



## Research Article

### Pollen Viability and Germinability of Gamma Irradiated M<sub>4</sub> Lines of Sesame

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#### Abstract

Pollen viability and germinability are indispensable criteria in plant breeding programmes. This research evaluated eleven M<sub>4</sub> mutant lines of gamma irradiated sesame (*Sesamum indicum*) for some pollen parameters. Seeds were obtained from the Department of Plant Biology, Federal University of Technology, Minna and were raised to maturity alongside their respective checks in a randomized complete block design (RCBD). The field experiment was conducted at the experimental field of the Upper Niger River Basin Development Authority, Minna. Pollen viability and diameter were determined using standard procedure. The germinability test was done using three different sucrose concentrations (10 %, 20 % and 30 %) with 1 % nutrient agar solution. The results revealed that all the M<sub>4</sub> mutant lines had adequate pollen viabilities (over 80%) with ML-10 having the highest (97.56 %) viability followed by ML-7 (95.61 %), ML-8 (95.01 %) and Check-2 (95.60 %). The highest pollen germinability was recorded at 20 % sucrose concentration for all the mutant lines with line ML-7 (39.70 %) having the highest percentage. Check-1 at 10 % sucrose concentration recorded the least percentage (11.46 %) across the concentration and treatments. Highest pollen diameter (169.52µm) was recorded in Check-1. Suboblate shapes with 10-13 colpi was observed in all the mutant lines and the checks. Pollens from all the lines comprised of circular and elliptic pollens except in ML-7 and the checks where the pollens were solely circular in polar view. The study revealed that gamma-irradiation could be a reasonable tool for inducing variability in sesame and advantageous in increasing the pollen viability.

**Keywords:** Gamma irradiation, Mutant lines, Pollen germinability, Pollen viability, Sesame

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#### Introduction

Sesame (*Sesamum indicum* L.) is the most traditional and the oldest oil seed crop valued for its high oil quality (Sruba and Amitava, 2017). Iqbal *et al.* (2018) opined that oil seed crops account for the major agricultural crop after food grains and sesame is among the most important edible vegetable oil crops and it has been nicknamed “Queen of oil seeds” due to its high quality oil. Sesame oil is used as edible oil in dishes, for making salad oils and margarine, cake, paste and for other confectionary purposes (Rizki *et al.*, 2015). The production of sesame is however relatively low (global production of 4.04 million tons annually) compared to other oil seed crops (Hota *et al.*, 2016). Attempt had been made to improve on the production rate using various

approaches such as improvement in cultural practices and development of improved cultivars through hybridization and mutation breeding.

Mutation breeding has been identified as a viable tool for improvement of crop plants (Girija and Dhanavel, 2013). However, in Africa and Nigeria in particular, the tool has not been adequately utilized. In fact, in the recently released lists of mutant varieties, no single mutant variety of sesame has been released in Nigeria (International Atomic Energy Agency, 2019). This might be due to lack of continuity in mutation breeding programmes.

Pollination and fertilization in self-pollinated crops like sesame are essential for fruits formation

(Abejide *et al.*, 2013) and pollen grains produced serve as one of the most valuable sources of evidence for phylogenetic studies and clarification of higher level relationships (Akhila and Beevy, 2015). Pollen viability connotes the ability of pollen to complete post pollination events and to effect fertilization while germination capability of pollen is the pollen's ability to elongate into a pollen tube (Khan and Perveen, 2008). Pollen viability may not automatically translate into pollen germinability as pollen germinability is dependent on so many factors (Abdullateef *et al.*, 2012). The quality of pollens lies in their ability to possess both traits. The extent of pollen viability and germinability in any plant is an indication of the effectiveness of its male parent or pollinator.

However, numerous works have been done on diversity of pollens in different mutated crops, such as direct irradiation of matured pollens like in sunflower by Aktas *et al.* (2018), in cotton by Aslam *et al.* (2018), Kiwi fruit by Lopes *et al.* (2020) and in Melon by Ivanova, (2020). Irradiation has been used to improve pollen germinability in sesame by Falusi *et al.* (2013). It has been used to cause variation in pollen viability of sesame by Bashir *et al.* (2013), Kumari *et al.* (2016), Ariharasutharsan *et al.* (2019) and Khah and Verma (2020). In most of these works, early generation of mutants have been the focus. In the present study, advanced generation (M<sub>4</sub>) lines were assessed for variation in pollen morphological parameters in respect to viability and germinability.

