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Effect of mutagens on growth parameters of Lentil [Lens culinaris (L.) Medikus]

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ABSTRACT:

Seeds of lentil [*Lens culinaris* (L.) Medikus] were treated with various doses of gamma rays (200, 300 and 400 Gy.), concentrations of ethyl methane sulphonate (EMS) (0.2, 0.3 and 0.4%) and sodium azide (SA) (0.02%, 0.03% and 0.04%). The effects on seed germination, seedling height (7 days), pollen sterility and plant survival at 45 days after sowing were investigated. Gradual reduction in seed germination, seedling height and plant survival was recorded with increase in dose/concentration of GR, EMS and SA.

Keywords: Mutagens, lentil, germination, pollen sterility.

INTRODUCTION

The lentil [*Lens culinaris* (L.) Medikus] is an annual, self pollinated plant with lens-shaped seeds. It is also known as Masur. Whole seeds or splits known as dal are cooked and eaten with rice or roti. It is bushy, erect, semi erect, or spreading herb belonging to family Fabaceae. The stem, slender with hairy branches. The leaves are alternate, stipulate, the upper leaves are converted into tendrils. The inflorescence is racemose and flowers are small, white, pink, purple, pale purple, or pale blue in colour. The pods setting takes place three to four days after opening of flower, the pods are oblong, containing two lens shaped seeds (Yadav et al, 2007).

Lentil is mainly cultivated in Madhya Pradesh, Uttar Pradesh, West Bengal, Bihar and Maharashtra. Global production of lentils in 2018 was 6.3 million tonnes and India with 25% (1.62 million tonnes) of the world (FAOSTAT) 2019. Lentils grow on various soil types such as sandy or clay loam, with moderate fertility. It can fix atmospheric nitrogen with specific rhizobia and grow well under low fertilizer input conditions (Erskine et.al., 2009). It is cultivated in *kharif* or *rabbi* season and improve the physical properties of soils by biological nitrogen fixation. (Yadav et al, 2007).

Lentil seeds can be eaten germinated, soaked, boiled, baked and fried. The most common preparation method is boiling (Erskine et al., 2009). Lentil curry is part of the everyday diet, eaten with rice and roti. Flour of lentil seeds is used to prepare papad and in many regional varieties of sweets. Raw grains of lentils contains 8% water, 63% carbohydrates including 11% dietary fiber, 1% fat and 25% protein (USDA National Nutrient Database, 2015). It contain the lutein, zeaxanthin, polyunsaturated fatty acids and carotenoids (Zhang et al., 2014). Masur also have antinutrient factors, such as trypsin inhibitors and high phytate content. According to Vidal-Valverde (1994) enzyme trypsin is involved in digestion and phytates reduce bioavailability of dietary minerals.

Mutation breeding is short cut method, which is beneficially utilized for inducing the desired characters of different crop plants. The use of induced mutations is to correct one or more undesired characters of a cultivar, without changing the genetic makeup (Bara, 2017). Mutations provides an opportunity to create until now unknown alleles, so that the plant breeder does not remain handicapped for limited allelic variation at one or more gene loci of interest (Dixit and Dube, 1984). To induce genetic variability in masur is necessary to isolate a mutants which may show some degree of tolerance or resistance against heat stress. In several breeding methods, mutation breeding is useful tool to increase the genetic variability and yield potentiality of lentil crop. Chemical and physical mutagens can induce mutants which increase yield and improve several other desired characters in lentil, Gaikwad and Kothekar (2004), Shah et al., (2011), Sinha and Lal (2007). The mutagen selection, their dose and procedure for mutation breeding in masur is an important step for creating new genetic variability (Ram Narayan et al., 2014). In this experiment, an attempt was made by using physical and chemical mutagens to inducing variability in lentil. These mutant lines will be used in future breeding programme to improve the yield and quality traits in lentil.

MATERIALS AND METHODS

The seeds of lentil [*Lens culinaris* (L.) Medikus] were obtained from local market of Manchar, Tal. Ambegaon, Dist- Pune-410503 (M.S.) India.

Gamma rays (GR), ethyl methane sulphonate (EMS) and sodium azide (SA) were used in present investigation for the treatments of seeds of lentil. 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 lentil with moisture content of 10-12 % were treated with gamma radiation 200, 300 and 400 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 lentil. Various concentrations of EMS (0.2% to 0.4%) 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 (0.02%, 0.03% and 0.04%) was prepared in distilled water.

The 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, 200, 300 and 400Gy, EMS 0.2, 0.3 and 0.4% while SA 0.02, 0.03 and 0.04% were finally selected for the seed treatment and the duration fixed was four hours. Selected seeds were soaked in distilled water for 10 hours and the wet seeds were treated with different concentrations of EMS (0.3, 0.4 and 0.5%) and SA 0.02, 0.03 and 0.04% for four hours. The untreated seeds served as control. For each treatment 255 seeds were used.

The treated seeds were washed thoroughly with tap water for one hour 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, with seed germination paper, were used for recording seed germination percentage, root and shoot length, seedling height on 7^{th} day. The remaining lot of treated seeds (225) from each treatment was used for raising M_1 generation in field.

Present investigation was carried out at experimental field, Department of Botany, Annasaheb Awate College, Manchar, Tal. Ambegaon, Dist- Pune (410503) (M.S.). The soil type of the experimental field was slightly deep and fine. The average minimum temperature was recorded as 18.24^0C and maximum 33.43^0C with average annual rainfall 712.05mm.

