SCIENCE DILIMAN
Received Date: February 15, 1993

Cytological Effects of Irradiated Guayabano Fruit Juice on Native Onion (*Allium fistulosum* L.)

Milagros S. Serrana* and Adoracion T. Arañez**

ABSTRACT

Thirty bottles of guayabano fruit juice were exposed to gamma ray doses of 0, 1, 2, 3 and 4 KGy. These bottles were then stored for 0, 4 and 8 days and used for treatment of Allium fistulosum *L. root meristems. For each treatment, 2000 cells were scored to obtain data on mitotic index and types and frequency of cytological aberrations. The newly irradiated juice did not inhibit mitosis but storage showed marked effects on the mitotic index. Both irradiated or unirradiated juice stored for 8 days caused a significant inhibition of mitosis. The irradiated juice induced the production of anaphase bridges, binucleate cells, cells, cells with elongated nucleus and cells with obliquely oriented equatorial plate.*

INTRODUCTION

The increasing demand for food has created a need for new methods of preventing microbial growth and enzyme activity in order to increase the shelf-life of food products. Among the food-processing technologies available today, the use of radiation is considered promising as it reduces the use of chemical preservatives whose residues have been found to be either carcinogenic or mutagenic. However, irradiation cannot be re-

* Miriam College Foundation, Inc., Diliman, Quezon City
** Institute of Biology, College of Science, University of the Philippines, Diliman, Quezon City

garded as a totally safe technology. Differences in chemical and physical nature of food, the type of microorganisms present, and the conditions of irradiation determine its effectiveness. Except for radiorized products which have been established as wholesome, the others have still to undergo series of tests before they can be commercially released.

In the Philippines, studies towards the possible use of radiorization are being carried out by the Philippine Nuclear Research Institute (formerly Philippine Atomic Energy Commission) and a number of individuals. Among the completed researches are the determination of effective radiation doses for native fruits and rootcrops [1], effects of gamma radiation on the chemical, physical, and organoleptic properties of mango [2] and banana [3], and mutagenicity of irradiated mango extracts on bacteria and mice [4].

This study is another contribution towards the understanding of the effects of irradiated food on organisms. Inasmuch as no study has been made on native fruit juices, the juice of guayabano (*Anona muricata* L.) was utilized to assess the wholesomeness/mutagenicity of irradiated juice based on its cytological effects on native onion (*Alium fistulosum* L.) root meristems. This study was also done to determine whether storage period influences the effects of irradiated juice on the test organism.

MATERIALS AND METHODS

Preparation and Irradiation of Juice

Ripe fruits of guayabano were purchased from Parang Market (Marikina). These were thoroughly washed, peeled, and the seeds removed with gloved hands. The fleshy pulp was homogenized with a fruit blender, and the mixture passed through a strainer. The collected juice was poured into 60-ml prescription bottles. Before use, the bottles were autoclaved for 15 minutes at 15 lbs pressure, while the strainer, fruit blender and the rest of the materials used for preparation were submerged in boiling water for 30 minutes. For the duration of the study, 30 bottles of the juice were prepared and treated at the Gamma Cell Facility of the Philippine Nuclear Research Insitute, Diliman, Quezon City using a Cobalt-60 source. Two trials were made with an interval of seven weeks using doses of 1, 2, 3, and 4 KGy. Three bottles per dose per trial, a total of 30 bottles for the two trials were made.

Immediately after irradiation, one set each from the irradiated and unirradiated juices were used for treatment and analysis. The other two sets were stored under laboratory conditions and used on the fourth and eight day of storage. During the study period, the temperature in the laboratory room ranged from 30°C to 38°C.

Cytological Technique

Sprouted bulbs of native onion (*Allium fistulosum* L.) were submerged in 25% solutions of irradiated guayabano fruit juice (GFJ) in sterile distilled H_2O. Treatment was done for six hours at room temperature (28°C–30°C). After treatment, they were washed in running water for another six hours to remove the juice totally from the onion root cells. Immediately after washing, the root tips were excised and fixed in freshly prepared Farmer's fluid (3:1 ethanol and glacial acetic acid) for twelve hours at low temperature. The materials were later transferred to 70% ethanol for storage. Sprouted bulbs soaked in 25% unirradiated GFJ were used as control.

