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Mutagenic Effects of Sodium Azide and Fast Neutron Irradiation on the Cytological Parameters of M_2 Lagos Spinach (*Celosia argentea* var *cristata* L.)

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Abstract The effects of fast neutron irradiation (FNI) and sodium azide (SA) on the pollen and cytological parameters of *Celosia argentea* was carried out. M_1 seeds of treated *C. argentea* plant with fast neutron and sodium azide were collected from the seed bank of Department of Biological Sciences, Federal University of Technology, Minna, Nigeria and raised on the field to maturity stage. Young flower buds were collected from the plants for cytological studies. Cytological analysis of the plants revealed heterogeneous size of pollen grains, with three distinct variant of 29.12, 34.31 and 39.21 µm. The least average pollen diameters (32.66 µm) was recorded in 6.00 mM SA and the highest (37.58 µm) in 4.00 µS FNI. Significant variation ($p \geq 0.05$) in the numbers of pollen production per flower and anther were obtained. Lower percentage pollen fertilities were recorded in all the treated plants when compared with the control (94.15 %). However, these values were insignificance ($p \leq 0.05$), except for 8.00 mM which had the least of percentage pollen fertility of 71.62 %. The phenomenon of pollen restitution caused by abnormal meiotic division resulted in the formation of dyad, triad and tetrad in higher irradiated doses plants. Cytological analysis of the plant indicated that 8.00 µS had the highest mitotic index with metaphase (56.56) being the most frequent stage followed by telophase (28.40). Meiotic chromosomal counts revealed n = 18 at metaphase, with the formation of dyad and tetrad in most of the treated plants and the control. Abnormal meiotic division in 4.00 mM and 12.00 µS resulted in triad division. Observation from this study therefore, revealed that pollen restitution coupled with high mitotic index in 8 µS confer greater reproductive advantages to the plant.

Keywords: *celosia, sodium azide, fast neutron, pollen parameters, restitution*

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1. Introduction

Celosia argentea, commonly known as Lagos spinach, is an edible leafy vegetable of the Amaranthaceae family. It is known as "*Soko Yòkòtò*" in Yoruba and "*Farar áláyyafó*" Hausa [1]. Two common varieties of this Genus are common cockscomb (*Celosia argentea* var. *cristata*) which has a wide comb-shaped inflorescence, and feathered amaranth (*Celosia argentea* var. *plumosa*) with feather-like flowers [2]. This leafy vegetable is an essential component of people's diet in Nigeria and other parts of West Africa. The leaves and young shoots of both forms of the vegetable are used in soups and stews. The leaves contain high levels of calcium, phosphorus and iron. This plant is an important source of proteins, calories, vitamins and minerals [3] that enrich the diet the people of West Africa [4].

Different uses of Lagos spinach has led to an increase in its demand among people and inevitably followed by increased production of the crop. However, *Celosia argentea* is one of the 24 vegetable species identified by IBPGR in 1979 which showed genetic erosion and is of local importance or ranked second in priority on global scale [5]. This evidently indicates the potentiality of the crop for improvement in yields, with the present varieties under cultivation having limited yield potential. Consequently, efforts are been shifted towards improvement of the crops through mutation breeding techniques. However, the impacts of this technique on the reproductive and cytological parameters of the plant have not been fully established. Therefore, this research aim at evaluation of the effects of fast neutron irradiation and sodium azide mutagens on the pollen and cytological parameters of the plant.

2. Materials and Methods

2.1. Collection, Mutagenic Treatments and Planting of Seeds

The seeds of M_1 generation of *Celosia argentea* were exposed to fast neutron irradiation (FNI) from Americium Beryllium source (^{241}Am/Be), of flux density 1.5 × 10^4n.cm^{-2}S^{-1} for 30, 60, 90 and 120min equivalents to 0, 4, 8, 12 and 16 µSv at the Centre for Energy and Research Training (CERT), Ahmadu Bello University, Zaria, Kaduna State, Nigeria and sodium azide (NaN_3) treated seeds with concentration of 0, 2, 4, 6 and 8 mM were collected from the seeds bank, Department of Biological Sciences, Federal University of Technology, Minna. Five (5) seeds each per treatment were sown in 7cm^3 experimental pot, filled to 6 cm^3 mark with supplementary sandy-loamy soil with animal manure, arranged in randomized completely block design (RCBD). The seedlings were thinned to three per pot and raised to maturity stage during the raining season.

