Effect Of Chemical Mutation Through Hydroxylamine Hydrochloride On Quantitative Traits Variation In *Phaseolus vulgaris* L.

Girma Mosisa, Manikandan Muthuswamy, Yohannes Petros

Abstract: Chemical mutagenesis is a simple technique used to create mutation in plants for their improvement of their potential agronomic traits. Experiments were conducted in experimental field stations at Haramaya University, Ethiopia. The objectives of the study was identifying the quantitative traits variation produced by induced chemical mutation; identifying the effect of mutagenic chemical on morphology, root nodules and determining the effectiveness of mutagenic chemicals in haricot bean growth. A mutagenic chemical (Hydroxylamine hydrochloride) were used as chemical mutagen. A variety of haricot bean (Haramaya G- 843) were used for inducing mutation and planted in Complete Randomized Design (CRD) factorial with three replications. A highly significant reduction at (P<0.0001) in seed germination of haramaya variety G-843 was observed at higher concentrations of Hydroxylamine hydrochloride when compared with the control. At a lower dose of mutagenic treatments, days required for flowering and days required for maturity were decreased in 0.02% concentration of Hydroxylamine hydrochloride in Haramaya variety G-843 when compared with control. The mutagenic effectiveness was found to be the highest at lower concentration in germination percentage, plant height, days of flowering and days of maturity, pods per plants, internodes length, and root nodules in Haramaya variety G-843 by hydroxylamine hydrochloride.

Key words: Induced chemical mutagenesis, haricot bean, hydroxylamine hydrochloride.

1 INTRODUCTION

Haricot bean is one of the most important food and export crop in Ethiopia and it is the source of protein and cash for poor farmers [1]. It has been an export crop for more than 40 years, and grown as a food crop for a much longer period. In many low lands of Ethiopia, haricot bean is considered as the main cash crop and protein source for the farmers [2]. At present, different verities of haricot beans are grown in the country. The average yield of haricot bean in Ethiopia is low, when compared to other African countries like Egypt where average yield is about 2,500kg/ha. This may be due to the lack of improved varieties for different agro-ecological zones [3]. Chemical mutagenesis is a simple technique used to create mutation in plants for their improvement of their potential agronomic traits. It is one of the most important tools used to study the nature and function of genes, which are the building blocks and basis of plant growth and development, thereby producing raw materials for genetic improvement of economic crops [4]. The main advantage of mutational breeding is the possibility of improving one or two quantitative characters without changing the rest of the genotypes. Induced mutations have great potentials and serve as a complementary approach in genetic improvement of crops [5].

- *Girma Mosisa is currently working in the Department of Biology, Metu University, Ethiopia*
- *Manikandan Muthuswamy and Yohannes Petros is working in the Department of Biology, Haramaya University, Ethiopia*
- *Manikandan Muthuswamy, corresponding author, Email: manikandanrejula12in@gmail.com*

This technology has been used to improve major crops such as wheat, rice, barley, cotton, peanut and cowpea, which are propagated through seeds. The various mutagenic agents that are used to induce favorable mutations at high rate include ionizing radiation and chemical mutagens [6]. Legumes are found to be well suited for genetic improvement through mutation breeding due to their evolutionary selection history [7]. It has been shown that most legumes have lost many alleles for high productivity, seed quality, pest and disease resistance in the process of adaptation to environmental stress. Valuable progress has, however, been reported on the improvement of nutritional quality of some legumes by induced mutation. The use of hydroxylamine to induce mutants has been reported by a number of workers [8]. The dynamics in the leaf number with varied concentration of hydroxylamine hydrochloride over the experimental period was presented [9]. Mutation techniques have been used almost exclusively for plant breeding. The outcome was indeed remarkable: about 2000 new varieties were developed in almost all countries except Africa, where progress was limited [10].

2 MATERIALS AND METHODS

2.1 Plant Materials and Mutagenic Chemical

The selection of the crop plant was based on economic importance as well as availability in Haramaya University, Ethiopia. Accordingly, the Haramaya variety (G-843) of haricot bean was selected. The induction of chemical mutation was achieved by using hydroxyl amine hydrochloride. The different concentrations of hydroxyl amine hydrochloride (0.01, 0.02, 0.03, 0.04 and 0.05 % V/V) were prepared in plastic beakers with distilled water.

