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Palynological Characters of *Solanum* L. Species grown in Sana'a University (New Campus), Yemen

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Abstract:

Morphological characters of a pollen grain are useful as any other characters in classifying and showing the relationships among different taxonomical groups. Earlier taxonomists declared that the leaf morphological characters show a limited taxonomical value in distinguishing between *Solanum* spp. This study deals with the Palynological Characters of four *Solanum* taxa (*S. coagulans* Forssk., *S. glabratum* Dunal, *S. incanum* L. and *S. villosum* Mill.), belongs to 2 different subgenera (Leptostemonum and Solanum); four sections (Monodolichopus; Oliganthes; Melongena and Solanum) grown in Sana'a University's new campus and its taxonomical significance. A total of 19 Palynological characters (6 qualitative features and 13 quantitative features) were observed in this study. Pollen size shows a high taxonomical significance in distinguishing between *S. coagulans* and the other three studied taxa; while aperture (Colporus) size shows a highly significant value in distinguishing between *S. villosum* and the other three studied taxa. On the other hand, the statistical analysis (Un-weighted pair group mathematical average clustering (UPGMA) and Principle Component Analysis (PCA)) demonstrate the taxonomical relationship which agrees with the taxonomical state of the studied *Solanum* taxa.

INTRODUCTION

The Solanaceae family is one of the most important families in Angiosperms due to its economical, ethnobotanical, and floristic properties (Olmstead *et al.,* 1999); it includes 2950 species in 94 genera (Mabberley, 1997), worldwide, occurring, but particularly in tropical regions (Hepper, 2002). In Yemen flora, Solanaceae is represented by 38 species (7 cultivated and 31 natives among them 4 endemics) belong to 10 genera (Al Khulaidi, 2013).

The genus *Solanum* L. is the type genus of the Solanaceae family (Jennifer and James, 1997), its habitat varies from annual herbs to perennial shrubs and sometimes scrambling or tree-like (Abedin *et al.*, 2001) that includes about 1500 species. Therefore, it is the largest and the most complex genus in this family (Jennifer and James, 1997), mainly distributed in tropical and warm temperate regions of the world with centers of diversity occurring in the Asian Southern Hemisphere (D'Arcy, 1991). Many species of this genus are cultivated for their economical importance as food plants such as potato (*S. tuberosum*) and eggplant (*S. melongena*) or as a medicinal plant such as *S. dulcamara* L. and *S. viarum* Dun., both are used as sources of corticosteroids (Jennifer and James, 1997).

In the Yemeni flora, the genus *Solanum* L. is represented by 15 species: 12 native species (*S. anguivi*, *S. coagulans*, *S. cordatum*, *S. forskalii*, *S. glabratum*, *S. incanum,* , *S. nigrum*, *S. pubescens*, *S. schimperianum*, *S. surattense*, *S. terminale* and *S. villosum*), 2 cultivated species (*S. melongena* and *S. tuberosum*) and one endemic species; S*. platacanthum* (Wood, 1997; Al Khulaidi, 2013), Furthermore; according to Dubaie et al. (1993) and Dubaie (1995) only 7 species were recorded from Sana'a city; 5 native species (*S. glabratum*, *S. incanum*, *S. nigrum*, *S. schimperianum*, *S. villosum*) and 2 cultivated species (*S. melongena* and *S. tuberosum*). In 1972 D'Arcy divided the genus *Solanum* L. into seven subgenera and numerous sections and series based on the morphological characters of the *Solanum* taxa.

