

STUDIES ON JOJOBA [*Simmondsia chinensis* (LINK)
SCHNEIDER]

III- POLLEN VIABILITY AND POLLEN ULTRASTRUCTURE.

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ABSTRACT

Fresh pollen of jojoba attained the highest pollen viability at the beginning of the flowering season, where pollen fertility percentages for all the tested male parents exceeded 90%. Pollen stored at 4°C showed a clear significant decline in viability percentages following the long-term storage, i.e. the longer the storage period, the lower the viability obtained.

The morphological characteristics of pollen grains of four selected jojoba male parents were investigated with respect to the main characteristics of pollen grains. The polar axis length (P) ranged from 32.34 to 33.32 µm, the equatorial diameter width (E) ranged from 24.54 to 27.01µm, while the ratio P/E ranged from 1.22 to 1.34. Pollen characteristics of all parents in equatorial view is spherical, tricolpate. Exine sculpturing tends to be scarbate. Furrows (colpi) are long and wide.

INTRODUCTION

Jojoba [*Simmondsia chinensis* (Link) Schneider. Family Simmondsiaceae] is an evergreen shrub native to the triangle of the Sonoran Desert whose corners are roughly California, Arizona and Mexico (National Research Council, 1985).

Jojoba is a dioecious plant; male and female flowers occur on different plants. The pollen is borne easily by wind which is the only method of pollination. (Gentry, 1958 and Hogan, 1979).

Gentry (1958) reported that jojoba is highly variable among individuals and populations. Some of its variability are hereditary and others are environmental. Lack of pollination due to low pollen viability was recorded for many plantations in some years which appeared to be due to environmental and genetic factors. Stone *et al.*, (1995) reported that pollen viability is known to decline, sometimes rapidly, with pollen age and exposure to environmental stresses. Generally, low temperatures (4°C to 20°C) combined with low humidity are known to lengthen the storage life of the pollen. However, the optimum moisture requirements and the maximum longevity of pollen viability under low temperature conditions vary greatly, depending on plant species (Johri and Vasil, 1961). Lee *et al.*, (1985) found that when jojoba pollen was stored at room temperature (22°C - 25°C), the initial pollen viability was decreased to 50% in three weeks and to zero% after ten weeks. Pollen stored at 4°C maintained its initial viability for ten weeks,

zero% after 22 weeks. Pollen stored at -196°C in liquid nitrogen for 2 years^{*} retained a germination percentage as high as that of fresh pollen. Hanna (1990) mentioned that pollen storage is a valuable tool in a plant breeding programme because it makes recurrent and breeding lines available on an as-needed basis regardless of flowering response and planting date. Rapid exchange and use of germplasm between scientists nationally and internationally is facilitated by pollen storage. The overall advantage of pollen storage is that it makes germplasm immediately available for crossing onto female parents, without having to continually plant recurrent parents, hoping they will flower at the same time as the female parents.

Ahmedullah (1983) reported that there are five morphological features important to the description of pollen: 1) shape; 2) furrow (colpa); 3) sculpturing of the exine; 4) size; and 5) P/E ratio (the ratio between pollen length and width). Gentry (1958) reported that *Simmondsia* is a monotypic genus with distinctive characters. The pollen is borne easily by the wind and fruiting pistillate plants have been observed a half mile or more from staminate bushes, indicating that pollen is borne such distances. No insects have been observed on the female flowers, which are "colourless" and without nectaries or scent glands. Kadish (1985) reported that jojoba genus; *Simmondsia* has no obvious relatives or ancestral taxa which might help in producing offspring segregations including pollen size and pollen morphology. Whitehead (1969) reported that for efficient wind pollination, pollen grains must be small. The majority of wind-pollinated species possess grains 20 to 40 nanometers in size. From these theoretical considerations, one can make certain assumptions concerning the kind of stigmatic adaptation necessary to capture grains efficiently. It is necessary to increase the area of the receptive surface of the stigma significantly to insure effective capture.

