International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Int. J. of Life Sciences, 2018; 6 (1):111-116
ISSN:2320-7817(p) | 2320-964X(o)
UGC Approved Journal No 48951

Original Article Open Access

Effectiveness and efficiency of mutagen on leaf chlorophyll in Hyacinth bean *Lablab purpureus* (L.) Sweet

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Manuscript details:

Received : 28.11.2017
Accepted : 12.02.2018
Published : 25.02.2018

Editor: Dr. Arvind Chavhan

Cite this article as:
Undirwade GP and Kulkarni GB (2018) Effectiveness and efficiency of mutagen on leaf chlorophyll in Hyacinth bean *Lablab purpureus* (L.) Sweet, *Int. J. of Life Sciences*, Volume 6(1): 111-116.

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Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

ABSTRACT

The seeds of Hyacinth bean (*Lablab purpureus* L.) Sweet varieties Konkan bhushan and Konkan Wal-2 were treated with various doses of chemical (EMS- 0.05%, 0.10%, 0.15% and SA- 0.010%, 0.015%, 0.020%) and physical (Gamma rays- 5KR, 10KR, 15KR) mutagen and were sown in the field with control and harvested in bulk to raise M1 generation to observe the characters and number of mutants in each population. to observe the chlorophyll mutation and leaf morphology. Chlorophyll mutation frequency was calculated on the basis of plant population. A distinct range of chlorophyll and morphological mutants were observed in the M1 generations. Four different types of spectrum of chlorophyll mutant's viz. albina, xantha, viridis and chlorina were identified in the treated population. Various morphological aberrations had been observe viz. Bilobed leaf, reduction in size of leaves, cotton shape leaf, obtuse and retuse apex, notch at margin, sinuate leaf margin, lanceolate leaf, wrinkled leaflet and triforked leaf. The frequency of leaf morphological changes noticed more in SA (0.020%) in Konkan bhushan and EMS (0.15%) in Konkan Wal-2 of Hyacinth bean variety.

Keywords: Hyacinth bean (*Lablab purpureus* L.) Sweet, EMS, SA, Gamma rays, chlorophyll mutation, leaf abnormalities

INTRODUCTION

Hyacinth bean (*Lablab purpureus* L.) Sweet, also known as field bean or dolichos bean, the member of family Fabaceae. It is one of the most ancient and originated crop among all the cultivated crops in India (Deka and Sarkar, 1990). It grows and cultivate throughout regions of tropical Africa and other countries like central and south America, East and West Africa, Caribbean, Sudan, Egypt, China, Philippines, Indonesia and Malaysia. As a field crop, it is cultivated in Tamil Nadu, Andhra Pradesh, Karnataka, Madhya Pradesh and Maharashtra as a pulses, vegetable and forage, in India. It had also been proved an excellent source of nitrogen fixer, to improve soil fertility, also

useful as antioxidants also (Bradley, 1999). Hyacinth beans show multipurpose utilization in rural areas of India, as green pods, dry seeds, foliage for vegetable purpose. It had plays a very significant role as a sufficient source of protein, minerals and vitamins for tribal. (Khan *et. al.*, 2005). The most important characteristic feature of this crop is adaptations to diverse climate condition and drought stress resistant. Hyacinth bean is a perennial plant, leaves are alternate and trifoliate with the habit of twining or bushy or semi erect. The main aim of present investigation, is to inquire the effect of different doses of chemical as well as physical mutagens on chlorophyll frequency and leaf morphological changes.

METHODOLOGY

The seed material of Hyacinth bean variety Konkan Bhushan and Konkan Wal-2 procured from the Dr. Balasaheb Savant Konkan Krishi Vidyapeeth, Dapoli, Dist. Ratnagiri (M.S.) India, were used and treated by various doses of Chemical and physical mutagen viz. EMS, SA and Gamma rays **[**Co^{60} 1000 curie, irradiated at the Department of Biophysics, Government Institute of Science, Aurangabad. (M.S)].

Healthy and uniform size of seeds were pre-soaked in distilled water for 6 hours, then theses seeds were immerse in the chemical mutagenic solution for 6 hours, with the different concentration 0.05%, 0.10% and 0.15% for EMS; 0.010%, 0.015% and 0.020% for SA, respectively, while for the physical mutagen the dose applied 5KR, 10KR and 15KR.

