IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

"Induced Genetic Variability in Black Turtle Bean (*Phaseolus vulgaris* L.)"

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ABSTRACT:

Mutagens may cause genetic changes in an organism, break the linkage and many new promising traits for the improvement of the crop plants are available. Induced genetic variability in crop plants is a valuable resource from which plant breeders can select and combine different desired characteristics to produce better crop plants. The desirable characters which have been bred through induced mutations. Various mutants have found wide acceptance, because the mutagenic treatment is needed only once to induce mutations and the varieties have the same attributes as those produced from conventional breeding techniques. In present investigation the physiological effects on seed germination and seedling height on 7th day after sowing were investigated. Gradual reduction in seed germination and seedling height was recorded with increase in dose / concentration of mutagens. Almost all the mutagenic treatments caused decrease in seed germination and seedling height.

Keywords: Mutagens, mutants, variability, seedling height.

Introduction:

Black Turtle Bean (*Phaseolus vulgaris* L.) locally known as *kala wal.* It is the most widely grown grain legume for human consumption and a major protein and mineral source. It is a source of filling fiber, disease fighting antioxidants, and numerous vitamins. It helps to protect against inflammation, heart diseases, weight gain, diabetes, certain cancers, and common nutrient deficiencies that we often see in people eating a typical western diet. The health benefits of black beans, their versatility, and their high nutrient content make them an excellent choice for both plant-based eaters and omnivores alike.

The green immature pods are cooked and eaten as a vegetable while dry beans furnish a large portion of the protein needs of low and middle class families. In some parts of the tropics leaves are used as a pot-herb, and to a lesser extent the green-shelled beans are eaten. Straw is used as fodder (James A. Duke. 1983).

Pulses are major sources of proteins among the vegetarians, and complement the staple cereals in the diets with proteins, essential amino acids, vitamins and minerals. Pulses provide significant nutritional, health benefits, and are known to reduce diseases such as colon cancer and cardiovascular diseases (Jukanti et al., 2012). The major pulses of the world are peas, beans, gram, mungbeans, horsegram, urdbeans, and cowpeas while the minor ones include like black turtle bean (*Phaseolus* vulgaris L.). The production share of each pulse clearly indicates the dominance of bean, pea and chickpea. Improvement in yield and productivity of black turtle bean is the need of the hour, but for this marginal land, aberrant rainfall, non availability of improved seeds and poor crop management are the main constraints. Amongst pulses black turtle bean is highly neglected crop throughout the world and hence require more emphasis on its improvement as it has nutritional and medicinal value too.

The major constraints are : Lack of high yielding varieties. Large area under rainfed cultivation. Biotic and abiotic stress conditions caused up to 30% losses. Poor crop management practices. Un availability of quality seeds. Deficiencies of major and micronutrients, low risk bearing capacity and resource poor farmers. On the technological front, pulses still need major break through in yield levels, through morphophysiological changes in plant type and development of multiple disease resistant varieties, with tolerance to abiotic stresses. Inadequate or no transfer of technologies, poor storage, lack of processing and marketing facilities (Massod Ali, 2005).

Black Turtle Bean : Black Turtle Beans are native to Mexico. The black seeds are resistant to disease and can tolerate hot conditions. Black Turtle Beans come to maturity about 90 days after germinating, and can be harvested when leaves are falling off or have lost most of their color. These beans are typically eaten as dry beans, soups, and other dishes (Choung, et al., 2003). Different types of legumes grown are consumed in tropical regions in the world. Legume growing areas in Tropical Africa include Nigeria, Senegal, Togo, Cameroun and in Tropical Asia include Indonesia and India (Borget, 1992).

Description : Black Turtle Beans is annual herb, erect and bushy, 20–60 cm tall, taproot system with root nodules, leaves alternate, green, trifoliolate, stipulate, petiolate, a pulvinus at base, leaflets ovate, entire, acuminate, 6–15 cm long, 3–11 cm wide, flowers axillary, racemes, zygomorphic, white, pink, or purplish, pods green, yellow, black, or purple, 7–18 cm long, seeds 4–6, usually glabrous, beak prominent; seeds grey or black, endosperm absent; 100 seeds weigh 10–67 g (James A. Duke. 1983).

