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GERMINATION, GROWTH RATES, AND ELECTRON MICROSCOPE ANALYSIS OF TOMATO SEEDS FLOWN ON THE LDEF

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Abstract—The purpose of the experiment was to determine cosmic rays long-term effects on living tissue. A batch of tomato seeds were flown in orbit aboard the Long Duration Exposure Facility (LDEF) for almost 6 y. During this time, the seeds received an abundant exposure to cosmic radiation. Upon the return of the LDEF to Earth, the seeds were distributed throughout the United States and 30 foreign countries for analysis. Our university analysis included germination and growth rates as well as scanning electron microscopy (SEM) and X-ray analysis of the control as well as space exposed tomato seeds.

In analyzing the seeds under the electron microscope, usual observations were performed on the nutritional and epidermis layer of the seed. These layers appeared to be more porous in the space exposed seeds than on Earth-based control seeds. This unusual characteristic may explain the increases in the space seeds growth pattern. (Several test results showed that the space-exposed seeds germinated sooner than Earth-based seeds. Also, the space-exposed seeds grew at a faster rate.) The porous nutritional region may allow the seeds to receive necessary nutrients and liquids more readily, thus enabling the plant to grow at a faster rate.

Roots, leaves and stems were cut into small sections and mounted. After sputter coating the specimens with argon/gold palladium plasma, they were viewed under the electron microscope. Many micrographs were taken. The X-ray analysis displayed possible identifications of calcium, potassium, chlorine, copper, aluminum, silicon, phosphate, carbon, and sometimes sulfur and iron. The highest concentrations were shown in potassium and calcium. As a result of the electron interaction and X-ray production within the open seeds, the traditional layers of the space-exposed seed gave peaks of Mg, P and S, while the Earth seed gave an iron peak, which was not detected in the space-exposed seed because of electron beam positioning difference. The space-exposed seed and the Earth-control seed specimens displayed high concentrations of copper. Copyright © 1996 Elsevier Science Ltd

1. INTRODUCTION

The long-term effect of cosmic environmental conditions on the normal growth and development of living plant tissue is a key component in understanding man's capabilities for space colonization. Rutgers California Supreme Tomato seeds (*Lycopersicon esculentum*, var. commune), obtained from NASA, were part of the LDEF (Long Duration Exposure Facility) satellite mission. The seeds were hermetically packaged at Park Seed Co., in Greenwood, South Carolina. A portion of the seeds remained at Park Seed Co., in a controlled climate of 21°C with 20% humidity. On 6 April 1984, the Space Shuttle Challenger placed an additional portion of seeds in orbit aboard the LDEF. During the LDEF's orbit, the seeds were kept in a scientifically controlled climate of 14 psi with 15% humidity.

The effective use of SEM, Digital Imaging Processing, and X-ray Microanalysis were primary techniques used in the understanding of internal and external structures as well as variation in tissue structure of the Rutgers tomato seeds, excluding water. Hamly (1932) believed that water exclusion was a property of the outer layers of the coat, that is, a suberized walls and caps of the Malpighian cells. More importantly, he showed that impermeability was lost when the highly stressed cells at the strophiole (lens) separated, thus forming a strophiolar cleft and permitting water entry.

The environmental conditions of space such as cosmic radiation, temperature, constant pressure and humidity are examples of abnormal conditions that cause additional stress on plant and animal tissue. This additional stress is thought to be responsible for creating strophiolar clefts in space-exposed tomato seeds that are larger in size than those created under Earth-based conditions. The two basic internal layers of the seed, which includes the embryonic layer (contains the ovary) and the endosperm (stores nutrients form growth), are visibly observed by digitally imaged micrographs taken with the SEM at very high magnification (see Figs 1 and 2). Moreover, note the relative thickness of the LDEF outer seed coat in comparison with the control seeds outer coat

Fig. 1. Outer seed coat for the control seed.

that is 75% thinner than the LDEF outer seed coat for the same position on the seed.

Potential risks to plant and humans in future space-based controlled ecological life support systems have not been addressed directly (Norman and Schuerger, 1990). The purpose of this study was to show structural changes, along with qualitative element identification and germination rate variations between Earth-based and space-exposed tomato plants. (Tables 1 and 2). Note the relative size of the strophiole clefts of the seed coat and the relative growth rate analysis for a 3 month period.

