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Studies on Gamma Rays Induced Cyto-Morphological Variations and Procurement of Some Induced Novel Mutants in Kalmegh [*Andrographis paniculata* (Burm. f.) Nees]

Kshama Dwivedi[a, b, *], Kamini Kumar[a], and Girjesh Kumar[b]

[a] *Cytogenetics, Plant Breeding, Molecular Biology, and Biotechnology laboratory, University Department of Botany, Ranchi University, Jharkhand, 834001 India*

[b] *Naithani Plant Genetics Laboratory, Department of Botany, University of Allahabad, Allahabad, 211001 India*

**e-mail: kshama.dwivedi@gmail.com*

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Abstract—In order to increase genetic variability for improvement and better adaptation in the present days' changing climate, present investigation has been done in *Andrographis paniculata* (Burm. f.) Nees (also known as Kalmegh in Ayurveda), an ethno-medicinal wild plant with highly immune-boosting properties. For the purpose, healthy, dry and homogeneous seeds were treated with four doses of gamma rays from Co-60 sources at the dose rate of 1.55 Gy per second; selected on the basis of LD_{50} i.e. 25 Gy (gray), 50, 100 and 200 Gy along with one set of non-irradiated seeds and sown in triplicates in a completely randomized block design (CRBD). Higher doses were detrimental hence not suggested for further mutation breeding experiments. However, lower doses (viz. 25 and 50 Gy) of gamma rays were stimulatory; had induced some significant ($p > 0.5$) variations in several lucrative traits of *Andrographis paniculata* (Kalmegh) viz. Plant height, leaf area, number of branches per plant, length of internodes, fruit length, and seeds per fruit. Some induced novel mutants (viz. bushy, dark green leaves mutant and tricotyledonous leaf mutant in M_2 generation) have also been procured. This is, to the best of our knowledge, first report on induction of tricotyledonous leaf mutant in *A. paniculata* using gamma rays.

Keywords: *Andrographis paniculata* (Kalmegh), chromosomal aberrations, gamma rays, genetic variation, meiosis, tricotyledon, wild medicinal plants

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1. INTRODUCTION

These days use of herbal and natural ancient medicines is gaining a gradual popularity, instead of isolated or synthesized compounds for their side effects and many hazards (Roy and Datta, 1988). Hence cure through herbal care has become the need of the day due to the many side effects of allopathic drugs. Owing to possessing narrow genetic base, increasing demand for herbal drug in various medicinal systems and excess exploitation of these wild medicinal plants from their natural habitat; these are becoming more prone to extinction. Moreover, exclusively, habitats of *Andrographis paniculata* (Kalmegh) plants have also been destroyed tremendously by common people owing to lack of awareness among people about the medical benefit of this wonder herb.

Andrographis paniculata (Burm. f.) Nees (Acanthaceae) popularly known as "Kalmegh" or "green chiretta", or "Bhuineem" and "King of Bitters" is a medicinal herb of immense therapeutic values in all three traditional medicinal systems of India—(1) Ayurvedic, (2) Unani, and (3) Siddha. It is also known as "Bile on earth" due to its extremely bitter taste and also possessed highly immune boosting properties. Every part of the plant are medicinally important thus suggesting the high therapeutic value and have been used in more than 25 polyherbal formulations as a hepatoprotective, mentioned in Pharmacopoeia (Lattoo et al., 2006). Kalmegh is extensively used as a remedy for various ailments i.e. common cold and fever, respiratory tract infections, jaundice, gonorrhoea, dysentery and dyspepsia, influenza, diarrhoea, loss of scalp hair, snakebite, malaria, Infertility. The severity of several chronic diseases (asthma, piles, gastric complaints, myocardial ischemia, diabetes, HIV) can be reduced by using this magical herb (Benoy et al., 2012). Kalmegh is also used as a blood purifying agent, antipyretic, anti-inflammatory, antibiotic.