2.2. Pollen Fertility

Five randomly selected plants from each treatment were used for pollen fertility study. Freshly opened flowers buds were randomly collected from the selected plants in the morning hour at around 8:00am. Matured anthers of the flowers were collected and squashed on a microscopic slide. A drop of two per cent acetocarmine stain was added and covered with a cover slip. Poorly stained, shrunken and empty mature pollens were considered as sterile and the deeply stained pollen grains were counted as fertile. Five slides were prepared for each concentration and viewed from the microscopic fields. Percentage pollen fertility (PF) and injury (PI) were calculated using the formulae below [6].

$$\mathrm{PF}(\%) = \frac{\text{Number of fertile(stained) pollen grains}}{\text{Total Number of pollengrains}} \times 100 \quad (1)$$

$$\mathrm{PI}(\%) = \frac{\text{Control} - \text{treated}}{\text{Control}} \times 100 \quad (2)$$

2.3. Pollen Diameter

Diameters of 30 randomly selected pollens were measured using the Eye Piece Graticle and recorded in micrometre (µm). Photograph of pollens were taken using a ×400 photomicrograph.

2.4. Pollen Production

Pollen production was carried out using modified method of [7]. Ten flower buds were used in this study. The flowers were divided into two groups, each group contained anthers from 5 flowers in a small glass vial. One (1) ml distilled water was added into the vials and the anthers were thoroughly crushed with a glass rod into suspensions. A drop of each suspension was placed on a two-counting area of haemacytometric slide (0.1 mm in depth) and the slide was covered with a special cover slip. Randomly placed pollens in the slide were counted from five large square areas of the haemacytometer counting area. Each treatment counted was replicated six times. The average pollen grains per flower (P/F) and per anther (P/A) were determined using the formula below:

$$\mathrm{P/F} = \frac{\left(\begin{array}{l}\text{Average pollens count} \\ \times \text{Volume of fluid}(\mathrm{mm}^3) \times \text{dilution factor}\end{array}\right)}{\text{number(10) offlower}} \quad (3)$$

Where Dilution factor = 0.10

$$\mathrm{P/A} = \frac{\text{Pollen per flower}}{\text{numberofantherperflower}} \quad (4)$$

2.5. Mitotic Studies

Treated and Control seeds were plated in petri dish, lined with moist tissue paper. The healthy root tips 1 to 1.5cm were cut and pre-treated with ice-packed for 2 hours to compact the chromosomes. The root tips were then fixed in 1:3 (v/v) glacial acetic acid and alcohol solution for 24 hours to arrest cell division. These root tips were preserved in 70 % alcohol until ready for viewing. The root tips were hydrolysed in 1N HCl (hydrochloric acid) for 15 minutes and squashed on a glass slide. They were then stained in 2 % acetocarmine for 1 hour and viewed under light microscope. Frequency of each phases of cell division was counted and the Mitotic index (MI) estimated. Five replicate slides were prepared for each treatment [8].

2.6. Meiotic Studies

For the meiotic studies, young flower buds of appropriate size were fixed in 1:3 acetic acid and absolute alcohol solutions for 24 hr, after which they were transferred to 70% alcohol and stored at4°C. Five replicated slides were prepared for each treatment and control using the anther squash technique and stained in 2% acetocarmine. The stained slides were observed under microscope and photomicrographs of the observable structures were taken [8].