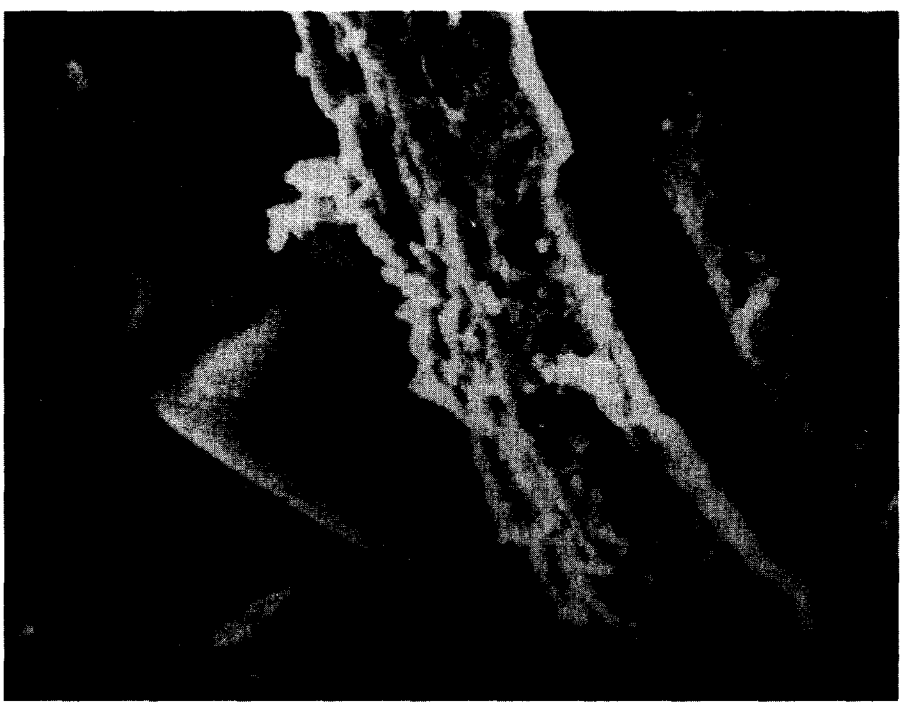

Fig. 2. Outer seed coat for the LDEF seed.

Table 1. Indicates the relative size of the strophiole clefts of the seed coat measured at a magnification of 11.1 KX. The heights of the controls show dramatic increases, while there is a modest increase for the strophiole widths

	Space		Earth	
	Height	Width	Height	Width
1.	12.4	4.4	7.1	3.2
2.	11.8	4.2	6.6	3.4
3.	11.4	5.2	6.2	4.1
4.	12.2	5.6	6.3	4.2
5.	13.1	5.1	5.7	4.2
6.	12.6	5.0	5.2	3.8
7.	12.4	4.7	6.1	3.4
8.	10.8	5.1	6.0	3.7
9.	11.9	4.5	5.3	3.1
10.	12.5	4.7	5.5	3.1
Mean value	12.1	4.85	6.0	3.61

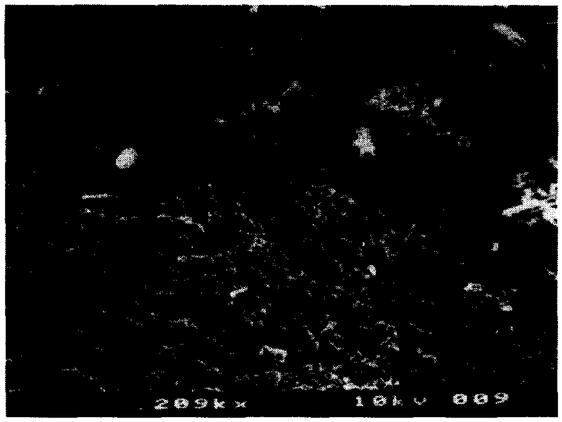

Fig. 4. Surface of LDEF exposed seed at 209X.

2. SURFACE COAT OF THE SEED

SEM studies have been conducted that demonstrate a unique difference between the LDEF seeds and the control seeds (see Figs 3 – 17). The control seeds have a wavelike surface with minimal surface pores. Moreover, both the LDEF and control seeds have cylindrical structures of some organic nature, but the LDEF-exposed seeds have cylindrical structures that are less symmetrical as shown in Figs 4 and 9. The most fascinating observation is that the LDEF-exposed seeds have a faster germination rate than the control seeds.

The seeds may have undergone some type of early germination while in the LDEF for the 69-month trip. The 3000X micrograph in Fig. 6 indicates the internal structure of the numerous pores observed for the LDEF seed. In Fig. 3 one can clearly see the pores

Table 2. A relative growth rate analysis over the 3 month germination period of the sample tomato plants. Note that the group growth rate for A, B, and C of the LDEF-exposed seeds have greater heights than Earth-based plant heights during the first month of growth. The initial measurements of the heights for the groups A and D show that group AÕs position had the highest rate of growth compared to group DÕs position. Subsequent height measurements indicate that Earth-based plant seeds in comparison with the LDEF seeds show minimal statistical variation in height after the third month

Date recorded	22 May	24 June	18 July
Earth based plants height (cm)	2.2	16.4	32.7
Spaced exposed plants height (cm)			
Group – A	4.41	21.0	30.5
Group – B	4.15	17.8	33.7
Group – C	3.71	18.5	32.8
Group – D	2.7	13.9	28.8