Moreover; some *Solanum* species have not been placed in a proper sub-generic or sectional rank and their phylogenetic relationships to other groups are still unknown (Bohs and Olmstead, 1997). On the other hand; in 1985 Jaeger declared that the leaf characters of the *Solanum* spp. are of limited diagnostic value except as an indicator of the likely habitat of an individual. Furthermore; Erdtman (1986) cited that the morphological structure of pollen grains offers many characters of taxonomical value that are useful as any other characters in the classification of plants and they best serve in distinguishing and showing relationships among the higher groups; such as families, tribes, and genera, but sometimes they are useful among the species level; thus pollen grain structure can be used to explain the obscure relationships which are difficult to demonstrate by other characters. Therefore; the present study aims to investigate the taxonomical relationship between *Solanum* L. taxa grown in the new compose of Sana'a University based on the morphological features of the Pollen grains and to determine the Pollen characters (qualitative and quantitative), that gives a high taxonomical significance in distinguishing between the studied *Solanum* L. taxa.

MATERIALS AND METHODS

Plant Materials collection

Four *Solanum* taxa belonging to two subgenera: Leptostemonum and Solanum were collected from Sana'a University's new campus (Table 1 and Figure 1) from April to September 2020. Plant material was identified by utilizing the available taxonomical and floristic literature (Wood, 1997; Collenette, 1999).

Palynology investigation

Pollen grains were obtained from the fresh anther of *Solanum* spp. samples collected from Sana'a University's new campus; Pollen grains were acetolyzed following the method of Avetissian (1950); pollen grain slides were observed by using a Letiz Dialux 20 microscope with a magnification of x1000 oil and

Photographs of Polar and Equatorial view of pollen were taken by Canon (IXUS255 HS) digital camera to investigate the qualitative and quantitative characters which were based on the terminology of Erdtman (1986), Agashe and Caulton (2009) and Hosney (2014), 19 characters (6 qualitative and 13 quantitative) were investigated (Table 2 and 3); due to the difficulties in studying the quantitative characters, only 12 pollen grain were measured in each taxon.

Table 1. Locality and Sample collection of the investigated *Solanum* taxa.

Solanum Taxa	Herbarium No.	Date	Coordinates		Altitude	Location
			Longitudenal	Latitudenal		
Solanum coagulans	BHSS:01555	16-04-020	44°11'2.05"E	15°21'53.97"N	2275 m asl.	Sana'a University, New Campus
	BHSS:01572	12-09-020	44°11'8.29"E	15°21'55.51"N	2267 m asl.	
Solanum glabratum	BHSS:01556	16-04-020	44°11'7.20"E	15°21'48.40"N	2270 m asl	
	BHSS:01557	04-05-020	44°11'1.40"E	15°21'53.00"N	2270 m asl	
	BHSS:01568	16-08-020	44°11'4.00"E	15°22'6.10"N	2267 m asl.	
Solanum incanum	BHSS:01558	11-05-020	44°11'14.00"E	15°21'49.00"N	2270 m asl	
	BHSS:01564	07-07-020	44°11'10.80"E	15°21'49.20"N	2270 m asl	
	BHSS:01565	12-07-020	44°11'9.50"E	15°21'48.90"N	2270 m asl	
Solanum villosum	BHSS:01560	16-06-020	44°11'16.33"E	15°21'48.80"N	2269 m asl	
	BHSS:01561	16-06-020	44°11'16.33"E	15°21'48.80"N	2269 m asl	

