

Effects of induced mutation on agronomic traits in barley (*Hordeum vulgare*L).

Abstract

Induced mutation 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 mutation 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 was analysed by Genstat software and mean separated by Duncan Multiple Range test. It was found that mutation 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 mutation 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.

Key words: mutation, number of tillers, height, weight of 1000 seeds, spike length, days to 50%heading

35 **Introduction**

36 The use of mutagens such as gamma rays for inducing variation is well established. Induced
37 mutations have been used especially by the FAO/IAEA division of nuclear techniques in
38 Agriculture, More than 1800 cultivars obtained either as direct mutants or derived from their
39 crosses have been released worldwide in over 50 countries.[1] Mutation breeding
40 supplements conventional plant breeding as a source of increasing variability and could
41 confer specific improvement without significantly altering its phenotype. The induced
42 mutations aid in development of many agronomic important traits in major crops such as
43 wheat ,barley rice and peanuts[2]. It has been demonstrated in many studies that genetic
44 variability for several desired characters can be induced successfully through mutations and
45 its practical value in plant improvement programmes has been well established [3]. The main
46 advantage of mutation breeding is the possibility of improving one or two characters with-
47 out changing the rest of the genotype[3].Barley (*Hordeum vulgare* L.) is an annual cereal
48 crop, which belongs to the tribeTriticeae of family Poaceae [4]. It is a diploid ($2n=14$) plant
49 with high degree of selffertilization.Barley is the most widelygrown crop over broad
50 environmentalconditions. It has persisted as a major cereal crop through many centuries andit
51 is the world's fourth important cerealcrop after wheat maize and rice[5]. The Agriculture
52 Sector, which is the backbone of Kenya's economy and its growth, is dependent on
53 increasing barley production amongst other crops and livestock [6]. The importance of barley
54 as a major crop in Kenya cannot be overlooked because it is the fourth most important cereal
55 in Kenya and the world after maize, wheat and rice [7]. Some of the Major barley producing
56 areas in Kenya includes; Timau, Moiben, Nakuru, on the wetter escarpment of Samburu
57 District near Maralal Town, Molo, and Mau Narok [8]. The total land area that is deemed
58 suitable for barley production in Kenya is 85,000 hectares. Only about 20,000 hectares of this
59 total land area suitable for barley production is under barley production, thus 65,000 hectares
60 has not been utilized [10]. Barley production in Kenya was estimated to be 75,000MT in
61 2007 [10].

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64 MATERIALS AND METHODS

65 Source of genotypes and Irradiation

66 Six hundred grams of M_0 seeds (non mutated seeds) of the barley variety Nguzo, obtained
67 from East African Maltings in Molo, Kenya and were sent to International Atomic Energy
68 Agency in Vienna and subjected to gamma radiation at an irradiation dose of 250 gy
69 (gray)[11] to obtain M_1 (mutated seed that gives rise to the first generation of mutants).

70 Site description

71 The study was conducted at the University of Eldoret in Kenya, The geographical coordinates
72 are $0^{\circ} 30' 0''$ North, $35^{\circ} 15' 0''$ East. The site is located 10 Km of Eldoret town, in Uasin
73 Gishu county of Kenya. It is located at an altitude of 2180m above sea level; it consists
74 primarily of an agro-ecological zone LH3. The site is among major wheat growing regions in
75 Kenya. University of Eldoret receives a unimodal rainfall which begins in March. The
76 average annual rainfall range is between 900 mm and 1100 mm and mean annual temperature
77 of 16.6°C . The soils are shallow, ferralsol, well drained, non humic cambisols with low
78 nutrient availability and moisture storage [12]

79 The study was also conducted at Kenya Agricultural Research institute, Njoro in Nakuru
80 county, ($0^{\circ}20'S$ $35^{\circ}56'E$), located in the lower highlands (LH3), at an altitude of 2166 meters
81 above sea level. The temperature ranged between $18-28^{\circ}\text{C}$ during the period of study, while
82 the average annual rainfall is about 1,000 mm. The soils are deep, well drained, fertile *Vitric*
83 *Mollic Andosols* [12]

