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Induced chemical and physical mutagenic studies in M_1 generation of French bean (*Phaseolus vulgaris* L.)

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Abstract
The seeds of French bean varieties Varun and Waghya were treated with chemical mutagens EMS and SA and physical mutagen gamma rays. M_1 generation was raised and studied with respect to different morphological parameters such as germination percentage, seedling height, leaf morphological changes, chlorophyll chimeras, pollen sterility and plant survival percentage at maturity. An increasing trend with an increase in mutagenic concentrations/doses could be recorded for leaf morphological changes, chlorophyll chimeras and pollen sterility while germination percentage, seedling height and plant survival percentage at maturity revealed decreasing trend with increasing mutagenic concentrations/doses.

Keywords: French bean, mutation breeding, chemical and physical mutagens

INTRODUCTION

French bean botanically described as *Phaseolus vulgaris* belongs to family Fabaceae. It is popularly grown for its edible immature pods as well as mature seeds. It is a low cost protein rich crop in many countries and also contains large quantities of complex carbohydrates, fibers and isoflavonoids [1].

A review of available literature reveals that such an important crop has got scant attention of scientists of our country regarding its improvement through induced mutation breeding. Therefore it was planned to initiate the work of induced mutation breeding in french bean. The present paper deals with the details of effects of physical mutagen gamma rays and chemical mutagens EMS and SA on different morphological parameters of M_1 generation of french bean varieties Varun and Waghya.

MATERIALS AND METHODS

Healthy seeds of french bean varieties Varun and Waghya were treated with physical (gamma-rays) and chemical mutagens (EMS and SA). Healthy and well dried seeds of varieties Varun and Waghya of french bean having uniform size and 10% moisture content were employed for irradiation with gamma rays. Seeds were packed in small polythene bags and sealed for gamma ray treatment. The seed samples were exposed to doses 05kR, 10kR and 15kR of gamma rays from Co^{60}, 1000 curie source of the gamma irradiation unit of the Department of Biophysics, Government Institute of Science, Nipat Niranjan, Aurangabad. (M.S.) India. The dose rate was 24,578 rads per hour. For chemical mutagenic treatment, healthy and uniform seeds of french bean varieties Varun and Waghya were surface sterilized with 0.1% mercuric chloride solution for about one minute and washed thoroughly with distilled water. They were presoaked in distilled water for 6 hours. The presoaked seeds were later immersed in the mutagenic solution for 4 hours in case of EMS and for 6 hours in case of SA and the treatments were given with intermittent shaking. The volume of the chemical mutagenic solution used was three times as that of seeds so as to facilitate uniform conditions. All the chemical mutagenic treatments were given at room temperature of 25±2°C. Seeds soaked in distilled water for 12 hours served as control. The different concentrations used for chemical mutagenic treatment were 0.05%, 0.10% and 0.15% for EMS and 0.010%, 0.015% and 0.020% for SA, respectively. Immediately after the completion of treatment, the seeds were washed thoroughly under running tap water to remove excess of mutagens. Later on treated seeds were post soaked in distilled water for 2 hours. The post soaked seeds were dried in folds of filter paper. 300 seeds were used for each treatment. 75 seeds from each treatment were kept on moist blotting paper in petriplates to record germination percentage. Another 75 seeds from each treatment were sown in pots to record seedling height. The remaining 150 seeds of each treatment were sown in the field following randomized block design (RBD) with three replications each consisting of 50 seeds along with control for raising the M_1 generation. The seeds were sown at a distance of 20 cm between the plants and 45 cm between the rows. The field experiments were carried out at the experimental field of Botany Department, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad during kharif season. The M_1 generation was thoroughly studied for following parameters.

Germination percentage

Germination percentage was recorded from the seeds germinated on moist blotting paper in petriplates when radical and plumule emerged out.

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Seedling height

Seedling height was measured on 10th day after germination of seeds, which were earlier sown in pots by randomly measuring the height of 10 seedlings from each treatment.

Leaf morphological changes

The frequency of leaf morphological changes was calculated by counting the number of plants carrying leaf abnormalities from the plants of each treatment.

Chlorophyll deficient sectors/chimeras

Plants with different types of chlorophyll deficient sectors in leaves were counted by screening the M_1 generation and frequency of such plants was calculated.

Pollen sterility

Pollen sterility was determined from 30 randomly selected plants of each treatment along with control. The anthers from the fresh flowers were removed and burst at one end. The pollen mass was smeared on a slide followed by addition of a few drops of 1.5% acetocarmine. Fully stained pollen grains were considered as fertile while empty, partially stained and shriveled ones were considered as sterile. The values were expressed as percentage.