### Materials and Methods

The research was conducted at the experimental field of Upper Niger River Basin Development Authority, Minna, Niger State, and Laboratory of Department of Plant Biology, Federal University of Technology, Minna, Nigeria. The experimental materials comprising of eleven (11) mutant lines and three (3) checks were obtained from breeding line in the Department of Plant Biology, Federal University of Technology, Minna, Nigeria.

Originally, three varieties (NCRIBEN 04E, NCRIBEN 01M and NCRIBEN 03L) (Table 1) were exposed to four different doses of gamma radiation (250 Gy, 350 Gy, 450 Gy and 550 Gy) to generate twelve treatment combinations. The

twelve treatments were raised alongside the three parental stocks as checks making it a total of fifteen treatment combinations to generate M<sub>1</sub> population. In M<sub>2</sub> generation, eight mutant lines were selected and evaluated to generate M<sub>3</sub> lines. Some of the M<sub>3</sub> lines segregated and a total of eleven M<sub>4</sub> lines were obtained and used as the experimental materials for this work (Table 2).

Seeds of the eleven (11) mutant lines were grown in progeny plots alongside their respective checks. For each entry, two hundred and eighty (280) plants were raised. Each plot size was 11.2m x 5m. Inter and intra row spacing were 40 cm and 25cm respectively. Four seeds were sown per hole and were later thinned to two plants per stand at two weeks after planting (WAP). The experiment was conducted between the months of August, 2019 and December, 2019 and the recommended agronomic and plant protection practices by International Plant Genetic Resource Institute and National Bureau of Plant Genetic Resources (IPGRI & NBPGR, 2004) were followed. At maturity, the flower buds of each mutant line and controls were collected for pollen viability and germinability test.

**Pollen viability test:** Pollen viability test was conducted following the method of Abejide *et al.* (2013). Ten flower buds were collected from five randomly selected plants of each mutant line as well as control. A drop of 2 % Aceto-carmin stain was placed on a cleaned, dried glass slide. Pollens from the flowers were carefully transferred unto the stain by tapping the flowers at a short distance above the stain layer. Three flowers were used per slide in order to transfer enough pollen for microscopic observation. The preparation was left for 30 minutes to allow pollens pick up enough stain. Afterwards, the slides were mounted on a light binocular microscope for observation and pollens were examined at magnification of ×40. The viability was scored according to the staining level. Pollens that were well stained were considered viable, those that were slightly stained were considered semi-viable and pollens that were not stained were considered non-viable. Percentage pollen viability (PPV) was calculated as:

$$PPV = \frac{\text{number of well stained pollen grain}}{\text{total number of pollen grains}} \times 100$$

**Pollen germinability test:** Pollen germinability test was conducted following the procedure of Abejide *et al.*, 2013. Different concentrations (10 %, 20 % and 30 %) of sucrose solution were prepared by adding 10 g, 20 g, and 30 g of sucrose to 1 g of nutrient agar and 100 ml of distilled water respectively. The prepared mixture was properly stirred and evenly spread on a petri dish and pollens were sprinkled onto the medium gently by tapping the flowers at a short distance above the petri dish. The petri dishes were properly covered to prevent loss of water and were kept at room temperature ( $\pm 28$  °C) for 24 hours. After the 24 hours, the petri dishes were stored in a refrigerator and the pollen's germinability was observed. Three petri dishes were used per sucrose concentration for each mutant line and the pollen germinability was expressed in percentages. Pollens with protrusions around the edges were considered to have germinated. Percentage pollen germinability (PPG) is calculated as:

$$\text{PPG} = \frac{\text{number of germinated pollen grain}}{\text{total number of pollen grains}} \times 100$$

**Pollen morphology (diameter and shape):** The diameter of thirty (30) different pollens selected at random were measured from each slide using the microscope eye piece graticule measuring glass (Abubakar *et al.*, 2015) and pollen shapes were determined by visual examination.

