

EFFECT OF GAMMA RADIATION ON THE MORPHOLOGY AND THERMOPHYSICAL PROPERTIES OF THE POLLEN WALL OF *PHOENIX DACTYLIFERA* L.

By

GAMAL EL-GHAZALY and M.E. KASSEM

Department of Botany and Department of Physics,
Faculty of Science, University of Qatar, Doha, Qatar

تأثير إشعاعات جاما على الشكل الخارجي والخواص الحرارية الفيزيائية لجدار حبوب لقاح نبات النخيل

جمال عبد المجيد الغزالي و محمد السيد قاسم

تمت دراسة تأثير جرعات مختلفة من أشعة جاما (٢٥٠ و ٥٠٠ و ١٠٠٠ راد و ١ ميجاراد) على جدار حبوب اللقاح لنبات النخيل بغرض معرفة التغيرات في الشكل الخارجي لجدار حبوب اللقاح وذلك باستخدام المجهر الالكتروني الماسح والتحليل الحراري التفاضلي. وعند استخدام جزء من هذه الحبوب المشعة وغير المشعة في التلقيح اليدوي لازهار النخيل المؤنثة، أظهرت الدراسات أنه عند معالجة حبوب اللقاح بجرعات جاما ٢٥٠، ٥٠٠ راد لم تحدث تغييرات واضحة في الشكل الخارجي للجدار على الرغم من وجود اختلافات في نتائج التحليل الحراري. وعند إجراء تلقيح باستخدام حبوب اللقاح المعالجة بهذه الجرعات لوحظ زيادة في حجم الثمار الناتجة بالمقارنة بالنباتات الملقحة بحبوب لقاح غير معالجة وكذا المعالجة بجرعات ١٠٠٠ راد و ١ ميجاراد. كما أنه شوهد تغير في الشكل الخارجي للعينات المعرضة لجرعة ١٠٠٠ راد مع زيادة في المقاومة الحرارية لجدار حبوب اللقاح وعند تعريض حبوب اللقاح لجرعة ١ ميجاراد لوحظ تغير كلي في الشكل الخارجي للجدار.

وقد أكدت الدراسات الفيزيائية الحرارية لجدار حبوب اللقاح المشعة بإشعاعات جاما أن طبقات جدار حبة اللقاح غير متجانسة.

Key words: γ -Radiation, Pollen morphology, Phoenix, Thermophysical

ABSTRACT

Pollen grains of *Phoenix dactylifera* were exposed to different doses of gamma radiation (250,500,1000 rad and 1 M rad). To access the effect of radiation on the external morphology of the pollen grains, they were examined with SEM after each treatment. In addition the differential thermal analysis technique was used to elucidate the stability of the chemical composition of the pollen wall to gamma radiation. A portion of the treated pollen was used to pollinate different inflorescences of two female palm trees. The pollen grains that were exposed to γ -radiation doses of 250,500 rad were morphologically similar, but showed differences in thermal behaviour. Pollination with these pollen grains resulted in setting fruits that were large in size compared with those developed after pollination with untreated pollen or pollen grains radiated with 1000 rad or 1 M rad. The treatment with 1000 rad induced in the morphology of the pollen, and increasing of the thermal resistance of the pollen wall. The 1 M rad dose caused obvious damage and collapse of the pollen wall. Thermophysical properties of various doses treated palm pollen indicate that materials of different layers of pollen wall are not homogenous.

INTRODUCTION

A number of studies have been carried out to clarify the influence of radiation on pollen grains [for example 1-4]. These studies were focused mainly on the effect of radiation on pollen tube formation and fertilization and on enzyme activities in pollen and pollen tubes. The effect of radiation on seeds, seedlings and whole plants has, also, been investigated extensively [5-8]. However the effect of radiation on the morphology of pollen grains and the structure of the pollen wall has been the subject of relatively few studies [for example 9].

