__Original Research Article__

Impact of papaya seed soaking in different BA, colchicine and EMS solutions on germination, growth and chromosomal behaviour

ABSTRACT (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

The present investigation was carried out during two consecutive seasons 2015 and 2016 in fruit nursery of faculty of Agriculture at Moshtohor, Benha University, in order to throw some spotlight on the impact of some chemical substances (Ethylmethanesulphonate – EMS in 10, 20 and 30 ppm); (colchicine at concentrations of 1%, 2% and 3%) and (benzyl adenine – BA, at concentrations of 1,2% and 3%) on seed germination %, seed germination rate, some seedling growth measurements and cytological examination of root tip of *Carica papaya* cv. Solo. The treatments were arranged in complete randomized block design with nine replicates (polyethylene bags), however, each replicate was represented by two papaya seedlings. The seedlings were divided into three categories according to their growth vigor, each category represented by three replicates for each treatment and subsequently each category sampled by 60 seedlings for all studied treatments. Seedling growth and chromosomal behavior as imported by the three studied chemical substances were evaluated on the 1st week of December. Data obtained revealed that both BA at 2% and BA 3% increased significantly germination %, germination rate and growth measurements. On the contrary, the least significant increase was always in concomitant to EMS at 3% and colchicine at 3 % during both experimental seasons. Moreover, EMS was more inhibitor of cell division followed by BA than Colchicine. This may be due to more damage resulted by BA and EMS affected on DNA replication during mitosis.

Keywords: [Carica papaya, germination %, seed germination rate, growth measurements, cytological examination, BA, Colchicine and EMS.]

1. INTRODUCTION (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

The papaya (*Carica papaya* L.) is cultivated for its ripe fruits, favored for people in the tropical region as breakfast fruit, and as ingredient in juice, jellies and preserves or cooked with young leaves and shoots as a vegetable plant. The fruit contains high level of papain; the proleolytic enzyme used for medical purposes and as a tenderizer for meat. The fruit, also, contains considerable quantities of vitamin A, B and C and about 10% sugar. Fruits and seeds extract have pronounced bactericidal activity against *Staphylococcus aureus*, *Bacillus cereus* and *Escherischia coli* and the latex is used to remove freckles. Other parts such as bark, are used for making rope while leaves are also used as a soap substitute supposed to remove stains.

Cytokinins can alter flower sex ratio in species with imperfect flowers. Cytokinins generally, increase the ratio of female flowers to male flowers which has implications for fruit production [1]. BA has also been used in the vegetable crop industry to alter flower sex ratios of monoecius and dioecious plants to increase the number of female flowers available to produce fruit [2]. Exogenous cytokinins can promote an accumulation of chlorophyll and promote the conversion of etioplasts into chloroplasts [3] even in dark grown seedlings. This may appear as a greening effect on ornamental crops which may be perceived as an increase in quality in green leaved crops and a decrease in quality in crops with other leaf colors. There is also some evidence that cytokinins can help increase the flower size of some plants. Cytokinins increased the size of petunia flowers [4]. In ferns however, cytokinins appear to induce maleness in the gametophytes [5].The

reduction in percentage of seed germination and survival was due to the disturbances caused at the physiological level coupled with chromosomal damage. Disturbance in the formation of enzymes involved in the germination process may be one of the physiological effects caused by mutagenic treatments particularly chemical mutagens [6].
Colchicine ($C_{22}H_{25}NO_6$), originally extracted from Colchicum autumnale, may induce some morphological, cytological and histological changes, and even changes in the gene expression level [7]. Chemical mutagens such as ethyl methane sulfonate (EMS), a compound of the alkaline sulfonate series, is most frequently used for chemical mutagenesis in higher plants due to its potency and the ease with which it can be used [8]. It usually causes high frequency of gene mutations and low frequency of chromosome aberrations [9].
The present investigation was planned and carried out to study the influence of some chemical substances i.e., (BA, colchicine, ethyl methane sulphonate) at different concentrations on some seed germination parameters, some vegetative growth measurements, as well as root till chromosomal behavior of papaya cultivar "Solo" through. the cytological examination of papaya seedling.

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

The present investigation was carried out during two consecutive seasons 2015 and 2016 in fruit nursery of faculty of Agriculture at Moshtohor, Benha University, in order to throw some spotlight on the impact of some chemical substances (Ethyl Methane Sulphonate – EMS; colchicine and benzyl adenine "(BA) on seed germination %, seed germination rate, some seedling growth measurements and cytological examination of root tip of *Carica papaya* cv. Solo.

In this regard, mature papaya fruits were collected from the trees which grown at fruit farm of Faculty of Agriculture, Moshtohor, Benha Univ., seed were extracted when the fruits have been ripened, and washed three times with tap water to get rid of fruit pulp residual. Finally, seeds were kept in shading place to be dried and stored in small coped glass contain calcium chloride to be ready for carrying out the investigation.

On the first week of March of both seasons, dried stored papaya seeds were soaked in tap water for 24h then taken out and placed in shade for 10 minutes to dry. Those seeds were divided into ten groups. Each group represented by two hundred seeds and subjected to one of the following treatments:

1- Soaking in tap water for 12 hours (control).

2- Soaking for 12 hours in benzyl adenine (BA) at 1 %.

3- Soaking for 12 hours in benzyl adenine (BA) at 2 %.

4- Soaking for 12 hours in benzyl adenine (BA) at 3 %.

5- Soaking for 12 hours in colchicine at 1 %.

6- Soaking for 12 hours in colchicine at 2 %.

7- Soaking for 12 hours in colchicine at 3 %.

8- Soaking for 12 hours in ethyl methane sulphonate (EMS) at 10 ppm.

9- Soaking for 12 hours in ethyl methane sulphonate (EMS) at 20 ppm.

10- Soaking for 12 hours in ethyl methane sulphonate (EMS) at 30 ppm.

The dried seeds were soaked in aqueous solutions of the three investigation chemical substances as well as control seed were soaked in tap water for 12 hours. Those seeds were re-dried for 10 minutes in shade after soaking in the investigation chemical substances and immediately sown on March 9th and 21st during 2015 and 2016 seasons, respectively, in black polyethylene bags (30 cm in diameter) filled with a mixture of sandy and clay soil (1:1 v/v) and kept under greenhouse conditions. The seeds were watered every other day in the morning till the appearance of plumule. Furthermore, fungicide was applied at the time of seed sowing as a tool protection against the fungal attack of *Rhizooctonia solani* and *Fusarim species*, as well as weeds were completely removed along with their roots as soon as they appear. The first appearance of plumule was recorded in the 1st week of April during both seasons of study.