The seeds treated with mutagens treatment were sown immediately, the randomized block design (RBD) applied with three replications along with control, to raise the M_1 generation. The seeds were sown at a distance of 45 X 30 cm spacing. The various chlorophyll deficient sectors and leaf abnormalities were noted dose wise and variety wise separately. The Mutagenic effectiveness and efficiency were calculated on the basis of formulae suggested by Konzak *et al.* (1965).

$$\text{Mutagenic effectiveness (}\textit{Physical mutagens)}= \frac{\text{Mf} \times 100}{\text{K rad}}$$

$$\text{Mutagenic effectiveness (}\textit{Chemical mutagens)}= \frac{\text{Mf} \times 100}{\text{C x t}}$$

RESULTS AND DISCUSSION

The screening of both the varieties of Hyacinth bean, in the M_1 generation revealed in majority of mutagenic treatments. The induction of chlorophyll deficient sectors observed in the leaves at all doses of mutagen. Such sectors were albino (white), xantha (yellow), viridis (dull green) and chlorina (light green) types. They were distributed throughout the leaf lamina. It had been observed that the various doses of the mutagens EMS, SA and Gamma rays have proved effective inducing the chlorophyll deficient sectors in both the varieties of Hyacinth bean (Table 1 and 2).

Table1. Effect of mutagens on frequency of plants carrying chlorophyll deficient sectors in M_1 generation of Hyacinth bean variety Konkan bhushan and Konkan wal-2.

	Variety	**Konkan bhushan**		**Konkan wal-2.**	
Treatment	**Dose (%)/ (KR)**	**Frequency of chlorophyll Deficient Sectors%**	**±SE**	**Frequency of chlorophyll Deficient Sectors%**	**±SE**
Control	--	--	--	--	--
EMS	0.05	1.99	0.12	2.42	0.15
	0.10	2.90	0.18	3.17	0.19
	0.15	3.54	0.22	3.61	0.22
SA	0.010	2.02	0.12	2.03	0.12
	0.015	3.04	0.20	2.78	0.17
	0.020	3.31	0.21	3.79	0.24
Gamma rays	5KR	1.58	0.09	2.01	0.12
	10KR	2.53	0.16	2.92	0.18
	15KR	3.64	0.23	3.29	0.21

Se= Standard error

Table2. Effect of mutagens on frequency of plants carrying leaf morphological changes in M_1generation of Hyacinth bean variety Konkan bhushanand Konkan wal-2

Variety		Konkan bhushan		Konkan Wal-2	
Treatment	**Dose (%) / (KR)**	**Frequency of leaf Morphological changes (%)**	**±SE**	**Frequency of leaf Morphological changes (%)**	**±SE**
Control	--	--	--	--	--
EMS	0.05	8.36	0.52	9.63	0.61
	0.10	10.44	0.66	11.95	0.75
	0.15	12.30	0.77	16.06	1.01
SA	0.010	11.33	0.72	10.08	0.64
	0.015	14.05	0.89	12.85	0.81
	0.020	16.04	1.02	14.57	0.92
Gamma rays	5KR	7.72	0.49	9.31	0.59
	10KR	9.38	0.59	10.61	0.67
	15KR	11.06	0.70	12.29	0.78