Therapeutic activities of Kalmegh is due to the presence of characteristic secondary metabolites encountered in this plant have considerably enhanced its importance in the arena of medicinal plants (Joselin and Jeeva, 2014). It contains several phytochemicals namely andrographolide and its related diterpene lactones (Srivastava et al., 2004). Andrographolide protects liver and gall bladder and could be better than any synthetic hepatoprotective drug.

Induced breeding has been in practice from a long ago and it has been demonstrated from several studies that induced mutation has practical values in the creation of enviable traits via genetic manipulation at macro level. Since then it provides unique opportunities to create new gene alleles, especially in narrow gene base plants (wild plants), that do not exist in germplasm pools. Ionizing radiations produce a wide range of effects on DNA either through free radical effects (indirect action) or direct action on DNA. Ionizing radiation induces direct DNA damage and indirect damage through the radiolysis of water. This damage is either eliminated or fixed in the cell as a mutation or chromosomal rearrangement by DNA repair processes (Morgan and Sowa, 2005).

Stimulatory impact of low doses of gamma rays have been extensively used by plant breeders for improving various crop varieties which were either deprived of or bearing poor qualitative, polygenic and other stress-tolerant traits. While in the present day, excess release of the radioactive contaminations by the nuclear reactors, nuclear weapons, etc. have been monitored and is matter of serious concerns.

Dicotyledons plants strictly bear two embryonic leaves or cotyledons just after germination. However for a given dicotyledonous plant species, the number of cotyledons can vary from two due to gene mutations (Liang et al., 2018). Such mutations are not very frequent in a natural population, but can also be induced using artificial mutagens and have been explored (Chen et al., 2006) as certain cotyledonous mutants confer better performance than normal cotyledon formations (Liang et al., 2018). Considering the above fact in mind, the present investigation has been planned to find out possible stimulatory doses of gamma rays for creation of novel genetic variation in a marvel wild medicinal plant (*Andrographis paniculata*) for better survival in the present scenario. The present piece of work will also help to aware people of the wonderful natural care properties of the undervalued wild plants for their wellbeing without any side effects.

2. MATERIAL AND METHODS

2.1. Material Procurement

Material for present investigation is *Andrographis paniculata*; an important medicinal wild plant which has been procured from Birsa Agricultural University, Ranchi, Jharkhand.

2.2. Treatment Methods

Dose ranges were selected on the basis of LD_{50} which included concentrations that range from little effective dose level upto 90% cell lethality. Healthy, dry and homogeneous seeds of *Andrographis paniculata* were irradiated with gamma rays at 25 Gy (gray), 50, 100 and 200 Gy from a Co-60 source (at the dose rate of 1.55 Gy per second)at National Botanical Research Institute (NBRI), Lucknow. Just after irradiation; seeds were soaked in distilled water for 6 hours along with one set of non-irradiated sets and sown in triplicates to raise population for mutation breeding. Replicates were arranged in completely randomized block design (CRBD).

2.3. Meiotic Preparation

Young flower buds of *Andrographis paniculata* were collected in vials at early morning and were fixed in a Clark's fixative(glacial acetic acid—absolute Ethanol, 1 : 3) v/v for 24 h at room temperature i.e. 27 ± 2°C and transferred to 70%alcohol and refrigerated at 4°C until use. After 24 h of incubation, slides were prepared by using anther squash technique with freshly prepared 2% standard aceto-carmine stain and observed under the microscope (Olympus CH20i). Photomicrographs of best slides were captured by Pinnacle PCTV software attached with *Nikon phase contrast research microscope* (Nikon Eclipse E200, Japan). Five plants were randomly selected per dose along with controls and approximately 900 pollen mother cells were examined for each dose.