The abovementioned ten investigated treatments were arranged in complete randomized design, where each treatment was replicated ten times (10 polyethylene bags) and each replicate represented by an individual polyethylene bag which contains twenty papaya seeds. Furthermore, the number of emerged seedlings was counted as soon as the appearance of first true leaves on the 4^{th} week at April of three days' intervals until seed germination was completely ceased, then the following seed germination parameters were calculated:

$$\text{1- Germination percentage} = \frac{\text{Total number of emerged seedling}}{\text{Total number of planted seeds}} \times 100$$

2- Germination rate according to equation [10] :

$$\text{Germination rate} = \frac{A1\ T1 + A2\ R2 + A3\ T3 + \ldots\ldots An\ Tn}{A1 + A2 + A3 \ldots\ldots\ldots\ldots An}$$

T1 = Number of days passed from soaking till first count 1.

T2 = Number of days passed from soaking till second count to Tn.

A1 = Number of germinated seeds at first count.

A2 = Number of germinated seeds at second count to An.

3- Number of days required for germination completion.

In order to study the impact of the three investigated chemical substances on some seedling growth measurements and chromosomal behavior of sprouted papaya seedlings, thin out of un-desirable seedlings (the weakest and the strongest ones) was done on the first week of July, while the nearly uniform seedlings in their growth vigor were remained in the polyethylene bags.

The treatments were arranged in complete randomized blocks design with nine replicates (polyethylene bags), however, each replicate was represented by two papaya seedlings. The seedlings were divided into three categories according to their growth vigor, each category represented by three replicates for each treatment and subsequently each category sampled by 30 seedlings for all studied treatments.

Seedling growth and chromosomal behavior as impacted by the three studied chemical substances were evaluated on the 1^{st} week of December through studding the following parameters:

A- Growth parameters: -

1- Seedling height.
2- Stem diameter (cm).
3- root length
4- Number of leaves/seedling.

B -cytological studies: -

Papaya (*Carica papaya* L.) reedling roots were used for bioassay. Papaya seeds were kindly supplemented from the research farm of Faculty of Agriculture, Moshtohor, Benha University to be used in this study. Seeds were soaked in three different concentrations of Benzyl adenine, EMS and Colchicine. Root meristem raised in water were fixed in a fixative solution (3:1) and kept in alcohol 70 % in refrigerator until used for cytological examination.

About 100 cleaned papaya seeds were set up in petri dishes and soaked for 24 hours here in tap water here in10 seeds were re-soaked in tab water and used as a control while the other 90 reads were picked out and divided into 3 groups, each one contain thirty reads and subjected to1%, 2% and 3% of Benzyl adenine (BA), Ethyl methane sulphonate (EMS) and Colchicine for 12 hours.

Mutagenic agents:

Ethyl methane sulfonate (EMS): The linear formula of EMS is $CH_3SO_3C_2H_5$. This formula was referred to the free chemical database: (ChemSpider ID: 5887). Seeds before germination were subjected to the following concentrations; 1%, 2% and 3% for twelve hours. Benzyl adenine (BA) or 6-Benzylaminopurine (BA) is $C_{12}H_{11}N_5$. Cyclophosphamide (Colchicine) at the concentrations of 1%, 2% and 3%. The linear formula of colchicine is $C_7H_{15}C_{l2}N_2O_2P$.

Fixation and storage solutions:

Root tips of the germinated Papaya seeds in the different investigated substances and ab water as control were excised and fixed in 1: 3 acidic alcohol consisted of a mixture of glacial acetic acid and ethanol respectively and later preserved in 70 % ethyl alcohol.

Staining agent (acetocarmine).

A carmine stain was prepared at the concentration at 1% by dissolving it in 45% acetic acid. Before adding the stain, root tips were put in a boiling acetocarmine for one minute for losing the tissue.

Root collection and slide preparation

Papaya seeds were germinated at lab temperature using petri dishes filled with enough tap water to top four to five weeks for root tips to grow. Seeds subjected to treatments were transferred to each concentration of BA, EMS and Colchicine after the length of the roots reached to 1-1.5 cm maximum. Roots were harvested at the morning. Root tips excised from treated and controlled materials were fixed in 1: 3 acidic alcohols and preserved in 70% ethyl alcohol. Root tips squashed were conducted using 1% Acetocarmine stain.

Mitotic index (MI) determination:

The slides were viewed under the light microscope, by using 40 objective lens. On one slide for each treatment dividing cells (prophase, metaphase, anaphase and telophase) were counted to determine MI. MI was expressed as the number of dividing cells per 1000 cells scored.

Chromosomal aberrations were characterized and classified in the following types: large chromosomal deletion or losing a hole chromosome, sticky chromosomes, anaphase bridge chromosomes, lagging chromosomes, disrupted chromosome segregation, star cluster chromosomes, clumped chromosomes in metaphase. These aberrations were saved in photographic pictures.

Statistical analysis:

All the obtained data during each season of this study were subjected to statistical analysis of variance according to the method described by [11]. However, the differences means were differentiated by using Duncan's multiple range test [12].

3. RESULTS AND DISCUSSION

Effect of seeds pre-sowing soaking in different BA, colchicine and EMS solutions on some germination measurements.

In this regard some germination measurements germination percentage and germination rate of papaya Solo cv. in response to pre-sowing soak in some BA, colchicine and EMS solutions were investigated during 2015 and 2016 experimental seasons are presented in **Table (1)**.

- Seeds germination percentage:

Data presented in **Table (1),** indicate that the seeds germination percentage of papaya "Solo" cv. after 4 weeks from planting as influenced by their soaking for 12 hours in different BA, colchicine and EMS solutions significantly increased during both experimental seasons. However, pre-sowing soak in the highest BA concentration surpassed significantly than investigated treatments. On the other side, the least concentration of colchicine and EMS solutions at (1 %) showed significantly the highest increase over control during two experimental seasons. In addition, other pre-sowing soak solutions (1% & 2%) of BA ranked statistically the second one. Moreover, BA as a growth promoter explain the function for activating growth and germination particularly cell division.

- Seeds germination rate:

Table (1) reveals obviously that germination rate followed typically the same trend previously discussed with germination percentage. Herein, all BA, colchicine and EMS solutions resulted in a significant increase over the tap water soaked seeds (control) during both experimental seasons. The highest BA solution were statistically the superior, while their lowest concentration (at 1% & 2%) ranked statistically second. In addition, tap water soaked seeds (control) was the inferior such trend was true during 2015 and 2016 experimental seasons.