Survival of plants at maturity

Survival of plants in each treatment and their respective controls were recorded in the field at the time of maturity. The values were expressed as percentage.

RESULTS AND DISCUSSION

Seed germination percentage

Observation regarding the germination percentage revealed decreasing trend of seed germination percentage with an increased mutagenic concentration/dose. Higher concentrations/doses of all the mutagens had shown maximum inhibition. The germination in control was found to be 93.33% in Varun whereas 83.33% in variety Waghya. In both the varieties the SA was found to be most inhibitory as compared to EMS and Gamma rays. Least inhibition in germination could be seen in gamma ray treatments as compared to EMS and SA in both the varieties. Maximum seed germination of 88.33% and 80% could be detected in 05kR gamma ray dose in Varun and Waghya, respectively while lowest germination was noticed in 0.020% SA concentration in both the varieties. The values recorded were 61.67% and 60% in Varun and Waghya, respectively. Reduction in germination is due to inhibition of genetic and physiological processes by mutagens which leads to lethality. Fadl (1983) [2] and Bajaj (1970) [3] have reported reduction in seed germination percentage in french bean after mutagenic treatments. The decrease in germination due to mutagenic action may be attributed to disturbances at cellular level (caused either at physiological level or at physical level) including chromosomal damages or due to the combined effect of both. Disturbance in the formation of enzymes involved in the germination process may be one of the physiological effects caused by mutagenic treatments particularly chemical mutagens like EMS and SA leading to decrease in germination. Decrease in percent seed germination in french bean caused by gamma rays, EMS and SA might be due to their effect on genetic, physiological and cytological processes.

Seedling height

Gradual decrease in seedling height in all mutagenic treatments as compared to control could be recorded in present investigation. Maximum reduction in seedling height was observed in treatments of SA in both the varieties. Higher concentrations/doses of all mutagens had affected seedling height appreciably. Lowest seedling heights of 5.22 cm and 5.14 cm have been detected in Varun and Waghya, respectively in case of 0.020% concentration of SA. Decreasing trend of seedling height with increased mutagenic concentration/dose has been reported in french bean by Ellyfa et al.(2007) [4] . Cheah and Lim (1982) [5] observed depressed seedling growth due to the effect of gamma irradiation in french bean. Reduced seedling height at higher mutagenic concentrations/doses may be due to gross injury caused at cellular level either due to acute chromosomal aberrations or gene controlled biochemical processes or sometimes both. Rao and Reddi (1986) [6] observed reduction in seedling growth by sodium azide due to inhibition of energy supply resulting in inhibition of mitosis which can be associated with seedling growth depression. Sparrow et al. (1961) [7] assigned the reduced seedling growth by radiations to inhibition of cell division and extra chromosomal damage. Conger and Stevenson (1969) [8] correlated increased seedling injury with chromosomal damage. Evans (1965) [9] considered growth reduction to be due to cumulative expression of mitotic cycle delay, creation of chromosomal structural changes and loss of proliferation capacity due to premature differentiation or cell death. However from the foregoing discussion it is evident that the reduction in seedling height in Varun and Waghya varieties of french bean might be due to gross injury caused at cellular level, reduced mitotic cell division, chromosomal damage or loss of proliferation capacity.

Leaf morphological changes

In comparison with trifoliolate leaves in control various types of leaf morphological changes were recorded. Variations in leaf size, shape, number of leaflets, margin and length of petiole were detected in all mutagenic treatments. Several plants exhibited large and unequal leaflets. Different types of shapes like linear, ovate, cotton leaf shape, narrow and oblong were observed. Number of leaflets increased as well as decreased in many cases. Unifoliolate, bifoliolate and tetrafoliolate leaves were seen in several treated plants. Presence of notch in apex of many leaflets could be noticed. Entire margin of some leaflets changed into dentate. Reduction in size of petiole also was noticed in several plants. Increasing frequency values with increasing concentration/dose of mutagens were recorded in both the varieties. Highest frequency of plants carrying leaf morphological changes was detected in Varun at 0.020% SA concentration and the value was 17.70% while 0.15% EMS induced highest frequency (17.13%) of leaf morphological changes in Waghya. Ellyfa et al. (2007) [4] detected leaf alterations in french bean after gamma irradiation. Silva and Barbosa (1996) [10] observed reduced leaf size, small and elongated leaflets and leaflets with folded margins in Milinionario 1732 variety of french bean after SA treatments. Joshua et al. (1972) [11] have correlated

the development of leaf abnormalities to the pleiotropic action of mutated genes. Gottschalk (1971) [12] opined that the leaf mutants and recombinants of *Pisum sativum* carry lot of taxonomic significance for discussing problems of leaf evolution in Leguminosae. In the present studies, the morphological alterations induced by mutagens might have developed due to changes in physiological and metabolic activities of the developing primordia and thus altering leaf morphology in both the varieties of french bean.