The outer pollen wall, the exine, consists mainly of sporopollenin, a very resistant material. The structure and biosynthesis of this polymer are still largely unknown. Ionization radiation is known to alter the internal structure of polymers and, hence, leads to a wide span of interrelated changes in their properties. It may result in crosslinking of the molecular chains [10-11], destruction and degradation of the macromolecules with the simultaneous formation of molecules with smaller chain lengths [11,12] and, possibly a change in the number and nature of double bond [13,14]. The object of this study was to demonstrate the effect of γ -radiation on the morphology of the pollen grain and on the chemical structure of the pollen wall. Another aim was to test the effect of irradiated pollen on fruit setting. The plant selected for this study was *Phoenix dactylifera*, a widely cultivated and an important economic plant in the Gulf area and other parts of the world.

MATERIAL AND METHODS

The palm trees used in this study are growing in the University of Qatar in a population of about 50 staminate and pistillate trees. All trees are ca. 5 years old. The stem is unidivided and ca. 1.5 meter long. The leaves are large, pinnate; leaflets folded upwards. The staminate trees are with small white flowers, each include 6 stamens and arranged on a richly branched spadix, surrounded by a solitary large spathe. The pistillate trees are with female flowers that contain 3 ovaries, but only one developing into fruit. Pollen grains were collected and divided into two parts. A part was used, directly after collection, in irradiation with gamma ray, while the other part was kept as control. Immediately after treatment with gamma ray irradiation, both untreated and treated pollen were hand transferred to the pistillate trees.

A- Radiation Facilities:

Samples were irradiated with γ rays at room temperature using a gamma cell 220 manufactured by Atomic Energy of Canada Ltd. The operating dose rate was 1.5 M rad/h and a set of samples spanning an integrated dose from 250 to 1000 rad and 1 M rad was prepared. The treated samples were used for thermal measurements, and scanning electron microscopy study.

B- Two female palm trees were used for the preliminary pollination test. Female inflorescences were isolated by plastic pages just before maturity. At maturity they were pollinated by pollen grains of different irradiation doses and by non

treated pollen for control. For each dose one inflorescence from each tree was used.

C- Thermal analysis technique:

The specific heat under constant pressure, C_p , was determined by using Shimadzu TA 30 thermal analyser. The instrument was calibrated with a pure indium standard. Measurements were performed by applying the base line method [15]. The heating rate was 2 K/min, and the samples masses ranged from 10 to 30 mg.

D- Scanning Electron Microscopy (SEM) preparation:

Pollen grains were dehydrated in an alcohol series, suspended in a drop of absolute alcohol and transferred to brass stubs. The pollen were then coated with gold-palladium for 5 minutes using a fine Coat ION Sputter JFC 1100. Scanning micrographs were taken with a Jeol JSM 25S-II.

RESULTS AND DISCUSSION

A typical example of temperature dependence of the specific heat for irradiated and nonirradiated pollen is shown in Fig. 1. It appears as a jump of $C_p(T)$ function at different temperatures, T_c . From the figure we observe that the value of specific heat, C_p^{\max} at transition decreases sharply with irradiation doses 250 and 500 rad. Beyond 500 rad the specific heat tends to increase only very slightly (Fig. 2). The initial decrease in C_p^{\max} for the lower radiation doses (<500 rad) was probably due to the formation of different types of radicals when irradiation carried out in air [16]. These radicals were probably associated with the surface of the pollen grains. Radical groups have been observed in irradiated spore coats of *Osmunda regalis* [17]. At relatively high doses, (>500 rad) the crosslinking is inhibited by oxygen which diffuses into the polymer where degradation takes place. Untreated pollen grains are prolate, heteropolar, equatorially biconvex, elliptic in equatorial view. They are monocolpate, colpus long with thick margin and rounded ends. Exine tectate, microreticulate, to perforate. Lumina are circular, rectangular or irregular in shape.