These results are in accordance with the findings of **[13]** reported that freshly extracted seeds of acid lime (*Citrus aurantifolia* swingle) were shade dried and were soaked in 15, 30, 45 or 60 mM EMS solution for 12 hours caused decrease of percentage seed germination (36%) with increasing of EMS concentrations to 60 mM. Despite**,** seeds of *L. esculentum* cv. Roma, were treated with 0.1, 0.5 and 1% ethyl methane sulphonate (EMS) and exposed for 3 and 6 hours, decrease in seed germination was observed with increasing EMS% **[14]**. Papaya seeds treated with colchicine at

0.5% or 1.0% and EMS at 200 ppm and 100 ppm improved germination parameters compared with untreated seeds (control) **[15]**. A clear effect of different EMS-treated on seeds germination percentage of *L. esculentum* (cv. Pusa – Early–Dwarf) showed that germination percentage increased with increasing EMS concentrations from 0.0150% to 0.1205%. Thereafter, decrease in germination percentage was observed at the highest concentration (0.2410%) **[16]**. Addition colchicine to cultured medium of *Solidago altissima* at 125 mg/l had an inhibition, while the other treatments (low concentration of colchicines) possessed the most promotion influences on survival capacity of explants (75-100%) **[17]**.

Seeds of water melon without coat during the seeding, nicking at radicle end with colchicine-treated showed high germination rates 84.3% and 77.1%, respectively **[18]**. The effect EMS and colchicine-treated seeds of Papaya at 0.1% and 0.5%, they found the stimulatory effects of low-dose colchicine treatment on seedling emergence and seed germination decreased with the increasing doses of colchicine **[19]**. Reduced seed germination due to the effect of increasing doses of chemical mutagens on the meristematic tissues of the seeds may be causing damage of cell constituents at a molecular level or to disturbance in the formation of enzymes involved in the germination process caused by EMS and colchicine. Impact of mutagenic treatments i.e., EMS-treated seeds at 0.25-0.30% of rice causing the reduction in percentage of seed germination and survival was due to the chromosomal damage and disturbance in the formation of enzymes involved in the germination process **[20]** and **[6].**

Table (1): Impact of papaya seed soaking in different BA, colchicine and EMS solutions on seed germination percentage and germination rate during 2015 & 2016 experimental seasons.

Parameters / Treatments	Germination percentage %		Germination rate	
	First season	Second season	First season	Second season
1.control	55.67 g	54.33 h	3.68 i	3.42 i
2. BA at 1 %.	77.00 b	79.33 b	5.10 c	5.04 c
3. BA at 2 %.	80.67 a	81.00 b	5.32 b	5.24 b
4. BA at 3 %.	81.67 a	83.33 a	5.43 a	5.33 a
5. colchi at 1 %.	68.67 d	68.00 e	4.09 f	3.96 f
6. colchi at 2 %.	73.33 c	71.67 d	4.24 d	4.11 e
7. colchi at 3 %.	75.00 c	74.33 c	4.28 d	4.13 d
8. EMS at 10 ppm	61.00 f	63.67 g	3.75 h	3.57 h
9. EMS at 20 ppm	65.33 e	65.67 f	3.89 g	3.78 g
10. EMS at 30 ppm	65.67 e	67.33 ef	4.15 e	4.10 e

Means followed by the same letter/s within each column during every season are not significantly at 5 % level.

- Impact of papaya seed soaking in different BA, colchicine and EMS solutions on some growth measurements during 2015 & 2016 experimental seasons.

In this concern average seedling height, stem diameter, root growth and average number of leaves/seedling in response to various treatments were investigated during two 2015 and 2016 experimental seasons are presented in **Tables (2)**.

- Average seedling height (sm):

Concerning the response of average seedling height to the differential treatments, it is quite clear as shown in **Table (2)**, that all investigated treatments with various solutions from BA, colchicine and EMS resulted in an increase in average seedling height of papaya "Solo" cv. translocated seedlings during both experimental seasons. Anyhow, the increase was more pronounced with (BA at 3%) treated seeds, descendingly followed by BA at 2%, BA at 1%, colchicine at 2% and colchicine at 3%. However, such increase was too few to reach level of significance either the investigated treatments were compared each other's or to tap water soaked seeds (control) only with few exceptions particularly with colchicine at 3% in the second season. Such trend of response was true during both 2015 and 2016 experimental seasons.

-Seedling diameter (cm):

Regarding the effect of different investigated treatments on stem diameter of papaya "Solo" cv. translocated seedlings **Table (2)** displays obviously that both (T3 & T4) treatments of BA solutions at 2% and BA 3% induced significantly the thickest stem. Such trend was true during two seasons of study. Moreover, (T10 and T2) treatments of (EMS at 3 % and BA at 1 %), respectively, ranked statistically second as their effect on stem diameter was concerned for papaya Solo cv. translocated seedlings during two experimental seasons. On the other side other investigated treatments increased significantly the average stem thickness during both seasons of study but T8 (EMS 1 %) showed statistically the least significant increase in stem diameter during 2015 and 2016 experimental seasons. In addition, other investigated treatments were statistically in between the aforesaid two extremes during two experimental seasons.

Moreover, BA as a growth promoter explain the function for activating growth specially stem diameter by increase cell division which gave more thickness for the stem.

- Root length (cm):

This is the response of root length to various investigated treatments during both 2015 and 2016 experimental seasons, and data obtained during both seasons for papaya Solo cv. translocated seedlings are presented in **Table (2)**. It is quite evident as shown from tabulated data that a noticeable grade of variance in trend of response could be observed between investigated treatments in this concern. Anyhow, the greatest length of root was significantly in closed relationship to BA at 3% during two seasons of study. Moreover, BA at 2% came statistically second. On the contrary, the least significant increase in root length was always in concomitant to EMS at 3% and colchicine at 3% during 2015 and 2016 experimental seasons of study. In addition, other treatments were statistically in between the aforesaid two extremes. Such trend was true during both seasons.

Moreover, the trend of response of root length of seedling may be attributed to the variance in biological and physiological roles could be played by BA pertaining shoot growth and root length and development.

- Number of leaves/seedling:

With regard to the response of leaves number per seedling an individual seedling to the differential investigated treatments, obtained data are presented in **Table (2)**. It is quite evident that the greatest leaves number of per seedling was significant in closed relationship to such seedling was subjected to BA at 3% during 2015 and 2016 experimental seasons. Moreover, BA at 2 % ranked statistically second. Anyhow, pre-sowing soaked in BA at 1 % solution ranked statistically 3rd, descendingly followed by soaking in EMS 1%, EMS 2% and EMS 3% during both 2015 and 2016 experimental seasons. On the contrary, the least significant leaves number per seedling that exhibited by three investigated treatments (colchicine at 3 %, control and colchicine at 2 %), respectively. Such trend was true during 2015 and 2016 experimental seasons. The seeds of two pea cultivars were treated with EMS at concentrations of 0.5%, 0.75% and 1.0%. In M1-generation, number of branches decreased with EMS at 0.75% and 1.0% **[21]**.