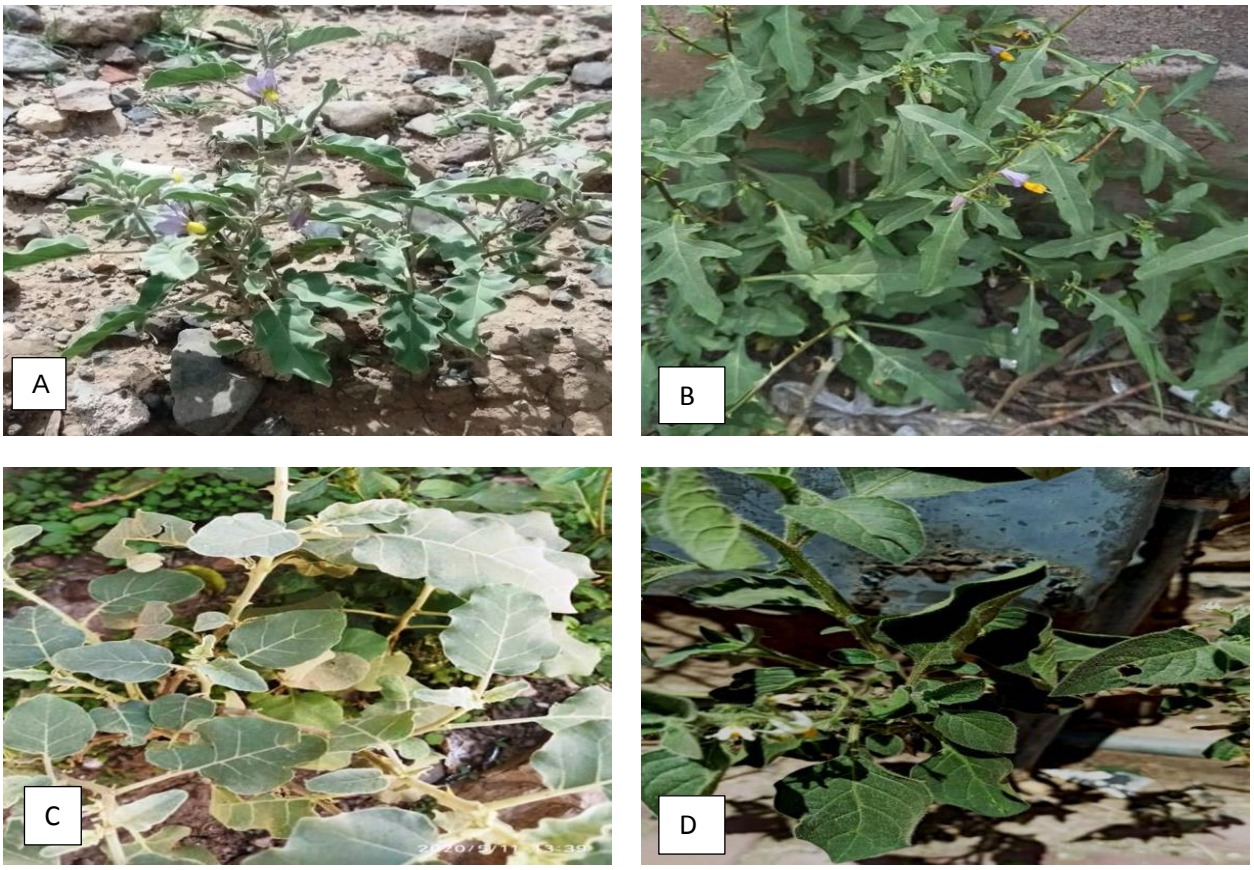

Fig.1. Investigated *Solanum* taxa: *S. coagulans*; B- *S. glabratum*; C- *S. incanum*; D- *S. villosum*.

Table 2. List of the Investigated Pollen grains Qualitative Morphological Characters of the four recoded *Solanum* taxa in Sana'a University new compose.

Sr. No.	Qualitative Characters			
1	Shape	Oblate spheroidal - Prolate spheroidal [1]	Prolate spheroidal [2]	Prolate spheroidal - Subprolate [3]
2	Size	Medium [1]	Medium -Large[2]	Large [3]
3	Polarity	Isopolar[1]	Not so [2]	
4	Shapes of Aperture	Colporus [1]	Not so [2]	
5	Pollen class	Trizonocolporus [1]	Not so [2]	
6	Symmetry	Radially symmetric [1]	Not so [2]	

Table 3. List of the Investigated Pollen grains Quantitative Characters of the four recoded *Solanum* taxa in Sana'a University new compose.