The root tips were treated with 1N hydrochloric acid until they became almost transparent. Each root tip was placed in a drop of 1% acetocarmine stain on a clean slide and flattened with a scalpel. A coverslip was placed over the material, and with a blotting paper on top of it, pressure was applied until all the cells were separated from each other. The slide was passed three to four times over the flame of an alcohol lamp without boiling the stain, and the cytological material was examined under a compound microscope.

A total of 2000 cells from each treatment were scored to obtain data on mitotic index and types and frequency of cytological aberrations.

Statistical Analysis

The data for the two trials were pooled and analyzed by a two-way analysis of variance (ANOVA). Means were compared using the Duncan's Multiple Range Test [5].

RESULTS AND DISCUSSION

The data (Table 1) on mitotic index in onion root tips treated with irradiated GFJ showed slight variations among the treatment groups. A two-way analysis of variance (Appendix A) indicates that only the storage time significantly affected the results and that no significant difference existed among the gamma radiation treatments. A Duncan's Multiple Range Test (DMRT) of the means showed that the juices stored for eight days both unirradiated and irradiated, significantly inhibited mitosis. Root tips treated with these juices have, on the average, a mitotic index of 0.12, while those treated with the unstored juices gave an index of 0.12.

Among the cytological changes observed in root tips treated with irradiated juice were oblique cell division, anaphase bridge, and binucleate cells (Table 2).

Oblique cell division (Fig. 1A and 1B) was different from the normal (Fig. 2A, 2B, and 2C) since the equatorial plate and the chromosomes were not oriented along the side of the cell. Instead, they were positioned toward the corners of the cell during metaphase. In subsequent anaphase, the chromosomes separated following the oblique orientation of the equatorial plate.

An anaphase bridge (Fig. 3) is characterized by the non-separation of sister chromatids during anaphase while binucleate cells (Fig. 4) are twice as large as the normal meristematic cells. These abnormalities occurred in higher frequencies in root tips treated with juices receiving 2 and 4 KGy gamma radiation.

When pooled, the frequency of cells exhibiting these three types of cellular abnormalities per gamma radiation dose was found to be 0.01% of the total mitotic cells.

Elongated nuclei (Table 3; Fig. 5) were observed in treated cells as well as in the control. Statistical analysis of the data (Appendix B) revealed, however, that the frequency of elongated nuclei was significantly higher in root tips treated with irradiated GFJ. The frequency was further observed to decrease with increasing period of storage.

Plasmolysis of cells, as evidenced by distorted appearance of the protoplasm was noted to be induced by the unirradiated and 1 KGy treated juice stored for four and eight days.

The result suggests that gamma radiation doses of up to 4 KGy do not induce the production of mitosis-inhibiting substances in GFJ, or if ever they are present, are in insignificant amounts to affect the rate of cell division in onion root tips. This varies from the findings of Swaminathan et al. [6] who observed inhibition of mitosis in barley embryos cultured in irradiated (20, 40 and 80 Krads of X-rays) potato mash. The discrepancy may be due to the differences in the test materials, as well as with the type of radiation used. Holsten et al. [7] observed inhibition in carrot explants exposed to coconut milk treated with 500 Krads. Sparrow et al. [8] have shown that the sensitivity of organisms to mutagens is dependent on the average volume of the interphase nucleus measured on root meristems and on chromosome number.

It is of interest to point out that the cytological abnormalities observed in the root meristems of onion, although in a very low frequency, have similarly been reported by previous investigators and have been attributed to the presence in the irradiated medium of preprophase-, spindle-, and cytokinesis inhibitors, as well as to the production of both long-lived and short-lived radiolytic products of sugars [7].

The presence of binucleate cells in onion root tips treated with irradiated GFJ is probably due to the production of substances that interfere either with the assembly of tubulins and actins to produce the phragmoplast, or to the cell metabolism itself [9]. The golgi complex might have been so affected that it became incapable of assembling pectin and hemicellulose components for the cell wall in its cisternae.

Anaphase bridges could have been formed from chromosomal breakage and by production of substances that could alter the nucleoprotein pattern of the chromosomes. The latter type of subchromatic aberration may have changed the surface properties of these chromosomes causing them to adhere to each other upon coming in contact [10]. Most workers, however, believe that anaphase bridges result from duplication of broken chromosomes whose sticky ends do not readily separate at the anaphase stage [11].