The cytokines promote shoot development through increased cell division, regulation of the cell cycle and the number of cycles that cells in the meristems [**22**]. After addition of 20 mg/l colchicine into the medium for one week, induced tetraploidy plants were subsequently added. Morphological observations showed that the stems and the leaves of tetraploid plants were thicker and larger than in diploid ones **[23] (1999)**. Also, BA treatment at 10 ppm increased growth characters i.e., plant height, total root length fresh and dry weights of shoots and roots of maize plants **[24]**. Foliar spray of soybean plants with benzyl adenine at 75 ppm significantly increased plant height, leaves number and branches per plant and dry matter of plant **[25]**. The effect beneficial of foliar application of soybean plants with benzyl adenine at 50 ppm significantly increased stem length, diameter, leaf area surface, branches number, leaves number per plant and fresh and dry weights of plant **[26]**. Similarity, the foliar application of pelargonium (Geranium) plants with BA at 20 and 40 mg/L significantly increased plant height and number of branches/plant finding by **[27]**. Egyptian lupine plants exposed to salt stress, observed that foliar application of benzyl adenine (BA) (1 & 100 ppm) has stimulating effect on all growth characters, i.e., plant height and number of branches/plant grown under normal and saline conditions [**28**]. In *Nigella sativa* plants which benzyl adenine (5 & 25 ppm) treatments as seed soaking increased root length and diameter, plant height stem diameter, number of leaves, total leaf area/plant and net assimilation rate **[29]**. Foliar spray of snap bean plants with benzyl adenine (BA) at 20 & 40 ppm and putrescine (Put) at 200 ppm significantly increased plant height, leaves number/plant and branches and fresh and dry weights of shoots **[30]**. The increased values of vegetative parameters due to the lower dose of colchicine might be due to enhance the action of auxin (indole-3-acetic acid) and the cells divided more actively in *Helianthus tuberosus* **[31]**. Higher doses of colchicine led to increased leaf size and number of leaves per plant in colchicine-treated plants over control in *Gossypium arboreum* L **[32]**. EMS-treated plants were also reported in papaya increased cell division, as well as activation of growth hormones such as auxin **[33]**. The effect of colchicine-treated seeds of *Phlox drummondi* has been found to increase the seed germination and morphological characteristics at low concentrations **[34]**. The effect of EMS-treatments on induced micro mutations and obtained on dwarf plant types. The minimum plant height in dwarf mutant was below 90 cm. The maximum frequency of dwarf mutants was observed in 30kr + 0.1% EMS followed by 40kr + 0.25% EMS treatment. The tallest mutant (155cm) was observed in 0.25% EMS treatment followed by a mutant with 131 cm in 30kr+0.25% EMS while the parent of rice *Akshaya cv*. possess 100-110cm height **[35]**.

Table (2): Impact of papaya seed soaking in different BA, colchicine and EMS solutions on some growth measurements during 2015 & 2016 experimental seasons.

Parameters / Treatments	No. leaves /seedling		Seedling height (cm)		Seedling diameter (cm)		Root length (cm)	
	First season	Second season	First season	Second season	First season	Second season	First season	Second season
1.control	9.33 f	7.67 f	52.33e	58.67f	2.53e	2.45de	14.73d	14.85d
2. BA at 1 %.	14.00 c	11.67 cd	99.00a	101.00cd	2.77d	2.83c	18.53c	18.63c
3. BA at 2 %.	15.33 b	13.00 b	97.00ab	103.00bc	3.13b	3.20b	21.38b	21.40b
4. BA at 3 %.	17.67 a	16.33 a	96.83ab	100.00d	3.37a	3.40a	23.80a	23.87a
5. colchi at 1 %.	10.33 e	13.00 b	75.00c	101.33cd	2.93c	3.13b	13.63ef	13.50f
6. colchi at 2 %.	10.67 e	8.00 ef	95.07b	105.00b	2.65de	2.62d	13.32fg	13.30f
7. colchi at 3 %.	7.67 g	8.67 e	97.00ab	113.33a	2.37f	2.27e	13.02g	13.07f
8. EMS at 10 ppm	12.67 d	11.00 d	70.00d	78.67e	2.50ef	2.45de	13.45fg	13.50f
9. EMS at 20 ppm	12.67 d	12.00 c	76.33c	80.00e	2.65de	2.57d	13.93e	13.92e
10. EMS at 30 ppm	12.67 d	13.33 b	69.00d	80.67e	2.97c	2.87c	14.10e	14.23e

Means followed by the same letters within each column during every season are not significantly at 5 % level.

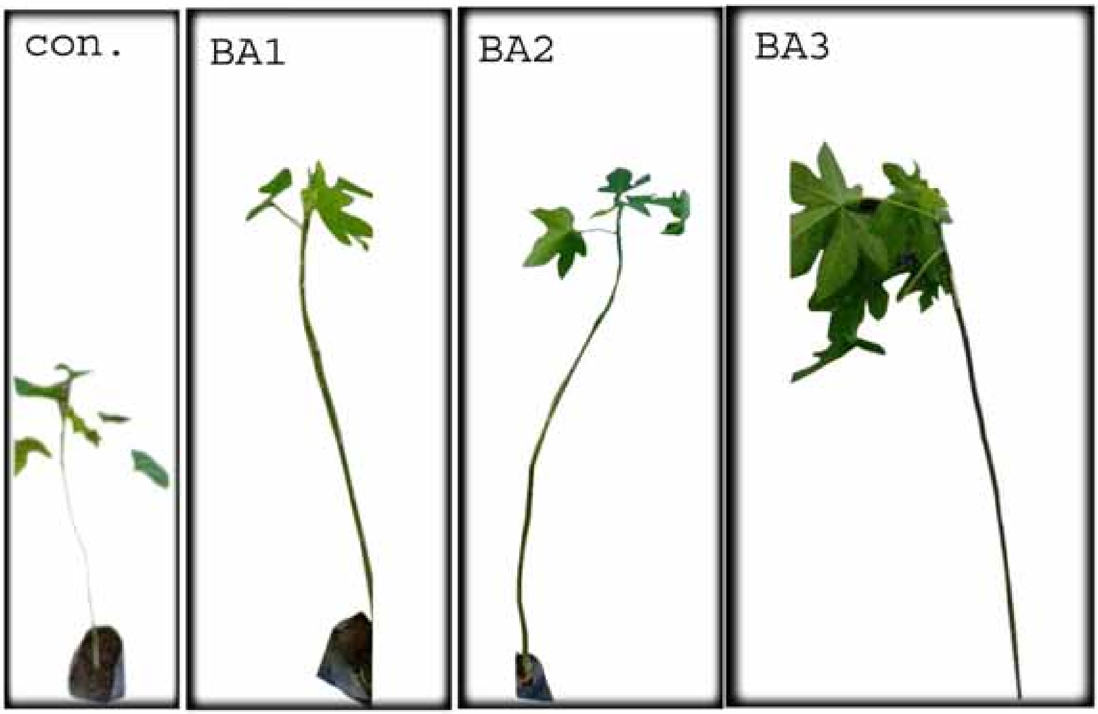


Photo (1) Impact of papaya seed soaking in different BA, solutions on vegetative growth during 2015 & 2016 experiment seasons.