View	Sr. No.	Quantitative Characters
Equatorial view	1	Polar axis (PA) µm
	2	Equatorial axis (EA) µm
	3	PA/EA
	4	(PA/EA) *100
	5	PA+EA/2
	6	Length of Aperture (Colporus) µm
	7	Width of Aperture (including the ora) from the Equatorial view µm
	8	Aperture (Colporus) size = (length*Width) μm^2
Polar view	9	Exine thickness µm
	10	Equatorial axis (EA) µm
	11	Mesocolpium diameter (MD) µm
	12	Number of Aperture (Colporus)
	13	Width of Aperture (Colporus) µm

Statistical Analysis

The taxonomical value of quantitative pollen grain morphological characters was determined by one-way ANOVA test using Graph Pad Prism 6.01 program, if P-value, $P < 0.05$ then the quantitative pollen grain features are significantly different. Moreover; the relationship between the four collected *Solanum* taxa have been demonstrated as a dendrogram, based on the obtained pollen morphological data (Table 2 and 3) by creating a data matrix for numerical analysis (Un-weighted Pair Group Mathematical Average clustering (UPGMA)) using Primer 5 software version: 5.2.2. Furthermore, Principal Component Analysis (PCA) was employed to confirm the results obtained from cluster analyses using Minitab software version 14.1.

RESULTS

The results showed that all studied pollen grains of the four *Solanum* taxa are Isopolar; Radially symmetric, Trizonocolporus aperturate (Table 4; 5 and Figure 2). On the other hand, the pollen grain of *S. coagulans* and *S. incanum* are prolate spheroidal in shape; while the pollen grains of *S. glabratum* shape ranged between oblate spheroidal and prolate spheroidal while; the pollen grains of *S. villosum* shape ranged between prolate spheroidal and subprolate. In addition, the size of *S. coagulans* pollen grain is large; however; the size of *S. incanum* pollen grain ranges between large to medium while the size of *S. glabratum* and *S. villosum* pollen grains are medium in size. Furthermore, the *S. coagulans* pollen grains have the longest Polar axis and Equatorial axis (Equatorial view) with mean length; 32 µ & 30 µ respectively followed by the Polar axis and Equatorial axis (Equatorial view) of *S. incanum* pollen grains (25.9 µ & 24.5 µ); *S. glabratum* (23.7 µ &23.9 µ) than *S. villosum* pollen grains with mean Polar axis length and Equatorial axis length 23.3 µ & 21.04 µ correspondingly; while the pollen grains of *S. villosum* have the largest Aperture (colporus) with mean size 285.6 μ^2 followed by the Aperture (colporus) size of *S. coagulans* pollen grains (107.6 μ^2); *S. incanum* pollen grains(67.7 μ^2) than *S. glabratum* pollen grains with mean size 63.2 μ^2. Moreover, the longest mean of Mesocolpium diameter was recorded in *S. coagulans* pollen grain (22.6 µ) followed by *S. incanum* pollen grains (19.4 µ); *S. villosum* pollen grains (19.3 µ) than *S. glabratum* pollen grains with mean Mesocolpium diameter 17.4 µ; although, the *S. villosum* pollen grains have the widest Aperture in the polar view with a mean width (6.6 µ) followed by *S. coagulans* pollen grains (3.3 µ); *S. glabratum* pollen grains (2.8 µ); then *S. incanum* pollen grains with mean width 2.4 µ.

According to Table 5; the pollen size (PA+EA/2) shows a high taxonomical significance in distinguishing between *S. coagulans* and the other three studied taxa; while Aperture (Colporus) size shows a highly significant value in distinguishing between *S. villosum* and the other three studied taxa.

Table 4. Data Matrix of Pollen grain Qualitative Morphological characters.

Sr. No.	Characters	*S. coagulans*	*S. glabratum*	*S. incanum*	*S. villosum*
1	Shape	2	1	2	3
2	Size	3	1	2	1
3	Polarity	1	1	1	1
4	Shapes of Aperture	1	1	1	1
5	Pollen class	1	1	1	1
6	Symmetry	1	1	1	1

Table 5. Pollen grain Quantitative Morphological Characters of the four recoded *Solanum* taxa in Sana'a University new compose.