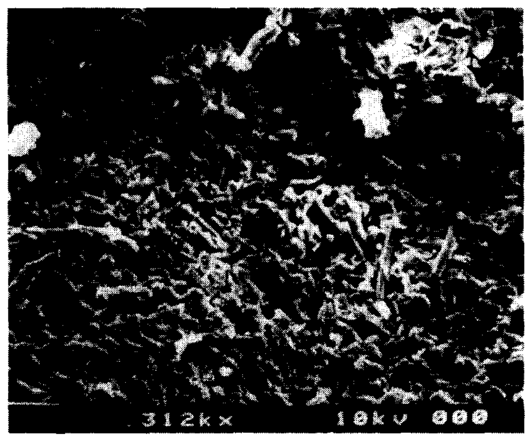

Fig. 5.Surface of LDEF exposed seed at 312X.

Fig. 3. Surface of LDEF exposed seed at 65X.

Fig. 6.Surface of LDEF exposed seed at 3150X.

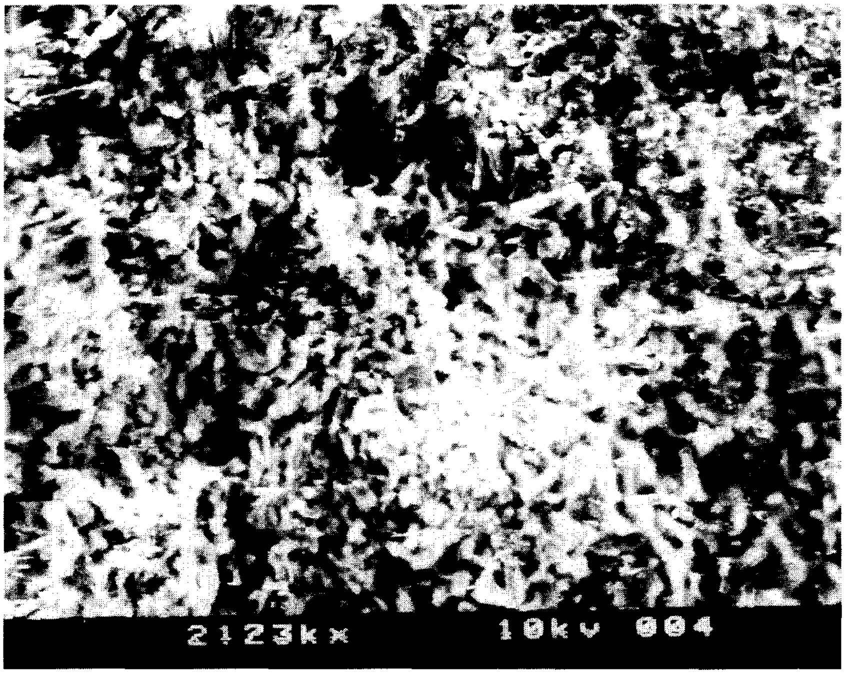

Fig. 7. Surface of control seed at 212X.

on the LDEF-exposed seeds. Figures 4, 5 and 6 indicate increased magnification of the pores region 209X to 3150X magnification. Figure 6, the highest magnification level, shows the organic matter of an individual pore in the LDEF-exposed seeds.

Figures 7 – 9 show increased magnification of the control seed whose surface has minimal porous openings. At 210X magnification, Fig. 9's surface shows minimal pores for the control seed.

3. COMPARATIVE ANALYSIS OF THE INTERNAL SEED STRUCTURE

The dramatic difference between the LDEF-exposed seed is observed when the seed is opened to expose the tomato plant embryo, the nutritional layer, and the outer seed coat. There is a unique and distinct separation between the embryo and the nutritional layer and between the nutritional layer and the outer seed coat. The LDEF-exposed seed

Fig. 8. Surface of control seed at 580X.