Con= control, BA1= BA solution at 1% , BA2=BA solution at 2% and BA3=BA solution at 3%.

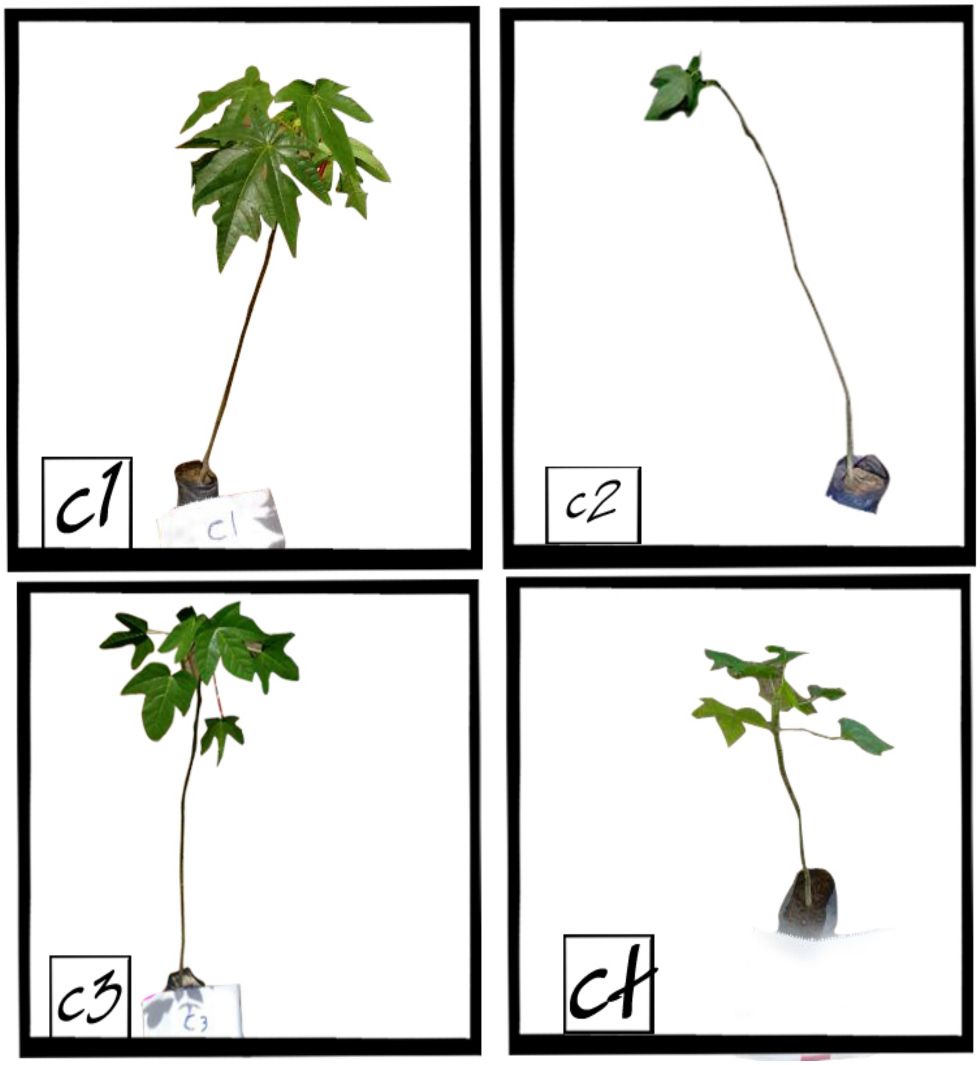


Photo (2) Impact of papaya seed soaking in different colchicine solutions on vegetative growth during 2015 & 20 experimental seasons.
C1= control , C2= Colchicine solution at 1% , C3= Colchicine solution at 2% and C4= Colchicine solution at 3%.

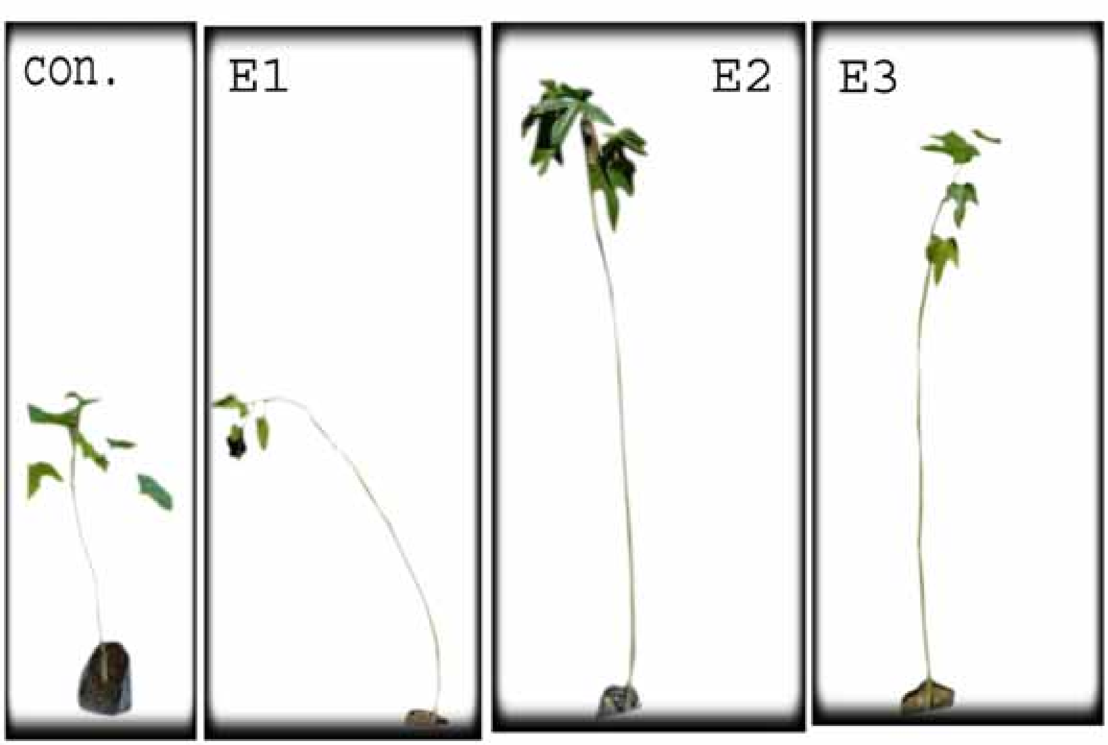


Photo (3) Impact of papaya seed soaking in different EMS solutions on vegetative growth during 2015 & 2016 experime seasons.