Oblique divisions, although occurring much less frequently than anticlinal and periclinal divisions, were reported to be present in vascular plants [12]. Very few cells in metaphase, anaphase and telophase stages were observed in the control and in the root tips exposed to 4 KGy irradiated juices, as compared to those receiving 1, 2 and 3 KGy of gamma radiation. It is possible that irradiation might have triggered the production of substances which caused the misorientation of the equatorial plate. Acute doses of gamma radiation (1.52 rads/hr for 14 days at 19°C) on *Vicia faba* roots disturbed the orientation of cell divisions and caused the disorganizations of cell lineages [13]. Such changes in the orientation of cell divisions may be regarded as the initial stages towards reestablishment of new meristems to replace the original meristem that have been destroyed by radiation.

The higher frequency of cells with elongated nuclei in root tips treated with irradiated juice may indicate the presence of radiolytic products that could have induced the change in nuclear morphology. The constancy in the shape of the nucleus has undoubtedly a genetic basis as indicated by the fact that certain cells have a characteristic nuclear shape [14].

The occurrence of chromosome and chromatid breaks are detected during anaphase wherein the broken segments may occur at or near the equatorial plate, while the rest of the chromosomes are migrating to the opposite poles. These broken segments are observed as micronuclei at late telophase or early prophase.

REFERENCES

1. Philippine Atomic Energy Commission. 1977. Progress Reports. PAEC Diliman, Quezon City.
2. Ahmad, M. 1972. "The Effects of Various Gamma Radiation on Spoilage and Physico-chemical Properties of Mango (*Mangifera indica* L. var. carabao) Puree." M.S. Thesis. University of the Philippines, Diliman, Quezon City.
3. Rillo, B.O. 1968. "Effects of Gamma Irradiation on Some Chemical, Physical, and Organoleptic Properties of Latundan Banana." M.S. Thesis. University of the Philippines, Diliman, Quezon City.
4. Luber, R.P. and R.C. Umaly. 1981. "Studies on Mutagenicity of Irradiated Mangoes Using Mice, Bacteria and Drosophila as Test Organisms." *Natural Applied Science Bulletin* 3(1&2)135–146.
5. Walpole, R.E. 1974. *Introduction to Statistics.* McMillan Publ. Co., Inc., New York.
6. Swaminathan, M.S., V.L. Chopra and S. Bhaskaran. 1962. "Cytological Abberations Observed in Barley Embryos Cultured in Irradiated Potato Mash." *Rad. Res.* 16:182–188.
7. Holsten, R.D., M. Sugii and F.C. Steward. 1965. "Direct and Indirect Effects of Radiations on Plant Cells: Their Relation to Growth and Growth Induction." *Nature* (Lond.) 208:850–856.

8. Sparrow, A.H., R.C. Sparrow, K.H. Thompson and L.A. Schairer. 1965. "The Use of Nuclear and Chromosomal Variables in Predicting Radio Sensitivities." In: *The Use of Induced Mutations in Plant Breeding.* (Rep. FAO/IAEA Tech. Meeting, Rome) Pergamon Press.
9. Karp. G. 1979. *Cell Biology.* Kosaido Printing Co., Ltd. Tokyo, Japan.
10. Pahl, G. 1980. *Radiation Biology.* Integrated Res. Ctr., De La Salle University, Manila.
11. Strickberger, M.W. 1976. *Genetics.* MacMillan Publ. Co., Inc. New York.
12. Esau, K. 1943. "Vascular Differentiation in the Pear Root." *Hilgardia.* 1(3):299–311.
13. Clowes, F.A.L. and E.J. Hall. 1966. "Reorganization of Root Apices after Irradiation." *Ann. Bot.* N. S. 23:205–210.
14. De Robertis, E.D.P., W.W. Nowinski and F.A. Saez. 1956. *General Cytology.* W.B. Saunders Co., Philadelphia, London.