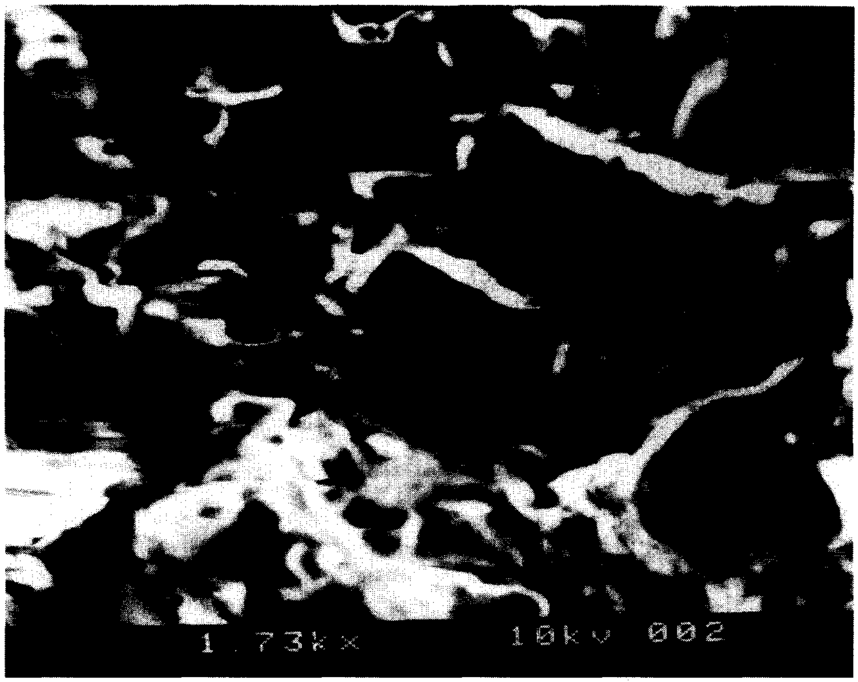

Fig. 9. Surface of control seed at 1730X.

have a porous material, flaky in nature, between the seed embryo and the nutritional layer. The space between the seed coat and the nutritional layer displays less of the porous filling than the inner seed layers. The control or Earth-bound seeds, when opened, have distinct absences of an organic material between the embryo and nutritional layer and between the nutritional layer and the outer seed coat.

4. ROOT STRUCTURE OF TOMATO PLANT AFTER GERMINATION

The root structure for the LDEF seeds and the control seeds do produce subtle changes in the cluster of materials and other weblike structures observed. Earth-control seeds have a higher density of material clusters and stringy weblike structure compared to LDEF seeds. An actual quantitative measure requires a study using statistical techniques to demonstrate the validity of the above proposition.

5. LEAF STRUCTURE AND CALCULATION OF COMPARABLE STRUCTURES

The leaf structure of the LDEF young plant versus the control young plant is remarkable because of the wavelike structure that permeates the young leaf surface. The micrograph of the control leaf was taken at 980X, while the space plant leaf was taken at 300X magnification. The leaves were removed at the same time from the young tomato plant. A calculation of similar structures in the LDEF leaf produces six times the length of the control germinated plant leaf structure that further confirms the increased germination rate of the plant from the LDEF-exposed seed.

Fig. 10. The control seed outer coat, nutritional layer, and seed embryo have distinct boundary separation at 37X.

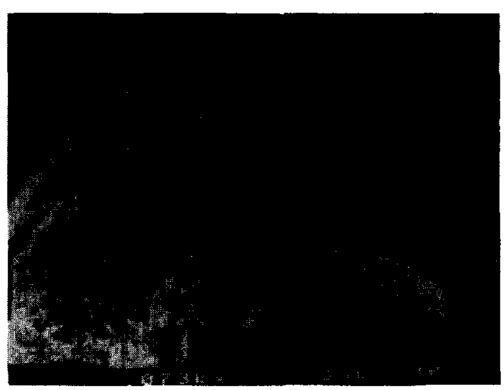

Fig. 11. The control seed boundary separation at 73X.

Fig. 12. The LDEF exposed seed has a porous flaky appearance at 22X.

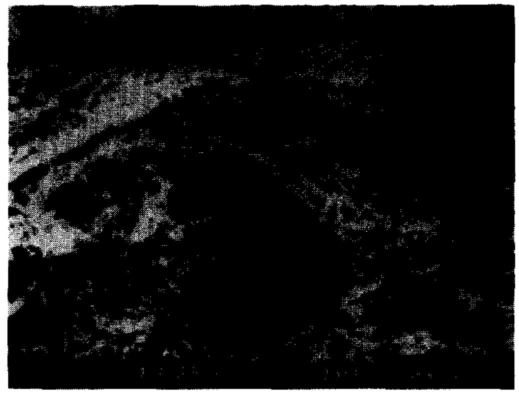

Fig. 13. A closer examination of the LDEF exposed seed at 113X.

The EDS X-ray microanalysis produces a list of eight elements that are common to the space as well as the Earth seeds. Aluminum and silver peaks are observed because the SEM mounts were made of aluminum and silver paint used to fasten the seeds to the stub. The peaks associated with carbon, potassium, copper, and calcium have been observed by other researchers including John N. Lott and colleagues. Elements such as chlorine and rhenium cannot be explained except that there may be another elemental peak masking it as those elements. The most unique observation is that iron is seen in the controls while the seeds aboard LDEF produced substantial peaks of magnesium, phosphorus, and sulfur. Other Lott research team reports assert that there are globoid crystals clusters in the embryo uregion containing P, K and Mg. The question remains why P, K, and Mg were found in the LDEF-exposed seeds, while the element, Fe, was observed in the controls. This may be a characteristic of the particular seed examined and electron beam concentration position.

6. DISCUSSION

The Strophiole region of the seed coat is considered to be the region more susceptible to permeability than any other region of the seed coat. The Strophiole clefts (pores of the strophiole region) of the space-exposed seeds, were larger in size than those clefts found in the strophiole region of Earth-based tomato seeds, thus allowing the rate of permeability and higher germination rates in the space-exposed seeds to occur at a higher rate than in Earth-based

Fig. 14. Young root from LDEF seed.