Chlorophyll chimeras

Four types of chlorophyll chimeras recorded were *albina* (white), *xantha* (yellow), *chlorina* (yellow green) and *viridis* (dull green). Maximum frequency of plants carrying chlorophyll chimeras could be detected in SA treatments in both the varieties. Highest frequencies (10.25%) and (8.15%) were detected in Varun and Waghya, respectively at 0.020% SA treatment. The lowest frequency values, 3.57% for Varun and 2.10% for Waghya were observed at 05kR dose in both the varieties. Less induction of chlorophyll chimeras was found in Waghya as compared to Varun in most of the treatments. Chlorophyll chimeras induced by mutagens embodies one of the prime M_1 biological effects. However, this effect is restricted to the M_1 generation only. Detail study of chlorophyll chimeras has been immensely helpful in rapid estimation of effectiveness of mutagenic agent and new treatment conditions quite early in M_1 generation itself. Stadler (1930) [13] was first to observe that mutations induced by seed irradiation appeared in the form of sectors in M_1 plants. Anderson et al. (1949) [14] demonstrated that the analysis of mutated sectors can greatly help in tracing the ontogeny of organs in M_1 plants. Motto et al. (1975) [15] reported chlorophyll chimeras in french bean after EMS treatments. Gaul (1958) [16] opined that differential response of the embryonic cells to mutagen causes chimerism. Developments of chlorophyll chimeras in plants become possible when a sector of the multicellular embryo becomes mutated. The embryo contains different sets of meristematic initials which are potentially capable of producing certain portion of the mature plant. A differential response of such embryonic cells to the mutagen causes chimerism [16].

Table 1. Effect of mutagens on different M_1 parameters in french bean variety Varun.

Treatment	Concentration (%) / Dose (kR)	Seed Germination %	Seedling height cm	Frequency of plants carrying leaf morphological changes %	Frequency of plants carrying chlorophyll chimeras %	Pollen sterility %	Plant survival %
Control	------	93.33±0.33	12.26±0.71	-------	-------	4.18±0.55	91.69±0.67
EMS	0.05	85.00±0.58	8.95±0.42	9.50±0.36	4.20±0.11	10.03±0.05	87.44±0.36
	0.10	81.67±0.88	7.96±0.49	11.82±0.57	5.30±0.65	10.88±0.13	81.31±0.50
	0.15	78.33±1.20	6.18±0.45	13.56±0.52	6.31±0.48	11.14±0.22	76.59±0.72
SA	0.010	71.67±0.88	7.13±0.09	12.47±0.71	7.49±0.33	12.88±0.48	82.28±0.86
	0.015	66.67±0.67	6.34±0.33	15.19±0.52	9.38±0.31	14.38±0.50	78.64±0.54
	0.020	61.67±1.20	5.22±0.12	17.70±0.85	10.25±0.16	16.32±0.29	73.75±0.39
Gamma rays	05kR	88.33±0.33	9.78±0.23	8.42±0.36	3.57±0.36	7.32±0.16	89.67±0.40
	10kR	83.33±0.67	8.32±0.39	10.45±0.20	3.83±0.12	8.27±0.14	84.62±0.77
	15kR	80.00±1.16	7.41±0.65	12.33±0.33	4.68±0.37	9.56±0.25	78.52±1.01

Table 2. Effect of mutagens on different M_1 parameters in french bean variety Waghya.