View	Sr. No.	Characters	*S. coagulans*	*S. glabratum*	*S. incanum*	*S. villosum*
Equatorial view	1	Polar axis length(PA) µm	26.2 (32 ± 5.1)41.6	20 (23.7± 2.9) 31.1 a^{****}	19.8 (25.9 ± 4.1) 33.7 a^{**}	19.2 (23.3 ± 2.6) 27.9 a^{****}
	2	Equatorial axis length (EA) µm	24.4 (30 ± 4.8) 40.2	20.2 (23.9 ± 3.1) 31.2 a^{***}	19.2 (24.5 ± 3.8) 31.4 a^{**}	16.3 (21.04 ± 2.9) 26.6 a^{****}
	3	PA/EA µm	1.01 (1.1 ± 0.1)1.2	0.9 (1± 0.1) 1.1 a^{*}	1.03 (1.06± 0.02)1.1	1 (1.1 ± 0.1) 1.2 b^{****}
	4	(PA/EA) ×100 µm	100.7 (106.8 ± 5.9)120.9	92.6 (99.6± 5.4) 109.9 a^{*}	103.1 (105.7± 2.4) 110.2	102.1 (111.4 ± 7.9) 124.8 b^{****}
	5	PA+EA/2 in µm	25.3 (31 ± 4.9) 40.4	20.4 (23.8± 2.9) 31.2 a^{***}	19.5 (25.2 ± 3.9) 32.6 a^{**}	18.3 (22.2± 2.7) 27.3 a^{****}
	6	Length of Aperture (AL) µm	18.1 (24.2± 5) 33.1	16.5 (19.8 ± 2.6) 26.1 a^{*}	16.6 (21.6 ± 3.8) 29.4	21 (25.9 ± 3.3) 31.2 a^{**}, b^{*}
	7	Width of Aperture (including the ora) from the Equatorial view (AW) µm	1.7 (4.4± 2.2) 8.2	1.8 (3.2± 0.9) 4.7	1.6 (3.1± 1.1) 5.7	8.3 (10.867 ± 2.3) 15.8 a^{****}, b^{****}, C^{****}
	8	Aperture size (AS) = (length× width) μm^2	38.5 (107.6± 62.3) 248.8	33 (63.2 ± 22.5) 121.3	34.5 (67.7± 24.7) 113	179.3 (285.6 ± 88.8) 477 a^{****}, b^{****}, C^{****}
Polar view	9	Exine thickness (EX) µm	0.7 (1.1 ± 0.4) 1.8	0.9 (1.4± 0.3) 2.1 a^{*}	1 (1.4± 0.3) 2	0.74 (1.2 ± 0.1) 1.7
	10	Equatorial axis (EAP) µm	22.7 (26.1 ± 2.6) 30.3	16.8 (20.6 ± 3.3)26.1 a^{***}	18.1 (22.5 ± 3.1) 27.8 a^{*}	15.4 (21.5 ± 4.1) 28.5 a^{**}
	11	Mesocolpium diameter (MD) µm	18.9 (22.6± 2.7) 28.6	14.4 (17.4 ± 2.5) 21 a^{***}	16.02 (19.4 ± 2.7) 23.7	13 (19.3± 3.9) 24.6 a^{*}
	12	Number of Aperture (Colporus)	3	3	3	3
	13	Width of Aperture (WAP) µm	1.3(3.3± 1.6) 5.7	2.1(2.8 ± 0.4) 3.6	1.5 (2.4± 0.6) 3.6	2.8 (6.6 ± 2.2) 9.8 a^{****}, b^{****}, C^{****}

a: Significant differences of species compered to *S. coagulans*; b: Significant differences of species compered to *S. glabratum*; c: *S. incanum*; *: P< 0.05; **: P< 0.01; ***: P< 0.001 & ****: < 0.0001.

