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Effects of Induced Mutagenesis on Agronomic Traits in Barley (*Hordeum vulgare L*)

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Authors' contributions

This work was carried out in collaboration between all authors. Author IJO provided the study concept and design, performed the statistical analysis and wrote the first draft of the manuscript. Authors MGK and OKK read and approved the final manuscript.

Original Research Article

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ABSTRACT

Induced mutagenesis has been extensively used to improve main crop species, particularly cereals including barley (*Hordeum vulgare L*).The main strategy in mutation-based breeding is to upgrade the well adapted plant varieties by improving a few desirable major traits such as yield component, tolerance to biotic and abiotic stress for consumer preferences. The present experiment was set out to determine the effects of induced mutagenesis on agronomic traits in mutant barley. The experiment was carried out in University of Eldoret research field in May –August 2012. One hundred and eighty three mutant lines were selected and used in the experiment with the parent (Nguzo) as a check. They were laid in a randomized complete block design with three replicates. The following parameters were used for the study; Number of tillers, plant height, a thousand seeds weight, spike length and days to 50% heading. The data obtained were analysed by Genstat software and mean separated by Duncan Multiple Range test. It was found that muta had a significant effect on number of tillers, plant height, weight of 1000 seeds, spike length and days to 50% heading at P<.001. From the results it is evident that mutagenesis had effects on agronomic traits in mutant barley lines hence can be used in creation of the much needed variation in breeding strategies in barley. The barley mutant lines that had superior characteristics in terms of the agronomic traits could be advanced and used in breeding programme or released as varieties.

Keywords: Mutagenesis; number of tillers; height; weight of 1000 seeds; spike length; days to 50% heading.

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1. INTRODUCTION

The use of mutagens such as gamma rays for inducing variation is well established. Induced mutagenesis has been widely used in agricultural research, especially by the FAO/IAEA division of nuclear techniques in Agriculture. More than 1800 cultivars obtained either as direct mutants or derived from their crosses have been released worldwide in over 50 countries [1]. Mutation breeding supplements conventional plant breeding as a source of increasing variability and could confer a specific improvement without significantly altering its phenotype. The induced mutations aid in development of many agronomically important traits in major crops such as wheat, barley, rice and peanuts [2]. It has been demonstrated in many studies that genetic variability for several desired characters can be induced successfully through mutations and its practical value in plant improvement programmes has been well established [3]. The main advantage of mutation breeding is the possibility of improving one or two characters with- out changing the rest of the genotype [3]. Barley (*Hordeum vulgare* L.) is an annual cereal crop, which belongs to the tribe Triticeae of family Poaceae [4]. It is a diploid (2n=14) plant with a high degree of self fertilization. Barley is the most widely grown crop over a broad range of environmental conditions. It has persisted as a major cereal crop through many centuries and it is the world's fourth most important cereal crop after wheat, maize and rice [5]. The Agriculture Sector, which is the backbone of Kenya's economy and its growth, is dependent on increasing barley production amongst other crops and livestock [6]. The importance of barley as a major crop in Kenya cannot be overlooked because it is the fourth most important cereal in Kenya and the world after maize, wheat and rice [7]. Some of the Major barley producing areas in Kenya includes; Timau, Moiben, Nakuru, on the wetter escarpment of Samburu District near Maralal Town, Molo, and Mau Narok [8]. The total land area that is deemed suitable for barley production in Kenya is 85,000 hectares. Only about 20,000 hectares of this total land area suitable for barley production is under barley production, thus 65,000 hectares has not been utilized [9]. Barley production in Kenya was estimated to be 75,000 MT in 2007 [10].

2. MATERIALS AND METHODS

2.1 Source of Genotypes and Iradiation

Six hundred grams of M_0 seeds (non mutated seeds) of the barley variety Nguzo, obtained from East African Maltings in Molo, Kenya, were sent to International Atomic Energy Agency in Vienna and subjected to gamma radiation at an irradiation dose of 250gy (gray) [11] to obtain M1(mutated seed that gives rise to the first generation of mutants).

