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Induction of morphological mutations in okra (*Abelmoschus esculentus* L.) through gamma rays and EMS

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Abstract
Induced mutagenesis has become an effective tool to improve a crop through creation of variability. Genetically pure, uniform and dry seeds of okra (*Abelmoschus esculentus* L.) variety P-8 were treated with physical mutagen gamma rays (35kR, 40kR, 45kR, 50kR, 55kR, 60 kR, 65kR, 70kR, 75kR, 80kR and 85kR) and chemical mutagen Ethyl Methane Sulphonate (0.2%, 0.4%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, 1.1%, 1.2%, 1.4% and 1.6%) and their combination treatments to assess the extent of macro-mutations induced in the M_2 generation and their scope for future use in plant improvement programme. The irradiated seeds were sown in the M_1 field and harvested in bulk to raise M_2 generation in Augmented Design. Wide spectrum of morphological macro mutants including variation in the leaf size, leaf shape and leaf colour etc were observed in M_2 generation. Among the morphological macro mutants (round, rosette and lobed leaf type); lobed leaf types was observed in highest frequency followed by rosette and round type. Lobed and round leaf types were maximum in 75kR whereas rosette leaf types were maximum in both the treatments ie. 75kR+1.4% EMS and 85kR+1.4% EMS.

Keywords: Morphological mutations, gamma rays, EMS

Introduction
Okra (*Abelmoschus esculentus* L. Moench.), lady's finger or bhindi is a multipurpose crop due to various uses of the fresh leaves, buds, flowers, pods, stems, and seeds; grown throughout the year in tropics and subtropics (Gemede *et al*., 2014) [4]. It belongs to the family Malvaceae and native to Ethiopia with secondary centre of origin in India (Vavilov 1951) [10]. Okra is sixth important and popular vegetable crop grown year round in India including low and mid hills of Himachal Pradesh. The somatic chromosome number of okra is 130 (2n) which is an amphidiploid of *Abelmoschus tuberculatus* with 2n=58; an unknown species with 2n=72 is also on record. Due to high nutritive value and long post harvest life, okra has captured a prominent position among export vegetables. It has a vast potential to earn foreign exchange and accounts for 60 per cent export of fresh vegetables excluding potato, onion and garlic, the destinations being the Middle East, United Kingdom, Western Europe and United States of America. Frozen bhindi is also exported to United Kingdom. Major exporting areas in India are Nasik, Ozar, Saikheda, Dindori, Kolhar, Naraingaon and Sholapur in Maharashtra (Thamburaj and Singh 2001) [9].
Despite the importance, the yield of okra in Ghana is low due to lack of improved varieties to mitigate climate change, diseases, pests, and edaphic factors. However, mutation breeding has proven to be a useful technique in plant improvement. Induced mutagenesis plays an important role in improvement of crops like okra where a large part of genetic variability has been eroded due to its continuous cultivation in marginal and sub-marginal land. The different physical and chemical mutagens vary in their efficacy in inducing the spectrum of mutations. Given that the mutagen induction process is independent and capable of interaction, combination of different mutagens has been used to increases the mutation frequency and alteration of the mutation spectrum (Wani 2009b) [11].

Based on above, the present experiment was undertaken to induce mutation using gamma irradiations and EMS to assess the frequency and spectrum of macro-mutations appeared in the M_2 generation and their scope of use in future crop improvement programme.

Materials and Methods

Experimental Material

Healthy seeds of P-8 variety of okra (*Abelmoschus esculentus* L.Moench) were procured from CSK HPKV, Palampur, Himachal Pradesh.

Mode of treatment with mutagenic agents

The seed materials were treated with physical (Gamma rays) and chemical mutagen Ethyl Methane Sulphonate.

