Effects of Induced Mutagenesis and Single Crossing on Agronomic Traits of Wheat (*Triticum Aestivum L.,)*

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Abstract

Wheat is a major food crop worldwide and ranks second after maize in Kenya. Mutation breeding is extensively applied as supplements to conventional plant breeding as a source of increasing variability and could confer a specific improvement without significantly altering its phenotype. The present experiment was set out to determine the effects of induced mutagenesis on agronomic traits in mutant wheat. The experiment was carried out at the University of Eldoret under green house conditions from May-August 2013. The experiment was laid in a randomized complete block design with three replicates. The following parameters were used for the study; number of tillers, plant height, number of spikelet's/spike, spike length and days to 50% heading. The data obtained were analyzed by Genstat software and mean separated by Duncan multiple range tests. It was found that mutation had a significant effect on number of tillers, plant height, spike length and days to heading at P<.001.

Key words: Mutagenesis, number of tillers; plant height; spike length; days to 50% heading.

1.0 Introduction

Wheat is the major food crop in the world and sustains the majority of the world population (USDA, 2014). In Kenya, wheat is the second most important crop in after maize and contributes significantly to food security (Singh *et al*., 2006). Largely dependent upon rain-fed agriculture and climatic conditions found at altitudes of 1, 500 m, Kenya wheat yield is low compared to world's top grower China, which produces 120 million metric tons a year (Aquino *et al*., 2002). Some of the Major wheat producing areas in Kenya include: Rift-valley regions of Uashin Gishu, Narok, Marakwet, Elgeyo marakwet, Londiani, Molo, Nakuru and Timau areas. Wheat falls into three natural groups based on chromosome number: diploid (2n=14), tetraploids (2n=28) and hexaploid (2n=42) plants with a high degree of self fertilization (Acquaah, 2012).

Induced mutagenesis has been extensively used in agricultural research as a key supplement to conventional plant breeding (IAEA, 1998). Induced mutation results to permanent change in the DNA sequence of a gene which can alter the amino acid sequence of the protein encoded by the gene without significantly altering its phenotype (IAEA, 2014). Induced mutations aid in development of many agronomically important traits such as improved local varieties of basic food crops for yield, quality, early maturity and tolerance to biotic and abiotic stresses in major crops such as wheat, barley, rice and peanuts (Jain, 2010). Previous studies have shown 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 (Al-Qurainy and Khan, 2009). A single cross is where two elite lines which possess adequate traits are done a one cross or a single cross (A × B).

The single cross hybrids are more uniforms and easier to produce (Acquaah, 2012). It is preferred mainly because it is a simple, rapid method when using greenhouses or growth rooms. It requires only small populations and it is inexpensive for achieving short term goals in wheat breeding. The best donor parent must possess the desired trait, but should not be seriously deficient in other traits (Pohlmen and Sleper, 1995). Morphological characterization is the first step in genetic relationship studies in most breeding programmes (Cox and Murphy, 1990). Phenotypic identification of plants has been used as a powerful tool in the classification of genotypes and to study taxonomic status, based on morphological traits recorded in the field (Van Beuningen and Busch, 1997).
In Kenya there is little data on effect of induced mutation on agronomic traits of two wheat varieties Njoro 2 (M_3A) and Chozi (M_3B) both at M_3 stage. Therefore, the objective of this study was to evaluate the effect of induced mutagenesis and single crossing on agronomic traits of two wheat varieties.

2.0 Materials and Methods

2.1 Source of Genotypes and Irradiation

Several Kenyan wheat varieties had been sent to the International Atomic Energy Agency (IAEA) laboratory in Vienna, Austria and subjected to gamma radiation at an irradiation dose of 300 gy (gray) to obtain M1 (mutated seed that gives rise to the first generation of mutants). The M1 seed was planted in an experimental field for advancement to the next generation (M2) then to subsequent generation (M3) and preliminary evaluation for positive effects of radiation done. Chozi and Njoro 2 mutants were selected since they had initially shown resistance to stem rust disease.