2.2 Site Description

The study was conducted at the University of Eldoret in Kenya, The geographical coordinates are 0° 30′ 0″ North, 35º 15′ 0″ East. The site is located 10km of Eldoret town, in Uasin Gishu county of Kenya. It is located at an altitude of 2180m above sea level; it consists primarily of an agro-ecological zone LH3. The site is among major wheat growing regions in Kenya. University of Eldoret receives an unimodal rainfall which begins in March. The average annual rainfall range is between 900mm and 1100mm and mean annual temperature of 16.6ºC.The soils are shallow, ferralsol, well drained, non humic cambisols with low nutrient availability and moisture storage [12]. The study was also conducted at Kenya Agricultural Research institute, Njoro in Nakuru county, (0º20'S 35º56'E), located in the lower highlands (LH3), at an altitude of 2166 meters above sea level. The temperature ranged between 18-28ºC during the period of study, while the average annual rainfall is about 1,000mm. The soils are deep, well drained, fertile *Vitric Mollic Andosols* [12].

2.3 Seed Multiplication and Selection

The plot was disc ploughed and harrowed to fine tilth suitable for barley planting. The irradiated M_1 seeds were planted in University of Eldoret for seed multiplication. The mutated seed were drilled on a plot measuring 125m by 40m. Drills were 5cm apart. All the agronomic practices like insect pest control, diseases control and weed control were done up to harvest time to ensure good crop establishment. One thousand plants were randomly selected from the M_1 population and two ears harvested from each selected plant and divided into two corresponding groups. One group planted at University of Eldoret experimental field while the corresponding group of a thousand ears was planted at KARI Njoro. Each ear formed a line (row).

2.4 Planting and Field management

The M_2 seed from each entry was sown in 1M rows. The experimental units were separated by 0.3m and 0.5m wide alleyways within and between the blocks, respectively. Sowing was done at an equivalent seeding rate of 125kg/ha. At planting time, Di-ammonium phosphate fertilizer was applied at an equivalent rate of 125kg/ha. Weeds growth were managed by applying both pre - and post - emergent herbicides. Stomp® 500 EC (pendimethalin) a broad spectrum, pre-emergent herbicide was applied at an equivalent rate of 2.5 l/ha. At tillering stage (Zadok's Growth stage 20-29), the plots were sprayed with Buctril MC (bromoxynil + MCPA) at an equivalent rate of 1.5l/ha to control broad-leaved weeds. The trial was top dressed with Calcium Ammonium Nitrate (CAN) at stem elongation stage (Zadok's GS 30).

2.5 Genotypes used in the Study

The genotypes used in the study were selections from a study on stem rust (Ug99) resistance. The selection was based on infection type [13] and Stem rust severity using the modified Cobb scale [14]. Seventy four lines were selected from Njoro whereas in University of Eldoret 109 lines were selected. These showed acceptable levels of resistance based on infection type and severity. Each harvested line was harvested and kept in a separate bag to avoid mechanical mixture. The rest of the materials were bulked together.

2.6 Experimental Procedure

The experiment was established on land previously used for wheat production and was disc ploughed and harrowed to fine tilth suitable for barley planting. Each entry was sown in double rows measuring 0.2×0.75m. The experimental units were separated by 0.3m and 0.5 m wide alleyways within and between the blocks, respectively. Sowing was done at an appropriate seeding rate. The entries were randomly assigned within a block. The experiment was laid out in RCBD replicated three times.

2.7 Data Collection

At physiological maturity 5 plants/plot were selected randomly and used to measure plant height (from plant base to the tip of spike excluding awns), spike length, number of tillers and days to 50% heading. Spike length was measured from the base of the ear to the tip of the spike (excluding the awns) based on an evaluation of all the spikes from the five plants. A sample of 1000 seeds per line was taken and weighed to give 1000-seed weight at harvest maturity.

2.8 Data Analysis

Analysis of variance for all agronomic traits was computed using GENSTAT and means separated using Duncan Multiple Range test. Correlation was done by SAS Pearson correlation Coefficients.

3. RESULTS

3.1 Effect of Mutagenesis on Number of Tillers on Mutant Barley Lines

Mutagenesis had a significant effect on the number of tillers in the various mutant barley lines as observed *P*=.001 (Table 1). From the table of means, mutant line 23 had the highest mean of 14 tillers and line 126 had the lowest mean of 6 tillers and the parent (non mutant-control) line 184 had a mean of 9.33 (Table 1).

Mutation had a significant effect on Plant height of the various mutant barley lines as observed from the Table 1 at *P*=.001. From the Table of mean values, line 156 had the highest mean of 110 cm and line 154 had the lowest mean of 75 cm and the parent (non mutant-control) line 184 had a mean of 99.33 cm, as from the above Table 1.