The data generated were subjected to one way analysis of variance (ANOVA) at  $P < 0.05$  to test for significant difference among the means and Duncan's Multiple Range Test (DMRT) was used to separate the means where there were significant differences.

## Results and Discussion

**Pollen viability:** Highest pollen viability was observed in ML-10 (97.56%) which was significantly different from ML-1 (86.70%) but was significantly the same with pollen viability observed in other mutant lines (Table 3). The least pollen viability was observed in ML-1 (86.70%). No significant difference was observed in number of semi-viable pollens. This result depicts that the pollens of irradiated  $M_4$  lines of sesame have a high level of viability varying from 86.70 % to 97.56 % (Table 3). Similar findings have been

previously reported by Falusiet *al.* (2001) in *Sesamum radiatum* and *Sesamum indicum*. Also, Abejide *et al.* (2013) who studied three sesame cultivars observed high pollen viability in irradiated sesame cultivars. On a contrary, Singh *et al.* (2018) has reported up to 50% reduction in pollen viability of  $M_1$  irradiated sesame. The differences in pollen viability percentages could be attributed to varietal differences. The fertility of male plants is dependent on its pollen viability and germinability (Tunistra and Wedel, 2000) and a monoecious plant with high pollen viability have greater tendencies of producing high seed set percentage. This finding also further explain that sesame mutant seeds still maintain their pollen viability and that certain doses of gamma-irradiation viz (350 Gy, 450 Gy and 550 Gy) did not hinder pollen viability.

**Pollen shape and size:** The pollen grains were suboblate in shape in all the mutant lines investigated (Figure 1c). No variations were observed in pollen shape of the mutant lines and control groups. This finding is in line with the work of Damaiyani *et al.* (2020) who reported that gamma irradiation as high as 600 Gy does not affect the pollen morphology of sesame plants.

Notable disparities were however observed in range of pollen grain size, the highest grain size was observed in Check-1 (169.52  $\mu\text{m}$ ) and was significantly different ( $p < 0.05$ ) from grain size of ML-1 (138.02  $\mu\text{m}$ ), ML-2 (144.00  $\mu\text{m}$ ), ML-6 (139.50  $\mu\text{m}$ ), ML-7 (106.52  $\mu\text{m}$ ), ML-8 (138.02  $\mu\text{m}$ ), Check 2 (138.02  $\mu\text{m}$ ) and ML-11 (147.02  $\mu\text{m}$ ). The least grain size was observed in ML-7 (106.52  $\mu\text{m}$ ). The large pollen grains observed had different number of colpi varying from 10-13 and were either circular or elliptic in polar view (Figure 1b). A few elliptic pollens were observed in all the mutant lines except in ML-7 and the checks. The range of pollen size recorded in this study is in line with findings of Akhila and Beevy (2015) who reported colpi range of 11-13 with circular and elliptic polar view characteristics in *Sesamum indicum*.

**Pollen germinability:** Based on the observations from this study, pollen germinability and pollen tube growth rate were not synchronous. Similar findings have been reported by Gaaliche *et al.*

(2013) who observed heterogeneous pollen germination rate and pollen tube growth in *Ficus carica* (L.) cultivars cultured in the same media. Varying germination rates observed indicates that germinability is influenced by various conditions such as nutrition conditions of the plants, suitable in vitro conditions and other environmental factors such as temperature and humidity. Similar claims have been made by Abejide *et al.* (2013). The least (11.46%) pollen germinability was observed in Check-1 at 10 % sucrose concentration and was significantly different ( $p < 0.05$ ) from germinability observed in ML-2 (19.80 %), ML-8 (22.81 %) and ML-9 (20.36 %). The highest pollen germinability at 10% and 20 % sucrose concentrations were recorded in ML-8 (22.81%) and ML-7 (39.70 %), respectively. The least pollen germinability at 20 % sucrose concentration was observed in Check 2 (24.23 %) and was significantly the same ( $p > 0.05$ ) with Check 1 (24.67 %) and Check 3 (24.40 %). At 30 % sucrose concentration, the highest pollen germinability was observed in ML-7 (21.24 %) while the least pollen germinability was observed in Check 1 (12.81 %) and was statistically the same with ML-10 (13.15) (Table 3). The least pollen germinability was observed in the checks: Check-1 with the value of 11.46 % at 10 % sucrose concentration, Check-2 (24.23 %) at 20 % sucrose concentration and Check-3 (12.81 %) at 30 % sucrose concentrations. These results revealed that gamma ray irradiation could result to increase in the germinability of the pollens.