2.7. Statistical Analysis

The data obtained on the pollen parameters were subjected to analysis of variance (ANOVA) to determine the level of significance among the treatment while the post hoc test were carried out using Dunan's Multiple Range Test (DMRT) to separate the means where necessary. Other data obtained were expressed in percentage and as average of the total (mean).

3. Results and Discussion

3.1. Effects of Mutagens (FNI and SA) on Pollen Parameters

The result of pollen production, diameter, fertility and percentage pollen injury are presented in Table 1 and Table 2 respectively; the results indicates that significant variations ($p \leq 0.05$) have been induced in some treatments by the mutagens. A decrease in pollen production was recorded in all the treatment plants when compared with the control. The control plant had pollen productions of 92,750.00 per flower and 15458.00 per anther. The highest pollen production (111,000.00 per flower and 18500.00 per anther) among the treated plant was recorded in 6.00 mM Sodium Azide plants while the least (33,000.00 per flower and 5500.00 per anther) was recorded in 12 µS. The significant difference in the number of pollen produced per flower and anther observed in this study might be attributed to slight changes in the genetic

composition of the plant due to mutagenic action. This result is similar to the work of [9]. They reported that the significant difference in pollen production among quince cultivars could be due to either genetic pattern and/or environmental growing conditions of the quince cultivars.

Table 1. Effects of Mutagens on Pollen Production and Diameter of *Celosia argentea*

Treatments	Pollen Production Per flower ($\times 10^2$)	Pollen Production Per anther ($\times 10^2$)	Pollen Diameter (µm)
Control	927.50±3.49^{cd}	154.58±2.63^{cd}	35.95±1.14^{bc}
Buffer	777.50±1.50^{c}	129.58±0.42^{c}	35.62±0.58^{ab}
2.0mM	380.00±4.19^{ab}	63.33±2.64^{ab}	34.30±0.49^{ab}
4.0mM	467.50±4.69^{ab}	77.92±2.78^{ab}	35.29±0.52^{ab}
6.0mM	1110.00±4.11^{d}	185.00±1.85^{d}	32.66±7.78^{a}
8.0mM	660.00±1.49^{bc}	110.00±0.51^{bc}	35.95±1.14^{bc}
4.0 µS	787.50±5.11^{c}	131.25±3.16^{c}	37.58±0.92^{c}
8.0 µS	400.00±3.54^{ab}	66.67±2.27^{ab}	35.29±1.28^{ab}
12 µS	330.00±4.16^{a}	55.00±2.53^{a}	34.97±1.25^{ab}
16 µS	470.00±2.12^{ab}	78.33±1.24$^{ab`}$	37.26±1.96^{bc}

Values are Means ± Standard Error, followed by the same letter(s) along the column are not significantly different at p> 0.05 as tested by DMRT.

The pollen diameter measurement showed that there were variation in the sizes of *C. argentea* pollens, with the three pollen sizes observed been 29.41 µm, 34.31 µm and 39.22 µm (Figure 1A). A significant variation ($p \leq 0.05$) in the mean diameters of pollen was obtained, with 4 µS irradiated plant having the highest (37.58 µm) while 6 mM sodium azide treated plants had the least (32.66 µm) as shown in Table 2. The variation in the mean size of pollens recorded is in agreement with the work of [10] who observed variation in the size and number of microspore in haxaploid *C. argentea*. He reported that the variations could be due to aberration in microsporogenesis resulting from high degree of meiotic irregularity. Also, in conformity with the results of this study, [11] reported impact of cytomisis on the variation of pollen grain size in wild population of wild Himalayan poppy (*Meconopsis aculeate* Royle).