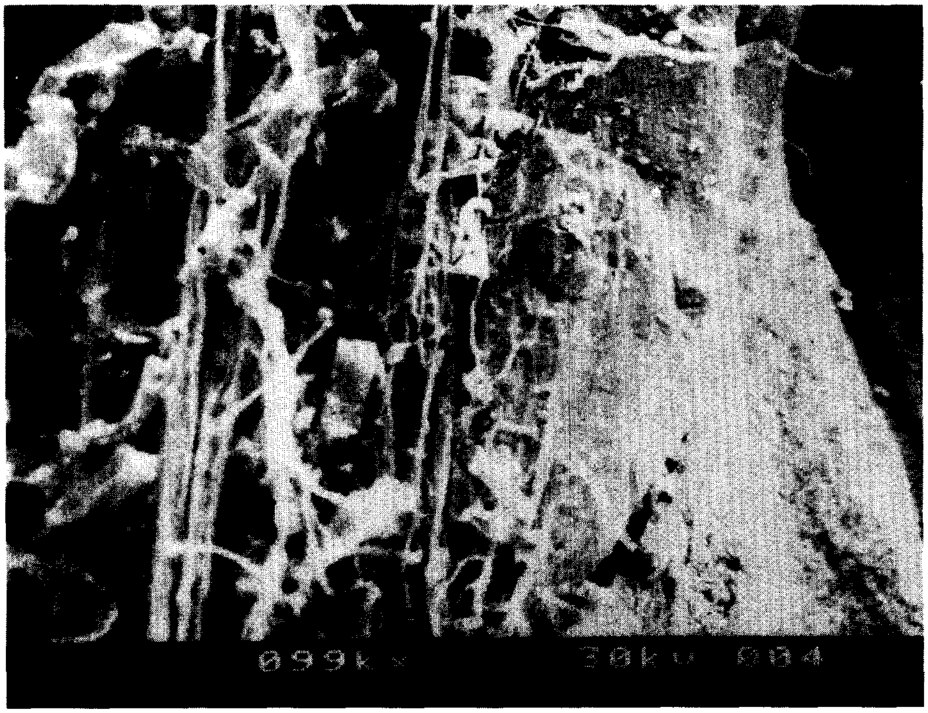

Fig. 15. Young root from control seed.

seeds. During the remainder of plant development, however Earth-based plants sprouted and grew to the approximate size of the space-exposed plants.

The visual observation of the internal and external structures of the Rutgers tomato seeds showed a greater separation of the seed coat from the endosperm/cotyledon layer of space-exposed seeds compared to Earth-based seeds.

The elemental composition found in the globoid crystals of the tomato seed contained P, K, and Mg, but some may also have had traces of Ca, Fe, and Mn (Spitzer and Lott, 1980). Throughout the embryo in

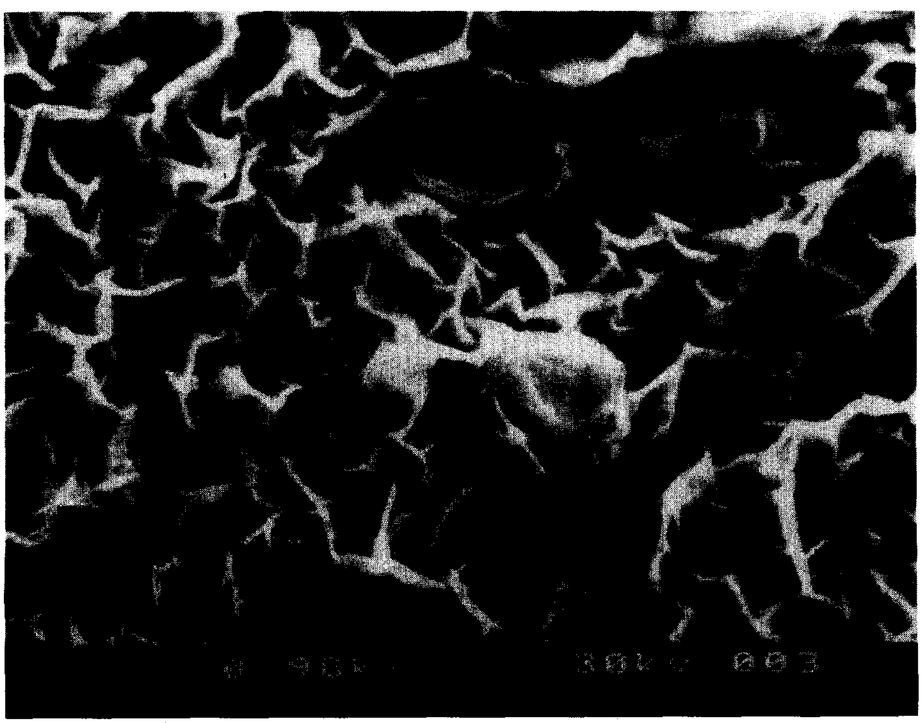

Fig. 16. Leaf from control seed.