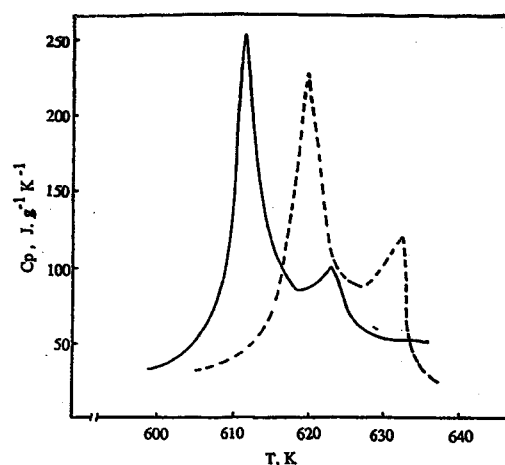


Fig. 1: Temperature dependence of specific heat of nonirradiated and 250 rad irradiated pollen

—— 0 rad ——— . ——— . ——— 250 rad.

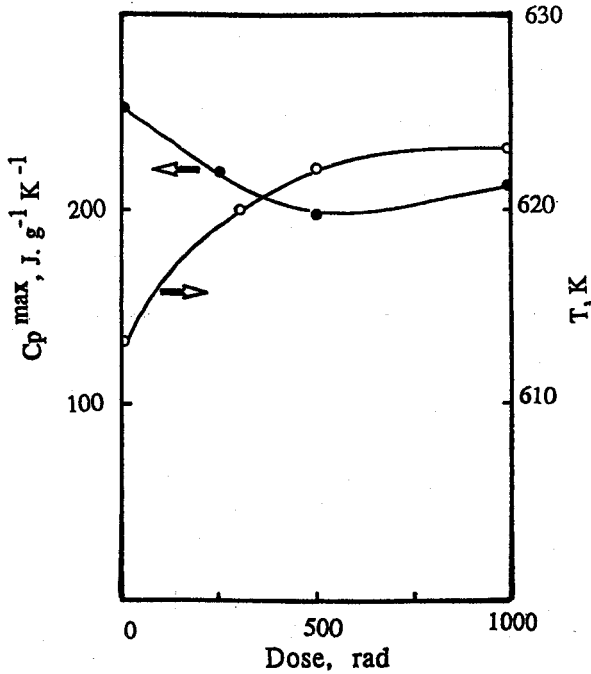


Fig. 2: γ -radiation dose dependence of C_p^{\max} and transition temperature T_c .

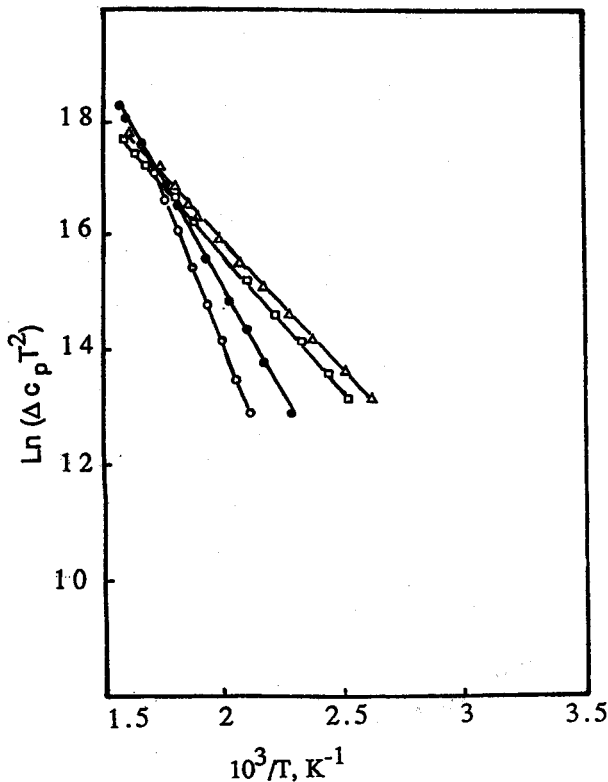


Fig. 3: Variation of $\ln D C_p T^2$ on $1/T$ for different γ -radiation doses 0 - O rad, • - 250 rad, Δ - 500 rad and \square - 1000 rad.

The pollen morphology after treatment with 250 rad γ -irradiation was characterised by a thin coat that obscured the details of exine reticulation (Figs. 7,8). In other areas of the pollen sur-

face the thin coat was removed (Fig. 7, arrow) revealing structural details. Treatment with 500 rad dose showed changes in reticulation patterns (cf. Figs. 5,6 and 9,10); Commonly tywo or more muri were fused forming irregular patterns (Figs. 9,10).