Although values obtained on pollen germinability were lower compared to pollen viability, 20 % sucrose concentration proved to be the optimum concentration for pollen germinability test in this study (Figure 2b). This is contrary to the work of Liqin *et al.* (2007) who reported high germinability of up to 58% at 35% sucrose concentration but in line with reports of Abejide *et al.* (2013). This could be due to varietal differences. Higher germinability percentages, than the checks, were observed in all the M<sub>4</sub> mutant lines at 20 % concentration. This could be attributed to the stimulatory effect of gamma-irradiation on sesame pollen germination. In line with the results of this study, Falusi *et al.* (2013) also reported an increase in pollen germinability percentages following Fast Neutron Irradiation (FNI) in three Nigerian sesame cultivars. On a contrary, Bashir *et al.* (2013),

Kumari *et al.* (2016) and Ariharasutharsanet *al.* (2019) had reported a reduction in pollen fertility of some irradiated sesame. Also in the works of Ariraman *et al.* (2018) on *Cajanus cajan* and Khah and Verma (2020) on Barley, similar reductions were observed.

The pollens germinability observed in all the mutant lines and the checks in all sucrose concentrations were generally low in this study. This could be attributed to pollen tube rupture, environmental factors and differences in the composition of the germination medium as against the in vivo environment. Similar reports have been made by Gilissen (1978) who recorded low pollen germination in vitro study of *Petunia* sp. and attributed this to possible differences in germination medium and stigmatic exudates between the in vivo and in vitro environment.

### Conclusion

In conclusion, this study has revealed that all the M<sub>4</sub> mutant lines had high percentage pollen viabilities which could be beneficial in the crop's improvement. This study has also revealed that 20 % sucrose concentration is the most suitable concentration for pollen germinability studies in sesame.

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**Table 1: Description of parental stock**

Agronomic traits	Variety		
	NCRIBEN 04E	NCRIBEN 01M	NCRIBEN 03L
Number of capsule/axil	Multicapsular	Unicapsular	Unicapsular
Number of carpels/capsule	Bicarpellate	Bicarpellate	Bicarpellate
Maturity	Early	Mid	Late
Nature of capsule	Dehiscent	Dehiscent	Dehiscent