2.4. Palynological Observations

Mature floral buds were collected and anthers having pollen grains were dusted over a glass slide and stained with freshly prepared glycerol-carmine mixture, covered and observed under an optical microscope. Frequency of fertile and sterile pollen grains was registered to estimate pollen fertility. Pollen viability was also recorded on the basis of pollen stainability test i.e. stained and filled pollen grains were considered as fertile however empty, small and shriveled were assumed as sterile.

2.5. Biochemical Analysis

For the extraction of chlorophyll (μg/mg FW), about 20mg of the third leaf was extracted in 80% acetone (v/v) and extract were centrifuged and supernatant was collected. The absorption spectrum of pigments from supernatant was recorded at wavelength corresponding to 663, 646 and 470 nm, respectively, using UV-Visible spectrophotometer (Lichtenthaler and Wellburn, 1983).

2.6. Statistical Analysis

Variations in the mean of various morphological and cytological traits were subjected to one-way analysis of variance (ANOVA) using post hoc Duncan's multiple range test (DMRT)test ($P < 0.05$) by using SPSS version 16 and graphs were prepared by using Sigma Plot version 14. In all experiments, three replicates were performed for each dose. Data presented in terms of mean values and standard error (±SE).

3. RESULTS

During experimentation, mutagenic potentialities of various doses of gamma rays have been evaluated in terms of significant variability on morphological, cytological, and palynological traits in M_2 generation. The control sets of *Andrographis paniculata* (Kalmegh) possessed the rate of germination percentage as 91.54 ± 0.56%, and survival percentage was recorded as 90.66 ± 0.66%. However, treated populations expressed a wide spectrum of variations in germination and survival percentages (Fig. 1). A significant higher germination rate has been recorded in case of lower doses (25 and 50 Gy) of gamma rays.

Data on significant morphological variation has been tabulated in Table 1. In the case of treated sets plant height varied greatly as compared to control depending upon the effectiveness of mutagens. Generally, dose dependent reduction in plant height has been observed while in case of *A. paniculata*, and the overall decrease in the mean plant height was recorded at 200 Gy dose of gamma rays. Significantly enlargement in the leaf area has been measured at 50 Gy dose of gamma treated set while other doses and sets showed the dose based reduction in leaf area. In the treated sets, the maximum number of branches per plant was evaluated at the lowest dose of gamma rays (25 Gy) as 24.00 ± 2.51 while length of the internodes was found to be highest at 25 Gy dose of gamma rays as 4.63 ± 0.18 cm. Here we find a reciprocal relationship between plant height and length of internodes hence plants obtained at 50 Gy dose was phenotypically bushy in nature (Fig. 2c) due to reduction in height along with undersized internodes. Estimation of fruit length in control and treated sets plants of *Andrographis* revealed an assortment of variability. A dose based decrease in fruit length was recorded in all the treatment sets except that of 50 Gy dose of gamma rays which was utmost and recorded as 1.57 ± 0.01 cm. Assessment of number of seeds per fruit in control and treated sets displayed much variability. In case of gamma-rays treatment set, maximum number of seeds per fruit was 12.66 ± 0.33; recorded at 50 Gy dose of gamma rays.

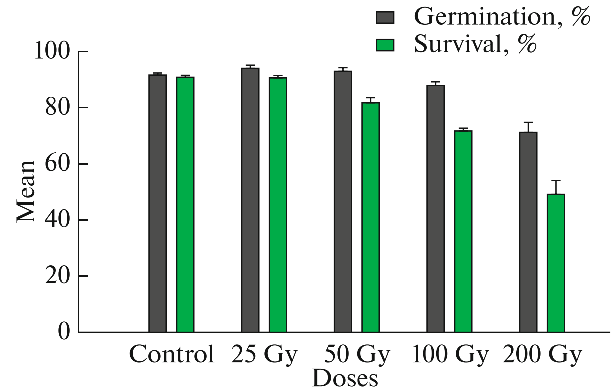

Fig. 1. Effect of gamma irradiation (γ-Rays) on germination and survival percentage of *Andrographis paniculata* (Kalmegh).