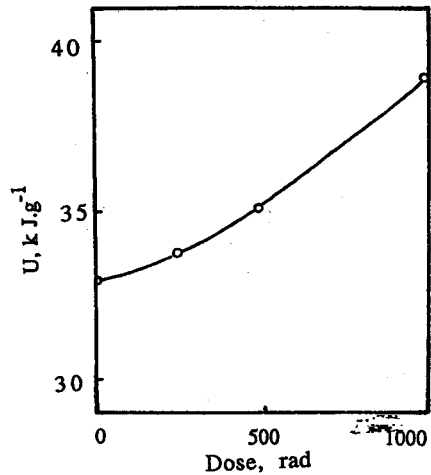


Fig. 4: The dependance of the ordering energy of transition in Pollen on γ -radiation doses.

A preliminary test of treated pollen grains on fruit setting and size indicated that pollen grains exposed to 250,500 rad doses resulted in setting of comparatively larger fruits than those formed by other treatments. This test needs to be repeated in large scale before linking any practical consideration of fruiting system of palm trees with pollination by γ -irradiated pollen grains.

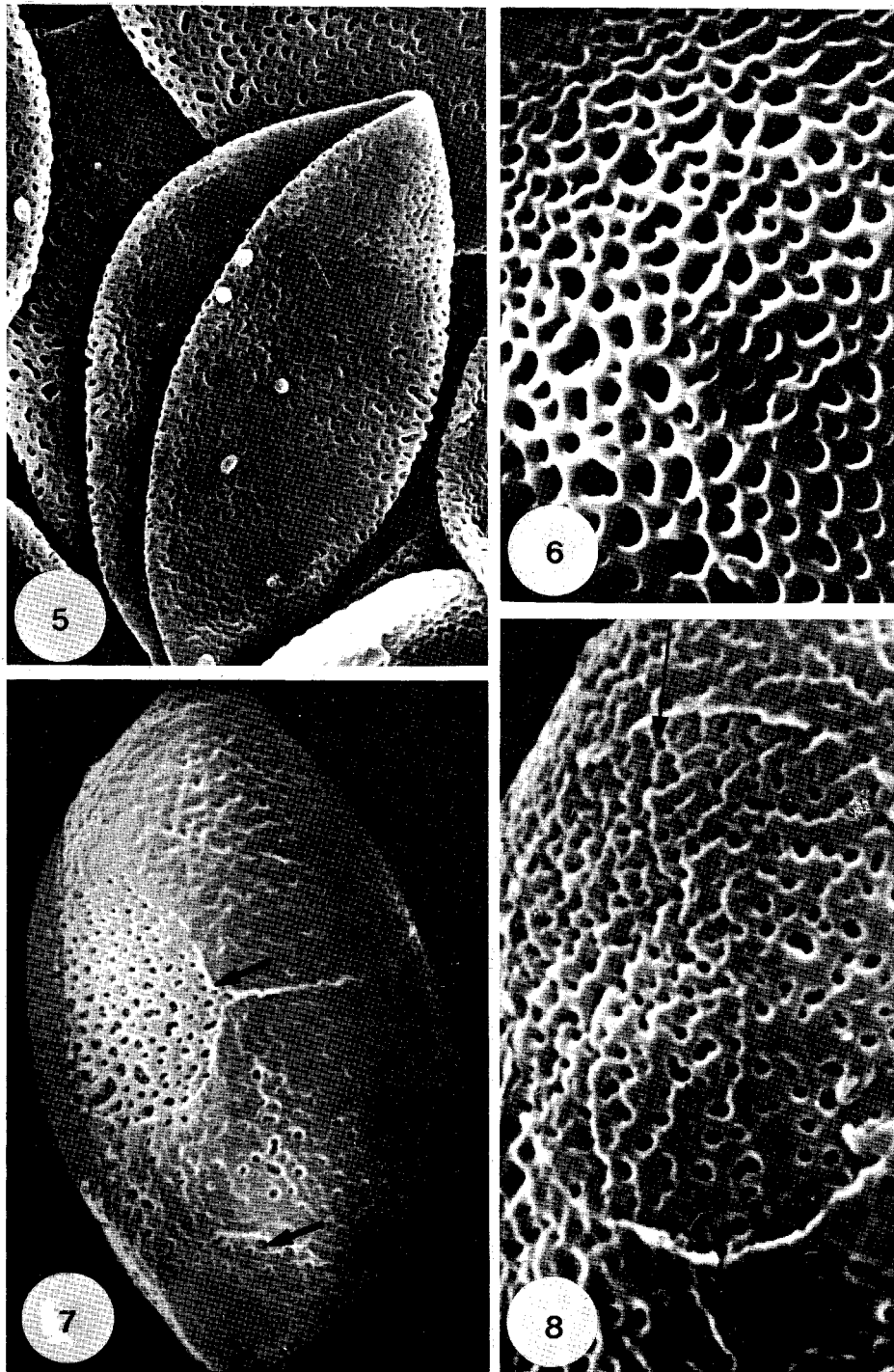
The value of fusion transition temperature (T_c) shifts upwards as the irradiation dose increases. In order to clarify the number of defects produced and the transition in the energy of ordering, we apply the following relation[18].