Numerical study

The statistical analysis shows the taxonomical relationship among the four studied taxa; The PCA (Figure 3) divides the studied taxa into two groups; the first group includes the taxa that belong to the subgenus Solanum (*S. villosum*); while the second group includes *S. coagulans*, *S. glabratum* and *S. incanum* which belongs to the subgenus Leptostemonum and this confirmed with the finding of UPGMA (Figure 4) where the studied taxa were divided into two different groups (subgenera); group I and group II at distance level 10.3, group I (subgenus Solanum, section Solanum) includes *S. villosum* and group II (subgenus Leptostemonum) include *S. coagulans*, *S. glabratum,* and *S. incanum*. Furthermore, group II (subgenus Leptostemonum) were divided into two

subgroups at distance level 5.9; subgroup A (section Monodolichopus) includes one species; *S. coagulans*; while, subgroup B includes two species *S. glabratum* (section Oliganthes) and *S. incanum* (section Melongena) which were separated at distance level 2.4.

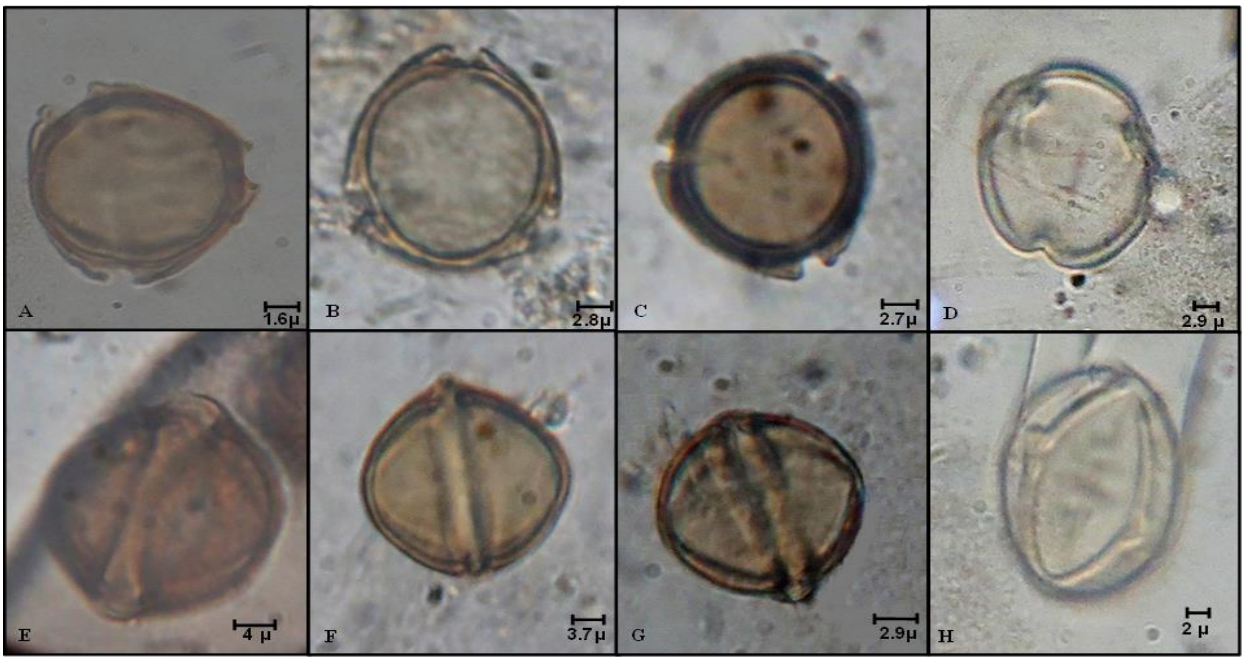

Fig. 2. Pollen grains of *Solanum* taxa.: A-D: Polar view; A: *S. coagulans;* B: *S. glabratum*; C: *S. incanum*; D: *S. villosum.;* E-H: Equatorial view; E: *S. coagulans*; F: *S. glabratum*; G: *S. incanum*; H: *S. villosum.*