SE= Standard Error

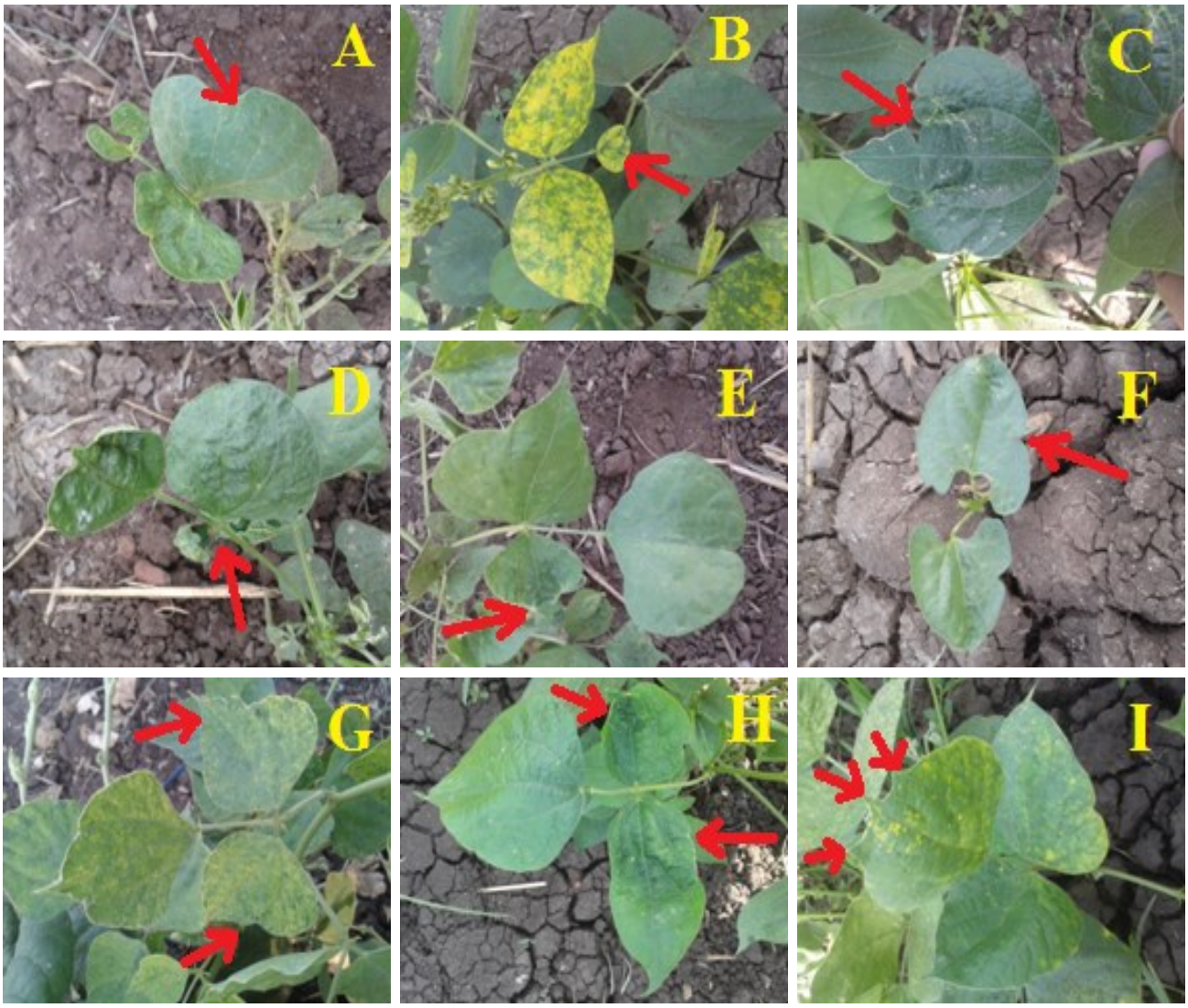

Fig.1. Leaf morphological changes of Hyacinth bean variety Konkan bhushan and Konkan Wal-2, found in M_1 plants: (a) bilobed leaflet, (b) reduction in size of leaf, (c) cotton shape leaf, (d) Obtuse apex with wrinkled leaflet, (e) retuse apex, (f) notch at margin, (g) cunniate leaf margin, (h) lanceolate shape with shrink leaf, (i) triforked leaf.