Con= control, E1= EMS solution at 10ppm, E2= EMS solution at 20ppm and E3= EMS solution at 30ppm.

Mitotic Index:

Means of mitotic index (MI %) resulted by BA, EMS and Colchicine are shown in Table 3. The means of mitotic index at three levels of Colchicine were close to each other and the same trend was also obtained by EMS. These results appeared that the differences between different levels of each agent were insignificant.

The means of dividing cells treated with Colchicine were significantly higher than of BA and EMS. This indicated that Colchicine did not interfere with mitosis and did not prevent cell division if compared with of BA and EMS which decreased the mitotic index and interfered with mitosis to greater extent.

Therefore, it can be concluded that EMS was more inhibitor of cell division followed by BA than Colchicine. This may be due to more damage resulted by BA and EMS affected on DNA replication during mitosis.

The figure shows the different chromosomal aberration as follows:

Sticky chromosomes at metaphase, but also laggards and lagging chromosomes, as well as polyploidy, were the main chromosomal aberrations or abnormalities during the cell division of papaya after treatment with the three mutagens with different ratio and different appearance.

Colchicine and EMS showed disrupted type of chromosomal aberrations which appeared during metaphase stage. It appeared that disrupted metaphase varied from Colchicine to EMS. In addition, EMS caused disrupted chromosomes in metaphase followed by anaphase which did not occur with Benzyl adenine.

Both Colchicine and EMS caused abnormal mitosis which appeared as sticky chromosomes. Colchicine caused sticky chromosomes in during metaphase and telophase. Similarly, EMS showed sticky with polyploidy chromosomes during metaphase, anaphase and telophase. These results indicated that colchicine had strongest effect on chromosomal behavior during mitosis and exerted more chromosomal damage. Indeed, sticky chromosomes would cause the death of those cells. Similar results were obtained by authors among them.

A chromatid bridge would occur as a result of the weakness of the spindle fiber. Bridge structure as an aberration occurs due to treatment by both EMS and Colchicine.

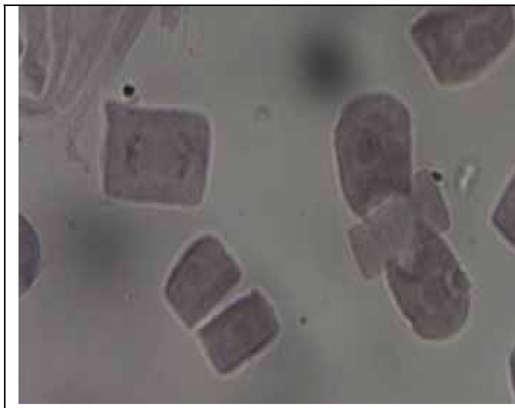
During abnormal chromosomal behavior of mitosis, spindle fiber can not to attract one chromosome, this chromosome remains near the middle of the cells. This phenomenon called lagging chromosome and resulted genome aneuploidy 2n-1. This kind of aberration did not occur among the chromosomal aberrations caused by Colchicine or EMS. Formation of chromosomes from “star type” was observed. Both Colchicine and EMS caused this aberration type.

In conclusion, the treatments by colchicine and EMS caused different types of chromosomal aberrations with variable percentages than the normal cells in control experiment the same time there were differences of the percentage ratio of each. This indicated that both chemical agents are dangerous. EMS was more dangerous than Colchicine because of cytotoxicity delaying mitosis and inducing mass chromosomal aberrations.

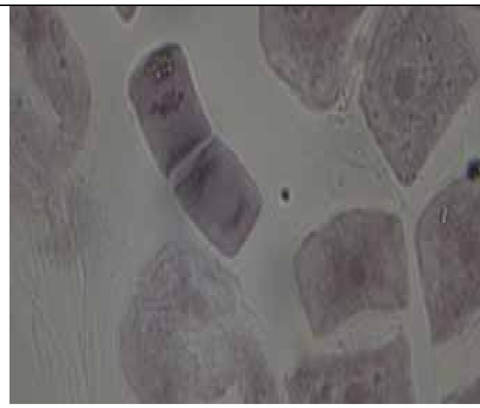
Sex determination in papaya (*C. papaya* L.) is due to a single gene with three allelic forms: *m, M1*and *M2*. The *mm*, *M1m*, and *M2 m* genotypes represent gynoecious, and roecious and hermaphrodite individuals, respectively. The *M1M1*, *M2M2* and *M1M2* genotypes are not found due to the zygotic lethality. The *m* homologous region is normal and the viable genotypes are *M1m* (male plant), *M2m* (hermaphrodite plant) and *mm* (female plant). A large concentration of genes for femaleness is in the sex chromosomes but genes for maleness are in the autosomes. Therefore, the *mm* genotype is distillated and its homozygote condition confers phenotypic stability **[36] and [37].** Small doses of colchicine enhanced the action of auxin (indole-3-acetic acid) because the cells divided more actively; instead, at higher doses, colchicine led to C-mitoses and inhibited cell multiplication in *Helianthus tuberosus* **[38]**.

The karyotype of *Carica papaya L*. consisted of eight medians (metacentric) four submedian, four sub terminal and two terminal-centromeric chromosomes, formed that the arm ratio value of eight median centromeric chromosomes range from 1.0 to 1.3 while the arm ratio value of four submedian centromeric chromosomes were very close to 3.1 the lowest extreme of the arm ratio range of the sub terminal centromeric chromosome **[15]**. The cells with a larger complement of chromosomes grow larger to maintain a constant ratio of cytoplasmic to nuclear volume, and express more proteins with the presence of more genes. This increase in size may translate to an increase in the plant and its organs **[32]**. Also, using several BAC clones that were explaning mapped to th papaya X/Y chromosomes, found that the presumed sex chromosomes of *J. spinosa* are homomorphic and pair completely. In other species, chromosomes had been counted with traditional means, and all were reported to have a diploid number of 2 *n* = 18. The remaining three genera have never been studied, yet are disproportionally important because, respectively, they represent the deepest divergence in the Caricaceae (*Cylicomorpha*) and the sister clade to *Carica* **[39]**. Gamma radiation, EMS, and their combinations are potent mutagens, well known for their action causing point mutations, enzyme inhibitions and chromosomal aberrations **[40]**. Sister to all New World Caricaceae is an African genus (*Cylicomorpha*) with two species. A draft of the papaya genome became available in 2008, and since then, considerable effort has gone into understanding the sex chromosomes of *C. papaya* **[41**]. All Caricaceae species are classified as diploids (2n=2x=18 chromosomes) and dioecious, except for C*. papaya*, *V. monoica* e *V. cundinarmacensis*. The plant sexual determination in papaya is due to one gene with three alleles. It was not observed sexual chromosome in their study. Thus, if there are sexual chromosomes in *C. papaya*, they are probably homomorphic **[42]**.