Table 1. Mitotic indices of onion root tips treated with irradiated and unirradiated guayabano fruit juice*

Days of Storage	Gamma Ray Dose (KGy)					Mean ± S.D.**
	0	1	2	3	4	
0	0.21	0.24	0.26	0.18	0.16	0.21a ± 0.04
4	0.23	0.23	0.20	0.16	0.13	0.19a ± 0.05
8	0.13	0.13	0.12	0.11	0.11	0.12b ± 0.01

*Average of two replicates

**Means followed by the same letter not significantly different by Duncan's Multiple Range Test (DMRT)

Table 2. Frequency of aberrant cells observed in meristematic cells of onion root tips treated with irradiated and unirradiated guayabano fruit juice

Gamma Ray Dose	Total Cells	Cytological Abnormalities					
(KGy)		Oblique Cell Division		Anaphase Bridge		Binucleate Cell	
		No.	Freq.	No.	Freq.	No.	Freq.
0	2000	0	0.0	0	0.0	0	0.0
1	2000	9	0.0045	2	0.0005	2	0.0010
2	2000	6	0.0030	5	0.0025	8	0.0040
3	2000	4	0.0020	3	0.0015	7	0.0035
4	2000	7	0.0035	6	0.0030	9	0.0045
TOTAL	1000	26	0.0130	15	0.0075	26	0.0130

Table 3. Number of cells in onion root tips with elongated nucleus after treatment with irradiated and unirradiated guayabano fruit juice*

		Gamma Ray Dose (KGy)					
Days of Storage	Total Cells	0	1	2	3	4	Mean ± S.D.**
0	100	6.00	18.50	24.00	18.30	15.50	16.50a ± 6.62
4	100	7.00	9.50	5.50	7.50	13.00	9.50b ± 2.89
8	100	4.50	5.50	8.00	7.00	8.00	6.60b ± 1.56

*Average of two replicates

**Means followed by the same letter not significantly different by Duncan's Multiple Range Test (DMRT)

APPENDIX

A. Analysis of variance of the mitotic indices of onion root tips treated with irradiated and unirradiated guayabano fruit juice

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	f
Gamma Dose (GD)	0.0207	4	0.0052	1.4054[ns]
Days of Storage (DS)	0.0370	2	0.0188	5.0000*
GD X DS	0.0058	8	0.0007	0.1892[ns]
Error	0.0056	15	0.0037	
TOTAL	0.1191	29		

B. Analysis of variance of the number of cells with elongated nucleus per 100 mature cells of onion root tip treated with irradiated and unirradiated guayabano fruit juice

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	f
Gamma Dose (GD)	175.4667	4	43.8667	5.2222**
Days of Storage (DS)	552.0667	2	276.0334	32.8611**
GD x DS	261.9333	8	32.7417	3.8978**
Error	126.0000	15	8.4000	
TOTAL	1115.4667	29		

Note:
ns—not significant
*significant at 5 percent level
**significant at 1 percent level

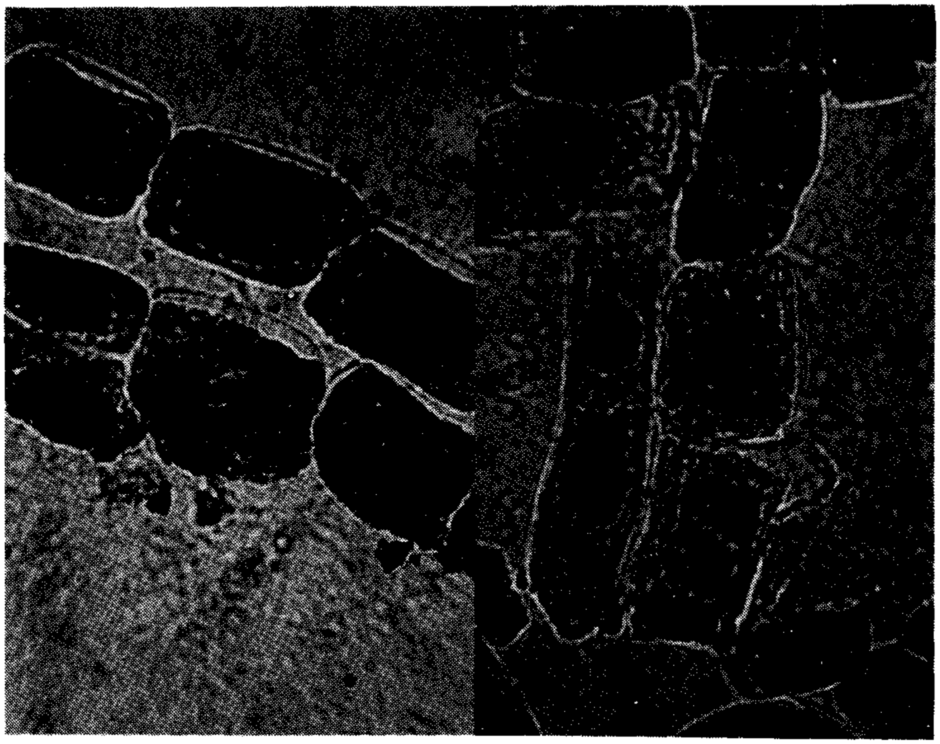

Figure 1. Cells with misoriented cell division at metaphase (A) *and telophase* (B) *stages of mitosis, X2315*