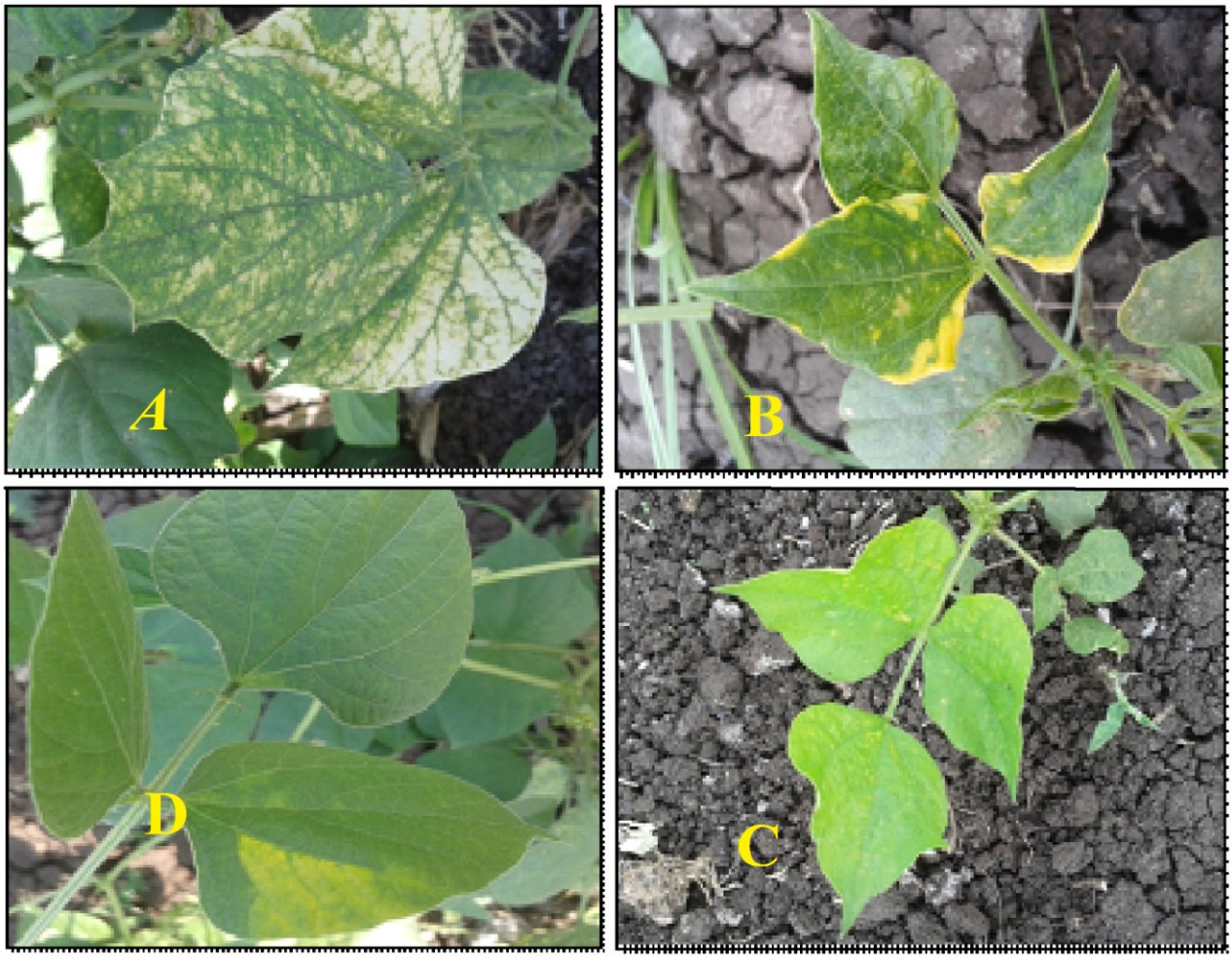

Fig.2. Chlorophyll deficient sectors found in M_1 plants: (a) albina, (b) xantha, (c) chlorina, (d) viridis.

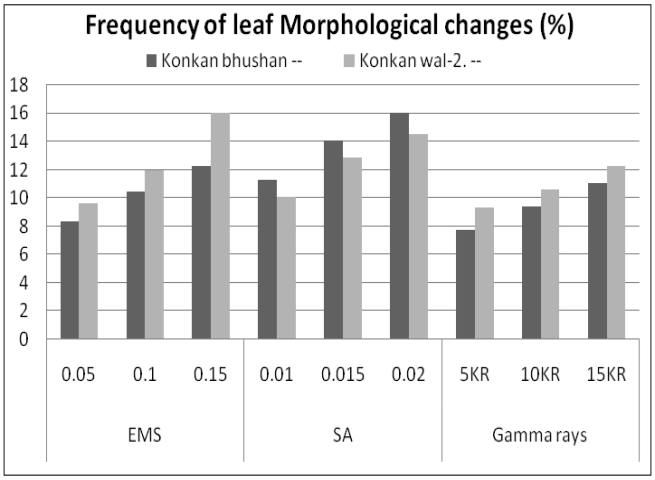

Fig. 3: Frequency of leaf Morphological Changes (%)

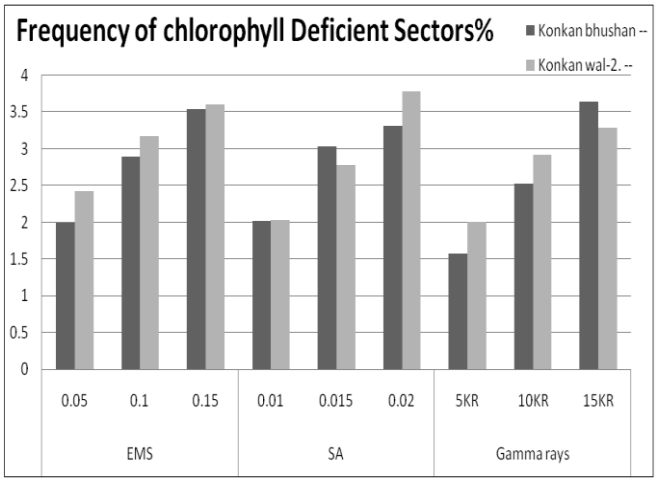

Fig. 4: Frequency of Chlorophyll Deficient Sector %

The frequency of sector plants was noticed highest in Konkan Bhushan by Gamma rays treatment. Whereas Konkan wal-2, the SA shows the highest frequency of chlorophyll deficient sectors of plants as compared to other mutagens (Table 1). The frequency of chlorophyll deficient sectors of plants ranged from 1.99% to 3.54% and 2.42% to 3.61% by EMS treatments, from 2.02% to 3.31% and 2.03% to 3.79% by SA treatments and 1.58%