$$\Delta C_p = (ZNU^2/RT^2)e^{-U/RT}$$

where N is the number of atoms displaced from the equilibrium position, U is the activation energy of ordering, R is the universal gas constant and Z is the coordination number.

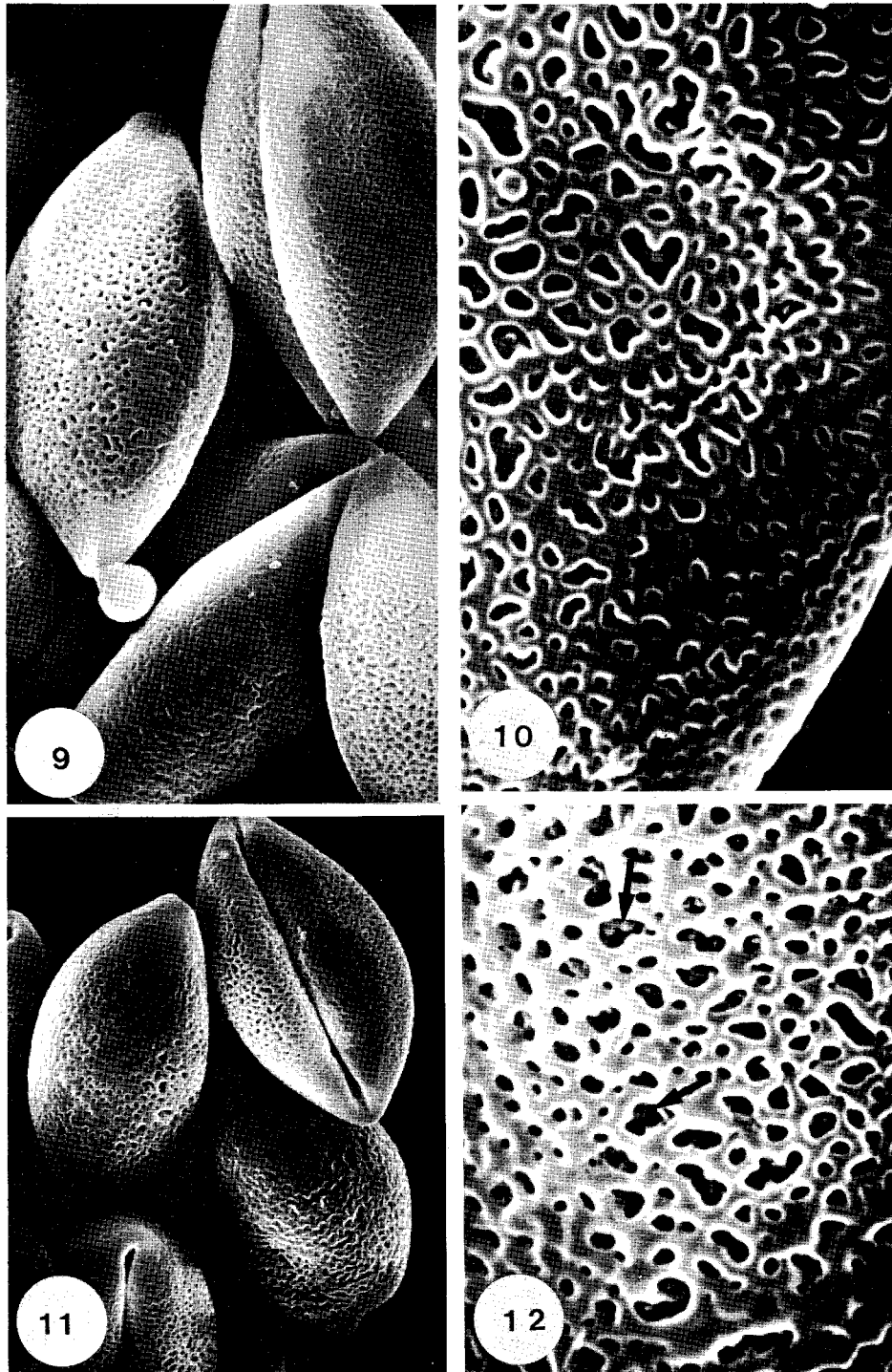
Figure 3 shows the dependance of $\ln \Delta C_p T^2$ on $1/T$ for irradiated and non irradiated samples. It is clear that the experimental point can be approximated by stragith line with exception of the temperature interval in the vicinity of the transition. The slopes of the straight lines give the activation energies which are plotted in Fig. 4. This figure shows that the ordering energy of transition (U) is increasing as the irradiation dose increase. This behaviour can be attributed to the fact that small irradiation dose causes degradation of the outer layer of the pollen. Upon increasing the dose to 1000 rad, the degradation effects the next layer of the pollen wassl and revealed subsurface structure, that have not been observed before (Figs. 11,12, arrows).

