

Original Research Article

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Induction of Morphological Leaf Mutations in *Lablab purpureus* (L.) Sweet through Chemical and Physical Mutagens

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ABSTRACT

Keywords

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A comparative study of morphological leaf mutations induced by the chemical mutagens like Ethyl Methanesulphonate (10mM, 20mM, 30mM and 40mM), Gamma radiations (100Gy, 200Gy, 300Gy and 400Gy) and Gamma rays and Ethyl methanesulphonate combination treatment (100Gy + 40mM, 200Gy+ 30mM, 300Gy+ 20mM and 400Gy+ 10mM). The morphological leaf variations were observed in the M₁ and M₂ generations. The variation was observed in the leaf lamina, leaf size, leaf apices, leaf shape etc. The normal leaf was with the acute apex but variation observed in the leaf apex with emarginated, acuminate apex. The bifurcated leaf, and Aristate, Lunate, elongated leaf lamina was also observed in the M₁ Generation.

Introduction

Lablab purpureus (L.) Sweet belonging to family Leguminosae sub-Family Papilionaceae. It is commonly known as *Dolichos* bean. It is widely distributed in tropical and subtropical regions. Where it is used as vegetable, grain legume and animal fodder. It is one of the most ancient crops among cultivated plant. In India *Lablab* was grown as a field crop and cultivated in Madhya Pradesh, Andhra Pradesh, Karnataka, and Maharashtra (Mahadevu and Byregowda, 2005). The crop is either cultivated as a pure crop or intercropped with groundnut, Castor or *Sorghum*. Despite

the many important traits the crop remained unexploited due to low productivity, photosensitivity and indeterminate growth habit. Development of new variety with the desirable traits depends upon the genetic variability present in the populations of the crop plant. Being the self fertilized plant the *Lablab purpureus* has limited genetic variability which affects the crop Improvement programme of this crop plant. The genetic variability could be achieved by the Conventional Hybridization programme or Mutation Breeding programme.

Mutation Breeding is an effective tool for the crop improvement (Acharya *et al.*, 2007). Mutagens like Gamma rays, Ethyl methane sulphonate, X-rays are known to influence plant growth and development by inducing cytological, genetically, biochemical, physiological, morphogenetical changes in cells and tissues (Girija and Danavel, 2009). Selecting the effective mutagenic dose important for the success of Mutation Breeding Programme.

The present investigation undertaken to estimate the effect of Physical and Chemical mutagens in induction of morphological mutation in *Lablab purpureus* (L.) Sweet, variety *Phule suruchi*.

Materials and Methods

Collection of seed material

The seeds of *Lablab purpureus* (L.) variety *Phule Suruchi* were collected from Mahatma Phule College of Agriculture, Shivajinagar, Pune. India.

Mutagens used

Chemical mutagen- Ethyl Methanesulphonate was obtained from Spectrochem Pvt. Ltd. Mumbai

Physical mutagen- For physical mutagen seed were irradiated with Gamma rays obtained from source CO⁶⁰. This facility was availed from Department of Chemistry, University of Pune, Pune.

Combination treatment- Combination (Ethyl Methanesulphonate and Gamma rays) of different concentration/dose of mutagens was used.

Mode of treatment

Each treatment 500 healthy seeds were selected and treated with different

concentration/dose for Ethyl Methanesulphonate (EMS) at concentration of 10mM, 20mM, 30mM, 40mM and Gamma rays doses with 100Gy, 200Gy, 300Gy, 400Gy and Combination of EMS and Gamma rays Concentration/dose at 100Gy+40mM, 200Gy+30mM, 300Gy+20mM, 400Gy+10mM. The concentration/dose was decided according to LD₅₀ treatments. For EMS concentrations seeds were pre-soaked with distilled water for four hours and treated with different concentrations of EMS for four hours followed by post soaking period of four hours. Also for EMS and Combination treatments seed material was post soaked for four hours. In combination treatment the seeds are first irradiated with the Gamma rays doses and then treated with different concentrations of EMS and followed by post soaking for 4 hrs.

Immediately after the treatments the seeds are sown in completely Randomized Block Design with 3 Replicates with the control. All the cultural practices were followed during the crop growth period. The morphological leaf variations were identified on the basis of morphological study.

Leaf abnormalities and Chimeras

Any change developed in the leaf morphology such as unifoliate, bifoliate, tetrafoliate etc. Chimerical plants showing sector or sectors in the leaves screen and record. The characters like-entire leaflet with albino, half leaflet albino and 2-3 sectors on the leaflets screen and record.