1. **Treatment with physical mutagen:** Gamma irradiation was performed by using gamma cell installed at Bhabha Atomic Research Centre (BARC), Trombay, Mumbai and the seeds were irradiated with ^{60}CO source. The different doses were 35 kR, 40 kR, 45 kR, 50 kR, 55 kR, 60 kR, 65 kR, 70 kR, 75 kR, 80 kR and 85 kR.
2. **Treatment with chemical mutagen:** The treatment of seeds with chemical mutagen Ethyl Methane Sulphonate (EMS) was done at Departmental laboratory of Vegetable Science and Floriculture, CSK HPKV, Palampur. Prior to chemical mutagenic treatments, six hundred well-filled, mature and disease free seeds were immersed in distilled water for 6 hours. The pre-soaking treatment enhances the rate of uptake of mutagen through increase in cell permeability and also initiates metabolism in seeds. Such pre-soaked seeds were later immersed in the mutagenic solution for another 6 hours with intermittent shaking. The different concentrations of EMS were 0.2%, 0.4%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, 1.1%, 1.2%, 1.4% and 1.6%. After the treatment, the seeds were washed in running water before sowing.

In the laboratory experiment, the treated seeds were sown in absorbent cotton -wet petridishes for recording the germination test. Based on the reduction of 50% seed germination, the LD_{50} values were calculated as 75kR and 1.4% for gamma rays and EMS, respectively. Three treatments of gamma rays (65kR, 75kR and 85kR), EMS (1.2%, 1.4% and 1.6%) around LD_{50} value and their combinations were fixed for further studies.

Recording of Data

The treated materials along with control (untreated) were immediately sown in Augmented Design at Experimental farm of Department of Vegetable Science and Floriculture, CSK HPKV, Palampur. The entire surviving M_1 crop was harvested individually to raise the M_2 generation population along with controls. Necessary cultural practices were adopted to raise a healthy crop. Observations for recording chlorophyll mutations were noted critically right from emergence to till the age of three weeks after germination. The detection of chlorophyll mutations were made as per the classification of Gustaffson. The mutations affecting gross morphological changes in growth habit, leaf, flower, pod, seed size and maturity etc., were scored as viable mutants. Viable leaf mutants were scored throughout the growing period as described by Blixt and the mutation frequency was estimated on M_2 plant basis.

Results and Discussion

Variability in shape, size and colour was observed in okra leaves. Different chlorophyll mutants were present in mutagen treated material. Different types of okra leaf shape mutants like rosette, lobed and round were observed as shown in plate 1.

In variety P-8 (control), leaves were lobed whereas different types of leaf mutants were found in other treatments. Maximum number of large leaves was observed in 75kR dose (9) whereas least large leaves were found in three treatments *viz*. 65kR, 1.6% and in combination of 75kR with 1.2% EMS (2). Large leaves were absent in 65kR+1.4% EMS, 65kR+1.6% EMS, 75kR+1.6% EMS, 85kR+1.2% EMS, 85kR+1.4% EMS and 85kR+1.6% EMS.

Maximum number of small leaves were observed in 75kR (6) followed by 1.6% EMS and 75kR+1.2% EMS (5). Single small leaf mutant was observed in 65kR and 75kR+1.6% EMS. In all the other treatments, small leaf mutant type was absent. The abnormalities observed in leaves is due to various causes such as disturbances in phytochromes, chromosomal aberrations, mitotic inhibition, disrupted auxin synthesis and mineral deficiencies, disturbance in DNA synthesis, enlargement of palisade, spongy and mesophyll cells (Raghuvanshi *et al*., 1974) [7].

Rosette leaf type mutants were maximum in 75kR+1.4% EMS and 85kR+1.4% EMS **(7)** followed by 1.4%EMS (5) and 75kR (4). Three rosette leaf mutants were found in 85kR and 1.6%EMS followed by two in the combination of 65kR+1.2% EMS and 75kR+1.2% EMS. Single rosette leaf mutant was observed in 65 kR and 85kR+1.2% EMS. Rosette leaf type mutants were absent in treatments *viz*., 1.2% EMS, 65kR+1.4% EMS, 75kR+1.6% EMS and 85kR+1.6% EMS.