Varieties (cultivars): Black Magic, Blackhawk, Domino, Nighthawk, Valentine and Zorro.

Nutritional value of blackgram: (Nutritional value per 100g (3.5oz) carbohydrate- 23.71, sugars- 0.32, dietary fibers- 8.7 and prtein-8.86. Vitamins- Vitamin A- 6IU, Thiamine (B1) – 0.244mg, Riboflavin (B2) – 0.059mg, Niacin (B3) – 0.505mg, vit. B6 – 0.069mg, vit. E- 0.87mg, vit. K- 3.3. Minerals- Ca-3%, Fe- 16%, Mg- 20%, P-20%, K- 8%, Na- 16, Zn-12%, Water- 65.40g (µg = micrograms, mg = milligrams, IU = International units, Percentages are roughly approximated using US recommendations for adults. Source- USDA Nutrient Database).

Health Benefits of Black Beans : Black beans improve cardiovascular health, protect against cancer, improve digestion. helps for keeping stable blood sugar, contains essential vitamins and minerals, proteins and helps for weight loss (Uzoechina O.B. (2009), Yude C. and Keqin R. (1993). 1) Maintaining healthy bones- Black beans are high in protein and fiber. The iron, phosphorus, calcium, magnesium, manganese, copper and zinc in black beans all contribute to building and maintain bone structure and strength. 2) Lowering blood pressure- Maintaining a low sodium intake it helps to decrease blood pressure naturally. 3) Managing diabetes- High-fiber diets have lower blood glucose levels. 4) Warding off heart disease- The fiber, potassium, folate, vitamin B6, and phytonutrient content of black turtle beans, coupled with its lack of cholesterol, all support heart health. 5) Preventing cancer- selenium plays a role in liver enzyme function and helps detoxify some cancer-causing compounds in the body. 6) Healthy digestion- fiber content in black beans help to prevent constipation and promote regularity for a healthy digestive tract. 7) Weight loss- Dietary fiber play important role in weight loss and weight management by functioning as a "bulking agent" in the digestive system. 8) Nutrition- According to the National Nutrient Database one-half cup (86g) of cooked black beans contains approximately: Energy- 114 kilocalories, Protein- 7.62 g, Fat- 0.46 g, Carbohydrate- 20.39 g, Fiber- 7.5 g, Sugars- 0.28 g, Calcium- 23 mg, Iron- 1.81 mg, Magnesium- 60 mg, Phosphorus- 120 mg, Potassium- 305 mg, Sodium- 1 mg, Zinc- 0.96 mg, Thiamin- 0.21 mg, Niacin- 0.434 mg, Folate- 128 mg and Vitamin K- 2.8 mg. 9) Diet- Black beans are available year-round and are often found in grocery stores either dried and packaged or canned. (https://draxe.com/black-beans-nutrition/).

MATERIALS AND METHODS

Seed material : The seeds of black turtle bean (*Phaseolus vulgaris* L.) were obtained from local market of Manchar, Tal. Ambegaon, Dist Pune-410503 (M.S.) India.

Mutagens used : Gamma rays (GR), ethyl methane sulphonate (EMS) and sodium azide (SA) were used in present study for the treatments of seeds of black turtle bean. Gamma radiation from ^{60}Co source fixed in the gamma cell 200 installed at Department of Chemistry, Savitribai Phule, Pune University, Pune was used in the present work. Healthy, dry and uniform seeds of black turtle bean with moisture content of 10-12 % were treated with 300, 400 and 500 Gy. Ethyl methane sulphonate ($CH_3SO_2OC_2H_5$) molecular weight 124.16, and 8% soluble in water, manufactured by Sigma chemical Co. Ltd. USA was used for the seed treatments of black turtle bean. Various concentrations of EMS (0.3% to 0.5%) were prepared in distilled water. Sodium Azide is inorganic compound. It is colour less salt, ionic compound, soluble in water and is highly toxic. Mol. Wt. is 65.0099g/mol. It is chemical mutagen and used for induction of mutations in the crop plants. Various concentrations of SA (0.15%,0.20% and 0.25%) was prepared in distilled water.