Treatment	Concentration (%) / Dose (kR)	Seed Germination %	Seedling height cm	Frequency of plants carrying leaf morphological changes %	Frequency of plants carrying chlorophyll chimeras %	Pollen sterility %	Plant survival %
Control	-----	83.33±0.88	11.93±0.49	-------	-------	5.55±0.54	84.90±0.92
EMS	0.05	75.00±0.58	8.24±0.27	10.96±0.41	2.67±0.57	10.25±0.45	82.79±0.42
	0.10	73.33±1.20	7.13±0.14	13.49±0.29	4.19±0.24	11.22±0.77	78.25±0.38
	0.15	68.33±0.67	6.04±0.04	17.13±0.25	5.20±0.69	12.11±0.92	74.46±0.30
SA	0.010	70.00±1.16	6.97±0.03	11.96±0.20	5.70±0.36	14.49±0.26	78.43±0.52
	0.015	66.67±0.88	6.01±0.15	14.20±0.28	6.88±0.44	16.00±0.40	75.00±0.58
	0.020	60.00±0.58	5.14±0.09	16.72±0.19	8.15±0.54	18.18±0.30	70.27±0.73
Gamma rays	05kR	80.00±1.00	9.68±0.26	10.38±0.54	2.10±0.11	8.24±0.29	82.17±0.98
	10kR	76.67±1.45	8.26±0.44	12.51±0.59	3.46±0.34	9.25±0.07	81.57±0.62
	15kR	71.67±1.86	7.14±0.65	14.72±0.56	5.42±0.56	10.22±0.17	76.12±0.59

Pollen sterility

Increasing trend of pollen sterility with increasing concentrations/doses of all the mutagens could be noticed in both the varieties. Maximum pollen sterility was induced by SA treatments in Varun and Waghya. Lowest sterility (7.32%) was recorded in

Varun at 05kR dose. As compared to Varun, maximum induction of pollen sterility could be detected in Waghya variety in all the mutagenic treatments. Different views have been expressed regarding mechanism of mutagens on induction of pollen sterility. According to Sudhakaran (1971) [17] and Konzak et al. (1961) [18] induction of pollen sterility is due to chromosomal abnormalities caused by the mutagens. According to Nilan et al. (1964) [19] gross injury due to gene controlled biochemical processes or acute chromosomal aberrations or both may be the reason for pollen sterility. The major cryptic changes in meiosis due to mutagenic treatments can be implicated for pollen sterility. Sato and Gaul (1967) [20] proposed that the radiation induced sterility in M_1 might be due to the detectable chromosomal aberrations and cryptic deficiencies while the sterility induced by EMS might be due to cryptic deficiencies and specific gene mutations. They classified the pollen sterility induced by EMS into three categories namely, (1) chromosomal (2) genetic and (3) purely physiological. Sudhakaran (1971) [17] has concluded that the pollen sterility might represent the cumulative result of aberrant meiotic stages as well as physiological and genetic damage induced by breakage of chromosomes through the formation of antimetabolic agents in the cells. Khan et al.(2009) [21] and Kumar and Rai (2009) [22] stated that frame shift mutations caused by MMS changed the protein product as a result of changes in amino acid sequences which might have affected morphology and fertility of pollen grains. Siddiq and Swaminathan (1969) [23] reported that chromosomal aberrations, especially high frequency of translocations was responsible for high sterility. Similar mechanism may be responsible for the observed increase in pollen sterility with increased concentrations of the mutagenic agents in french bean.

Plant survival percentage at maturity

A negative correlation was observed between concentration/dose of the mutagens and survival percentage. Increase in concentration of all mutagens reduced plant survival percentage at maturity in both the varieties of french bean. Reduction in plant survival at maturity was more pronounced in SA as compared to EMS and gamma rays. The survival percentage of plants ranged from 82.28% to 73.75% in Varun for SA treatments and from 78.43% to 70.27% in Waghya. Gamma rays affected the survival of plants to less extent as compared to EMS and SA. Values of survival percentage in Varun were 89.67%, 84.62% and 78.52% while 82.17%, 81.57% and 76.12% in Waghya for gamma ray treatments could be noted. Plant survival at maturity is one of the most reliable parameters to evaluate the effect of any mutagen. An inverse relation between concentration of the mutagen and survival of plants at maturity has also been reported in french bean by Yankulov et al. (1980) [24] and Ellyfa et al. (2007) [4]. Gaul (1964) [25] opined that chromosomal and extra chromosomal injury might lead to disturbances at physiological and cytological levels resulting in decrease in survival percentage. Nilan et al. (1964) [19] proposed that different types of chromosomal aberrations or physiological imbalances might be the prime cause of lethality. Swaminathan et al. (1962) [26] noted decrease in survival of plants at maturity due to rapid infusion of chemical mutagens and their ability to produce chromosomal aberrations. All these factors and increase in lethality of cells, which is usually observed at higher mutagenic concentrations/doses might be responsible for reduction in plant survival rate in french bean.

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