Table 2. Effects of Mutagens on Pollen Restitution, Fertility and injury of *Celosia argentea*

	Actual Pollen	Pollen Restitution (%)	Pollen Fertility (%)	Pollen Injury (%)
Control	140	-	94.15±2.92^{b}	-
Buffer	69	-	90.11±3.32^{ab}	4.27±1.52^{ab}
2.0Mm	112	-	75.82±4.20^{ab}	19.44±3.65^{cd}
4.0mM	88	-	89.90±1.15^{ab}	4.52±0.22^{ab}
6.0Mm	117	-	92.99±2.89^{b}	1.23±0.07^{a}
8.0Mm	93	-	71.62±3.12^{a}	23.93±4.62^{d}
4.0 µS	118	-	84.51±4.57^{ab}	10.28±4.02^{ab}
8.0 µS	128	24.56±3.12	76.88±4.65^{ab}	18.49±5.11^{c}
12 µS	467	32.56±4.26	79.02±3.02^{ab}	16.06±3.33^{c}
16 µS	65	19.56±3.32	85.72±2.57^{ab}	8.53±1.80^{b}

Values are Means ± Standard Error, followed by the same letter(s) along the column are not significantly different at p> 0.05 as tested by DMRT.

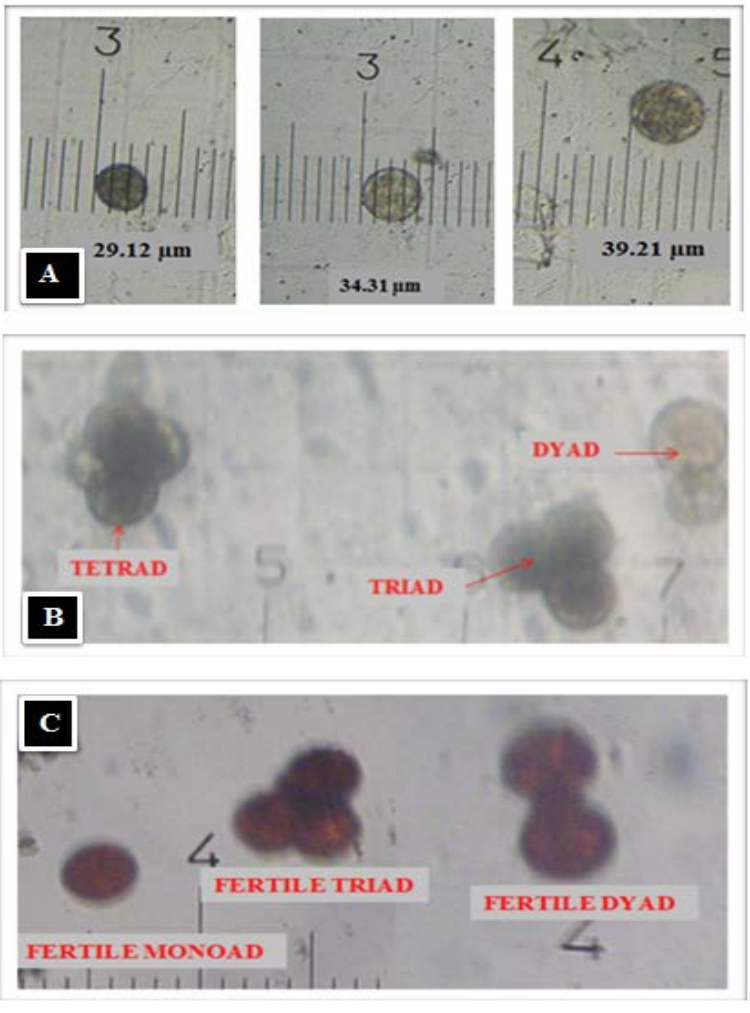

Figure 1. (A) Variation in Pollen Sizes (Diameter), (B) Pollen Restitution 8 µS and 12 µS FNI (C) Fertile Pollen Restitution of 8 µS and 12 µS FNI

A reduction in percentage fertility was recorded for all the treated plants when compared with the control. With the exception of 8 mM which had the least percentage fertility (71.62%), statistical analysis showed that there were no significant differences ($p \geq 0.05$) among the treated plants and the control. Estimations of pollen injury showed that there was significant variation ($p \leq 0.05$) in the level of damages caused on the pollen by Sodium Azide mutagen. The highest pollen injury (23.93) was recorded in 8 mM Sodium Azide while the 6 mM had the least (1.23). However, 16 µS FNI causes the least (8.53) damages on the pollen among the irradiated exposed plants (Table 2). These results conform with the work of [12] who studied pollen in mung bean. He opined that mutagenic treatments bring about a reduction in pollen fertility. Also, in agreement with this work, [13] reported that maximum pollen sterility was induced by SA treatments. Reference [14] affirm that radiation induced sterility, might be due to the detectable chromosomal aberrations and cryptic deficiencies while the sterility induced by chemical (EMS) might be due to cryptic deficiencies and specific gene mutations.