3.1. Procurement of Novel Mutants

As we know that the all the flowering plants or angiosperms are basically classified in to two groups i.e. monocotyledons (monocot) and dicotyledons (dicots). *A. paniculata* belongs to dicot group and is one of the typical characteristics of the group, which suggest that seed bears two embryonic leaves or cotyledons after germination that further formed two true leaves at each node. However in our morphological observations of gamma irradiated plants in M_2 generation, seedling with three cotyledons was found which had distinctly borne trimeric true leaves at each node as shown in Fig. 2f. Table 3 clearly shows the morphological and biochemical variations in tricotyledon leaf as compared to control. Here an insignificant increase in the seeds/fruit and 100 seed weight (100 SW) while a moderate significant increase in biochemical traits was observed.

Table 1. Effect of gamma irradiation on some morphological traits of *A. paniculata* (Kalmegh)

Dose	Plant height, cm	Leaf area meter, cm^2; at 125 cm^2 plate	No. of primary branches/plant	Length of internodes, cm	Fruit length, cm	Seeds/fruit
Control	$63.00 \pm 0.57^{ab*\#}$	$61.20 \pm .17^{ab}$	$22.66 \pm .66^{a}$	$4.03 \pm .27^{a}$	1.48 ± 0.01^{b}	12.00 ± 0.00^{ab}
25Gy	67.00 ± 4.35^{a}	$60.03 \pm .31^{ab}$	24.00 ± 2.51^{a}	$4.63 \pm .18^{a}$	1.48 ± 0.02^{b}	11.33 ± 0.33^{b}
50Gy	59.33 ± 4.40^{b}	$63.66 \pm .33^{a}$	22.00 ± 1.15^{a}	$3.40 \pm .05^{b}$	1.57 ± 0.01^{a}	12.66 ± 0.33^{a}
100Gy	55.66 ± 0.66^{c}	$57.76 \pm .43^{bc}$	19.33 ± 1.76^{ab}	$4.33 \pm .16^{a}$	1.40 ± 0.32^{bc}	8.66 ± 0.33^{cd}
200Gy	41.33 ± 1.20^{d}	$54.50 \pm .76^{dc}$	$14.66 \pm .66^{c}$	$3.16 \pm .16^{b}$	1.30 ± 0.06^{c}	6.66 ± 0.33^{e}

* Mean ± SE, # ($p < 0.05$) by Duncan's Multiple Range Test (DMRT) One way ANOVA.

Table 2. Effect of gamma irradiationon meiotic behavior and pollen fertility of *A. paniculata* (kalmegh)

Dose	Tco	Metaphase I/II Ab %						Anaphase I/II Ab %					T Ab %	Pollen fertility, %
		Pm	Un	Sc	St	Mn	Oth	Bg	Lg	St	Ds	Oth		
Control	900	–	–	–	–	–	–	–	–	–	–	–	–	99.97 ± 0.19
25Gy	900	0.30	0.12	0.33	0.43	0.08	0.60	0.63	0.65	0.28	0.10	0.12	3.64	99.45 ± 0.22
50Gy	900	0.08	0.18	0.30	0.15	0.60	0.18	0.32	0.75	0.24	0.53	0.33	3.66	99.43 ± 0.56
100Gy	900	0.24	0.22	0.54	0.14	1.31	0.42	1.45	1.22	0.33	0.24	0.22	6.33	88.12 ± 0.43
200Gy	900	0.33	0.45	0.99	0.48	1.69	0.52	1.54	2.14	0.79	0.46	0.35	9.74	78.28 ± 1.32

Abbreviations: Pm—Precocious chromosomes, Un—Unorientation, Sc—Scattering, St—Stickiness, Mn—micronuclei, Oth—Other, Bg—Bridge, Lg—Laggards, St—Stickiness, Ds—disturbed spindle, Tco—Total cell observed, T Ab—Total abnormalities.

