GMP Journals
Journal of contemporary agricultural practices
Vol.1, No 1, P.1-8, 2019
www.gmpjournals.com

Research Paper

Evaluation of drought tolerance in some Kenyan bread wheat (*Triticum Aestivum* L.) at seedling stage

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Submitted on 20th October 2019
Published on 02nd November 2019

ABSTRACT
Wheat (*Triticum aestivum* L) is the second largest contributor to food security in Kenya. However, water deficiency retards plant growth and productivity significantly. With few drought tolerant varieties available, there is need to develop more drought tolerant wheat varieties. Objective of this study was to screen for drought tolerance in the mutant wheat lines at seedling stage. Seeds of two wheat varieties were subjected to gamma radiation at an irradiation dose of 300 gy (gray). The mutants and two local varieties – Chozi and Duma (controls) were sown in polythene bags in a complete randomized design and screened for various seedling traits. Data were analysed using ANOVA and Pearson correlation. Results indicated that Mutant 1 and Mutant 2 had high emergence percentage, emergence index, energy emergence and per cent seedling recovery compared to local varieties Chozi and Duma. Emergency percentage showed a positive correlation with the following parameters; emergence index ($r = 0.965$); Energy of emergency ($r = 0.990$) and Percentage seedling recovery ($r = 0.941$). It was recommended that the mutant wheat be screened for other biotic and abiotic stresses that affect wheat production in Kenya.

Key words: Wheat, induced mutations, drought tolerance, seedling

1. INTRODUCTION

Wheat (*Triticum aestivum* L.) is an annual crop that is widely cultivated as a small-grain cereal. It is the major food crop in the world and sustains the majority of the world population (Kurata et al., 1994). Approximately 600 million tons of wheat is produced annually, throughout the world roughly half of which is produced in developing countries (Goyal and Manoharachary, 2014). In Kenya, wheat is among the most important cereal crops grown that contributes significantly to food security in the country. It ranks second after maize in Kenya (CIMMYT, 2015). Wheat yield is significantly influenced by global climate change and water resources scarcity in the environment (Al-Ghamdi, 2009). Drought is one of the environmental stresses seriously limiting crop production in the majority of agricultural fields of the world (Abedi and Pakniyat, 2010) and recent global climate change has made this situation more adverse (Anand et al., 2003). About one fifth of the developing world's wheat (*Triticum aestivum* L.) is grown in the arid and semi-arid lands (ASALs) (Ndiema et al., 2011).

In Kenya, ASALs represent 83% of the total land area (56.9 million ha), which experience frequent crop failure due to drought stress. Approximately 300,000 hectares of these areas are arable land and are not fully utilized because annual rainfall is low (200-400mm), unreliable (40-50 days) and highly erratic (Kinyua et al., 2000). Lack of widely adapted drought resistant wheat varieties and unfavourable weather patterns pose a significant problem to wheat production in these areas (Kimurto et al., 2003). Consequently, there is need to develop improved plant materials that have drought tolerance and allow efficient utilization of limited rainwater.

Breeding for drought resistance is complicated by the lack of fast, reproducible screening techniques and the inability to routinely create defined and repeatable water stress conditions when a large number of genotypes are to be evaluated efficiently (Ghasemi, and Farshadfar, 2015). Induced mutation is highly instrumental in plant biology to induce genetic variability in a great number of crops. The technology is simple, relatively cheap to perform and equally usable on a small and large scale (Siddiqui and Khan, 1999). Mutagenesis has been widely used as a potent method of enhancing variability for crop improvement (Singh and Singh, 2001). Induced mutation, using physical and chemical mutagen, is a way to generate genetic variation, resulting in the creation of new varieties with better characteristic (Wongpiyasatid et al., 2000). Mutation has been successfully employed in breeding of several food crop varieties, ornamentals and export crops (Mohamad et al., 2005). Gamma rays are the most energetic form of electromagnetic radiation. Their energy level is from ten to several hundred kilo electron volts, and they are considered as the most penetrative compared to other radiations (Kovacs and Keresztes, 2002). Gamma radiation can be useful for the alteration of physiological characters (Kiong et al., 2008).

The objective of this study was to screen for drought resistance in the selected mutant wheat at seedling stage.

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 subsequent generations for preliminary evaluation for positive effects of radiation. Mutant 1 and Mutant 2 were selected since they had initially shown resistance to stem rust disease.