Mutation had a significant effect on spike length of the various mutant barley lines as observed from the table at *P*=.001. From the table of mean values, line 156 had the highest mean of 11.5 cm and line 116 had the lowest mean of 8.5 cm and the parent (non mutant-control) line 184 had a mean of 9.233 cm as shown in Table 1.

Mutation had a significant effect on weight of 1000 seeds per mutant line of the various mutant barley lines as observed from the table at *P*=.001. From the table of means, line 173 had the highest mean of 52.5 g and line 163 had the lowest mean of 33.4 g and the parent (non mutant-control) line 184 had a mean of 44.33 g (see Table 1).

Mutation had a significant effect on days to 50% heading of mutant barley lines as observed from the table at *P*=.001. From the table of means, line 90 took 58 days to 50% heading while line 5 took 80 days to 50% heading. The control non mutant parent line took 68 days to 50% heading, shown in the above Table 1.

From the above Table 2, height was positively and significantly correlated to the number of tillers, and so was to spike length. Spike length had a positive correlation with the number of tillers at *P*=.05, with also weight of 1000 grains of mutant barley lines at *P*=.001 and negatively correlated with height and days to 50% heading of mutant barley lines at *P*=.001.Weight had a positive correlation with spike length at *P*=.001,weight of 1000 grains per mutant line had a negative correlation with days to 50%heading of the mutant barley lines at *P*=0.001. Height positively correlated with tillers at *P*=.01, height also had a positive correlation with days to 50% heading at *P*=.001.height had a negative correlation with spike length at *P*=.001. Days to 50% heading had a positive correlation with height at *P*=.001 and a negative correlation with spike length and weight of 1000 grains per mutant barley lines at *P*=.001

Table 1. Summary table of means of agronomic traits

Mutant lines	Tillers	Height	Spike length	Days to 50% heading	Weight of 1000 seeds
23	14a	96.33a-k	11a-b	72b-p	44.5a-l
7	12.67a-b	92.33b-l	9.5a-h	77.33a-c	50.87a-c
154	12a-c	75m	6.33j	64.67r-A	46.03a-l
1	11.33a-d	93.33a-k	9.667a-h	77a-d	45.7a-l
36	8b-d	97.33a-j	9a-j	64s-A	44.97a-l
124	6.67c-d	89.67b-m	9a-j	70.67c-u	40.13a-l
126	6d	92.33b-l	9.5a-h	71.67c-r	39.2c-l
156	9.67a-d	110a	11.5a	75a-i	42.17a-l
161	9.33a-d	104a-c	10.167a-g	71.67c-r	42.23a-l
27	11a-d	105.33a-b	9.7a-h	66.67l-x	42.8a-l
21	11.67a-d	89b-m	8.9a-j	72b-q	35.6i-l
8	11.33a-d	96.33a-k	10.43a-e	77a-d	42.8a-l
41	11.67a-d	98a-j	8.667b-j	71c-t	37.87d-l
44	12a-c	92.33b-l	7.67e-j	66.33m-x	47.6a-j
95	9a-d	94a-k	10.9a-c	68.33g-x	40.97a-l
90	8b-d	95.67a-k	8.13c-j	58Ya	38.57c-l
5	9.33a-d	93.33b-k	10.33a-e	80.33a	35.43j-l
2	10a-d	97a-j	9.67a-h	79.33ab	43a-l
34	9.33a-d	101a-h	9.5a-h	63v-A	48.9a-f
62	8.33a-d	97a-j	9.2a-j	62x-A	43.07a-l
173	8.33a-d	87.67c-m	8.6b-j	72b-q	52.5a
163	10a-d	89b-m	9.5a-h	75.67a-g	33.4l
165	9a-d	97.67a-j	9.5a-h	66.67l-x	50.4a-d
130	8b-d	89.33b-m	7.1h-j	65p-A	36.67f-l
54	11a-d	99a-j	7.93d-j	69f-x	51.93a-b
9	12.67a-b	95.33a-k	9a-h	75.67a-g	44.03a-l
26	8.33a-d	97a-j	8.9a-j	70c-w	35.8h-l
49	10.67a-d	92.33b-l	9.5a-h	65.33o-y	48.13a-i
55	11.33a-d	96.67a-k	9.53a-h	66n-x	40.3a-l
58	8.67a-d	96.67a-k	8.17c-j	68.67g-x	46.27a-k
69	9a-d	96a-k	8d-j	66n-x	43.3a-l
76	8b-d	99a-j	9.47a-h	67.33j-x	47.13a-j
59	11a-d	94.33a-k	9.5a-h	66.67l-x	45.77a-l
78	9.33a-d	95.67a-k	9.27a-i	67k-x	45.2a-l
184(Nguzo)	9.33a-d	99.33a-i	9.23a-i	67.67i-x	44.33a-l
SED	1.23	2.92	0.60	3.54	2.75
CV%	16.1	5.2	8.2	4.6	7.7