A novel bushy plant mutant of *A. paniculata* has been procured which also possessed significant increase in coloration patterns of leaves from green (at control, Fig. 2a) to dark green (at 50 Gy treatment dose, Fig. 2b) leaf. Table 3 clearly shows the significant increase in fruit length, seeds/fruit and 100 SW. Further significant increase in biochemical contents (chl a, chl b and carotenoid content as shown in Table 3) as compared to the control clearly supports our observation of dark green coloration in the bushy mutant. These mutants may provide ample scope for the selection of better mutants with higher yields of important medicinal plants.

3.2. Cytological Effect of Gamma Rays on Andrographis paniculata (Kalmegh)

Andrographis paniculata (Kalmegh) exhibits 50 chromosomes as normal somatic complement i.e., $2n = 50$ (Roy and Datta, 1988). Meiotic behavior of chromosomes was almost normal during microsporogenesis of control (Figs. 3a, 3b, 3i, 3l).Kalmegh belongs to advance family hence the size of the chromosomes was very small that had made chromosome analysis extremely tough during the investigation. PMCs (pollen mother cells) of untreated plants showed 25 bivalents at diakinesis (Fig. 3b) and metaphase-I and separation of bivalents into 25 : 25 at anaphase-I. Table 2 presents the occurrence characteristics of normal and disturbed phases of the cell cycle during meiosis. Collinear relationship between abnormality percentages and the treatment doses has been registered in the pollen mother cells of the mutagens treated population. Several significant meiotic aberrations were recorded in the gamma treated sets (Table 3, Figs. 3c–3h, 3j, 3k) namely bridges, stickiness, laggards, micronuclei, precocious chromosomes, unorientation, multivalent, asynchrony while scattering, forward movement of chromosomes were noticed in erratic frequency in gamma rays set. Analysis of PMCs of gamma irradiated sets at varying stages of division viz. metaphase-I/II and anaphase-I/II showed a copious count of cytological anomalies. Their frequency of occurrence was asymmetrical and varied from moderate to high depending upon the extent of doses. The lowest value (3.64%) was found at 25 Gy dose of gamma ray which elevated significantly upto 9.74% at 200 Gy dose. Overall the abnormalities that prevailed in gamma rays treated sets were laggards followed by chromatin bridge formation at Anaphase I/II while the least scored abnormalities were precocious chromosomes followed by unorientation of metaphase plate at Metaphase I/II.

3.3. Palynological Observation

The rate of pollen fertility in the mutagen treated population was observed to vary significantly, and on the whole, it had been observed to be ranged from 99.45 ± 0.22% to 78.28 ± 1.32% in gamma treated sets. Control plants possessed 99.97 ± 0.19% pollen fertility rate. A comparative account of pollen fertility estimation suggests that it is directly influenced by the mutagenic treatment. Besides pollen grains sterility, post-meiotic products also revealed various abnormalities at a significant percentage. Abnormal tetrads (Fig. 3j) such as dyad, triad and polyad were observed.

4. DISCUSSION

Induced mutations have been used to enhance genetic variability via genetic manipulation, which was utilized not only to increase the productivity of economic important plants but also for basic studies in various plants (Chopra and Sharma, 1985). Besides, it also provides the background for the improvement of several genetic features without halting the other developmental processes of treated ones. Thus, the present piece of work has been performed for the creation of novel genetic variation in the wild medicinal plant (Kalmegh). Considering seedling germination, here higher doses of gamma ray showed maximum inhibition and also delayed germination. A group of scientist (Kleinhofs et al., 1978) has also been reported the delay in the initiation of metabolism following germination, resulting in a uniform delay in mitotic activity and hence seedling growth.