2.2 Method of Chemical Mutagenesis

The seeds of the selected variety of Haramaya (G-843) were surface sterilized with 0.1% mercuric chloride for 1 minute to remove the fungal spores on the surface of the seeds. Then the seeds were washed with distilled water several times to remove the mercuric chloride. After this the seeds were pre soaked in plastic beaker which contains distilled water for six hours and then treated with hydroxyl

amine hydrochloride at different concentrations (0.01, 0.02, 0.03, 0.04 and 0.05%) for 6 hrs [11].

2.3 Bioassay Studies

The bioassay studies were carried out following the method of [12]. Five seeds from each of the treatments were placed on whatman filter paper in petriplates (9cm X 2cm). Each petriplate was moistened with 2ml/ plate of distilled water and the control were normal seeds (untreated) and incubated at room temperature. The germination percentage, root and shoot length were measured after 8 days. The germination percentage was calculated by using the following formula.

$$\text{Germination (\%)} = \frac{Number\ of\ seeds\ germinated}{Number\ of\ seeds\ placed\ for\ germination} x100$$

2.4 Green house experiments

The seeds that were treated with chemical mutagenic agent in the laboratory were sown in earthen pots (24 cm x 24 cm) in green house by CRD factorial design with three replications along with their respective control. Then the experimental plants were grown under the green house condition in Haramaya University, Ethiopia. The control plants were grown from the untreated seeds. The agronomic traits such as plant height, number of branches on the main axis, seed length, internode length, days to 50% flowering, days to 90% maturity, pods per plants and above ground biomass were measured and determined.

2.5 Statistical Analysis

Inference and descriptions were made on the data collected. Analysis of Variance (ANOVA) was done for comparing the treatments with control using SAS version nine Program. Mean separation were carried out by using least significant difference (LSD) at 5% level of significance

3 RESULTS

Hydroxylamine hydrochloride was observed to reduce the seed germination percentage in all the concentrations, though there were no significant differences between germination at 0.01% and 0.02% treatments and the controls. A gradual decrease was observed in seed germination percentage with increasing concentrations. Highly significant reduction in seed germination was observed at higher concentration when compared with control at (P<0.0001) level (Table 1). Root growth was significantly high in 0.01% and 0.02% concentrations, when compared to control plants. The result indicates that the shoot growth was stimulated at 0.02%, concentration when compared to the control plants (Table 1).

TABLE 1
BIOASSAY STUDY OF HYDROXYLAMINE HYDROCHLORIDE ON SEED GERMINATION AND SEEDLING GROWTH OF THE HARAMAYA VARIETY HARICOT BEAN

S.N	Concentration	Germination (%)	Root Length (cm)	Shoot Length (cm)
1.	Control	93.33	7.59	6.31
2.	0.01%	80.00	10.	7.39
3.	0.02%	80.00	11.1	9.43
4.	0.03%	73.33	6.02	6.51
5.	0.04%	66.67	6.55	6.43
6.	0.05%	53.33	5.74	5.43
	LSD	14.03	2.33	2.99

Means in the same column followed by the same letters are not significantly different at 5% p level

A comparison of plant height in the treatment and control showed that the mean values significantly increased in all concentrations of hydroxylamine hydrochloride except in 0.04% and 0.05%. The stimulatory effect was highly significance in 0.02% concentration when compared with the control. Significant variations were observed in the number of branches especially in 0.02% concentrations when compared with control plants. The result also indicates that the internodes length was increased in 0.01%, 0.02% and 0.03% concentration when compared with control (Table 2). Root nodule numbers were increased in 0.01%, 0.02% and 0.03% concentration and highly significant difference were observed at (P<0.0001) level when compared with the control (Table 2)

TABLE 2
EFFECTS OF VARIOUS CONCENTRATIONS OF HYDROXYLAMINE HYDROCHLORIDE ON PLANT HEIGHT, NUMBER OF BRANCHES ON MAIN AXIS, INTERNODE LENGTH, AND ROOT NODULES OF THE HARICOT BEAN (HARAMAYA VARIETY)