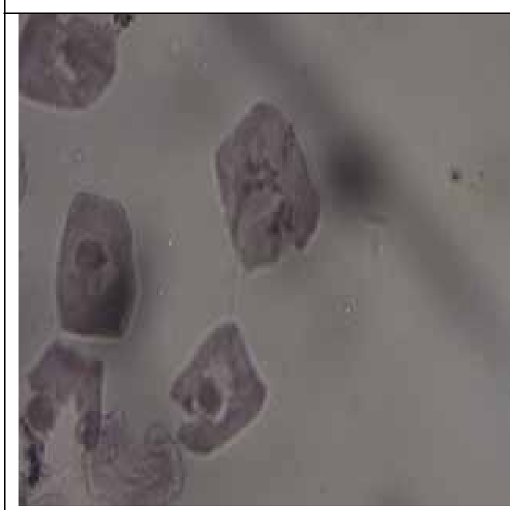
photo (4): Normal metaphase without any treatment in the mitotic cell of papaya.



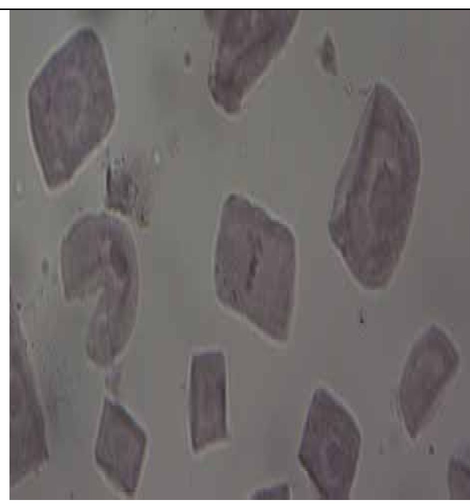
A



B



C



D

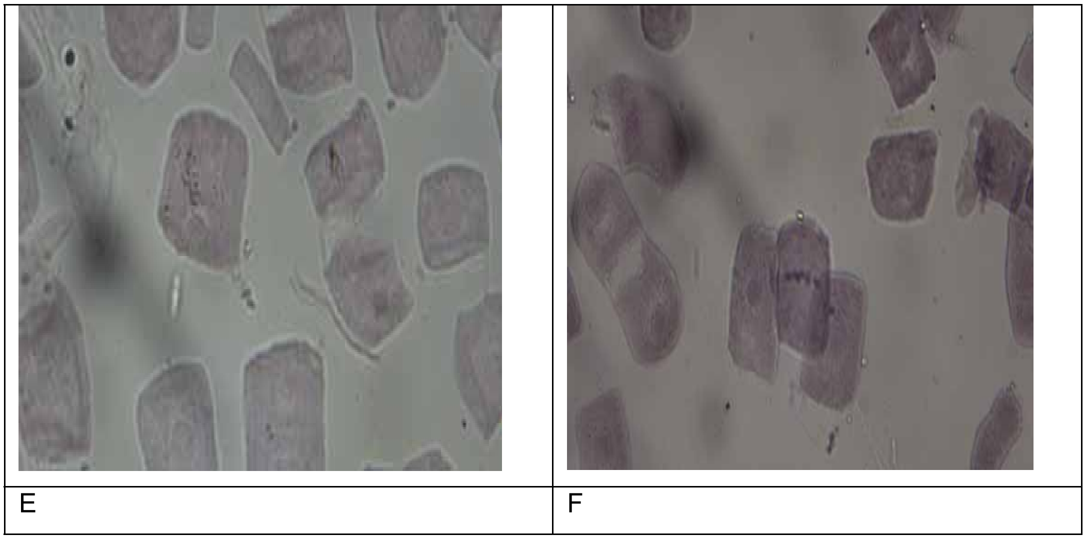


photo (5): The effect of Benzyl adenine with three different concentrations on the mitotic cells of papaya.

photo (5): The effect of Benzyl adenine with three different concentrations on the mitotic cells of papaya. photo 5-A and B anaphase with irregular distribution of chromosomes between the two poles. photo 5-C three star groups of scattering of chromosomes in a dividing cell of a root tip at the beginning of telophase. photo 5-D one fragment at the equator of the metaphase. photo 5-E irregular distribution of chromosomes at the metaphase. photo 5-F Two laggards at metaphase.