Mean separated by duncan multiple range test

Table 2. Correlation coefficient between the agronomic traits

	Tillers	Height	Spike length	Weight of 1000 seeds	Days to 50% heading
Tillers	1	0.11**	0.08*	0.02	0.03
Height		1	-0.26***	-0.02	0.16***
Spike length			1	0.26***	-0.41***
Weight of 1000 seeds				1	-0.35***
Days to 50% Heading					1

=significant at P=.05, **=significant at P=.01, *=significant at P=.001*

4. DISCUSSION

4.1 The Effects of Mutation on Agronomic Traits

The results of the present study illustrated that mutagenesis is an efficient tool for increasing genetic variability in barley. It produced variability among the mutant barley lines in terms of height. This ranged from tall plants to short plant compared to the check, the parent material (non mutant).The short plants could be positively selected and be used in breeding for against lodging in crossing programmes. Similar results in terms of variation in plant height were observed in irradiated wheat population [15]. It was also reported the variation in terms of number of tillers observed in mutant lines upon usage of the various doses of gamma radiation. In the present study, mutant line 156 had the highest mean of 110 cm and line 154 had the lowest mean of 75 cm. Mutation had a significant effect on spike length of the mutant barley lines as observed from the above results. The effects were in comparison to the control or wild-type (the non mutant material: parent).These results are in agreement with those of [15] they concluded that gamma radiation induced greater variability and improvements in different traits such as spike length and yield per spike. In the present study, mutant line 156 had the longest spike length of 11.5cm and mutant line 116 had the shortest spike length of 8.5 cm. Mutation had significant effect on the weight of 1000 seeds per mutant barley line as observed from the results, and this was in comparison with the wild-type (non mutant parent material). The lines with a higher weight as compared to the non mutant material could be positively selected and advanced. These results are in agreement with those of [16] who found that gamma radiation produced a greater variability on yield related parameters in wheat. Rahimi et al. [17] also reported similar results on the effects of gamma radiations on 1000 grain weight and grain yield of wheat crop. The variation in terms of weight varied from 52.5 g to 33.4g, this were shown by line number 173 and 163, respectively. Mutation had a significant effect on 50% heading as observed from the results. Some lines showed ear lines in heading compared to the parent line (control) while others showed a delayed heading compared to the non mutant parent lines; similar results were reported by [18]. Radiation intensity delays heading and increases maturity period [19]. In the present study, variation in terms of days to 50% heading was from 58 days to 80 days shown by mutant line number 90 and 5, respectively. Gustafson et al. [20] developed a high yielding and early maturing barley by mutation breeding methods.

4.2 Correlation Coefficient among Traits

Spike length showed a positive correlation with weight of 1000 grains per mutant barley lines, which means that the longer the spike length the more the weight of the 1000 grains per mutant barley line. These results are in agreement with those of [21,22,23].This could mean that the lines with longer spike length could be positively selected and advanced. There was a negative correlation between days to 50% heading and weight of 1000 grain weight per mutant line, these results are in agreement with those of other authors [15] who found a negative correlation between days to 50 % heading and weight of 1000 grain in durum wheat. Days to 50% heading had a positive and significant correlation with height of mutant barley lines. This meant that the more the days, the more height of a plant increases.

5. CONCLUSIONS

Mutagenesis by gamma irradiation was successfully applied and generated the much needed variability. It had an effect on the agronomic traits; hence by this the superior mutant lines could be positively selected for advancement. The following lines did show superiority; 1,2,7,9,21,23,26,27,34,44,49,54,55,58,59,62,69,76,78,90,95,130,156,161,165 and 173.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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