MATERIALS AND METHODS

This experiment was carried out during two successive seasons of 1998 and 1999, at the Research Stations of the Desert Development Center, belonging to the American University in Cairo. In order to study the effect of seasonal and genetical variations on pollen viability of jojoba, pollen grains were collected from ten branches of each of the pollen parents (grown at Sadat Research Station) :SM1, SM2, SM3, SM4 and SM5. The pollen from each parent was scraped off, thoroughly mixed and equally divided into three small glass bottles (three replications) to be freshly tested. The procedure was repeated five times during the flowering season : February 15, March 1, March 15, April 1 and April 15 for the first season of 1998 and February 18, March 4, March 18, April 1 and April 15 for the second season of 1999. This experiment was carried out in the form of Randomized Complete Block Design containing three replications (Snedecor and Cochran, 1990). Pollen viability was investigated in the current study using the acetocarmine technique as described by Darlington and Lacour (1962). Ten microscopic fields were examined for each experimental unit. The turgid and darkly-stained pollen grains were considered fertile, while the shrunken and colourless grains were considered sterile (Baksh, 1979).

In order to study the effect of storage conditions and duration on pollen viability, the pollen of each clone was collected in the same way described earlier and was divided into 18 glass bottles (6 storage periods \times 3 replications) for both the first season (1998) and the second one (1999). Ten microscopic fields were examined for each experimental unit. The experiment was also carried out in the form of a Randomized Complete Block Design. Glass bottles containing pollen grains were either tested as fresh pollen (control) or after storage, at 4°C for different periods; 2,4,6,8 and 10 weeks.

As for the study of the morphological characteristics of pollen grains, representative flowering branches of four selected jojoba males grown at Sadat Research Station (SM1 and SM3) and South Tahrir (TM4 and TM22) were collected during the active flowering season of 2000. For the scanning electron microscopy (SEM), a small quantity of pollen was placed on a metallic stub with double-sticking tape. Specimens were sputter coated with gold using a Joel JFC-1100E ion sputtering device and studied at 15kv beam voltage using a Joel JSM 5300 scanning microscope. SEM studies were carried out at the EM-Unit, Faculty of Science, Alexandria University. At least three specimens of each male parent were photographed at 350X and individual pollen grains at 2000X. The polar axis length (P) and equatorial diameter width (E) of representative pollen grains were accurately measured from photographs, and the ratios P/E were determined for each clone after Ahmedullah (1983) and Kosenko (1991). This experiment was also carried out in the form of Randomized Complete Block Design containing three replications (three photographs).

RESULTS AND DISCUSSION

1- Effect of environmental and genetically variations on pollen viability

Pollen viability percentages of some selected jojoba clones were taken throughout the flowering period of 1998 and 1999. Generally, it is obvious that fresh pollen of jojoba attained the highest pollen viability at the beginning of the flowering season, where pollen fertility percentages for all the tested male parents exceeded 90% (Tables 1 and 2 and Figures 1 and 2). High values were obtained during February / March period except for the fifth clone; SM5 whose pollen viability began to deteriorate earlier. Reduction of pollen viability started to occur with the beginning of April in both seasons. Pollen grains lost more than 50% of their viability at the middle of April, i.e. after about two months of flowering. Some differences between the 5 pollen parents as well as between the two seasons could also be detected (Figures 1 and 2). Deterioration rate of pollen viability differed greatly among shrubs. At the end of the flowering period, it was found that the male parent SM1 lost about 26% and 46% of its initial viability (in the first and second season, respectively) compared to SM2 which lost about 49% and 73%. The variability of pollen fertility appeared in the present study has been confirmed and recorded for many jojoba plantations in some years which has been attributed to environmental and genetic factors (Gentry, 1958). The role of environmental conditions; or seasonal variability was as illustrated in Figure

3. However, is considered to be another main factor affecting pollen viability. Pollen viability is known to decline, sometimes rapidly, with pollen age and exposure to environmental stresses (Stone *et al.*, 1995). Changes in solar radiation, temperature, relative humidity, and rainfall might be significantly associated with the seasonal variation of pollen viability (Ortiz *et al.*, 1998).

Table 1: Pollen viability percentages of some jojoba clones grown at Sadat station taken throughout the flowering period of the first season; 1998.

Clones	Feb. 15	March 1	March 15	April 1	April 15
SM1	93.17 b	92.09	94.05 a	90.04 a	69.03 a
SM2	93.40 b	92.22	95.28 a	82.53 bc	47.49 b
SM3	99.03 a	94.07	94.60 a	89.82 a	43.79 b
SM4	97.11 a	95.05	95.43 a	87.15 ab	44.93 b
SM5	97.37 a	93.76	89.28 b	78.19 c	40.79 b
L.S.D.0.05	3.23	N.S.	2.92	4.88	7.05

• Values followed by the same letters are not significantly different at 0.05 level of probability.