When pollen grains were subjected to high dose of irradiation (one mega rad), their overall shape appeared collapsed and the pattern of the exine appeared irregular. Pollination with these pollen grains resulted in setting of small fruits. It is generally known that high doses of gamma irradiation inhibites pollen germination and pollen tube growth[8,19].



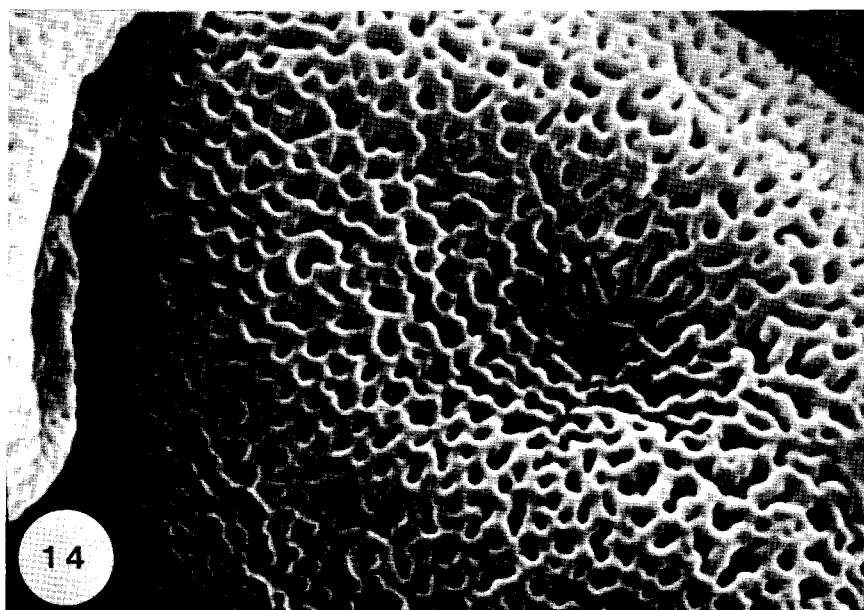
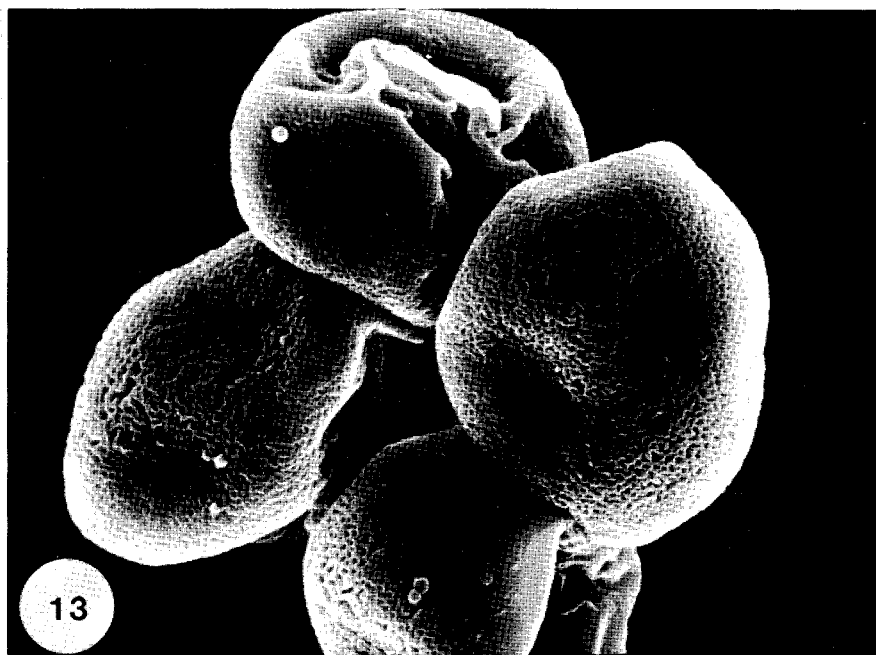
Figs. 5, 6: SEM-micrographs of untreated pollen (Fig. 5 X 4800). Fig. 6 showing details of sexine pattern (x 10000).

Figs. 7, 8: (x 4800, x 10000) SEM-micrographs of pollen grain irradiated with 250 rad. Note thin coat obscuring parts of pollen sexine, while other parts appear etched (arrows).



Figs. 9, 10: (x 3400, x 10000) SEM-micrographs of pollen irradiated with 500 rad. Note irregularities in the reticulate pattern of the sexine, cf. Figs. 5,6.

Figs.11, 12: (x 2200, x 10000) SEM-micrographs of pollen irradiated with 1000 rad. Note the appearance of granular layer (arrows) beneath the reticulate tectum.



Figs.13,14: (x 3200, 10000) SEM-micrographs of pollen irradiated with one mega rad. Note that the pollen grains are generally collapsed.

The effect of irradiated pollen on fruit setting and fruit size in *Phoenix dactylifera* could be of economic importance. More investigations should be carried to find out the most suitable irradiation dose and the size and quality of fruit setting after pollination with treated pollen.

The treatment of the exine with different doses of γ -radiation, followed by measurement of its thermophysical properties indicates the existence of substructural features. Similar results were obtained by treatment of the exine with a variety of chemical reagent [20-22].

The results obtained from measurements of thermophysical properties of various doses treated palm pollen may also indicate that material of different layers of pollen wall are not homogenous. Recently, the high resolution ^{13}C NMR spectra obtained from exine of pollen grains of different plants showed that these materials are distinct substances. Sporopollenin is therefore considered as a class of biopolymers rather than a single homogenous macromolecule^[23,24].