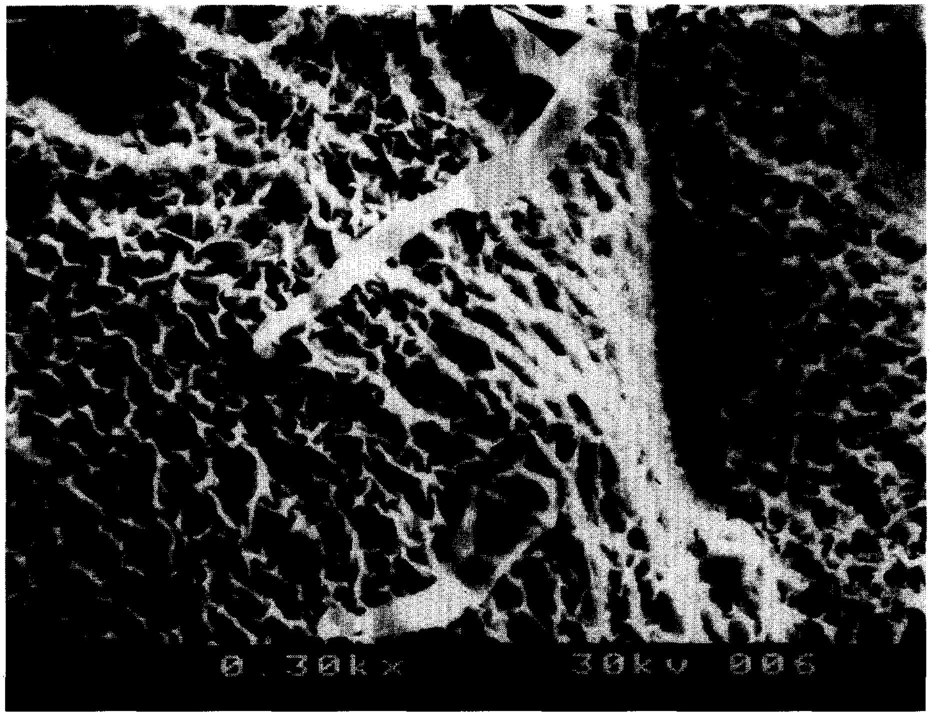

Fig. 17. Leaf from LDEF exposed seed.

the endosperm, occasional cells contained Ca in its globoid crystals (Murray and Roxburgh, 1984). In this study we found that the elemental composition of both Earth-based and space-based seeds contained the elements that were found in previous studies although there were additional elements found in this study that had not been cited previously. The relationship of these elements to the growth and development of the seeds in this study has not yet been concluded.

Future research should refine the technique of analyzing the organic material on the seed coat, the germinating root structure, and the leaf and stem structure. It is hoped that higher magnification will be achieved utilizing the scanning electron microscope and ultimately the scanning tunneling microscope

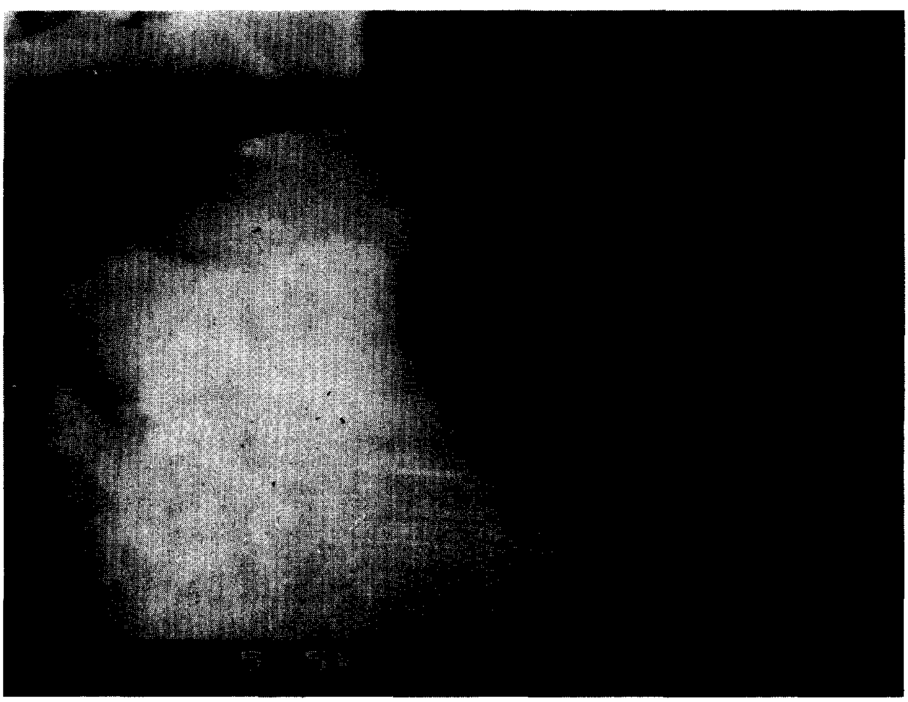

Fig. 18. Isolated pore in LDEF exposed seed.

