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Mutagenic efficiency of gamma-rays, ethyl methane sulphonate and its combination on microsperma lentil (*Lens culinaris* Medik)

S. P. Singh*, R.P. Singh, N.K. Singh, J.P. Prasad and J.P. Shahi

Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, B.H.U., VARANASI (U.P.) INDIA

ABSTRACT

Seeds of microsperma lentil (*Lens culinaris* Medik) variety Pant L-406 were selected for the mutagenic treatment. Fresh healthy seeds were irradiated with 10kR, 15kR, 20kR, 25kR, and 30kR doses of gamma-rays. Healthy seeds of this variety were presoaked in distilled water and treated with different concentration of ethyl methane sulphonate (0.02M, 0.03M, 0.04M and 0.05M). Half of the irradiated seeds were also treated with 0.03M ethyl methane sulphonate (EMS) for combination treatments. Observations were recorded for percent germination, seedling height, pollen fertility, plant survival and chromosomal abnormalities in M_1 generation. Frequency and spectrum of chlorophyll and viable mutations were scored in M_2 generation. Higher doses of mutagen either alone and / or in combinations severely reduced the percentage of germination, seedling height, pollen fertility and plant survival than their corresponding lower doses. The percent chromosomal abnormalities *viz*. fragments, bridges, laggards and micronuclei were increased with increase in dosage/ concentration of mutagens. Mutagenic efficiency was computed on the basis of seedling injury, lethality and pollen sterility. Lower dose of EMS (0.02M) was found to be most efficient in inducing more chlorophyll and viable mutations. Various types of chlorophyll and viable mutations were identified from all the treatments.

Key words : Lentil, Mutation frequency, Chromosomal aberratious, Mutagenic efficiency.

INTRODUCTION

Lentil is grown as a winter crop all over India either as sole or mixed crop. The species *Lens culinaris* Medik has been divided into two subspecies macrosperma and microsperma on the basis of seed size and cotyledon colour (Barulina, 1930). The seeds of macrosperma are bold with yellow cotyledon colour and seeds of microsperma are small with red cotyledon colour. The choice of mutagen holds great importance in changing the spectrum of mutations in a predictable manner. The physical and chemical mutagens cause three types of effects i.e. physical damage, gene mutation and chromosomal aberration (Swaminathan 1965). Among all the mutagens, the most effective physical and chemical mutagens are x-rays, gamma-rays and EMS respectively. The information available on the relative potency of the frequency of chlorophyll and viable mutations induced by these two major groups of mutagens in microsperma lentil is insufficient. Therefore, a comparative study of the frequency and spectrum of chlorophyll and viable mutations induced by gamma rays, EMS and their combinations was undertaken in microsperma lentil.

MATERIALS AND METHODS

Healthy seeds (100g) of lentil variety Pant L-406 were irradiated with gamma-rays at 10 kR, 15 kR, 20 kR, 25 kR and 30 kR doses (irradiation source was ^{60}Co gamma cell with capacity to release 3000 Ci delivery 7200 r/min.). Half of the irradiated seeds were used for combination treatment with 0.03 M EMS. Separate seed lots of this variety were presoaked in distilled water for 6 hrs. The soaked seeds were treated with 0.02 M, 0.03 M, 0.04 M and 0.05 M EMS for 6 hrs. (pH 7.0).The treated seeds were washed thoroughly in running tap water. The hundred seeds from each treatments along with control were placed in Petri dishes in the laboratory (25 ± 1° C) for taking observations on seed germination and seedling height. Rest of the treated seeds was sown in the field to obtain M_1 generation (2001-02). Observations on seed germination and seedling height were recorded on 8th and 12th day of incubation on treated and untreated (control) seeds. The fifty root tips from primary roots were excised from germinated seeds from each treatment including control. The root tips were fixed in Cornoy's solution (1:3:6, glacial acetic acid: chloroform: absolute alcohol) for 24 hrs for cytological studies. The root tips were boiled in acetocarmine solution (1g in 45% acetic acid) and smear were prepared in acetocarmine solution. The preparation were observed under microscope for cytological analysis (*viz.* laggard, bridges, fragments and micronuclei).The plant survival and pollen fertility were recorded from field grown plants to estimate mutagenic effect in M_1 generation. The mutagenic efficiency was determined as per the formula suggested by Konzak *et al*. (1965). All the M_1 plants were harvested individually and separately treatment-wise and M_2 generation was raised in the field in the next season (2002-03). The M_2 population were screened for both chlorophyll and viable mutations throughout the lifespan and presented as per 1000 M_2 plants.

RESULTS AND DISCUSSION

The doses of gamma-rays, EMS and their combinations cause corresponding decrease in germination per cent and plant survival in this variety (table 1). Lowest germination and plant survivals were found in combination treatment of 0.03 M EMS + 30 kR Gamma rays. Combination treatments in general, permitted low plant survival than all gamma-rays and EMS treatment individually. Similar relationship has been reported in lentil (Sarkar and Sharma, 1989; Gaikwad and Kothekar, 2004) and in other pulses (Waghmare and Mehra, 2001; Sharma *et al.,* 2005). The

* Author for corrospondence.