Table 2: Pollen viability percentages of some jojoba clones grown at Sadat station taken throughout the flowering period of the second season; 1999.

Clones	Feb. 18	March 4	March 18	April 1	April 15
SM1	91.72	95.78 ab	93.12	87.55	49.25 a
SM2	92.56	95.17 ab	98.16	61.24	24.82 c
SM3	97.44	92.57 b	95.69	87.65	48.97 a
SM4	95.14	97.52 a	95.00	79.27	46.43 a
SM5	97.48	90.21 c	83.99	74.67	35.98 b
L.S.D.0.05	N.S.	4.57	N.S.	N.S.	2.91

• Values followed by the same letters are not significantly different at 0.05 level of probability.

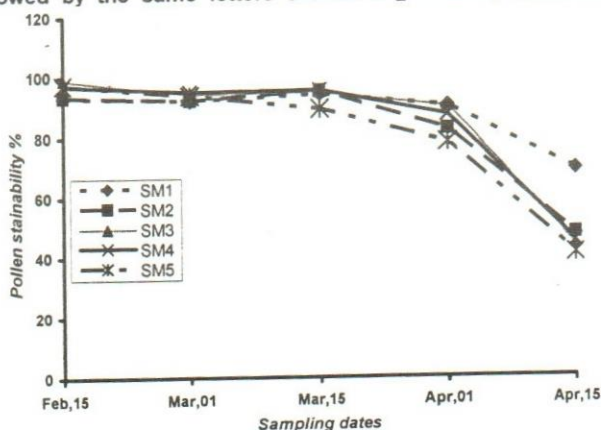


Figure 1: Pollen viability percentages of some jojoba clones grown at Sadat station taken throughout the flowering period of the first season 1998.

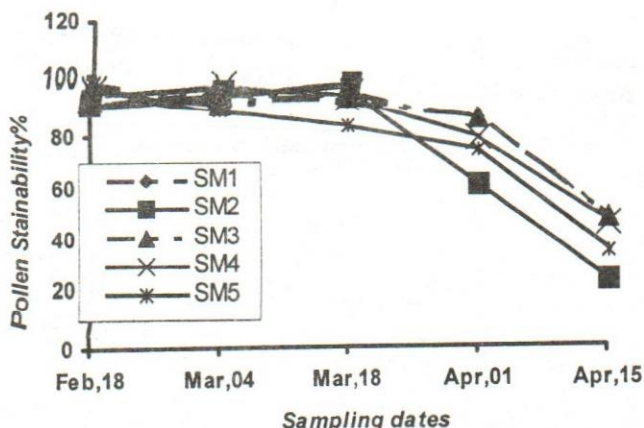


Figure 2: Pollen viability percentages of some jojoba clones grown at Sadat station taken throughout the flowering period of the first season 1999.

2- Effect of storage conditions and duration on pollen viability

Data presented in Tables 3 and 4 and illustrated in Figures 4 and 5 revealed that pollen viability of all the studied shrubs greatly responded to the storage period. It was found that pollen stored at 4°C showed a clear significant decline in viability percentages following the long-term storage, i.e. the longer the storage period, the lower the viability obtained. Most of the studied shrubs maintained their pollen viability until the fourth week of cold-storage without being greatly affected. Three clones, however, maintained some viability after six weeks of storage; SM2 (61.98%), SM3 (61.60%), and SM4 (62.52%), in the first season, others lost their viability directly after six weeks. At the end of the storage period (after ten weeks), SM2 proved to be the only pollen parent which still capable to produce some viable pollen in both seasons (18.59% and 13.29%, in the first and second seasons respectively).

Table 3: Pollen viability percentages of some jojoba genotypes grown at Sadat station as affected by cold-storage duration in the first season; 1998.

Clones	Storage period (weeks)					
	Zero	2	4	6	8	10
SM1	93.17 b	92.74 a	85.19	41.83 b	0.70 c	0.70 b
SM2	93.40 b	85.38 b	80.95	61.98 a	37.72 a	18.59 a
SM3	99.03 a	95.05 a	86.03	61.60 a	13.69 b	0.70 b
SM4	97.11 a	93.70 a	81.28	62.52 a	6.54 b	0.70 b
SM5	97.37 a	85.17 b	79.09	35.40 b	0.70 c	0.70 b
L.S.D.0.05	3.23	3.61	N.S.	7.73	12.35	0.77

Table 4: Pollen viability percentages of some jojoba genotypes grown at Sadat station as affected by cold-storage duration in the second season; 1999.