Pollens restitution was also observed in the 8 µS and 12µS FNI, with the pollens clumping-up into dyad, triad and tetrad. However, these pollens were found to be fertile when stained with acetocermain (Figure 1B & C). The observation of pollens clumping in monoads, dyads, triads and tetrads could be due to abnormal meiotic activities. This result conform with the report of [15] who discovered abnormalities such as chromosomal bridges, lagging chromosomes, micronuclei, monads, dyads and triads during cytological analysis of the microsporogenesis on *Agave tequilana* and *A. angustifolia*. This occurs due to abnormal meiotic divisions. The first division restitution, abnormal meiosis takes place with the formation of many univalents, and according to [16], it is a cellular mechanism for terminating the prolonged first division. Nevertheless the resultant restitution formed unreduced pollen. The restitution following the second meiotic division in pollen formation yields 2n pollen. Triad formation occurs as a result of second division restitution [17]. Often, such pollens are fertile which agrees with the result of this study [18].

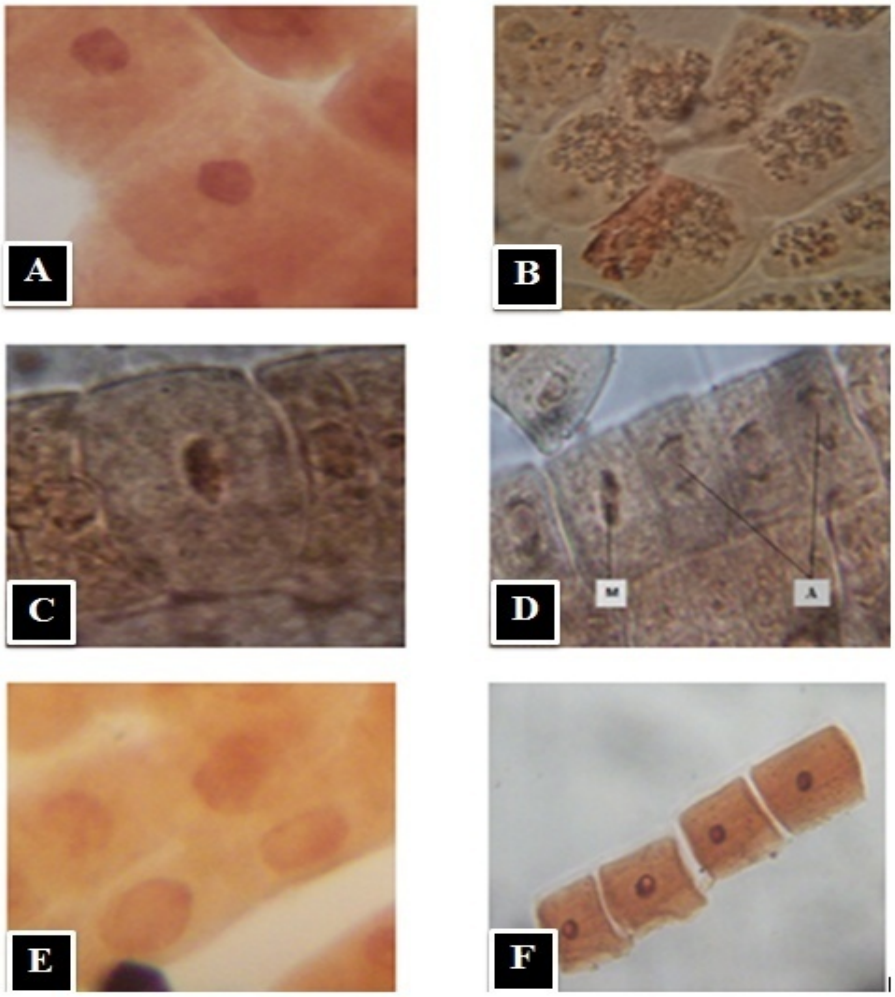

Figure 2. Mitotic Division Stages of *Celosia argentea* (A) Interphase, (B) Restitution Prophase, (C) Metaphase, (D) Late Metaphase and Anaphase stages, (E) Early Telophase, (F) Late Telophase

3.2. Cytological Studies of Effects of Fast Neutron Irradiation and Sodium Azide