The crop of lentil was grown in *Kharif* season. All the experiments were carried out in triplicate following RBD design. Each plot had 75 plants. The distance between two rows and two plants was 30 X 15 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

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. On 7^{th} 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. Reduction in the mean seedling height as compared to the control was regarded as seedling injury and expressed as percentage. The seedling injury was calculated as follows

% seedling injury = [(Control seedling height - Treatment seedling height)/ Control seedling height] X 100

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 percentage was calculated by scoring the total number of plants attaining maturity (45 days) in each treatment and expressed as percentage over the control. All the surviving M_1 plants were harvested individually and seeds of 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 lentil 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 (93.57%). It has clearly indicated that the mutagens had exerted negative effects on seed germination. Seed germination percent was decreased with the increase in doses/ concentrations of the mutagens. The percent seed germination decreased from 81.32% to 57.18% in gamma radiation, 75.23% to 55.46% in EMS and 86.52% to 67.75% in SA. Maximum reduction in germination was recorded 55.46% in 0.4% EMS.
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 Singh et al. (2007) in lentil, Bolbhat and Dhumal (2009) in horsegram, Kumari and Singh (1996) in pea. They concluded that the effect of mutagens on seeds is expressed through delayed emergence of roots, reduction in vigour, low metabolic and enzymatic activity, losses of membrane integrity and finally loss of germinability.

Table 1 : Effect of mutagens on seed germination, seedling height, seedling injury, pollen sterility and plant survival at maturity in M_1 generation of lentil.

Treatments	Germination (%)	Shoot length (cm)	Root length (cm)	Seedling height (cm)	Seedling injury (%)	Pollen sterility (%)	Plant survival at maturity (%)
Control	93.57±13.10	3.19±0.45	2.95±0.41	6.14±0.86	00.00±0.00	04.11±0.58	90.11±12.62
200Gy	81.32±6.51	2.87±0.23	2.49±0.20	5.36±0.43	12.70±1.02	12.19±0.98	76.23±6.10
300	69.91±7.69	2.33±0.26	2.05±0.23	4.38±0.48	28.66±3.15	19.32±2.13	65.48±7.20
400	57.18±7.43	2.12±0.28	1.76±0.23	3.88±0.50	36.81±4.79	25.46±3.31	54.69±7.11
0.2 % EMS	75.23±10.53	2.68±0.38	2.31±0.32	4.99±0.70	18.73±2.62	14.37±2.01	73.37±10.27
0.3	64.31±4.50	2.19±0.15	1.87±0.13	4.06±0.28	33.88±2.37	21.64±1.51	59.75±4.18
0.4	55.46±4.44	2.03±0.16	1.43±0.11	3.46±0.28	43.65±3.49	26.72±2.14	48.03±3.84
0.02%SA	86.52±12.98	3.11±0.47	2.71±0.41	5.82±0.87	5.21±0.78	08.93±1.34	82.72±12.41
0.03	78.65±11.01	2.91±0.41	2.32±0.32	5.23±0.73	14.82±2.07	13.53±1.89	73.90±10.35
0.04	67.75±6.10	2.52±0.23	2.12±0.19	4.64±0.42	24.43±2.20	17.15±1.54	61.34±5.52
SEM±	7.33	0.26	0.22	0.48	2.13	1.54	6.97
F-value	5.72	5.19	8.20	6.39	8.75	4.35	7.00
P-value	0.01	0.01	0.01	0.01	0.01	0.01	0.01
$LSD_{0.05}$	15.97	0.57	0.48	1.05	4.64	3.36	15.19

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

Data recorded in Table-1 indicated that mutagen treatments showed reduction in seedling height. Maximum decrease in seedling height (3.88cm) was noted in 400Gy, 0.4%EMS (3.46cm) and 0.04%SA (4.64cm). 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. EMS treatments had caused highest seedling injury, followed by the GR and SA. The seedling injury increased with the increase in doses or concentrations of mutagenic treatments. Maximum seedling injury (43.65%) was observed in 0.4%EMS. In the present investigation, the seedling injury increased with the increase in conce. or dose of mutagenic treatments in lentil. Similar results has been reported by Sinha and Lal (2007) in lentil, Bolbhat et al., (2020) in black beans. Chromosomal aberrations or biochemical process responsible for reduction in seedling growth.

Data on pollen sterility is depicted in table. From the table it is clear that all mutagens were effective in inducing pollen sterility in lentil. EMS was the most effective as compare to other mutagens in inducing pollen sterility. It was followed by the GR and SA. The highest pollen sterility was recorded 0.4%EMS (26.72%). Among the chemical mutagens EMS has induced higher sterility. Results obtained on pollen sterility revealed that all the mutagens used in the present investigation are effective in inducing pollen sterility in M_1 generation. The pollen sterility rate increase with increase in the conc/ dose of the mutagens. These results are in agreement with earlier researchers like Sagade et al., (2008) in urdbean, Barshile et al., (2006) in chickpea, Bolbhat et al., (2020) in black beans, Bolbhat and Wagh (2020) in horsegram, Uttarde et.al., (2020) in sesame. 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 0.4%EMS (48.03%), (54.69%) in 400Gy, and 0.04%SA (61.34%) as compared to control (90.11%). All mutagens reduced the rate of survival at maturity, Barshile et al., (2006) in chickpea, Bolbhat et al., (2020) in black beans, Bolbhat and Wagh (2020) in horsegram supported the above findings.

CONCLUSION

The present study revealed that the growth parameters such as germination (%), seedling height (cm), pollen sterility and survival % at maturity was inhibited due to increasing doses/ concentrations of mutagens.

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