Treatment details : The pilot experiments were conducted to determine the lethal dose (LD_{50}), suitable concentrations of EMS, SA and duration of seed treatment. The doses of gamma rays, 300, 400 and 500Gy, EMS 0.3, 0.4 and 0.5% while SA 0.15, 0.20 and 0.25% were finally selected for the seed treatment and the duration fixed was four hours. Selected seeds were soaked in distilled water for 8 hours and the wet seeds were treated with different concentrations of EMS (0.3, 0.4 and 0.5%) and SA 0.15, 0.20 and 0.25% for four hours. The untreated seeds served as control. For each treatment 330 seeds were used.

The seeds treated with various concentrations of EMS and SA were washed thoroughly with tap water for two hours to terminate the reaction of chemical mutagen and to leach out the residual chemicals. A total of 30 seeds from each treatment was used for seed germination in laboratory. Three replications with 10 seeds / replication kept in petri dishes, containing seed germination paper, were used for recording seed germination percentage, root as well as shoot length, seedling growth on 7th day. The remaining lot of treated seeds (300) from each treatment was used for raising M_1 generation in field.

Experimental site : Present investigation was carried out at experimental field, Department of Botany and Ph. D. Research Center in Botany, Rayat Shikshan Sanstha's Annasaheb Awate Arts, Commerce and Hutatma Babu Genu Science College, Manchar, Tal. Ambegaon, Dist- Pune (410503) (M.S.). The soil type of the experimental field was slightly deep, fine and calcareous with good drainage. The average minimum temperature was recorded as 17.63^{0}C and maximum 32.73^{0}C with average annual rainfall 641.03mm.

Experimental design for field experiments: The field experiments were conducted on the experimental field at Department of Botany. The crop of black turtle bean was grown in *Kharif* season under uniform conditions. All the experiments were carried out in triplicate following RBD design. Each plot had 100 plants. The distance between two rows and two plants was 30X 30 cm and the distance between two adjacent plots was one meter. A total of 10 treatment combinations in M_1 generation including untreated dry seeds were used as control. Treated and control seeds were sown in field in randomized block design replicated thrice.

Observations on M_1 generation

Germination percentage: The number of seeds showing emergence of the radical and plumule was counted from the seeds kept in Petri plates lined on moist germination paper, data was used to calculate percent seed germination.

Root and shoot length: On 7th day of sowing, 10 seedlings from control and each treatment were randomly selected for measuring the root and shoot length and the average values were recorded in table.

Seedling injury: Seedling height was recorded on 7th day. Reduction in the mean seedling length as compared to the control was regarded as seedling injury and expressed as percentage. The seedling injury was calculated as follows

$$\% \text{ seedling injury} = \frac{\text{Control seedling height - Treatment seedling height X 100}}{\text{Control seedling height.}}$$

Pollen sterility : Pollen sterility was determined from 10 randomly selected plants per treatment, along with control. The pollen grains from freshly dehisced anthers were stained with 1% aceto-carmine. Pollen grains stained as uniform deep red colour were counted as fertile and others as sterile.

Survival of plants at maturity : Survival percentage was calculated by scoring the total number of plants attaining maturity (45 days) in each treatment and expressed as percentage over the control.

Harvesting of seeds from M_1 plants : All the surviving M_1 plants were harvested individually and seeds of single plant from each treatment were kept separately for raising M_2 generation.

Statistical analysis

The data were summarized as the means of three replicates with standard deviation as the measures of variability. One-way ANOVA test was performed to determine significant differences due to various treatments. Fisher's LSD (Least significant difference) was used as post hoc test to as certain significant differences among treatments at p= 0.05. Statistical analysis and graphical data presentations were carried out by using Sigma stat (ver.25).