Morphological traits also displayed significant results at lower doses of gamma rays. Plenty of reports,

Fig. 2. (a) Control plant leaves, (b) dark green leaves of bushy mutant, (c) dark green and bushy plant mutant (full view) at 50 Gy dose compared to control and 25 Gy dose of gamma rays, (d) a tricotyledon mutant (red arrow) and a normal dicotyledon seedlings, (e) first trimeric true leaves (red arrow), and (f) second trimeric true leaves (black arrow) of a tricotyledon mutant.

however, are available which showed the stimulatory effects of the lower dose of ionizing radiation (Luckey, 1980; Kim et al., 2004) due to a phenomenon "hormesis" and similar finding has reported in the present case as well. More importantly, 50 Gy dose of gamma rays found more potent among all the treatment of gamma rays, since the impact of these dose was significant ($p > 0.5$) as well as up to the mark in inducing several stable lucrative traits in *Andrographis paniculata* (Kalmegh) viz. leaf area, number of

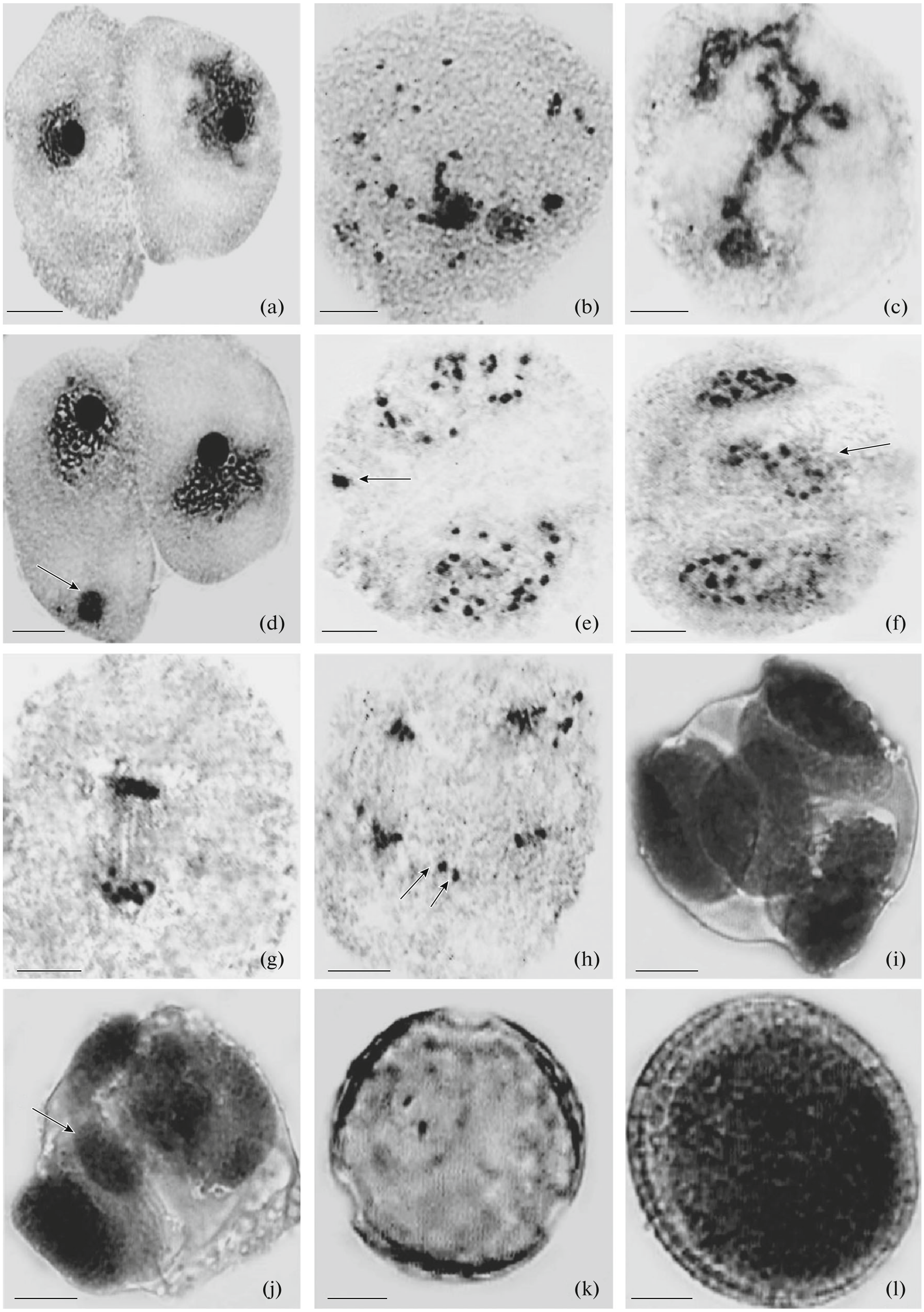

Fig. 3. (a, b) Normal division during cell cycle (a) Prophase I, (b) a diakinesis ($n = 25$), (c–h) abnormal division during cell cycle: (c) a pollen mother cells carrying irregular translocation heterozygote, (d) prophase I with extra micronucleus (arrow), (e) Scattered anaphase with laggard, (f) disturbed spindle at anaphase I, (g) stickiness of chromosomes at anaphase I, (h) several laggards at anaphase II, (i) normal tetrad with equal shaped microspores, (j) abnormal tetrad with various shaped microspores, (k) sterile Pollen grain (empty), (l) fertile pollen grain (filled). (bar = 10 μm).

Table 3. Comparative morphological and biochemical analysis of control and mutant plants of Kalmegh