2.2 Experimental Site and Crop Establishment

The experiment was conducted in a greenhouse at Mimea International, Kitengela. Kitengela is located in Kajiado County which borders Nairobi

County. It is situated between Longitudes 360 5′ and 370 5′ East and between Latitudes 10 0′ and 30 0′ South. Two mutant wheat lines were obtained from University of Eldoret while two drought resistant varieties (Chozi and Duma) were obtained from KALRO-Njoro. The experiment was laid out in complete randomized design (CRD) with three replications. The seeds were sown in 18x9 cm polythene bags filled with a measured quantity of normal field soil (450 g/bag) as described previously by Noorka and Khaliq (2007). The bags were arranged in iron trays, each genotype comprising three bags per replication. Two seeds of each variety were sown in the three bags at a uniform depth of 3 cm to ensure full crop stand. The following parameters were recorded for further analysis; emergence percentage, emergence index, emergence rate index, energy of emergence, mean emergence time, desiccation tolerance index and per cent seedling recovery.

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2.2.1 Emergence Percentage (E%)

Data collection began after the emergence of the first seedling in any bag and onwards measurements were made on daily basis at 1600 h. Eighteen days after sowing, the number of visible seedlings was recorded. Data collection continued until there was no further emergence. Emergence percentage was then calculated according to the formula derived by Smith and Millet (1964).

$$E\% = \frac{\text{Total number of seedlings emerged 18 DAS}}{\text{Total number of seedlings grown}} \times 100$$

DAS = Days after Sowing

2.2.2 Emergence Index (EI)

Emergence index is the estimate of emergence rate of seedlings and was calculated by the formula as delineated in Association of Official Seed Analysis (AOSA) (1983). Each day, seedling at least 1.5 cm long from the different wheat varieties were counted and removed until no further seeds germinated. This total was then divided by the sum of the number of days after sowing for each day. The emergence index was then calculated as a percentage.

$$EI\% = \frac{\text{No. of seeds emerged at first count} + \ldots\ldots + \text{No of seeds emerged at final count}}{\text{Days of first count} + \ldots\ldots\ldots\ldots + \text{days of final count}}$$

2.2.3 Emergence Rate Index (ERI)

Emergence rate index for each treatment and replication was calculated as follows:

ERI = Emergence index/Emergence percentage

2.2.4 Energy of Emergence (EE)

Energy of emergence was computed according to the method as outlined by Ruan *et al*. (2002). It is the percentage of emerged seedlings three days after sowing. This was calculated by counting the number of seedling that emerged three days after sowing for the different wheat varieties. This was then divided by the total number of seeds planted for each variety and then calculated as a percentage.

2.2.5 Mean Emergence Time (MET)

Mean emergence time was calculated by the equation of Ellis and Roberts (1981) as under:

$MET = \Sigma Dn / \Sigma n$

where n is the number of seeds germinated on day D and D is the number of days counted from the beginning of emergence.

2.2.6 Desiccation Tolerance Index (DTI)

To check the potential of the genotype to bear the water stress condition, the plants were watered until they sprouted two to three leaves. This is considered the best stage to evaluate the genotype for their water stress tolerance and susceptibility as suggested by International Seed Testing Association (ISTA) (Anonymous, 1997). In order to induce artificial water stress condition, the water provision was withheld for two weeks a result of which most of the seedlings died. Irrigation was started again and survival was noted on re-growth of plants in each replication. The number of live as well as dead seedlings was counted daily following the procedure described by Noorka and Khaliq (2007). Desiccation tolerance index was done by counting the number of dead plants after the resumption of irrigation and dividing it by the number of live seedlings in all the wheat varieties.

Desiccation tolerance index = Final number of dead seedlings/Final seedlings emergence number

2.2.7 Per cent Seedling Recovery (PSR)

After resumption of the irrigation water, drought tolerant genotypes will recover. Per cent recovery or re-growth of seedlings after desiccation is calculated by the formula used by Noorka and Khaliq (2007).

$$\text{Percent seedling recovery} = \frac{\text{Number of plants resuming growth}}{\text{Total number of seedlings}} \times 100$$

2.3 Data analysis.

Data was subjected to analysis of variance (ANOVA) at 95% level of significance using GENSTAT 12th edition and means separated using Duncan Multiple range test. The relationship among the seedling traits was determined using Pearson Correlation. The statistical model used was: $X_{ij}=\mu+t_i+e_{ij}$

Where, Xij= observation, μ=overall mean, ti=treatment effect and eij=experimental error.

3.0 Results

There were significant differences in emergency percentage (EP), emergency index (EI), energy of emergence (EE), desiccation tolerance index (DTI) and percentage seedling recovery (PSR) among mutants and the non-mutant controls. Mutant 1 and Mutant 2 showed a significant difference with Duma variety in respect to emergence rate index (ERI) and mean emergence time (MET).