Mitotic activities observed in the cells of the treated plants and the control are presented in Table 3. Estimated mitotic index values showed that with the exception of 12 µS irradiated plants which had the highest percentage of 95.68%, mitotic index values obtained where lower in all the treated cells than that of the control (83.33). 6 mM sodium azide treatements had the least value (64.71). The

most occurring mitotic stages is the metaphase (56.50) followed by the telophase (28.40). Figure 2 showed some normal mitotic stages; interphase, prophase, metaphase, anaphase and telophase observed. The decreased in mitotic index of the treated plants compared with the control corroborate with the reports of [19,20]. It was reported that there were inhibitory effects of NaN_3 on the mitotic index. This means that NaN_3 can have a genotoxic and mutagenic effects. Similar effect of NaN_3 was observed on barley seedlings by [19]. They opined that ATP deficiency caused by sodium azide may be one of the reasons for the decrease in the mitotic index and demands for ATP of dividing cells are much higher as compared to non proliferating cells.

Table 3. Effects of Sodium Azide and Fast Neutron Irradiation on Mitotic Activities of *Celosia Argentea*

Conc	Interphase	Prophase	Metaphase	Anaphase	Telophase	Total Dividing Cell	Total Cell	Mitotic Index
Control	12	2	59	3	24	100	120	83.33
Buffer	-	5	39	2	36	82	108	75.93
2Mm	37	4	42	5	23	111	154	72.08
4Mm	-	2	82	-	9	93	123	75.61
6Mm	9	1	17	4	13	44	68	64.71
8Mm	-	2	32	4	39	77	97	79.39
4.0 µS	14	-	43	-	51	108	134	80.60
8.0 µS	32	3	139	20	29	223	297	75.09
12 µS	52	-	76	10	17	155	162	95.68
16 µS	7	3	36	2	43	91	118	77.12
Mean	23.29	2.75	56.50	6.25	28.40	108.40	138.10	77.95