Traits	Fruit length, cm	Seeds/fruit	100 seed weight, g	Chlorophyll a, μg/mg FW	Chlorophyll b, μg/mg FW	Carotenoid, μg/mg FW
Control	1.48 ± 0.013$^{b*\#}$ (0.02)**	11.33 ± 0.33^{b} (0.58)	0.18 ± 0.001^{a} (0.004)	1.55 ± 0.02^{c} (0.02)	0.60 ± 0.02^{b} (0.035)	0.66 ± 0.02^{b} (0.032)
Tricotyledon mutant	1.48 ± 0.006^{b} (0.01)	11.67 ± 0.33^{b} (0.58)	0.19 ± 0.002^{a} (0.002)	1.67 ± 0.012^{b} (0.02)	0.64 ± 0.016^{ab} (0.029)	0.70 ± 0.01^{ab} (0.015)
Bushy and dark green leaf mutant	1.52 ± 0.012^{a} (0.02)	13.67 ± 0.66^{a} (1.15)	0.21 ± 0.007^{a} (0.012)	1.92 ± 0.015^{a} (0.17)	0.75 ± 0.03^{a} (0.065)	0.77 ± 0.0^{2a} (0.025)

* Mean ± SE, ** Standard Deviation, # ($p < 0.05$) by Duncan's Multiple Range Test (DMRT) One way ANOVA.

branches per plant, Length of internodes, fruit length and seeds per fruit.

However, the higher doses at and above 100 Gy were not found beneficial (Table 1), and as also evident with the work of (Hanafy and Akladious, 2015); they too explained that the highest implement gamma ray dosage had negative and hazardous effects on fenugreek morphology and growth compared to the control plant.

Further few novel variant/mutants viz. dark green and bushy leaf mutant (Figs. 2b, 2c—a whole plant) has also been found at 50 Gy dose of gamma rays treatment. Lately genetically verified leaf color mutants (regulated by a single recessive gene mutant) of "Changchunmici" from M_2 generation have been procured by (Song et al., 2018) after EMS mutagenesis. This leaf color mutant could be the model for several physiological (photosynthesis and chloroplast ultrastructure), genetic (the study of types of a gene involved in their expression) and biochemical studies (chlorophylls and carotenoid biosynthetic pathway) as stated by (Stern et al., 2004).