RESULTS AND DISCUSSION

Results obtained in the present investigation on seed germination, seedling injury, pollen sterility and survival of plant at maturity in M_1 generation of black turtle bean are illustrated in Table- 1. Data obtained on mean percent seed germination in control and mutagen treatments presented in Table-1 clearly indicated that the seed germination percent was decreased in all the treatments as compared to control. It has clearly indicated that the mutagens had exerted negative effects on seed germination. Percent seed germination was decreased with the increase in doses/ concentrations of the mutagens. The percent seed germination decreased from 74.17% to 53.31% in gamma radiation, 78.24% to 58.12% in EMS and 81.69% to 54.07% in SA. The maximum (50%) decrease in percent seed germination was observed with gamma radiation treatment 500Gy (53.31%), EMS 0.5% (58.12%) and in SA 0.25% (54.07%). Thus 500Gy treatment was very effective in reducing percent seed germination in black turtle bean to almost 50%.

Table 1 : Effect of GR, EMS and SA on seed germination, seedling height, seedling injury, pollen sterility and plant survival at maturity in M_1 generation of black turtle bean.

Treatments	Germination (%)	Shoot length (cm)	Root length (cm)	Seedling height (cm)	Seedling injury (%)	Pollen sterility (%)	Plant survival at Maturity (%)
Control	86.43±12.10	4.82±0.67	4.65±0.65	9.47±1.33	00.00±0.00	07.61±1.07	78.49±10.99
300Gy	74.17±5.93	4.57±0.37	4.26±0.34	8.83±0.71	06.76±0.54	18.25±1.46	67.31±5.38
400	61.29±6.74	3.04±0.33	3.29±0.36	6.33±0.70	33.16±3.65	23.56±2.59	55.83±6.14
500	53.31±6.93	2.9±0.37	2.56±0.33	5.40±0.70	42.98±5.59	32.11±4.17	44.57±5.79
0.3 % EMS	78.24±10.95	4.35±0.61	4.10±0.57	8.45±1.18	10.77±1.51	21.83±3.06	65.14±9.12
0.4	66.81±4.68	3.62±0.25	3.55±0.25	7.17±0.50	24.08±1.69	29.09±2.04	54.76±3.83
0.5	58.12±4.65	3.14±0.25	3.05±0.24	6.19±0.50	34.64±2.77	35.17±2.81	46.92±3.75
0.15%SA	81.69±12.25	4.71±0.71	3.95±0.59	8.66±1.30	08.55±1.28	15.87±2.38	70.63±10.59
0.20	69.52±9.73	4.02±0.56	3.53±0.49	7.55±1.06	20.27±2.84	20.24±2.83	61.18±8.57
0.25	54.07±4.69	3.28±0.30	3.11±0.28	6.39±0.58	32.52±2.93	27.42±2.47	53.25±4.79
SEM±	6.86	0.39	0.36	0.74	2.25	2.14	6.01
F-value	6.08	7.50	6.36	6.73	8.18	29.50	6.37
P-value	0.01	0.01	0.01	0.01	0.01	0.01	0.01
$LSD_{0.05}$	14.94	0.85	0.78	1.61	4.90	4.66	13.10

Data are means of three replicates ± standard deviation. Significant difference due to treatments was assessed by Fisher's LSD as a post-hoc test.

Results indicate that, percent seed germination decreased with increasing doses or concentration of GR, EMS and SA in black turtle bean. This clearly indicates that the mutagens have exerted an inhibitory effect on seed germination. Similar inhibitory effect on seed germination reported earlier by Toker and Cagiragan (2004) and Barshile *et al*., (2006) in chick pea, Sharma *et al*., (2005) in urdbean, Samiullah Khan *et al*., (2006) in mung bean, Uttarde et.al., (2020) in sesame, Bolbhat and Dhumal (2011) in horsegram. The reduction in germination may be due to genetic and physiological processes inhibited by the mutagens resulting in cell maturity. Presoaking of seeds increases the sensitivity to chemical mutagens. This is subjected to various reasons such as change in metabolic processes (Sharma, 1969).