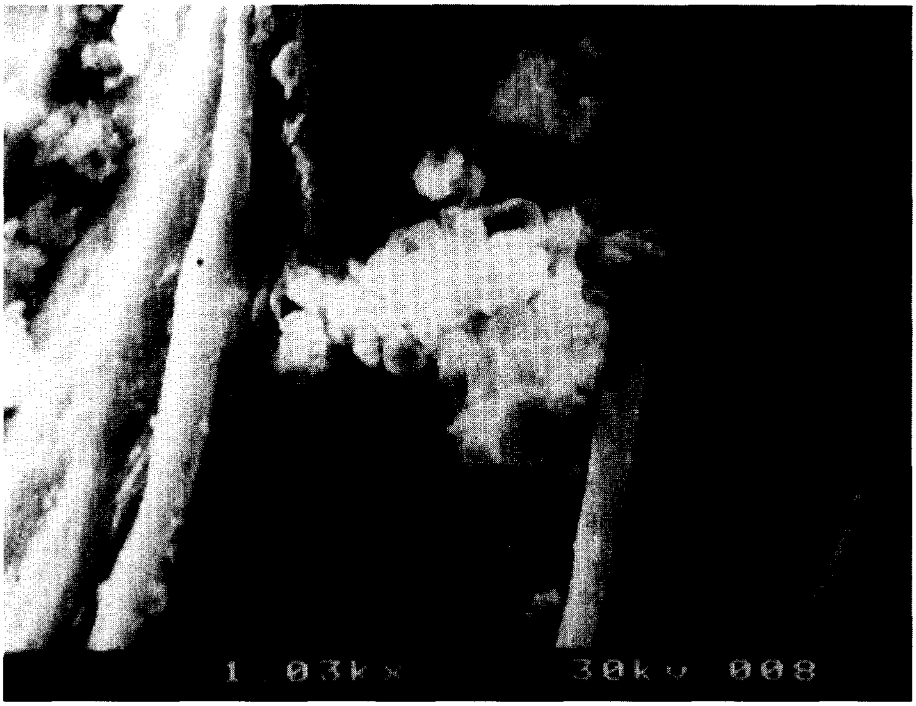

Fig. 19. Globoid cluster at 1030X magnification.

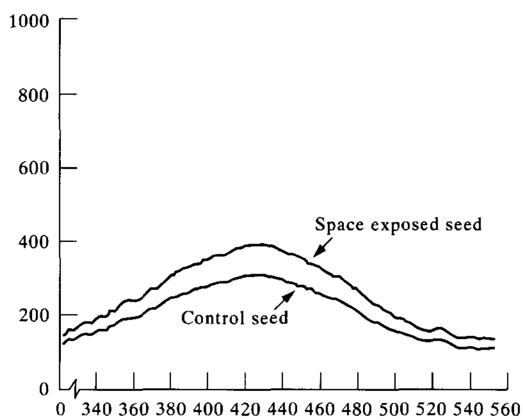

Fig. 20. Comparative emission spectra of the control and space bound seed.

and atomic force microscope to look at biological structures of seeds at atomic resolution levels.

An in depth picture of one of the pores within the space-exposed seeds surface at approximately 5000X magnification is shown in the micrograph in Fig. 18. An example of microscopic limits using the tomato seeds is shown in Fig. 19, which pictures globoid clusters at magnifications of 1000X, X-ray analysis can reveal its elemental composition with accuracy. Moreover, fluorescence studies of the LDEF-exposed seeds in comparison to the controlled seeds using excitation frequency of 300 nm and emission maximum frequency to 587 nm indicate that the surface of the LDEF seeds have a 30% increase in

POSSIBLE IDENTIFICATION

R KA OR AG LA LB2
A KA KB
L KA
I KA OR RB LA?
L KA OR BR LA?
U KA
KA OR ZR LA?
KA OR MO LA? OR TL MA
KA
G KA

PEAK LISTING

	ENERGY	AREA	EL. AND LINE	
1	0.260	303	C	KA
2	1.210	294	MG	KA
3	1.467	1589	AL	KA OR BR LA?
4	1.739	2054	SI	KA OR RB LA?
5	2.013	642	P	KA OR ZR LA?
6	2.300	541	S	KA OR MO LA?
7	2.619	2550	CL	KA
8	2.981	8450	AG	LA
9	3.313	3909	AG	LB2
0	3.692	5457	CA	KA
1	4.039	525	CA	KB
2	8.047	1056	CU	KA