**Table 2: Description of research materials**

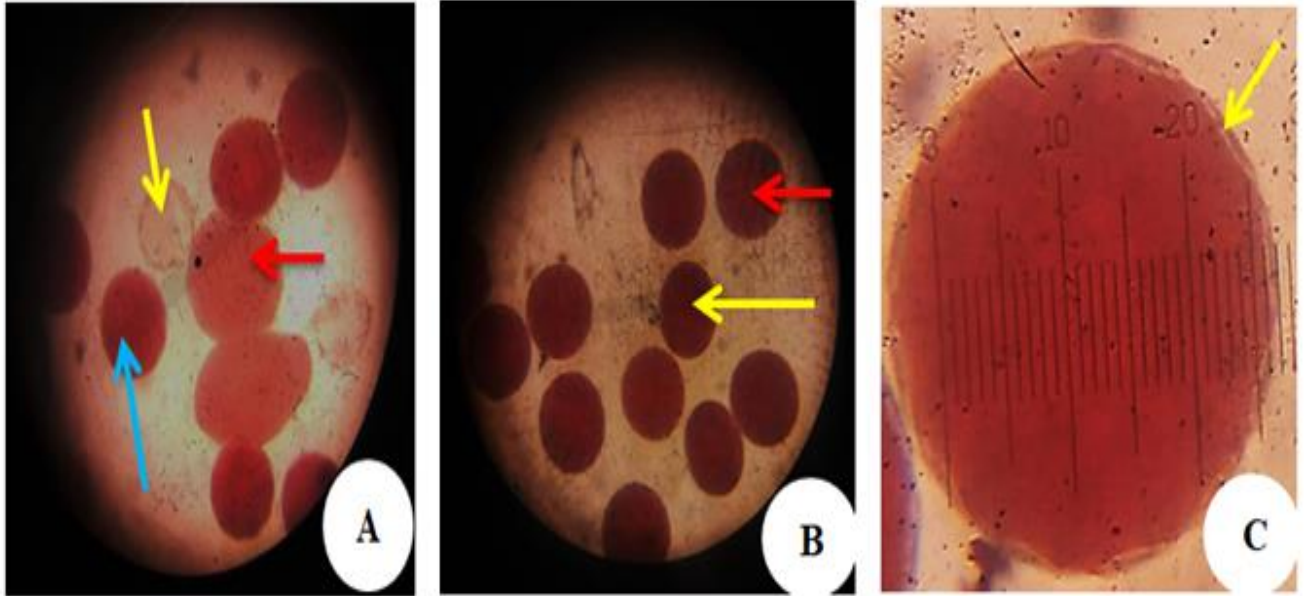
Mutant lines	Mutant Name	Major Features
ML-1	04E450G <sub>1-3</sub>	3 carpels/capsule, single capsule per leaf axil
ML-2	04E450G <sub>2-3</sub>	2 carpels/capsule, single capsule per leaf axil
ML-3	04E450G <sub>3-3</sub>	2 carpels/capsule, 2-3 capsules per leaf axil
ML-4	01M350G2-22	2 carpels/capsule, 2-3 capsules per leaf axil
ML-5	01M350G <sub>1-21</sub>	3-4 carpels/capsule, 2-3 capsules per leaf axil
ML-6	01M550G <sub>2-2</sub>	2 carpels/capsule, single capsule per leaf axil
ML-7	01M350G <sub>1-2</sub>	3 carpels/capsule, single capsule per leaf axil
ML-8	03L550G <sub>1-2</sub>	2 carpels/capsule, single capsule per leaf axil
ML-9	03L450G <sub>2-2</sub>	3 carpels/capsule, single capsule per leaf axil
ML-10	03L250G <sub>1-1</sub>	2 carpels/capsule, single capsule per leaf axil
ML-11	03L250G <sub>1-11</sub>	3 carpels/capsule, single capsule per leaf axil