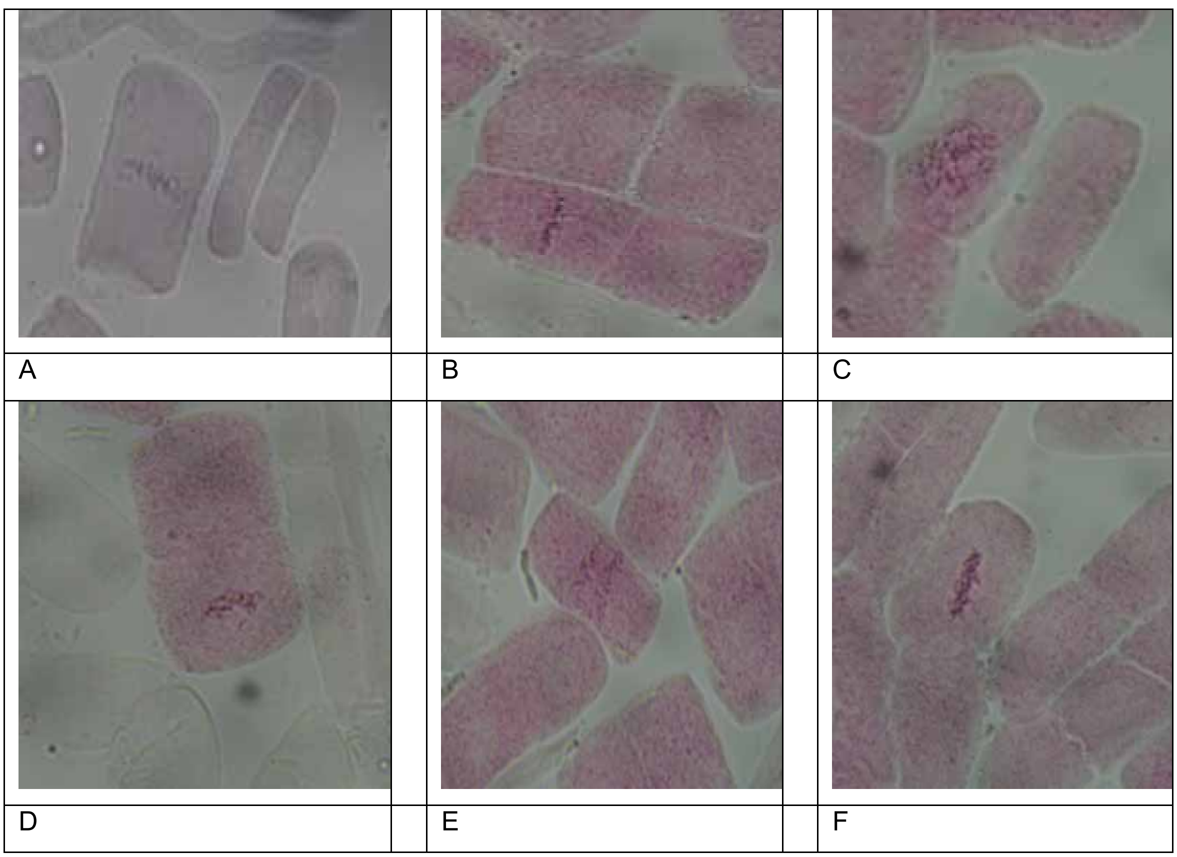


photo (6): The effect of EMS with three different concentrations on the mitotic cells of papaya.

photo (6): The effect of EMS with three different concentrations on the mitotic cells of papaya. photo 6-A and B metaphase with one lagging chromosome. photo 6-C Scattering of chromosomes in a dividing cell of a root tip at metaphase. photo 6-D one lagging chromosome at metaphase. photo 6-E irregular distribution of chromosomes at the beginning of anaphase. photo 6-F clear polyploidy in metaphase with tetraploid number of chromosomes and C-metaphase.

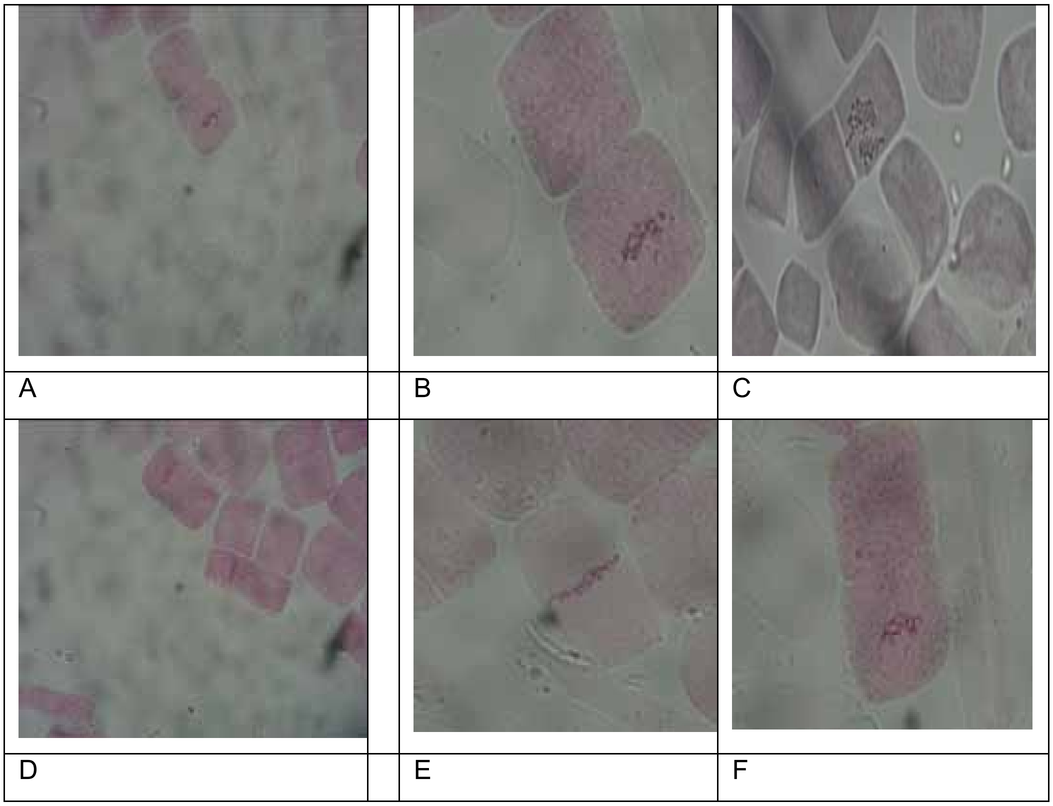


photo (7): The effect of Colchicine at three different concentrations on the mitotic cells of papaya.

photo (7): The effect of Colchicine with three different concentrations on the mitotic cells of papaya. photo 7-A metaphase with one lagging chromosome. photo 7-B metaphase with two lagging chromosomes. photo 7-C Unequal distribution of chromosomes in anaphase with polyploidy. photo 7-D sticky chromosomes at metaphase. photo 7-E metaphase with tetraploid number of chromosomes. photo 7-F scattering of chromosomes in a dividing cell of a root-tip exposed to 3% colchicine.

Table (3): Type and percentage of mitotic abnormalities in the root tips of papaya exposed to the Benzyl adenine, Ethylmethanesulphonate and colchicine with three different concentrations.

Conc. Ppm of mutagen	Total cells scors	No.of Divid. cells	MI %	Number of cells in the different phases of the cell cycle				
				Interphase	prophase	Metaphase	Anaphase	Telophase.
Control	500	92	18.4%	15.9%	2.20%	0.12	0.5	0.13
BA 1%	500	47	9.40%	8.02%	1.09%	0,10	0.04	0.15
BA 2%	500	32	6.40%	5.10%	0.98%	0.09	0.11	0.12
BA 3%	500	18	3.60%	2.11%	1.20%	0.07	0.02	0.20
Control	500	87	17.4%	14.8%	2.00%	0.22	0.08	0.0.8
EMS 1%	500	40	8.00%	6.01%	1.35%	0.16	0.04	0.28
EMS 2%	500	24	4.80%	3.00%	0.80%	0.25	0.49	0.26
EMS 3%	500	20	4.00%	2.90%	1.10%	0.00	0.00	0.00
Control	500	97	19.4%	17.95%	1.06%	0.20	0.09	0.11
Colchicine 1%	500	81	16.2%	15.05%	0.85%	0.40	0.05	0.30
Colchicine 2%	500	69	13.8%	12.00%	1.12%	0.23	0.30	0.15
Colchicine 3%	500	53	10.6%	9.00%	0.95%	0.16	0.30	0.19

4. CONCLUSION

According the results of the current study, both BA at 2% and 3% increased significantly germination %, germination rate and growth measurements. Moreover, in the presence of BA EMS inhibited the process of cell division, unlike addition of Colchicine. This effect might be explained with affected DNA-replication during mitosis by more damages, caused by BA and EMS.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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