EMS and gamma rays are potent mutagens well known for their action in inducing point mutations, enzyme inhibitions and chromosomal aberrations. The results of present study have clearly shown that the black turtle bean was sensitive to all the mutagens. Amongst the mutagens used, gamma radiation treatments were more effective in reducing percent seed germinations, followed by EMS and SA.

Data recorded in Table-1 indicated that doses of gamma radiation and concentrations of EMS and SA treatments showed inhibitory effect on seedling height. Maximum decrease in seedling height (5.40cm) was noted in 500Gy, 0.5%EMS (6.19cm) and 0.25%SA (6.39). Data on the effect of gamma radiation, EMS and SA on seedling injury at M_1 shown in Table-1 revealed that all mutagenic treatments were highly injurious to the seedlings. GR treatments had caused highest seedling injury, followed by the single treatments of EMS and SA. The seedling injury increased with the increase in doses/ concentrations of mutagenic treatments. Maximum seedling injury (42.98%) was observed in 500Gy.

In the present investigation it was reported that the seedling injury increased with the increase in concentration or dose of mutagenic treatments in black turtle bean. Similar increase in seedling injury with increased dose/concentrations of mutagens has been reported by Khan et al., (2006) in mungbean, Sharma et al., (2005) and Sagade et al., (2008) in urdbean, Uttarde et.al., (2020) in sesame.

The reduction in seedling height by chemical and physical mutagens was ascribed to different factors. According to Kawsar and Markeen (2007) reduction in seedling growth with higher dose may be due to the gross injury caused at cellular level either due to gene controlled biochemical process or acute chromosomal aberrations or both. According to Sparrow et al., (1961) the chief cause of rays in seedling growth is the extra-chromosomal damage or inhibition of cell division. According to Bhamburkar (1981) the reduced seedling growth is due to reduction in number of cells contributing to seedling growth.

Data on pollen sterility is depicted in tables 1. From the table it is clear that all mutagens were effective in inducing pollen sterility in black turtle bean. EMS was the most effective of all the three mutagens in inducing pollen sterility. It was followed by the GR and SA. The highest pollen sterility was recorded 0.5%EMS (35.17%). Among the chemical mutagens EMS has induced higher sterility.

Results obtained on pollen sterility revealed that all the mutagens employed in the present investigation (GR, EMS and SA) are effective in inducing pollen sterility in M_1 generation. The rate of pollen sterility increased with increase in the concentration or dose of the mutagens. These results are in agreement with earlier researchers like Khan et al., (2004) and Auti (2005) in mungbean, Sharma et al., (2005), Sharma et al, (2006) and Sagade et al., (2008) in urdbean, Barshile et al., (2006) in chickpea, Uttarde et.al., (2020) in sesame.

According to Konzak et al., (1961) and Sparrow and Woodweel (1962) induction of the pollen sterility is due to chromosomal irregularities caused by the mutagen. Gunkel (1957) proposed that gross injury at cellular level is due to acute chromosomal aberrations. Various types of chromosomal abnormalities such as translocation, anaphase bridges and laggards were found in the progenies obtained from treated seeds.

The results on the effects of gamma radiation, EMS and SA revealed that in all the mutagenic treatments, survival % was decreased than the control (Table- 1). There was linear decrease in the survival % with increasing dose/ conc. of gamma radiation, EMS, and SA. The lowest survival % at the higher treatments was noted (44.57%) in 500Gy, 0.5%EMS (46.92%) and 0.25%SA (53.25%) as compared to control (78.49%). All mutagens reduced the rate of survival at maturity, Kavithamni *et al.*, (2008) in soybean supported the above findings.

CONCLUSION

Percent seed germination and seedling growth was inhibited due to increasing doses/ concentrations of mutagens. All mutagens (GR, EMS and SA) were effective in inducing pollen sterility in M_1 generation. The rate of pollen sterility increased with increase in dose/ concs. of the mutagens and the survival rate was highly reduced with increasing dose/concs. of mutagens.

ACKNOWLEDGEMENT

Authors are thankful to Principal Dr. N.S. Gaikwad for providing library, laboratory and research field facilities.

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