**Table 3: Pollen parameters of M4 mutants of gamma-irradiated sesame**

Mutant lines	Pollen Physical Parameters				Pollen Germination Percentage		
	VP (%)	SVP (%)	PDC ( $\mu\text{m}$ )	PDE ( $\mu\text{m}$ )	10%	20%	30%
MI-1	86.70 $\pm$ 3.80 <sup>a</sup>	4.28 $\pm$ 0.27 <sup>a</sup>	138.02 $\pm$ 0.88 <sup>b</sup>	117.00 $\pm$ 2.00	11.65 $\pm$ 2.26 <sup>a</sup>	32.11 $\pm$ 3.29 <sup>abc</sup>	19.05 $\pm$ 1.76 <sup>b</sup>
MI-2	94.53 $\pm$ 0.79 <sup>ab</sup>	1.06 $\pm$ 0.05 <sup>a</sup>	144.00 $\pm$ 1.15 <sup>ab</sup>	109.49 $\pm$ 0.88	19.80 $\pm$ 5.26 <sup>b</sup>	36.03 $\pm$ 1.98 <sup>bc</sup>	19.16 $\pm$ 4.81 <sup>b</sup>
MI-3	93.26 $\pm$ 2.63 <sup>ab</sup>	1.87 $\pm$ 0.10 <sup>a</sup>	156.02 $\pm$ 1.45 <sup>abc</sup>	109.49 $\pm$ 0.88	15.29 $\pm$ 3.01 <sup>ab</sup>	38.23 $\pm$ 0.81 <sup>bc</sup>	20.83 $\pm$ 1.18 <sup>b</sup>
Check1	92.62 $\pm$ 2.24 <sup>ab</sup>	1.83 $\pm$ 0.31 <sup>a</sup>	169.52 $\pm$ 1.76 <sup>c</sup>	**	11.46 $\pm$ 0.24 <sup>a</sup>	24.67 $\pm$ 2.40 <sup>a</sup>	12.81 $\pm$ 2.14 <sup>a</sup>
MI-4	92.97 $\pm$ 2.84 <sup>ab</sup>	1.86 $\pm$ 0.12 <sup>a</sup>	165.02 $\pm$ 1.20 <sup>bc</sup>	108.00 $\pm$ 1.15	12.15 $\pm$ 3.35 <sup>a</sup>	34.13 $\pm$ 3.70 <sup>abc</sup>	20.58 $\pm$ 1.92 <sup>b</sup>
MI-5	90.63 $\pm$ 2.46 <sup>ab</sup>	1.44 $\pm$ 0.09 <sup>a</sup>	156.02 $\pm$ 1.45 <sup>abc</sup>	99.00 $\pm$ 0.58	15.17 $\pm$ 1.84 <sup>ab</sup>	35.77 $\pm$ 1.13 <sup>bc</sup>	16.05 $\pm$ 4.43 <sup>ab</sup>
MI-6	91.90 $\pm$ 3.04 <sup>ab</sup>	0.77 $\pm$ 0.08 <sup>a</sup>	139.50 $\pm$ 0.58 <sup>b</sup>	117.00 $\pm$ 1.15	15.60 $\pm$ 7.06 <sup>ab</sup>	30.57 $\pm$ 3.13 <sup>abc</sup>	14.02 $\pm$ 1.68 <sup>ab</sup>
MI-7	95.61 $\pm$ 1.03 <sup>b</sup>	0.97 $\pm$ 0.06 <sup>a</sup>	106.52 $\pm$ 0.33 <sup>a</sup>	**	15.00 $\pm$ 1.40 <sup>ab</sup>	39.70 $\pm$ 4.75 <sup>c</sup>	21.24 $\pm$ 7.80 <sup>b</sup>
Check2	95.60 $\pm$ 1.18 <sup>b</sup>	0.96 $\pm$ 0.14 <sup>a</sup>	138.02 $\pm$ 0.67 <sup>b</sup>	**	15.31 $\pm$ 1.83 <sup>ab</sup>	24.23 $\pm$ 1.55 <sup>a</sup>	15.63 $\pm$ 3.70 <sup>ab</sup>
MI-8	95.01 $\pm$ 1.69 <sup>b</sup>	1.55 $\pm$ 0.27 <sup>a</sup>	138.02 $\pm$ 0.67 <sup>b</sup>	117.00 $\pm$ 1.15	22.81 $\pm$ 1.66 <sup>b</sup>	38.71 $\pm$ 4.48 <sup>bc</sup>	19.71 $\pm$ 1.30 <sup>b</sup>
MI-9	89.35 $\pm$ 1.33 <sup>ab</sup>	2.21 $\pm$ 0.12 <sup>a</sup>	151.52 $\pm$ 0.88 <sup>abc</sup>	117.00 $\pm$ 1.15	20.36 $\pm$ 5.18 <sup>b</sup>	38.84 $\pm$ 1.32 <sup>bc</sup>	15.37 $\pm$ 0.76 <sup>ab</sup>
MI-10	97.56 $\pm$ 0.89 <sup>b</sup>	0.57 $\pm$ 0.07 <sup>a</sup>	157.50 $\pm$ 3.05 <sup>abc</sup>	121.50 $\pm$ 0.57	18.98 $\pm$ 2.70 <sup>ab</sup>	29.05 $\pm$ 5.94 <sup>abc</sup>	13.15 $\pm$ 2.00 <sup>a</sup>
MI-11	91.77 $\pm$ 4.63 <sup>ab</sup>	2.07 $\pm$ 0.15 <sup>a</sup>	147.02 $\pm$ 1.20 <sup>ab</sup>	111.02 $\pm$ 1.20	16.39 $\pm$ 2.39 <sup>ab</sup>	28.19 $\pm$ 4.42 <sup>ab</sup>	18.70 $\pm$ 2.08 <sup>b</sup>
Check3	92.12 $\pm$ 3.48 <sup>ab</sup>	1.75 $\pm$ 0.10 <sup>a</sup>	163.49 $\pm$ 2.33 <sup>bc</sup>	**	14.55 $\pm$ 1.73 <sup>ab</sup>	24.40 $\pm$ 0.50 <sup>a</sup>	15.60 $\pm$ 2.40 <sup>ab</sup>

