorders. These previous observations can explain the variations in the pollen pheno-characters within the studied stages. From the worth noticing observation is the differences in the sulcus width which can be referred to the hydration state of the pollen grains as mentioned by Lu *et al.* (2016). There is a remarkable explanation by Bozic and Siber (2020) as they attributed the infolding of the aperture to the desiccation process to reduce the rate of water loss. This explanation can be satisfying to understand the variations in the sulcus width between the studied catkin positions and directions.

Pollen mineral contents are indicators to the environmental disorders and their developmental stage. From the most important element in the processes of pollination and fertilization is Calcium, which is located in the intine layer. This element was recorded in the 2nd., 6th. and 7th. catkin stages only and this indicates that the pollen grains dispersed from these catkin stages are ready for pollination than the other catkin stages. The lack of Calcium in the other stages means that either their pollen grains are already ejected, or they are still in their developmental stages. The importance of the Calcium in pollination and fertilization has been mentioned by Lu *et al.* (2016). While the presence of some heavy metals such as Copper and Zinc in the 1st, 8th and 9th catkin stages are important elements in pollen fertility and pollen tube emergence as pointed by Azouaou and Souvre (1993). In an interesting work on the effect of both elements (Copper and Zinc) on the plant productivity of *Vicia faba* is that of Alhasany *et al.* (2019) as they found significant increase in the plant yield and productivity. The presence of both phosphorous and Sulfur in the catkin stages from two to seven are due to their importance in the pollen development and pollen tube growth. Both elements are essential micro-elements for plants in general but the increase in their amount decrease the quality of the pollen grains as mentioned by Pers-Kamczyc *et al.* (2020).

Our observation showed that *G. biloba* trees growing in Alexandria city are adapted to the city climate and release their pollen grains gradually to ensure successful pollination. Not all the microsporangia mature at the same time; accordingly, their pollen grains are in different developmental and hydrolytic states. The obtained results proved the high adaptation processes which shown in both the phenol-morphological states beside their mineral contents of the *G. biloba* pollen grains. This conclusion supports Dmuchowski *et al.* (2022) and Bidak *et al.* (2022) who pointed to the specific strategy of *G. biloba* tree to accommodate with the environmental and climatic conditions and how it uses this strategy to sustain and protect himself throughout all the history.

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Conflict of interest

There is no conflict of interest.

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