Tricot seedling has also been reported in sunflower by (Hu et al., 2006), who stated that a phenotype controlled by few recessive genes, the most often genotype of the mutations and usually masked by dominant traits, but sometimes might be able to express themselves (such as in present case) due to lethality of masking genotypes. That seedling that possessed tricotyledon leaves also bears three true leaves at each internode. These plants could be potentially useful for faster establishment of seedling after planting because of the larger photosynthetic area in the early growing stages and may serve as a morphological characteristic for distinguishing cultivars (Hu et al., 2005).

Evaluation of genetic manipulation in terms of chromosomal variations has been performed during microsporogenesis of *Andrographis paniculata*, a wild medicinal plant. These effects are due to the cytological changes such as chromosomal rearrangement, damages, altered meiotic division, degeneration of nuclei, cell enlargement etc., as reported by (Pollard, 1964; Karpate and Choudhary, 1997). Half life of radiation induced free radicals is few microseconds in water (Foyer and Harbinson, 1994) which affects chromosomal behaviour immediately and therefore maximum number of chromosomal abnormalities was recorded at the initial stages (viz. prophase I and anaphase I); if not repaired at different checkpoints of division may persist up to later stages of cell cycle causing severe damage to the microtubules. Microlubules are highly dynamic and polar structures; they are involved in a variety of cellular processes in plants, including chromosome segregation, cell plate formation, cytoplasmic organization and intracellular transport (Smertenko et al., 1997). Alterations in microtubule structure and tubulin polymerization are a part of the cellular response to DNA damage, (Porter and Lee, 2001) thus proper functioning of microtubule apparatus is a prerequisite for proper chromosome arrangements. A dose based increase in chromosomal abnormalities was evident in the present case as well which might be due to the effect of mutagens and have also been studied by various investigators like (Ahmad and Yasmin, 1992; Chatterjee et al., 2010; Dixit et al., 2013). However the frequency of mutants is dependable on the genotype of the plant and also on different effectiveness of mutagens as suggested by (Gustafsson, 1963). Formation of chromatin bridges might be due to the failure of chiasmata in bivalent to terminate and chromosome gets stretched between the poles. Disturbed spindle might be due to spindle dysfunction induced by a higher dose of radiations. However the ultimate fate of laggards, precocious chromosome might result as micronuclei in the late phases of division which may be able to generate aneuploids with some novel and desired traits.

Reduction in pollen fertility is because of the formation of sterile pollen due to the side effects of mutagens on male reproductive organs. Pollen viability is considered to be an important parameter of pollen quality (Dafni and Firmage, 2000). As reviewed by (Aizen and Harder, 2007), many genetic studies demonstrate that poor-quality pollen can also reduce seed production, which is interpreted as a "quality limitation" and results in decreased fruit and seed set. The effect of mutagens (radiation) on reduction in pollen fertility has been studied by (Katiyar, 1978;

Dixit et al., 2013; Liu et al., 2018) also reported the positive correlation between increased meiotic aberrations with those of pollen sterility.

Conclusively, the lower doses were found to stimulatory rather than being deleterious; may create several potential desired mutants (viz. tricotyledon mutant) without any deleterious impact on mutated plants. However, the utmost dose of gamma rays was found significantly inhibitory. The data are consistent with other irradiation results reported by (Majeed, 2010; Ali et al., 2015; Hanafy and Akladious, 2018). This might be due to effect of high-dose irradiation that exerted a negative impact on growth; attributed to the cell cycle arrest at the G2/M phase during cell division and/or various damages in the entire genome (Preussa and Britta, 2003). Thus, from the practical breeding point of view, the mutagenic treatments that induce high mutation rates with the least accompanying deleterious effects are desirable (Kumar and Yadav, 2010). The genetic structure of our material was manipulated (i.e. induced genetic variation) favouring novel genetic changes in the wild medicinal plant *Andrographis paniculata* for the better survival in the present day changing climate.

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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