Clones	Storage period (weeks)					
	Zero	2	4	6	8	10
SM1	91.72	95.73	84.02	0.70	0.70 b	0.70 b
SM2	92.56	84.92	92.16	10.48	23.81 a	13.29 a
SM3	97.44	93.75	93.25	14.11	0.70 b	0.70 b
SM4	95.14	88.40	94.33	8.80	0.70 b	0.70 b
SM5	97.48	85.40	93.29	0.70	0.70 b	0.70 b
L.S.D.0.05	N.S.	N.S.	N.S.	N.S.	12.82	2.16

• Values followed by the same letters are not significantly different at 0.05 level of probability.

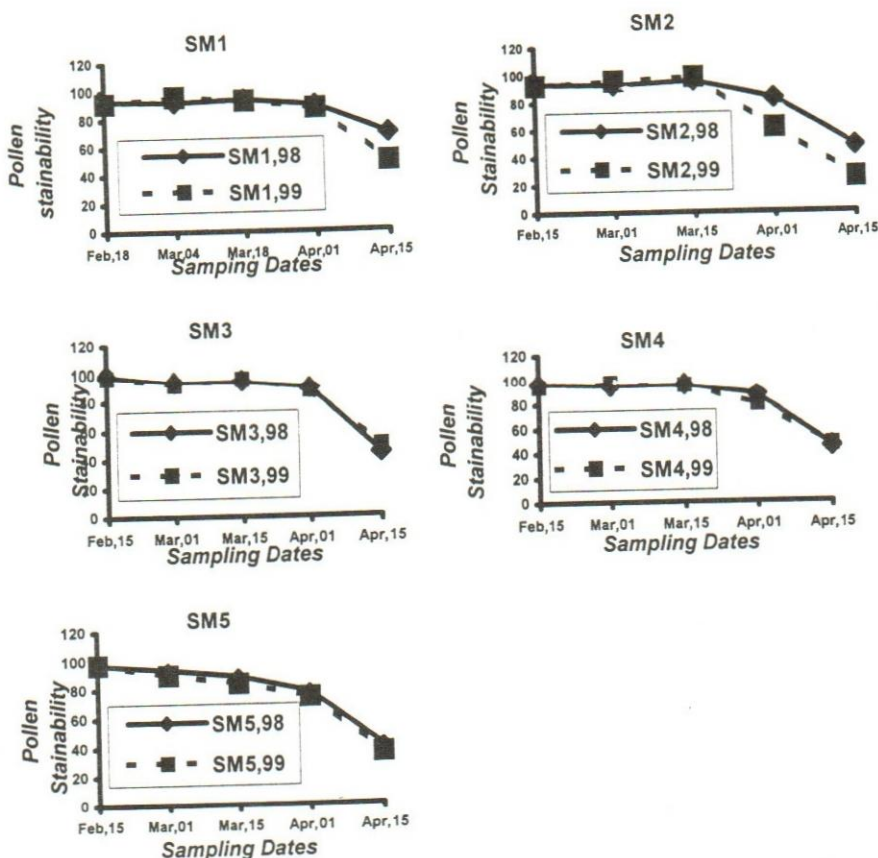


Figure 3: Seasonal variability of pollen viability percentages of some jojoba clones grown at Sadat station taken throughout the flowering period of the two seasons; 1998, 1999.

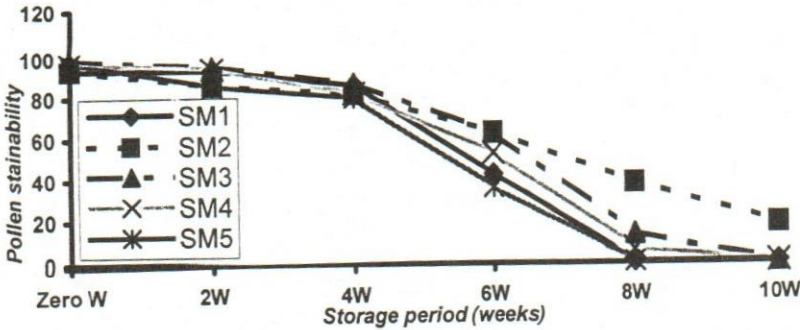


Figure 4: Pollen viability percentages of some jobba clones grown at Sadat station as affected by cold-storage duration in the first season 1998.