to 3.64% and 2.01% to 3.29% by Gamma ray treatments, respectively in Konkan Bhushan and Konkan Wal-2 varieties. Result shows the maximum chlorophyll and viable mutation frequency in both the varieties of *Lablab purpureus*. Chlorophyll mutation is shows the directly relation of the mutagenic effect. The occurrence of chlorophyll deficient mutant was noticed due to change in gene and a set of genes responsible for chlorophyll mutations (Monika and Seetharaman, 2017). That was the confirmed by earlier reports of Kumar *et al*. (2009) in Mungbean, Not any such mutations were observed in the controls.

The occurrence of chlorophyll mutations had reported earlier by several researchers, in Lentil (Sharma and Sharma, 1981), in Chickpea (Kharkwal, 1998, Khan *et.al* 2005, Shah *et al.* 2008, Bara *et al.* 2017), in *Lathyrus* (Prasad and Das, 1980) and (Waghmare and Mehra, 2001), in *Vicia* beans (Bhat *et al.* 2007), Cowpea (Girija and Dhanavel, 2009 and Kumar *et al.* 2010), in Black Gram (Thilagavathi and Mullainathan, 2009; Lal *et al*. 2009), in Mungbean (Kumar *et al.,*2009), in green gram (Das and Baisakh, 2010, Vikram *et al*., 2014), in Urdbean (Goyal and Khan, 2010), in two varieties of *Trigonella* **(**Vasu and Hasan, 2011), in horse gram (Kulkarni and Mogle, 2013), in *Lablab* (Monika and Seethraman, 2017).

The two varieties of Hyacinth bean treated various doses was revealed the some abnormal shapes and size of leaves (Fig.1). The abnormalities of leaf variations comprised bilobed leaf, reduction in size of leaf, cotton shape leaf, obtuse apex, retuse apex, notch at margin, sinuate leaf margin, tri apex leaflet, lanceolate and wrinkled leaflet. The frequency of plants showed the leaf morphological changes observed in various doses of mutagens (Fig. 1 and Fig. 2). Among the three mutagens, the SA specially its 0.020% dose succeeded in inducing the highest morphological frequency (16.04%) in Konkan bhushan and 0.15% concentration of EMS inducing the highest frequency (16.06%) in Konkan Wal-2. The lowest frequency of leaf abnormality changes carrying plants (7.72% and 9.31%) could be noted at 5KR Gamma rays in variety Konkan bhushan and Konkan Wal-2 respectively.

The changes in leaf shape and size have been reported by some investigators earliar, in Pea (Gelin, 1954), Soyabean (Zacharias, 1956), in Safflower Satpute (1994), in mungbean (Singh *et al.* 2000 and Sangsiri *et al.* 2005), Chickpea (More *et al.* 2011, Bara *et al.* 2017) and French bean (Mahamune and Kothekar, 2012), in horse gram (Kulkarni and Mogle, 2013), in green gram (Vikram *et al.* 2014), in *Lablab purpureus* (Monika and Seethraman, 2017).

In the present study the leaf morphological changes may develop due to developmental alterations produced in both the varieties of Hyacinth bean. The disturbances in phytochromes causes due to abnormalities in leaves is such as, chromosomal aberrations, mitotic inhibition, disrupted auxin synthesis and mineral deficiencies, disturbance in DNA synthesis, enlargement of palisade, spongy and mesophyll cells (Kashid and Slave, 2014). The formation of triforked leaves might be developed by the death of apical cells at meristematic zone which have specific influences on the development of leaves and leaf shape (Bolbhat *et al.,*2012).

CONCLUSION

The effectiveness and efficiency of mutagen on leaf chlorophyll in Hyacinth bean are useful in identifying the genetic effect of mutagen. Mutations in these chlorophyll genes might be reflected in subsequent generations in the form of different mutants. The morphological mutants as well as frequency of chlorophyll deficient sectors induced in the present investigation included agronomical desirable characters which may possibly be utilized in future breeding programme.

Acknowledgement:
Authors are thankful to the Principal JES College, Jalna for providing research facilities. First author is grateful to UGC, New Delhi for giving financial support in the form of RGNF Fellowship. Authors are extremely grateful to the Department of Biophysics, Government Institute of Science, Aurangabad (M.S.) for providing Gamma Rays Irradiation facility.

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