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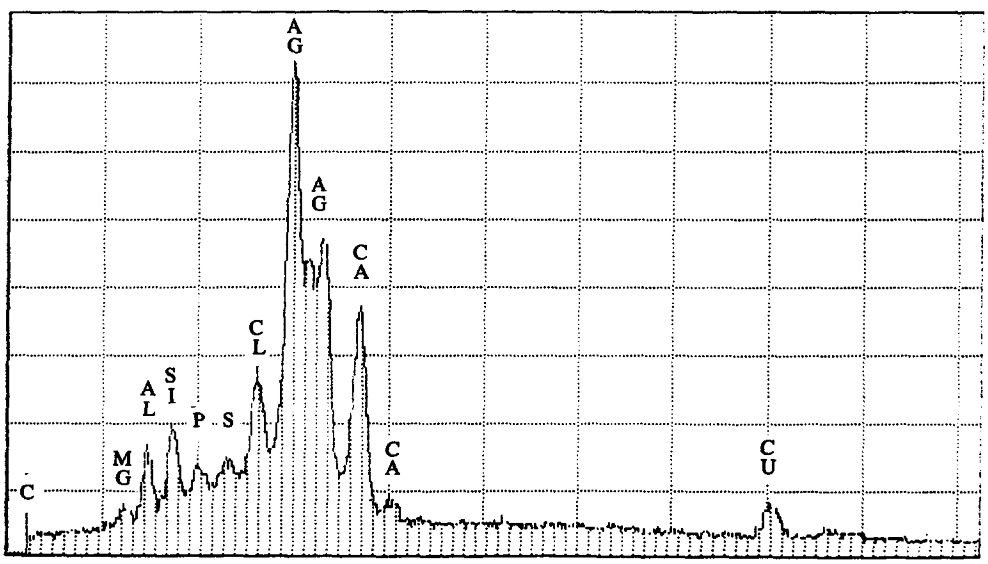

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QUALITATIVE ELEMENT IDENTIFICATION

Fig. 21. Emission spectra of the control seed.

PEAK LISTING

	ENERGY	AREA	EL. AND LINE	
1	0.235	271	C	KA
2	1.236	376	MG	KA OR AS LA?
3	2.006	1138	P	KA OR ZR LA?
4	2.300	488	S	KA OR MO LA?
5	2.616	1755	CL	KA
6	3.314	3929	K	KA OR IN LA?
7	3.691	5982	CA	KA
8	4.031	585	CA	KB
9	8.040	497	CU	KA

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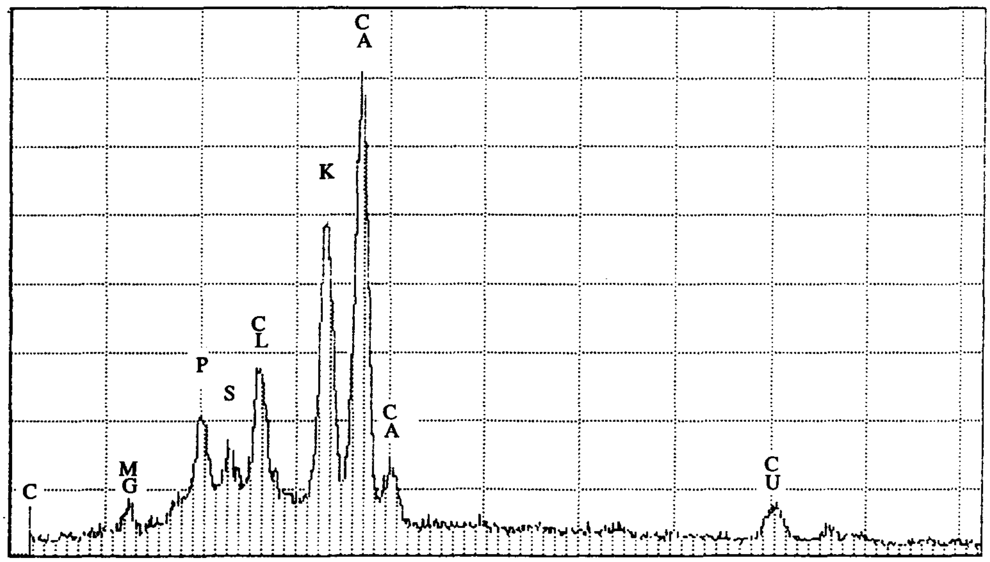

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QUALITATIVE ELEMENT IDENTIFICATION

SAMPLE ID: EXEC (7-C) DATA LABEL

POSSIBLE IDENTIFICATION

CA KA KB

Fig. 22. Emission spectra of the LDEF exposed seed.

fluorescent-intensity compared to the control seed fluorescence. There is also a slight shift toward the higher wavelength for the control seed that has the reduced intensity. See Figs 20 – 22. The peak shifts on the EDS X-ray analysis chart indicate an electron beam interaction variability caused by the elemental concentration within and between the outer coat, nutritional layer, and the embryo. Also, these peaks shifts are caused by variability of the density of the elements within any of the seen divisions.

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