2.2 Site Description

The study was conducted at the University of Eldoret in Kenya, on geographical coordinates 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 region in Kenya. University of Eldoret receives a 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 high nutrient availability and moisture storage (Jaetzold *et al.,* 1983)

2.4 Crop establishment and field management

The two mutant wheat varieties Njoro 2 and Chozi (M_3A and M_3B) which had earlier shown resistance to stem rust disease were sown under green house condition alongside their parental wheat varieties Njoro 2 and Chozi as positive check. 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 growths 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, 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 (Maling'a, *et al.,* 2004)

2.1 Single Backcrossing

Mutant wheat from two varieties which had earlier shown resistance to stem rust disease were planted in 2013 in a green house alongside their parents (Chozi and Njoro 2). Temperature was controlled between 21 to 25^{0}C and normal daylight hours used. A randomised complete block design was used with three replicates. Direct anther emasculation technique was used as described by Acquaah, (2012) and spikes bagged to prevent pollination from foreign pollen. Emasculated flowers were then pollinated within 2–4 days and then tagged with the following information: date of emasculation, date of pollination and parentage.

2.2 Data Collection

At physiological maturity 10 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 ten plants.
Spikelet's per spikes and number of tillers at booting was done by actual count of individual spikeletes and tillers respectively. Days to 50% heading was the number of days counted from planting up to 50% heading.

2.3 Data analysis.

Data on agronomic traits was subjected to analysis of variance using GENSTAT 12th edition and means separated using Duncan Multiple range test. Correlation was done by Pearson correlation Coefficients to determine significant associations among the agronomic variables. Statistical model used was: Xij=μ+ti+βj+eij Where, Xij= observation, μ=overall mean, ti=treatment effect, βj=block effect and eij=experimental error

3.0 Results

There was a significant difference in the plant height between the different wheat varieties. Chozi parent (CP) was the tallest with a mean height of 110.05 cm while Chozi Single Cross (CSC) was the shortest with a mean height of 29.13 cm. Spike length differed significantly between the different wheat varieties with Chozi parent (CP) having the longest average spike length of 11.73 cm and Chozi Single Cross (CSC) the shortest average length of 9 cm. Spikelets per spike differed significantly between the different wheat varieties. Chozi Single Cross (CSC) had the lowest mean number (9.0) while Chozi Parent (CP) had the highest (32.2). The number of tillers was counted from all the wheat varieties. There was a significant difference between the different wheat varieties. Mutant a (M_3A) had the highest average number of tillers while Njoro 2 single cross (NSC) had the lowest average number of tillers (Table 3.1). There was a significant difference in the days to 50% heading between the different wheat varieties. Chozi Single Cross (CSC) had the longest number of days to 50% heading (63 days) while Chozi Parent (CP) and Mutant B (M_3B) had the shortest days to 50% heading (57 days). Number of grains per spike differed significantly between the different wheat varieties. Mutant A (M_3A) had the highest average number of grains per spike (65.85) followed by Chozi Parent (CP) 62.05, while the least was Chozi Single Cross (CSC) 55.05.

Table 3.1: Summary table of means of agronomic traits of wheat varieties

Variety	Plant height	Spike length	Spikeletsper spike	No. Of tillers	Days to heading	Grains per spike
NP	85.80 c	10.44 b	26.10 b	4.6 bc	59.0 c	55.45 ab
M_3A	90.20 cd	10.50 bc	31.85 d	6.15 d	58.0 b	65.85 e
NSC	71.90 b	10.7 bc	28.15 c	3.35 a	59.0 c	58.95 bc
CP	110.05 e	11.73 c	32.20 d	5.00 c	57.0 a	62.05 cd
M_3B	90.60cd	9.75 ab	28.00 c	7.00 e	57.0 a	57.15 abc
CSC	29.13 a	9.0 a	9.00 a	5.00 c	63.0 c	55.05 ab
SE	6.07	1.275	5.496	0.8434	0.0	5.496
CV%	7.8	12.4	9.3	16.6	0.0	9.3
F value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
P value	0.001	0.001	0.001	0.001	0.001	0.001