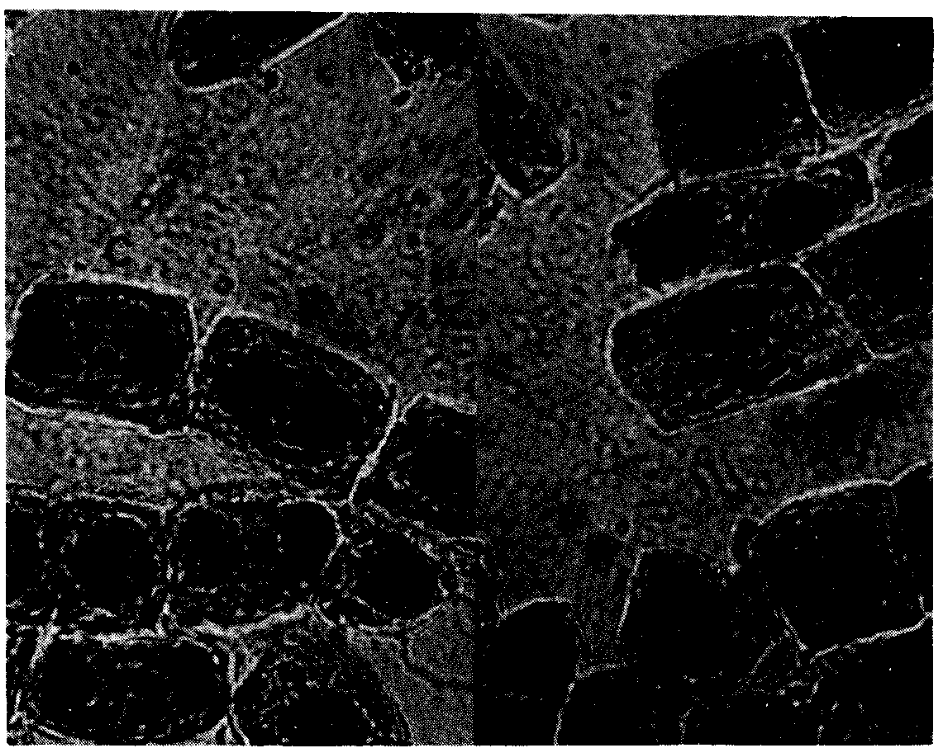

Figure 2. Cells with normal cell division at metaphase (A), *anaphase* (B), *and telophase* (C) *stages of mitosis, X2315*