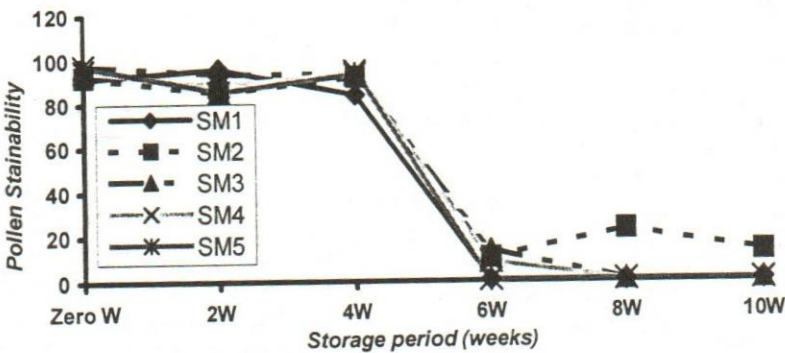


Figure 5: Pollen viability percentages of some jobba clones grown at Sadat station as affected by cold-storage duration in the first season 1999.

From the previously mentioned information, some facts could be obtained :

- 1- The response of the different clones used throughout this study to the cold-storage period significantly differed between shrubs referring to the possible effects of genotype and / or genotype × environment interaction as explained by Parfitt and Almehdi (1984), Devi and Deka (1992), Van der Walt and Littlejohn (1996) and Martinez-Gomez *et al.*, (2002).
- 2- The gradual decline of pollen viability related to longer periods of storage is considered a normal phenomenon in plant species. The percentage of decline appeared to depend on the pollen parent. The loss of pollen viability occurred during cold-storage has been described and interpreted for a wide range of plant species such as jobba (Lee *et al.*, 1985), pecan (Yates *et al.*, 1991), protea (Van der Walt and Littlejohn, 1996), banksia

(Maguire and Sedgley, 1997) and almond (Martinez-Gomez *et al.*, 2000 and 2002). As a rule, in most plants, pollen loses viability soon after dehiscence of the anthers, especially where temperature and humidity are unusually high. It is well known that stored pollen usually becomes dry and shriveled, and shows poor viability. The probable causes of the loss of pollen viability after long periods of cold storage may be desiccation, utilization of reserve food and inactivation of enzymes, causing failure of metabolic processes, which may be responsible for pollen germination (Linskens, 1964).

- 3- Lee *et al.* (1985) succeeded to keep the jojoba pollen completely viable (as fresh pollen) at 4°C for ten weeks followed by a gradual decrease to 70% after 17 weeks. Whereas, in the current investigation, it was not possible to keep pollen viability high for more than six or eight weeks although the same storage temperature was used. This is due to the low humidity storage conditions carried out during the experiment of Lee *et al.*, (1985), while in the current investigation, pollen humidity was comparatively high. Low temperature combined with low humidity are known to lengthen the storage life of pollen (Johri and Vasil, 1961).
- 4- According to the results obtained in the current investigation, pollen of SM2 could possibly be used for long term cold-storage. The pollen viability of this selected pollen parent, however, might be prolonged and enhanced if the cold storage of pollen is carried out after pollen partial desiccation.

3- Pollen ultrastructure

The morphological characteristics of pollen grains of four selected jojoba male clones grown at Sadat Research station (SM1, SM3) and South Tahrir (TM4, TM22) are presented in Table 5 with respect to the three main characteristics of pollen grains; polar axis length (P), equatorial diameter width (E) and the ratio P/E (Ahmedullah, 1983), it was found that no significant differences were statistically detected between the different clones. All of them behaved as a single variety sharing the same genetic background controlling pollen size and shape. This is may be due to that jojoba is a monotypic genus with distinctive characters (Gentry, 1958), which has no obvious relatives or ancestral taxa (Kadish, 1985) which might help producing offspring segregations including pollen size or morphology. The polar axis length ranged from 32.34 to 33.32 μm , the equatorial diameter width ranged from 24.54 to 27.01 μm , while the ratio P/E ranged from 1.22 to 1.34.

Pollen characteristics of all clones in equatorial view is spherical, tricolpate with rotational symmetry in which it is possible to identify the polar axis and equatorial width since the P/E ratio is more than one (1.22 to 1.34). Exine sculpturing tends to be scarbate. Furrows (colpi) are long and wide (Figures 6 to 8). The pollen of all four clones showed a great deal of similarity in surface morphology and exine sculpture. The study proved also that jojoba pollen grains are generally smooth and small and do not adhere to one another as a characteristic of most of the wind pollinated flowers (Raven *et al.*, 1981).