Mutant variety 2 (M2) recorded lowest DTI compared to the other varieties hence had the highest survival rates as shown by the PSR, closely followed by Mutant 1, Duma and Chozi had the lowest PSR. It was observed that the two mutant varieties (M1 and M2) recorded high emergency percentage (EP) and emergency index (EI) as compared to the non-mutant controls (Chozi and Duma). It was also evident that MI, M2 and Duma possessed high EP, EE, PSR and low MET that enabled them to escape the hazards of stress conditions as shown by low DTI. Chozi on the other hand had low EP, EI, EE and high MET that resulted in low PSR and high DIT (Table 3.1)

Emergency percentage recorded a positive correlation with the following parameters; emergence index (r = 0.965); energy of emergency (r = 0.990) and per cent seedling recovery (r = 0.941) at 0.01 level of significant. Hence as the emergency percentage increased, there was an increase in the three seedling traits described. It was noted that emergency percentage negatively correlated with following seedling growth parameters; emergency rate index (r = -0.465); mean emergency time (r = -0.694) and desiccation tolerance index (r = -0.864). Increase in emergency percentage, therefore, resulted in a decrease in ERI, MET and DTI (Table 3.2)

4.0 Discussion

The results of the present study illustrated that mutagenesis is an efficient tool for increasing genetic variability in wheat. It produced variability among the varieties in the various seedling traits. Mutated varieties (M1 and M2) recorded high emergency percentage, the energy emergence and per cent seedling recovery as compared to the non-mutant. The variability could have been due to the effect of the mutagens on the meristematic tissue of the seed that increased the physiological and biological processes necessary for seed germination. These metabolic processes could lead to increased enzymatic activity, hormonal balances and increased mitotic processes. The findings are in agreement with early studies by Kiong *et al.*, (2008) who reported that gamma radiations were useful for the alternation of the physiological disorders of the citrus seeds.

Table 3.1: Effects of mutagenesis on various seedling traits on mutated and non-mutated wheat varieties

Variety	EP	EI	ERI	EE	MET	DTI	PSR
Mutant 1	77.8 d	91.7 d	1.2 a	27.8 d	11.9 a	0.4 a	57.1 d
Mutant 2	72.2 c	77.4 c	1.1 a	22.2 c	12.0 a	0.5 b	46.2 c
Chozi	38.9 a	49.4 a	1.3 b	0 a	13.4 b	0.7 d	28.6 a
Duma	66.7 b	72.0 b	1.1 a	16.7 b	12.3 a	0.6 c	41.7 b
F-value	0.17	1.25	2.59	1.65	2.36	0.18	1.62
P-value	0.844499	0.301487	0.092273	0.209573	0.112306	0.836196	0.215299

Within the columns, means followed by the same letter are not significantly different from each other. Means separated using Duncan Multiple Range test at $\alpha=0.05$

Key: EP-emergence percentage, EI-emergence index, ERI - emergence rate index, EE-energy of emergence, MET- mean emergence time, DTI-desiccation tolerance index, PSR - per cent seedling recovery

Table 3.2 Correlation coefficient among various seedling traits of mutated and non-mutated wheat varieties

	variety	EP	EI	ERI	EE	MET	DTI	PSR
variety	1	-0.495**	-0.638**	-0.039	-0.596**	0.343*	0.633**	-0.699**
EP		1	0.965**	-0.465**	0.990**	-0.694**	-0.862**	0.941**
EI			1	-0.365*	0.985**	-0.680**	-0.903**	0.995**
ERI				1	-0.406*	0.259	0.276	-0.311
EE					1	-0.701**	-0.895**	0.972**
MET						1	0.644**	-0.663**
DTI							1	-0.906**
PSR								1

**. Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

Key: EP-emergence percentage, EI-emergence index, ERI - emergence rate index, EE-energy of emergence, MET- mean emergence time, DTI-desiccation tolerance index, PSR – per cent seedling recovery

Mutant variety M1 and M2 had increased survival rate as shown by low Desiccation index tolerance of 0.4 and 0.5 respectively and high per cent seedling recovery. These findings are in agreement with Dalvi et al., (1990) who reported a decrease in survival per cent due to mutagenic treatments in horsegram, Dhanavel et al., (2008) and Kavithamni et al., (2008) who reported a decrease in survival rate in various pulse crops.

It was observed that varieties with higher emergence percentage, emergence index and energy of emergence and lower mean emergence time showed earlier and rapid germination. These findings support the earlier work on Canola (*Brassica compestris*) by Zheng *et al* (1994), wheat (*Triticum aestivum* L.) by Nayyar *et al*., (1995) and rice (*Oryza sativa*) by Basra *et al*., (2003). These studies reported an inverse relation between emergence percentage, emergence index, energy of emergence and mean emergence time.

5.0 Conclusions and recommendations

Mutagenesis had positive effects on emergence percentage, emergence index and per cent seedling recovery hence the two mutant varieties (M1 and M2) can be grown in ASALs regions and can be selected for wheat breeding in Kenya. We recommended the two varieties be further screened for other biotic and abiotic stresses that affect wheat production in Kenya, for purposes of determining their suitability as potential varieties.

Acknowledgements

The authors wish to thank all the participants who took part in this study.

Financial Support

We wish to thank IAEA and University of Eldoret for their support.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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