Mean separation using Duncan Multiple Range test at α=0.05; means followed by the same letter are not significantly different from each other. Key: NP = Njoro parent; M_3A = Mutant variety A at M3 generation; NSC = Njoro 2 single cross; CP = Chozi parent; M_3B = Mutant variety B at M3 generation; CSC = Chozi single cross Results obtained from this study showed that there was a positive correlation (p=0.05) between spikelets per spike and the different wheat varieties. Plant height was positively and significantly correlated to the number of to spike length (r = 0.503; P =0.01) and spiketets per spike (r = 0.442; p=0.01). However, plant height negatively correlated to days to 50% heading (r = -0.782; P=0.01). Spike length and spikelets per spike also had a negative correlation with days to 50% heading P =0.01. There was a positive correlation (p=0.01) between grain per spike and plant height, spike length and spikeletes per spike (Table 3.2).

Table 3.2: Correlations coefficient between the different agronomic traits

	wheat Variety	Plant height (cm)	Spike length (cm)	Spikelets per spikes	Days to heading	Number of tillers	Grains per spike
wheat Variety	1	-.046	-.032	.230**	.077	-.331**	.196*
Plant height (cm)		1	.503**	.820**	-.755**	.238**	.442**
Spike length (cm)			1	.408**	-.311**	-.072	.488**
Spikelets per spikes				1	-.871**	.095	.410**
Days to heading					1	-.199*	-.289**
Number of tillers						1	.115
Grains per spike							1

* Correlation is significant at the 0.05 level. ** Correlation is significant at the 0.01 level.

Discussion

4.1 Agronomic traits

4.1.2 Plant height

The mutation had a positive effect in the plant height in Njoro 2. Single crossing, however, had a negative effect on Njoro 2 plant height with a reduced height of 71.9 cm. The mutant wheat might have had the genes controlling height altered during mutation and hence the difference in the height. These results are similar to those found by Badigannavar and Mondal (2010) who worked on mutant groundnut and found that plant height was either increased or decreased depending on the cultivar used. For both Chozi and Njoro 2, the single cross reduced the plant height and the results of this study are contrary to those of Rahman & Kronstad (1992) who reported high heritability for plant height in wheat. Possible reason could be that the mutant wheat being in the M3 population stage could still be segregating (IAEA, 2014) at this stage and therefore the plant height trait was not homozygous.

4.1.2 Spike length

Both mutation and single crossing had a positive effect on Njoro 2 as compared to the parent wheat variety. The Njoro 2 mutant variety (M_3A) produced the longest spike with a mean of 65.85 cm, single cross with a mean of 58.95 cm while Njoro 2 parent had the least mean spike length of 55.45 cm. These results are similar to those of Mohammad *et al*. (2004) who also reported highly significant differences for spike length in bread wheat. This kind of improvement is expected since single crossing and use of mutation are all plant breeding techniques used in the improvement of plant varieties (Acquaah, 2012). However, both mutation and single crossing of Chozi variety reduce the spike length relative to the Chozi parent variety. Possible reason could be that the mutant wheat being in the M_3 population stage could still be segregating (IAEA, 2014). After crossing the mutant with the parent it is possible that a heterogeneous variety could have been formed which had shorter spike length than the parent plant.

4.1.3 Spikelets per spike

Results show that there was a significant difference in the number of spikelets per spike. Previous studies by Zaheer, (1991) showed that yield could be increased through selection of plants with taller plant height and more spikelets per spike. The results from this study are in line with those of Kirby and Appleyard (1984) who found the number of spikelets per spike varying from 20 to 30. The results also concur with those of Degewione and Alamerew (2013) who studied genetic diversity in bread wheat genotypes and found that there was a significant difference between genotypes.

4.1.4 Number of tillers

Tillers are an important component of wheat yield because they have the potential to develop grain-bearing heads. The results from this study show that there was a significant difference in the number of tillers in the two genotypes. These results are similar to those of Singh *et al.,* (2014) who studied Indian wheat genotypes. The number of productive tillers depends on genotype and environment and is strongly influenced by planting density (FAO, 2001). Tillers per plant may be used as effective selection criteria for yield as Khan *et al*., (2005) reported that tillers per plant had the highest positive direct effect on grain yield.