REFERENCE

- [1] Brewbaker, J.L. and G.C., Emery, 1962. Pollen radiobotany, *Radiat. Bot.* 1: 101-154.
- [2] Denissen, C.J.M. and A.P.M. Den Nijs, 1986. Effect of gamma irradiation on in vitro pollen germination of different *Cucumis* species, *Euphytica* 36: 651-658.
- [3] Georgieva, I.D., 1987. Cytochemical investigation of pollen and pollen tubes after gamma irradiation. II. Effect of the irradiation on Quinone formation. *Phytomorphology*, 37: 159-163.
- [4] Cuny, F. and A.C. Roudot, 1991. Germination et Croissance pollinique in vitro du pollen de melon (*Cucumis melo* L.) après irradiations gamma, *Environmental and Experimental Botany* 31: 277-283.
- [5] Vora, A.B., H.K. Shah and A.I. Vyas. 1974. Biochemical changes associated with gamma rays irradiation of *Phaseolus mungo* seeds in roots during juvenile differentiation. *Use Radiat. Radioisot. Stud. Plant. Prod. Bombay* 200-208.
- [6] Popa, D., M. Trifu, D. Cachit-Cosma and O. Henergarik, 1982. Modificera unor indici la plantulele de prurumb iridiate Cu rase gamma si tratate Cu pro caina. *Studia Univ. Babeş. Boyali. Biol.* 27: 18-23.
- [7] Nor Arevyan, N.G., S.P. Senerzyan and K.A. Vardanyan, 1982. On the role of quinanes in the mechanism of radiosensitivity of plants (in Russia). *Radiosensitivity of plants (in Russia)*, *Radiobiologia USSR* 22: 567-571.
- [8] Georgieva, I.D. and A.I. Atanassov, 1986. Cytochemical investigation of pollen and pollen tubes after γ -irradiation: effect of the γ -irradiation on some dehydrogenases and hydrolases, *Phytomorphology* 36: 337-346.
- [9] Chanda, S. and S. Sahay, 1970. Some effects of X-irradiation on the pollen of *Trigonella*, *Grana* 10: 136-140.
- [10] Ray, C.R. and J.E. Willard, 1972. *J. Phys. Chem.* 76: 1405.
- [11] Lim, S.L., A.G. Fane and C.J. Fell, 1990. *J. Appl. Polym. Sci.* 41: 661.
- [12] Shintani, N., H. Kikuchi and A. Nakamura, 1990. *J. Polym. Sci.* 41: 661.
- [13] Charlesby, A., 1960. *Atomic radiation and polymers*. Pergamon Press, London.
- [14] Dole, M., 1972. *The radiation chemistry of macromolecules*. Academic press New York.
- [15] Daniels, T., 1973. *Thermal analysis*. Kogan page limite, London pp 127.
- [16] Truerdale, R.D., H.A. Faroch and C.P. Poole, 1980. Hysteresis effects in X-irradiated KH_2PO_4 , KD_2PO_4 and RbH_2PO_4 Ferroelectric single crystals observed with electron spin resonance, *Phys. Rev. B* 22: 305.
- [17] Dodd, N.J.F. and M. Ebert, 1970. Effects of ionizing radiation on dried spores of *Osmunda regalis*. I. Electron spin resonance study of spores, *Int. J. Radiat. Biol.* 17: 1-11.
- [18] Kassem, M.E. and M. El-Muraikhi, 1991. The influence of γ -irradiation on the thermophysical properties of potassium sulphate crystals, *J. Thermal Anal.* 37: 55.
- [19] Zhang, Yx. and Y. Lespinasse, 1991. Pollination with gamma-irradiated pollen and development of fruits, seeds and parthenogenetic plants in apple, *Euphytica*. 54: 101-109.
- [20] Rowley, J.R. and B. Prijianto, 1977. Selective destruction of the exine of pollen grains, *Geophytology* 7: 1-23.
- [21] Rowley, J.R., A.O. Dahl and J.S. Rowley, 1981. Substructure in exines of *Artemisia vulgaris*, *Rev. Palaeobot. Palynol.* 35: 1-38.
- [22] Claugher, D., 1986. Pillen wall structure, a new interpretation, *Scanning Electron Microscopy* 1: 291-299.
- [23] Guilford, W. J., D. M. Schneider, J. Labovitz and S. J. Opella, 1988. High resolution solid state ^{13}C NMR spectroscopy of sporopollenins from different plant taxa, *Plant Physiol.* 86: 134-136.
- [24] Wehling, K., Ch. Niester, J. J. Boon, M. T. M. Willemse and R. Wiermann, 1989. p-Coumaric acid- a monomer in the sporopollenin skeleton, *Planta* 179: 376-380.