According to Whitehead (1969), the majority of wind-pollinated angiosperms possess grains are 20 to 40 μm long, while the general range

for pollen is 5 to 250 μm . The size of jojoba pollen grains (Table 4.3.11) ranged from 32.34 to 33.32 μm which lies in the range mentioned by Whitehead (1969). The disadvantage of small pollen grains in wind pollination is that as pollen size decreased, the stigma becomes increasingly less efficient in catching pollen as found in Phoenix and described by Tisserat and DeMason (1982). Unlike phoenix, the stigmatic surface of jojoba is such bigger since jojoba pistils have a large stigma with three long arms (Figure 9) covered with dense hairs (Figure 10) as a kind of modification to help trapping much more pollen efficiently.

Table 5: Morphological characteristics of pollen grains of four jojoba male clones grown at Sadat station (SM1, SM3) and South Tahrir (TM4, TM22).

Pollen clones	Pollen size (μm)		P/E ratio
	Polar axis length (P)	Equatorial diameter width (E)	
SM1	33.32	25.44	1.32
SM3	32.34	24.54	1.33
TM4	32.58	27.01	1.22
TM22	33.10	24.98	1.34
L.S.D. _{0.05}	N.S.	N.S.	N.S.

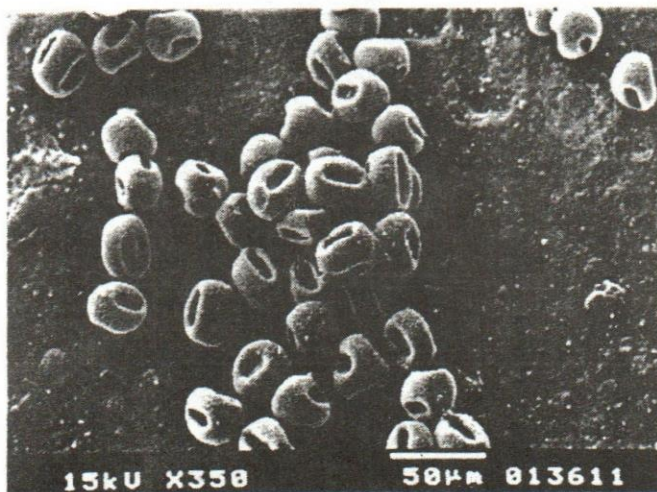


Figure 6: Scanning electron micrograph (SEM) showing the general shape of jojoba pollen.



Figure 7: SEM polar view of jojoba pollen showing the scarbate sculpturing of the exine. The long and wide colpi also appears.

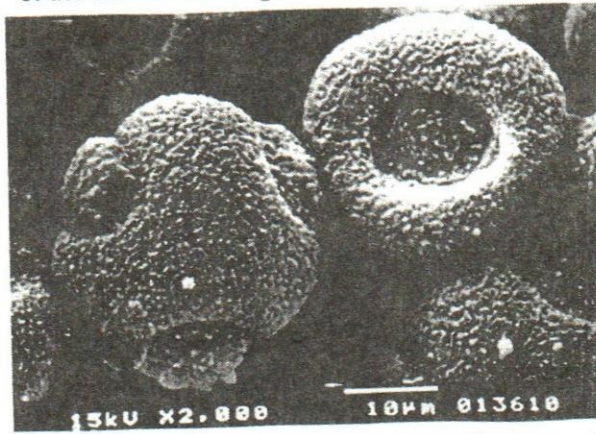


Figure 8 : SEM equatorial view of jojoba pollen showing the three colpi and the early emergence of three pollen tubes (left).

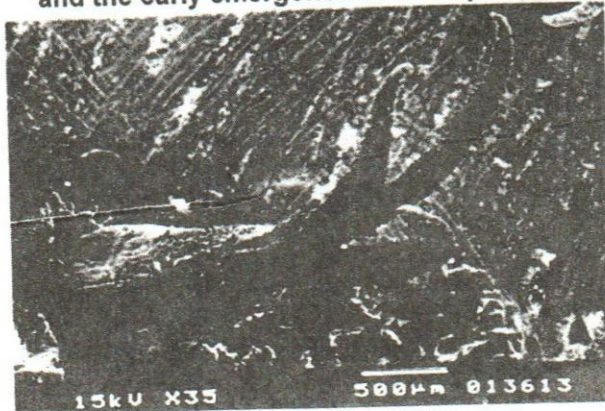


Figure 9 : Scanning electron micrograph showing the general shape of jojoba pistil. The three long stigmas appear clearly.