4.1.5 Days to 50% heading

Mutation had a significant effect on days to 50% heading as observed from the results. Njoro 2 mutated variety (M_3A) recorded a delayed heading compared to the parent line Njoro 2 (NP) and resulted in increase in yield per spike. However, there was no significant difference in days to heading between mutated Chozi variety (M_3B) and the Chozi parent line (CP) the results of this study are in agreement to what was reported by Saleem et al. (1988) and Jamil and Khan, (2002) who reported that radiation intensity delays heading and increases maturity period in wheat varieties. These results are also similar to that of Mollasadeghi *et al.,* (2012) who compared 12 bread wheat genotypes based on number of phonological and morphological traits and found that there was a significant difference (p=0.05) in the days to heading.

4.1.6 Number of grains per spike

The number of grains in each spike were counted and the results show that there was significant difference (p= 0.05). In the Chozi variety, all treatments, that is, mutation and single crossing had a negative effect on the number of grains per spike.

In both Mutated and backcrossed crossed Njoro 2 variety (M_3A and NSC respectively) showed an increase in number of grains per spike as compared to the parent variety (NP). These results collaborate with those of Ahmad et al. (2011) who found a highly significant difference ($p \leq 0.01$) in mixed genotypes showing diverse types of wheat and triticale genotypes. This could be attributed to the different genetic makeup of the two wheat varieties. In the Njoro 2 variety, the improvement in the number of grains per spike was expected since backcrossing and use of mutation are all plant breeding techniques used in the improvement of plant traits (Acquaah, 2012).

4.2 Correlation Coefficient among Traits

There was a positive correlation between the plant height and spike length with the taller a plant was the longer the spike length was. These results are in agreement with those of Zaheer (1991) and Khan et al. (2005) who reported plant height of various wheat varieties had positive correlation spikelets per spike. Haq *et al.* (2010) observed that spike length, spikelets per spike, grains per spike, tillers per m^2, 1000-grain weight had positive correlation with grain yield. The results demonstrated that genotypes with larger spikes should be selected under irrigated condition to increase grain yield. Guohua *et al.*, (2000) and Okuyama *et al*., (2004) suggested the same strategy for increasing grain yield.There was a negative correlation between days to heading with the plant height, spike length and spikelets per spike ($p=0.01$). These results are similar to those of Obare *et al.*, (2014) who studied the effects of mutation on barley.

4.3 Effects of Mutation

Generally mutation resulted in a reduction of plant height as all the mutants were shorter than the parent because mutation altered the genetic sequence of the mutant lines. Besides variation in height of the plant observed in the mutant population, there were also other observable variations in the phenotypic characteristics of mutant wheat. Some of the notable characteristics included variation in days to 50% heading, plant height, and spike length, spikelets per spike and grains per spike. It is generally and widely accepted that in mutation breeding induced traits become fixed during M2-M4 generations (Azad *et al.*, 2010; Hamid *et al.*, 2006; Shamsuzzaman *et al.*, 1998; Azam and Uddin, 1999). But it has been reported that it is possible to isolate fixed mutants even in M1 generation of heavy ion irradiated sweet pepper (Honda *et al.*, 2006). The results of this study are similar to those of Obare *et al.*, (2014) who studied variations in mutant barley and found out that mutation had an effect on the agronomic traits such as tillers, height length, spike length and days to heading.

5. Conclusions and Recommendations

Mutagenesis by gamma irradiation generated the much agronomic variability between the two varieties. Njoro 2 mutant variety (M_3A) had super quality in all morphological traits evaluated including plant height, spikelength, and spiklets per spike, increased number of tiller, decreased days to heading and resulted in increase in yield per spike as compared to the parent variety. Hence, this mutated variety could be positively selected for advancement in plant breeding programs. However, we recommend more studies to be done to compare the mutant lines with a larger variety of Kenyan wheat varieties.

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