Results and Discussion

The chemical and physical mutagens induced the mutation in the plant result into the morphological changes in the leaf. The variation was observed in the leaf lamina,

leaf size, leaf apices and leaf shape. The normal leaf was with the acute apex but variation was observed in the leaf apex with emarginated, acuminate apex. The bifurcated leaf, and lunate, elongated leaf lamina was also observed in the M₁ generation. The chlorophyll mutants were also observed during the treatment.

In the M₁ generation of *Lablab purpureus* (L.), the chlorophyll deficient sectors were observed in all of the mutagenic treatments. Chlorophyll chimeric plants can be originated when sector becomes mutate. The embryo consists of the different meristamatic tissues and which have ability to develop a certain parts of the mature plants. A response of such embryonic cells to the mutagen causes chimerism (Gaul, 1958). In the present investigation, the chlorophyll chimeric plants cannot breed true. But the chimeras may be developed due to a certain changes at initial level at the embryos in addition to the physiological change may also add to the formation of leaf chimeras. In the frequencies of chimera

plants shows very low values and narrow range of variations in a given mutagenic treatment.

The M₁ population of plants have chlorophyll chimeras demonstrated a few changes or variation in the shape and size of the leaflets. The variation in leaflets like linear, broad, dark green and torn leaflet apex. These variations of leaflet were found maximum in EMS mutagenic with respect to all treatments. The extension of leaf morphology has resulted in the family Fabaceae due to spontaneous gene mutation and DNA recombination during evolution. The tetrafoliate condition of leaves has been evolved by lobing and gradual bifurcation of a simple leaf in to the tetra condition. The change in morphology of leaflets has been developed due to the alteration in metabolic and physiological activities of the developing primordial after mutagenic treatment (Prasad 1967). Chimerism was attributed the leaf abnormalities to the pleiotropic action of mutated gene were reported by (Joshua *et al.*, (1972).

Photo plate No.1 Morphological leaf mutations



- a) Leaf with emarginated apices
- b) Leaf with acute apex but oblique at one side
- c) Leaf with acute apex



d) Tapering leaf lamina with acute apex and leaf broad than length



e) Leaf apex bifurcated with distinct acuminate apex



f) The odd leaflet



g) Elongated leaf lamina

In present investigation the broad spectrum of leaf mutations was observed in the M₂ population of *Lablab purpureus* (L.). Variation in number of leaflets like bifoliate, tetrafoliate and pentafoliate were recorded. Large number of variations in shape and size of leaf were observed. The changes in leaves are due to chromosomal aberrations produced in plant because of the action of mutagens. Chlorophyll synthesis in the mutant seemed to be environmentally dependent i.e. on light intensity during the growing period, under less light intensity

mutant produced more chlorophyll and became greener. The morphological variation in M₂ generation of *Lablab purpureus* (L.) using Gamma rays, large leaflet shape showed high yield advantage over the narrow leaflet. Large leaflet area have high photosynthetic rate which leads to high metabolic activities in plant resulting in high yield. .

The induction of chimeric variation in leaflets after treatment of Gamma rays in *Phaseolus vulgaris* L. was reported by

(Kawai, 1983). Biological damage in M₁ generation o was observed in two cultivars of French bean treated by EMS, SA and Gamma rays. The maximum frequency of plants carrying chlorophyll chimeras was reported in SA treatments in both the varieties of French bean by (Mahamune and Kothekar, 2012). Many researchers have observed the same results in different plants like Alfalfa by (More, 1992), in Winged bean by (Hakande 1990), Urdbean by (Sagade and Apparao, 2011), in Cowpea by (Gaikwad, 2013), in Cluster bean by (Shinde, 2013) and (Bhosale, 2013) in *Withania*, (Salve, 2013) in *Coriandrum* and (Ramezani, 2014) in Grasspea.

In conclusion, the number of variants inducing the shape and size of leaflets were recorded in M₁ generation of *Lablab purpureus* (L.) Var. *Phule suruchi*. These includes enhancement in the size of leaflets, reduction in size of leaflet, broad leaflet, linear leaflet, dark green color, trifoliate, tetrafoliate and pentfoliate. These were found at the margin of the leaflets or entire leaflet lamina. It was found that the Combination treatment succeeded in producing the highest number of chlorophyll deficient sectors followed by EMS and least number was found in Gamma rays.

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