84 Seed Multiplication and Selection

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

94 **Planting and field management**

95 The M₂ seed from each entry was sown in 1M rows. The experimental units were separated
96 by 0.3m and 0.5m wide alleyways within and between the blocks, respectively. Sowing was
97 done at an equivalent seeding rate of 125 kg/ ha. At planting time, Di-ammonium phosphate
98 fertilizer was applied at an equivalent rate of 125kg/ha. Weeds growth were managed by
99 applying both pre - and post - emergent herbicides. Stomp® 500 EC (pendimethalin) a broad
100 spectrum, pre-emergent herbicide was applied at an equivalent rate of 2.5 l/ha. At tillering
101 stage (Zadok's Growth stage 20-29) the plots were sprayed with Buctril MC (bromoxynil +
102 MCPA) at an equivalent rate of 1.5l/ha to control broad-leaved weeds. The trial was top
103 dressed with Calcium Ammonium Nitrate (CAN) at stem elongation stage (Zadok's GS 30).

104 **Genotypes used in the study**

105 The genotypes used in the study were selections from a study on stem rust (Ug99)resistance
106 .The selection was based on infection type [13] and Stem rust severity using the modified
107 Cobb scale [14] Seventy four lines were selected from Njoro whereas in University of Eldoret
108 109 lines were selected. These showed acceptable levels of resistance based on infection type
109 and severity. Each harvested line was harvested and kept in a separate bag to avoid
110 mechanical mixture. The rest of the materials were bulked together.

111 **Experimental Procedure**

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

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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.

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.

142 **RESULTS**143 **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	11ab	72b-p	44.5a-l
7	12.667ab	92.33b-l	9.5a-h	77.33a-c	50.87a-c
154	12abc	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	6.667c-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	9.667a-d	110a	11.5a	75a-i	42.17a-l
161	9.33abcd	104abc	10.167a-g	71.67c-r	42.23a-l
27	11a-d	105.33ab	9.7a-h	66.67l-x	42.8a-l
21	11.667a-d	89b-m	8.9a-j	72b-q	35.6ijkl
8	11.33a-d	96.33a-k	10.433a-e	77a-d	42.8a-l
41	11.667a-d	98a-j	8.667b-j	71c-t	37.87d-l
44	12a-c	92.33b-l	7.667e-j	66.33m-x	47.6a-j
95	9a-d	94a-k	10.9a-c	68.33g-x	40.97a-l
90	8bcd	95.67a-k	8.133c-j	58Ya	38.57c-l
5	9.33a-d	93.33b-k	10.33a-e	80.33a	35.43jkl
2	10a-d	97a-j	9.667a-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	7.933d-j	69f-x	51.93a-b
9	12.667a-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	10.667a-d	92.33b-l	9.5a-h	65.33o-y	48.13a-i
55	11.33a-d	96.67a-k	9.533a-h	66n-x	40.3a-l
58	8.667a-d	96.67a-k	8.167c-j	68.67g-x	46.27a-k
69	9a-d	96a-k	8d-j	66n-x	43.3a-l
76	8b-d	99a-j	9.467a-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	9.267a-i	67k-x	45.2a-l
184(Nguzo)	9.33a-d	99.33a-i	9.233a-i	67.67i-x	44.33a-l
SED	1.2317	2.922	0.6021	3.543	2.748
CV%	16.1	5.2	8.2	4.6	7.7

Effect of mutation on number of tillers on mutant barley lines

Mutation had a significant effect on number of tillers on 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) 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 anova table at $P=.001$. From the table of mean line 156 had the highest mean of 110cm and line 154 had the lowest mean of 75cm and the parent (non mutant) line 184 had a mean of 99.33cm, as from the above table 1.