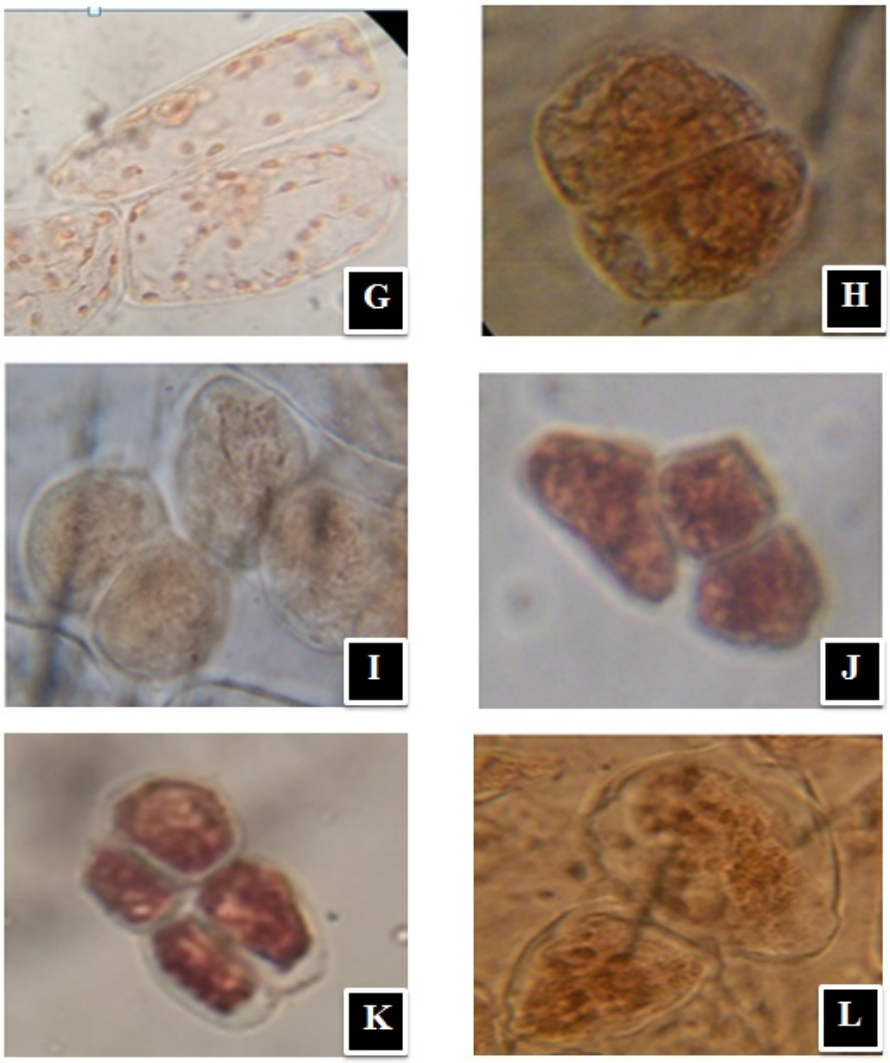

Figure 3. Meiotic Division of *Celosia argentea* (G) Meiotic Chromosomes n = 18 at metaphase stage, (H) Telophase I stage, (I) Dyad chromosome, (J) Triad chromosomal division due to abnormal meiotic division at 4 mM and 12 µS FNI, (K) Tetrad chromosome and (L) Nuclear restitution at 12 µS

Meiotic chromosomal counts of n = 18 at metaphase stage of 8 µS FNI plant was observed (Figure 2). This agrees with the result of [10] who reported that parental species of *C. argentea* exhibited typical meiotic behaviour with 18 and 36 bivalent chromosome at metaphase. The tetrad chromosomal stages was observed in most of the

treated plants with the exception of 4 mM and 12 µS FNI where triads was observed. This indicated that chromosomal division was normal; without any aberration in most of the treated plants and control Figure 3. In addition, meiotic nuclear restitution observed in 12 µS is an indication of abnormalities in meiotic divisions. The nuclear restitution observed in 12 µS FNI confirmed the formation of 2n pollen resulting to dyad and triad. This conformed to the results of [21]; who reported that meiotic nuclear restitution mechanisms occurred following the failure of the reductional wall or, alternatively, of the equational wall. They further stated that analysis of sporads evidenced tetrads as well as dyads, triads and rare monads and poliads. Also, the formation of restitution nuclei in *C. argentea* was attributed to the chromosomes remaining on spindle at telophase after division [10].

4. Conclusion

This study revealed that sensitivity of *Celosia argentea* to different levels of SA and FNI had positive effects on pollen and cytological parameters of the plant. Significant valuable variability was induced in the pollen size and fertility. Cytological characterization of the plant confirms 2n=32 (diploid) chromosome of the plant. The occurrence of meiotic restitution mechanisms coupled with higher mitotic index in 8.00 µS is an evidence of the formation of balanced gametes which contribute to increase the pollen fertility. The study further affirm that pollen restitution and reduction in mitotic index in mutagenised plants might be an indication of disturbance at cellular level or physiological activities of the cell due to mutagenic action.

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