Table 1: Different types of leaf mutants in okra

Treatment	Leaf Type			Total mutants
	Lobed	Round	Rosette	
65 kR	2	1	1	4
75 Kr	9	6	4	19
85 Kr	4	-	3	7
1.2% EMS	5	-	-	5
1.4% EMS	8	-	5	13
1.6% EMS	2	5	3	10
65kR+1.2% EMS	5	-	2	7
65kR+1.4% EMS	-	-	-	-
65kR+1.6% EMS	-	-	-	-
75kR+1.2% EMS	2	5	2	9
75kR+1.4% EMS	3	-	7	10
75kR+1.6% EMS	-	1	-	1
85kR+1.2% EMS	-	-	1	1
85kR+1.4% EMS	-	-	7	7
85kR+1.6% EMS	-	-	-	-
Control (P-8)	Lobed			

Rosette Round Lobed

Plate 1: Different types of okra leaf mutants (Rosette, Lobed and Round)

Similar type of leaf mutants were also studied by Monica and Seetharaman (2017) [5] and Surendran and Udayan (2017) in *Lablab purpureus* L. and okra, respectively. Monica and Seetharaman (2017) [5] also concluded that EMS induced higher proportion of chlorophyll and viable mutants than gamma rays and the highest mutation frequency was induced in 30mM of EMS followed by 25KR of gamma rays.

The presence of lobed leaves could be explained by the death of cells in the center of the meristematic regions which have specific influences on the development of leaves and leaf shape. Assuming that physiological activity has started in the meristematic regions toward the formation of new plants, gamma radiation may have adversely affected certain embryonal mechanisms which resulted in the non-development of leaf apices. These mutants have been reported by Bolbhat *et al*. (2012) [2] in Horse gram and Navnath *et al*. (2014) [6] in Okra.

References

1. Blixt S. Quantitative studies of induced mutation in peas, Chlorophyll mutations. Agriculture Horticulture Genetics. 1961; 19:402-447.
2. Bolbhat SN, Gawade BB, Bhoge VD, Wadavkar DS, Shendage VS, Dhumal KN. Effect of mutagens on frequency and spectrum of viable mutations in Horse gram (*Macrotyloma uniflorum* (Lam.) Verdc). International Journal of Agriculture and Veterinary Sciences. 2012; 1(1):45-55.
3. Gustafsson A. The mutation system of the chlorophyll apparatus. Lunds. Univ. Arsskr. 1940; 36:1-40.
4. Gemede HF, Ratta N, Haki GD, Woldegiorgis AZ, Beyene F. Nutritional Quality and Health Benefits of Okra (*Abelmoschus esculentus*), Food Science and Quality Management. 2014; 33:87-96.
5. Monica S, Seetharaman N. Induced chlorophyll and viable mutations in *Lablab purpureus* (L.) sweet var. *typicus* through gamma rays and ethyl methane sulphonate. International Journal of Pharmacognosy and Biological Sciences. 2017; 8(2):240-249.
6. Navnath Kashid G, Slave US. Effect of induced mutation on leaf morphological changes in Okra (*Abelmoschus esculentus* L. Moench). International Journal of Recent Advances in Multidisciplinary Research. 2014; 1(1):1-2.
7. Raghuvanshi SS, Singh AK. Studies on the effect of gamma rays on Trigonella foenum graceum. Cytologia. 1974; 39:473-482.
8. Surendran S, Udayan PS. Gamma Rays Induced Mutations on Morphological and Yield Attributing Characters in M2 Generation of Okra [*Abelmoschus esculentus* (L.) Moench]. International Journal of Current Microbiology and Applied Sciences. 2017; 6(10):3623-3628.
9. Thamburaj S, Singh N. *Vegetables, Tuber crops and Spices*. Directorate of information and publications of agriculture, ICAR (Indian Council of Agricultural Research), New Delhi, 2001, 222.
10. Vavilov NI. The origin, variation, immunity and breeding of cultivated plants. Chronical Botany. 1951; 13:1-364.
11. Wani AA. Spectrum and frequency of macromutations induced in chickpea (*Cicer arietinum* L.). Turkish Journal of Biology. 2009b; 35:221-231