Mutation had a significant effect on spike length of the various mutant barley lines as observed from the anova table at $P=.001$. From the table of mean line 156 had the highest mean of 11.5cm and line 116 had the lowest mean of 8.5cm and the parent (non mutant) line 184 had a mean of 9.233cm shown from above 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 anova table at $P=.001$. From the table of means line 173 had the highest mean of 52.5g and line 163 had the lowest mean of 33.4g and the parent (non mutant) line 184 had a mean of 44.33g. Shown from above table 1.

Mutation had a significant effect on days to 50% heading of mutant barley lines as observed from the anova 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 check non mutant parent line took 68 days to 50% heading, shown in the above table 1.

Table 2: Correlation coefficient between the agronomic traits.

	Tillers	height	spike length	weight	days 50%
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				1	-0.35***
days 50%					1

*=significant at $P=0.05$, **=significant at $P=0.01$, ***=significant at $P=0.001$

From the above table height were positively and significantly correlated to the number of tillers, and so was to spike length. Spike length had a positive correlation with number of tillers at $P=0.05$, with also weight of 1000 grains of mutant barley lines at $P=0.001$ and negatively correlated with height and days to 50% heading of mutant barley lines at $P=0.001$. Weight had a positive correlation with spike length at $P=0.001$, 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 $P=0.01$, height also had a positive correlation with days to 50% heading at $P=0.001$. Height had a negative correlation with spike length at $P=0.001$. Days to 50% heading had a positive correlation with height at $P=0.001$ and a negative correlation with spike length and weight of 1000 grains per mutant barley lines at $P=0.001$.

195 Discussion

196 The effects of mutation on agronomic traits

197 The results of the present study illustrated that mutation is an efficient tool for increasing
198 genetic variability in barley. It produced variability among the mutant barley lines in terms of
199 height. This ranged from tall plants to short plant compared to the check, the parent material
200 (non mutant). The short plants could be positively selected and be used in breeding for against
201 lodging in crossing programmes. Similar results in terms of variation in of plant height were
202 observed in irradiated wheat population[15]. It was also reported the variation in terms of
203 number of tillers observed in mutant lines upon usage of the various doses of gamma
204 radiation. In the present study mutant line 156 had the highest mean of 110cm and line 154
205 had the lowest mean of 75 cm. Mutation had significant effect on spike length of the mutant
206 barley lines as observed from the above results. The effects were in comparison to the check
207 (the non mutant material: parent). This results are in agreement with those of [15] they
208 concluded that gamma radiation induced greater variability and improvements in different
209 traits such as spike length and yield per spike. In the present study, mutant line 156 had the
210 longest spike length of 11.5cm and mutant line 116 had the shortest spike length of 8.5cm.
211 Mutation had significant effect on the weight of 1000 seeds per mutant barley line as
212 observed from the results, and this was in comparison with the check (non mutant parent
213 material). The lines with higher weight as compared to the non mutant material could be
214 positively selected and advanced. These results are in agreement with those of [16] who
215 found that gamma radiation produced greater variability on yield related parameters in wheat.
216 Rahimi et al[17] also reported similar results on effects of gamma radiations on 1000 grain
217 weight and grain yield of wheat crop. The variation in terms of weight varied from 52.5g to
218 33.4g, this were shown by line number 173 and 163 respectively. Mutation had significant
219 effect on 50% heading as observed from the results. some lines showed ear lines in heading
220 compared to the parent lines while others showed delayed heading compared to the non
221 mutant parent lines, similar results were reported by [18]. Radiation intensity delays heading
222 and increases maturity period [19]. In the present study variation in terms of days to 50%
223 heading was from 58 days to 80 days shown by mutant line number 90 and 5 respectively.
224 Gustafson et al. [20] developed a high yielding and early maturing barley by mutation
225 breeding methods.

Correlation coefficient among traits

Spike length showed 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] and [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.

CONCLUSIONS

Mutation by 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. In the present study, the following lines did show superiority. mutant line 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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