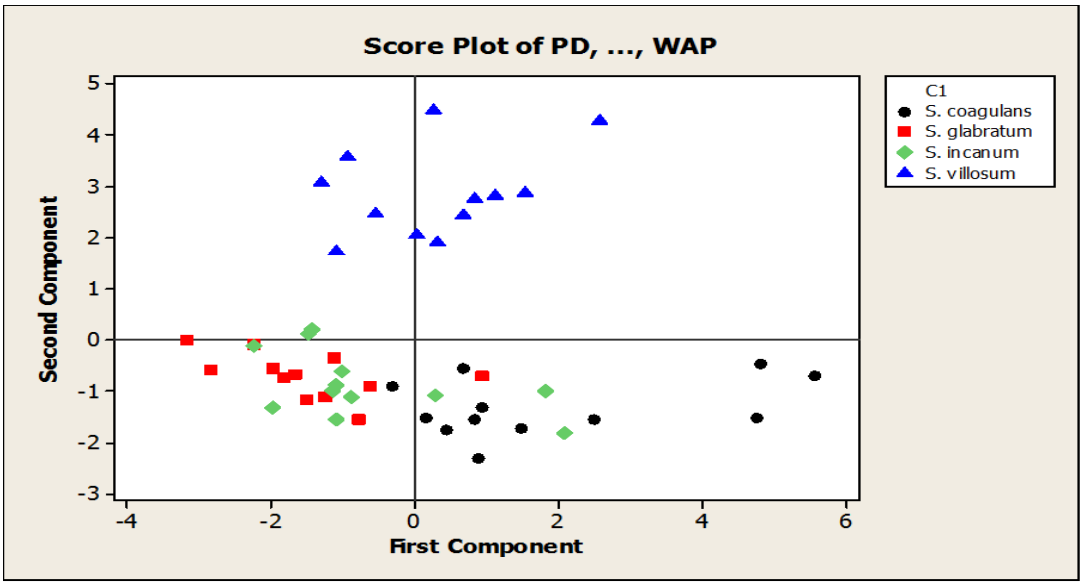

Fig. 3. Principle component analysis illustrates the relationship among the four recoded *Solanum* taxa in Sana'a University new compose based on 11 quantitative morphological features.

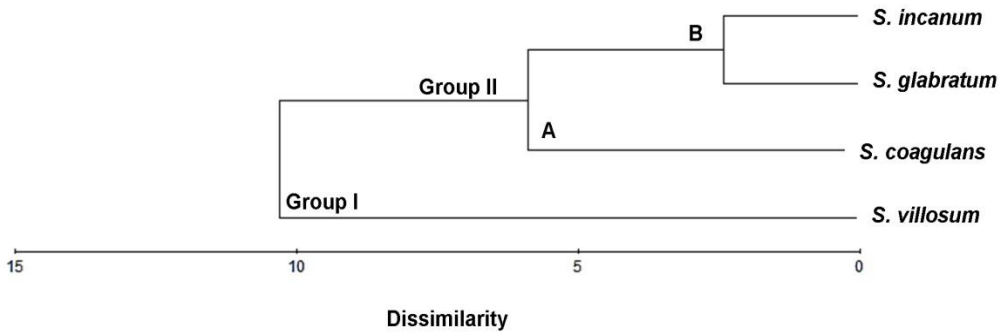

Fig. 4. Cluster analysis illustrates the relationship among the four recoded *Solanum* taxa in Sana'a University new compose based on 19 Pollen grain morphological features by using the UPGMA method.

DISCUSSION

This work investigates the comparative morphological structures of the pollen grains of four *Solanum* species grown at the new campus of Sana'a University. Palynological data of the investigated *Solanum* taxa indicated that all pollen grains are Isopolar, Radially symmetric, Trizoncolporates and this agrees with other researchers (El-Ghazali 1993; Al-Wadei and Lashin, 2007; Lashin, 2011; Mahdy *et al.*, 2019).