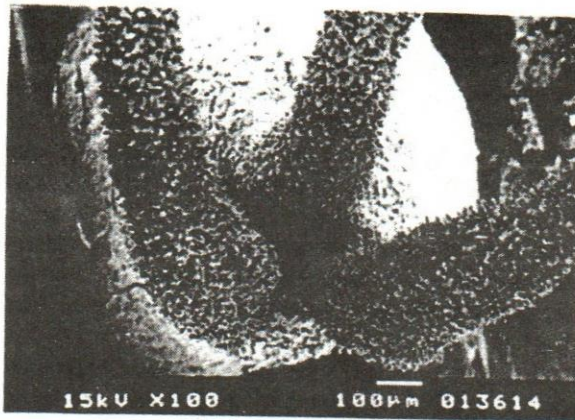


Figure 10 : SEM equatorial view of jojoba pistil showing the dense hairs covering all stigmatic surfaces.

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دراسات على نبات الجوجوبا ٣ - حيوية حبوب اللقاح وتركيبها الدقيق.
محمد جمال التركي^١ وعبد الفتاح حامد شاهين^٢ وعلا عبد العزيز الشناوي^١ وإيمان
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تم إجراء هذه التجارب خلال موسمي ١٩٩٨ و ١٩٩٩ بمحطات بحث مركز تنمية الصحراء، التابع للجامعة الأمريكية بالقاهرة.

حققت حبوب اللقاح الطازجة أعلى نسبة حيوية عند بداية موسم التزهير من منتصف شهر فبراير حيث تعدت تلك القيمة ٩٠% لجميع الآباء المدروسة. هذا وقد تم الحصول أيضا على نسب حيوية مرتفعة لحبوب اللقاح خلال فبراير/مارس ماعدا السلالة SM5 والتي بدأ تدهور حيوية حبوب لقاحها مبكرا عن الباقيين. وبدأ الانخفاض في حيوية حبوب اللقاح مع بداية أبريل في كلا الموسمين حيث فقدت حبوب اللقاح أكثر من ٥٠% من حيويتها في منتصف أبريل. وبالإضافة إلى ذلك، فقد تأثرت حيوية حبوب اللقاح لجميع السلالات المدروسة بفترة التخزين حيث وجد أن حبوب اللقاح التي تم تخزينها على درجة ٤°م أظهرت انخفاضا واضحا في حيويتها عقب التخزين لفترة ٦-١٠ اسبوعا، حيث وجد أنه كلما طالت فترة التخزين كلما انخفضت حيوية حبوب اللقاح. ووجد عموما أن اختلافات استجابة الآباء للتخزين المبرد ترجع إلى التأثيرات المحتملة للتركيب الوراثي لها أو للتفاعل بين التركيب الوراثي والظروف البيئية معا.

تم دراسة التركيب المورفولوجي الدقيق لحبوب اللقاح لأربعة سلالات هوهوبا منتخبة سواء من محطة بحوث السادات (SM1 و SM3) أو محطة بحوث التحرير (TM4 و TM22) وذلك بالنسبة للثلاث صفات الرئيسية لحبوب اللقاح وهي : الطول والعرض والنسبة بينهما. تراوح طول حبة اللقاح من ٣٢,٣٤ إلى ٣٣,٣٢ ميكرون، والعرض من ٢٤,٥٤ إلى ٢٧,٠١ ميكرون في حين تراوحت النسبة بين الطول والعرض من ١,٢٢ إلى ١,٣٤. وثبتت بدراسات الميكروسكوب الإلكتروني أن حبوب اللقاح في الهوهوبا تبدو في المقطع العرضي كروية الشكل ذات ثلاث بروزات موزعة بانتظام ويوضح منها أنه من الممكن التعرف على أي من الطول أو العرض بسهولة حيث أن نسبة الطول إلى العرض دائما أكبر من الوحدة (١,٢٢ إلى ١,٣٤). هذا وقد تم التعرف على طراز الشكل الخارجي لحبة اللقاح وأنه من النوع scarbate وأن الحبة مقسمة طوليا بثلاث شقوق.