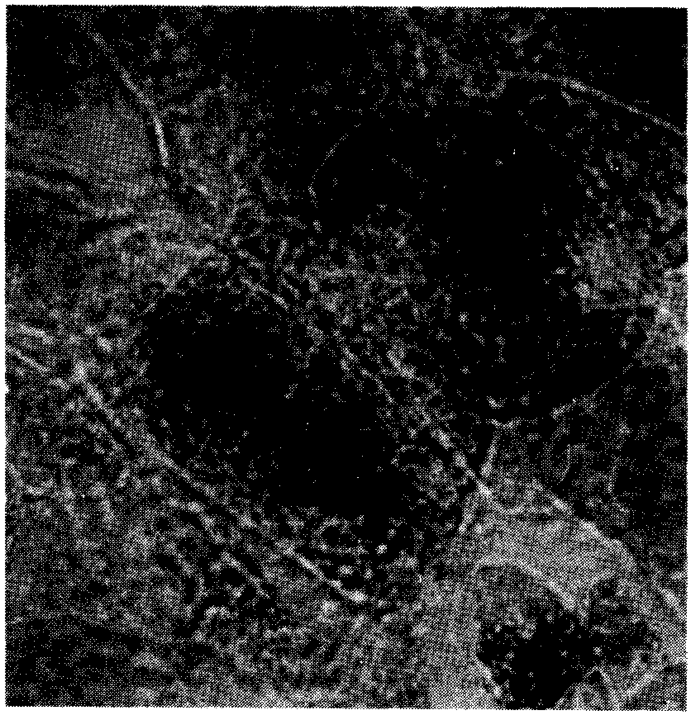

Figure 3. Left cell with an anaphase bridge, X2315

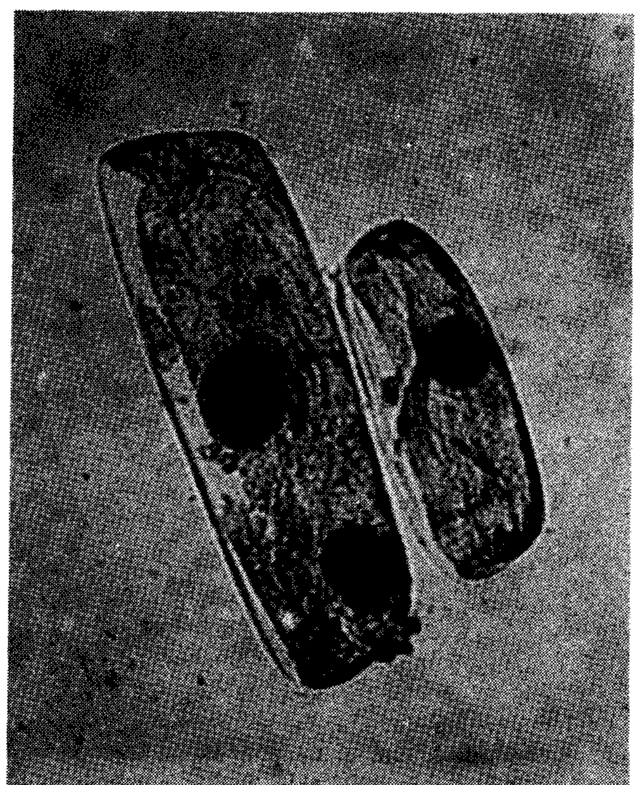

Figure 4. A binucleate cell compared with the normal, X2315

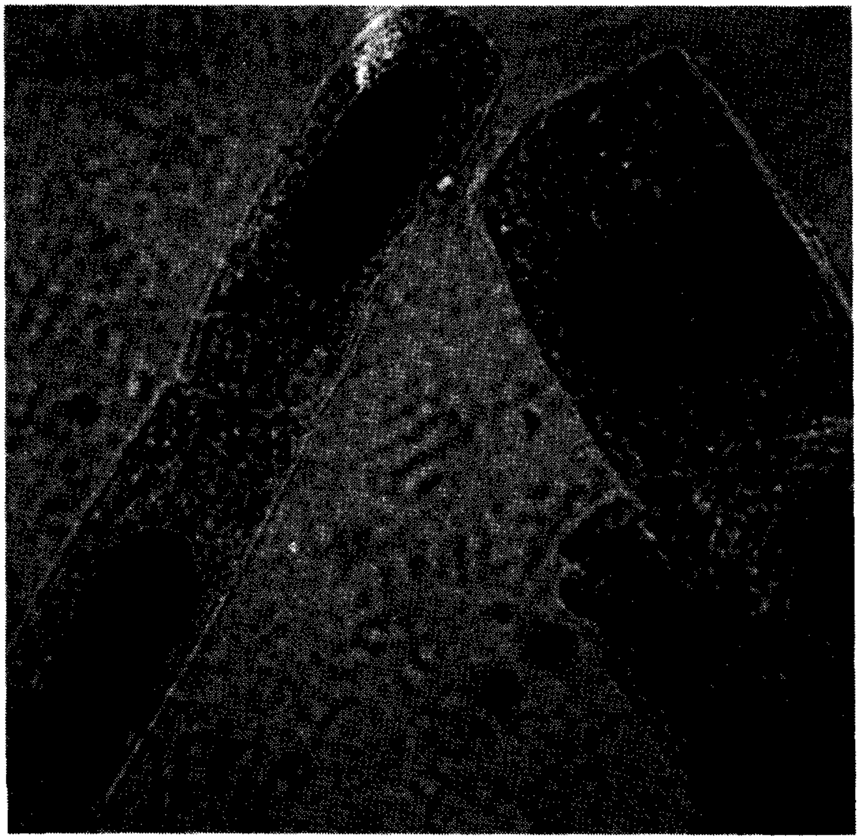

Figure 5. Cells with elongated nucleus compared with the normal, X2315