Our Morphological results showed an overlapping in pollen grain size, this confirmed with the findings of early studies (Erdtman, 1986; Al-Wadei and Lashin, 2007) where they mentioned that the Solanaceae family is a eurypalynous family and the variation in its pollen size might be due to indiscriminate mating leading to hybridization, which may be operating in this genus. Moreover; the mean length of the polar axis of *S. coagulans* and *S. incanum* pollen grains and the mean length of the equatorial axis of *S. incanum* pollen grains are closely related to what Mahdy et al. (2019) cited. Our results showed that *S. villosum* has the smallest pollen grains in size followed by *S. glabratum* and *S. incanum* while *S. coagulens* has the largest pollen grains, this consistent with the findings of Mahdy et al. (2019). Our findings indications that the shape of pollen grains ranging from oblate spheroidal to prolate spheroidal (88-114) and this agrees with the findings of Erdtman (1986) where he cited that the shape of pollen grains of Solanaceae taxa ranged between oblate to prolate (50 – 200). However; the differences in pollen morphology among the studied species agree with previous observations (Gbile and Sowunmi, 1979; Mahdy *et al.*, 2019) where they conducted separately a palynological study in *Solanum* spp. and they found highly significant differences in pollen grain size and shape within the studied groups. Furthermore; the mean aperture lengths of *S. coagulans*, *S. incanum,* and *S. villosum* pollen grains are closely related to the findings of Mahdy et al. (2019). On the other hand, the Static analysis; Un-weighted Pair Group Mathematical Average clustering (UPGMA), and Principle Component Analysis (PCA); show that the studied taxa were divided into two groups. The first group includes *S. villosum,* and the second group includes the other three taxa: *S. coagulans*, *S. glabratum,* and *S. incanum*. Based on the UPGMA the second group is divided into two clades at distance level 5.9, where the first clad includes S. *coagulans* and the second clade includes *S. glabratum* and *S. incanum* which were separated at distance level 2.4; this agrees with D'Arcy (1972) who classified the four studied taxa into two subgenera: Solanum which includes S. *villosum* (section solanum); while the three taxa; *S. coagulans*, *S. glabratum*, *S. incanum* where placed under the subgenus Leptostemonum. Moreover; the Statistical analysis; Un-weighted Pair Group Mathematical Average clustering (UPGMA) and Principle Component Analysis

(PCA) results confirm with Fawzi and Habeeb (2016) where they divided the wild species of the genus *Solanum* L. grown in Egypt into 2 groups based on their morphological characters, the first group comprises all members of Solanum subgenus grown in Egypt (including *S. villosum*), while the second group comprises all members of Leptostemonum subgenus (including *S. coagulans* and *S. incanum*). Furthermore, *S. coagulans*, *S. glabratum*, *S. incanum* were placed under three different sections: Monodolichopus; Oliganthes, and Melongena respectively (Jaeger, 1985, Bohs, 2005; Fawzi and Habeeb, 2016), and this agrees with the Un-weighted Pair Group Mathematical Average clustering (UPGMA) results.

CONCLUSION

The pollen size shows a high taxonomical significance in distinguishing between *S. coagulans* and the other three studied *Solanum* taxa; while Aperture (Colporus) size shows a highly significant value in distinguishing between *S. villosum* and the other three studied *Solanum* taxa.

Moreover, the statistical analysis exhibit the taxonomical relationship among the four studied taxa; The PCA separates the studied taxa into two groups and this confirmed the finding of UPGMA, where the studied taxa were divided into two different clads (subgenus) at distance level 10.3. The first clad (subgenus Solanum), includes *S. villosum* and the second clad (subgenus Leptostemonum) includes *S. coagulans, S. glabratum,* and *S. incanum*. Furthermore, the second clad (subgenus) was divided into two groups at distance level 5.9; the first group (section) includes one species; *S. coagulans* which belongs to section Monodolichopus, while the second group includes two species *S. glabratum* and *S. incanum* which were separated at distance level 2.4 where those two species belong to two different sections: Oliganthes and Melongena respectively.

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CONFLICT OF INTEREST

There is no conflict of interest.

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