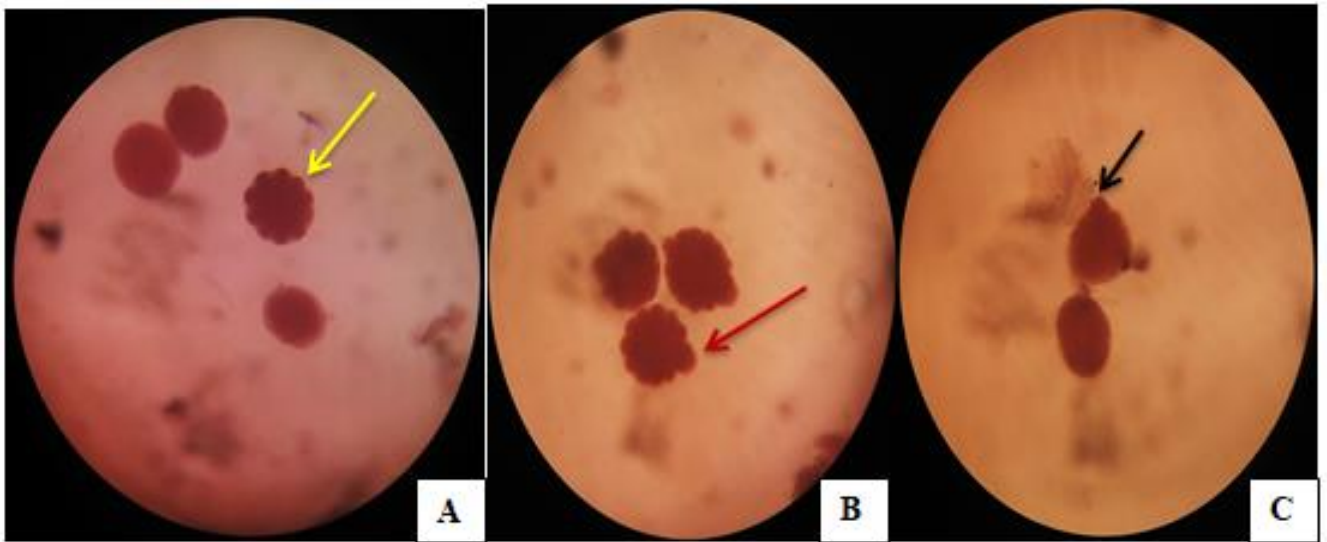
Values are mean  $\pm$  standard error of mean. Values followed by different superscript along the same column are significantly different at  $P \leq 0.05$ . VP =Viable pollen, SVP= semi viable pollen, PDC= Pollen diameter of circular pollens, PDE= Pollen diameter of elliptic pollens.

\*\* : No elliptic pollen structure



**Figure 1:** Pollen viability and pollen shape of  $M_4$  gamma-irradiated *Sesamum indicum*.

A - Pollen viability: yellow arrow showing non-viable pollen, red arrow showing a semi-viable pollen and blue arrow showing viable pollen. B - Pollen shape in polar view: red arrow showing a circular pollen and yellow arrow showing elliptic pollen in polar view. C - Pollen shape described as suboblate with yellow arrow showing the colpi.



**Figure 2:** Pollen germinability of  $M_4$  gamma-irradiated *Sesamum indicum*

A - Pollen germinability at 10% sucrose concentration, yellow arrow showing protrusions for tube growth, B - Pollen germinability at 20% sucrose concentration, red arrow showing pollen tube growth, C - Pollen germinability at 30% sucrose concentration, black arrow showing pollen tube growth