S.N	Concentration	PH	NB	IL	RN
1.	Control	29.7	3.20	2.73	130
2.	0.01%	34.2	3.40	4.04	196
3.	0.02%	36.5	3.67	4.10	182.
4.	0.03%	33.8	3.60	3.90	159
5.	0.04%	32.8	3.40	3.42	135
6.	0.05%	29.7	2.87	3.07	110
	LSD	3.62	0.74	0.95	15.8

Means in the same column followed by the same letters are not significantly different at 5% p level

PH= Plant height, NBMA=Number of branches on main axis

IL= Internodes length, RN =Number of root nodules

In the lower doses of mutagenic treatments days required for flowering were found to be decreased in 0.01%, 0.02% and 0.03% concentration. Days required for maturity were found to be decreased in all concentration. Highly

significant at (P<0.0001) level were observed in days of flowering and days of maturity when compared the control (Table 3). The result indicates that the number of pods per plants were highly significant at (P<0.001) level (Table 3) in 0.01% and 0.02% concentration. There was no significant difference observed in seed length of mutagenic treatment with the control (Table 3). The aerial biomass was larger in 0.02% concentration when compared with control and other concentrations. The drastic decreases in biomass were observed at 0.04% and 0.05% concentration.

TABLE 3
EFFECTS OF VARIOUS CONCENTRATIONS HYDROXLAMINE HYDROCHLORIDE ON DAYS TO FLOWERING, PODS PER PLANT, DAYS TO MATURITY, SEED LENGTH AND ABOVE GROUND BIOMASS OF THE HARICOT BEAN (HARAMAYA VARIETY)

S. N	Concentration	DF	PPP	DM	SL	AGB
1.	Control	54.3	2.47	107.3	10.2	26.3
2.	0.01%	49.0	4.1	94.3	10.4	28.50
3.	0.02%	46.67	5.0	89.67	10.5	30.83
4.	0.03%	51.00	3.13	94.6	10.7	25.13
5.	0.04%	52.67	2.50	95.33	10.6	22.67
6.	0.05%	52.67	1.96	101.0	10.6	20.00
	LSD	2.82	0.98	3.38	0.8	3.36

Means in the same column followed by the same letters are not significantly different at 5% p level

DF=Days of flowering, PPP= Pods per plant, DM=Days of maturity, SL=Seed length, AGB= above ground biomass

4 DISCUSSION

The present study showed mutageic effect of hydroxyl amine hydrochloride on haricot bean (Haramaya variety). The mutagenic effect was observed by bioassay and green house experiment. In the bioassay study, revealed that the reduction in seed germination percentage was associated with the increase in the dose/concentration of mutagens. Similar results were also reported by[13,14] in Soybean. In green house experiment. The stimulatory effect on plant height was highly significance in 0.02% concentration of mutagenic agent hydroxyl amine hydrochloride when compared with the control. A similar trend was observed by [15], whereas the low dosage of hydoxylamine hydrochloride led to increased plant height in *Vigna unguiculata.* Regarding increased root nodules number. This finding is similar with that of [16]. They found out higher nodulation on black gram treated with gamma radiation followed by EMS treatment. Days required for maturity were found to be decreased in all concentration of mutagenic chemical. Similar result was suggested in the seeds of pre-soaked chilli variety in methyl methane sulphonate [17]. In addition to this use of induced mutations for obtaining early maturing cultivars has been a frequent breeding objective [18].

5 CONCLUSION

In bioassay studies the analysis of variance showed highly significant difference in seed germination of Haramaya varieties of the treatment by Hydroxylamine hydrochloride when compared with the control plants. In Haramaya variety the root and shoot growth was stimulated by hydroxylamine hydrochloride in 0.01% and 0.02% concentration. There was a significant change in number of branches, internodes length, root nodule number, aerial biomass and pods per plant of Haramaya variety in 0.02% concentration of hydroxylamine hydrochloride when compared with the control plants. The results showed that, chemical mutagen hydroxyl amine hydrochloride showed positive effects Haramya variety of haricot beans on some quantitative traits and this shows that chemical mutagens are essential in the improvements of crop plants. However, there is a need to repeat the experiment for more than one generation to arrive at conclusive recommendation.

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