Drought Tolerant Wheat Varieties Developed Through Mutation Breeding Technique

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ABSTRACT

In search for higher yielding drought tolerant wheat varieties, one of the Kenyan high yielding variety 'Pasa' was irradiated with gamma rays (at 150, 200, and 250gy) in 1997 so as to induce variability and select for drought tolerance. Six mutants ((KM10, KM14, KM15, KM18, KM20 and KM21) were selected at M4 for their drought tolerance screening. The six mutants and 2 checks, Duma and Pasa were tested for their performance in a National performance trial. The study was carried out as a National Dryland Wheat Performance Trial in 4 sites in Kenya and selection done for two seasons, 1999 and 2000. The sites were Katumani, Naivasha, Lanet and Mogotio, which represent marginal rainfall areas in Kenya. Randomised complete block design was used and replicated three times. Data on plant height, yield and hectolitre weight were taken. The results showed that mutant line KM21 and KM14 performed significantly ($P<0.05$) better than the other elite lines in yield performance. They also yielded significantly higher ($P<0.05$) than the parent 'Pasa' in most of the sites while KM14 had the highest average yield across sites in both years. In Lanet and Katumani they performed better than variety 'Duma', which was used as the check variety in the dryland. The two mutants were presented to the National technical release committee in the year 2001, to be considered for release. KM14 was accepted and has now been released for commercial production in the marginal areas of Kenya such as Njoro BW1. This study clearly demonstrated the usefulness of mutation as a tool of creating variability in wheat especially for complex traits like drought tolerance.

KEY WORDS: Mutation, drought tolerance, bread wheat and yield stability.

1.0 INTRODUCTION

Wheat improvement by traditional methods, which involves collection, hybridization and inbreeding, has been practiced since the beginning of 20th Century. However, it has now been realized that these methods are insufficient to make much further break through or cope with the increasing demand for improvement in crop varieties (Njau, 2001). Some of the limitations of conventional breeding may be the exhaustion of diversity of the wheat gene pool, low response to drought tolerance of the introduced materials and the low combining ability especially with complex character like drought tolerance. Interest in the use of mutation technique for generating and selecting desired genetic variation in crop species has significantly increased over the past decade. This has been mainly due to (1) Successful application of *in-vivo* mutation techniques in the breeding of new improved crop varieties (2) new opportunities for induced mutation using *in-vitro* technique for improving propagated crops (3) emerging possibilities for applying *in-vitro* selection of mutergenised cells and tissues and (4) increasing application of doubled haploids (DHs) for the rapid selection and shortening of the breeding cycle of improved varieties from desired mutants and for the development of F_1 performing DH lines from hectoric hybrids. (Maluszynski *et al,* 1995). Most of the available genetic variation used in breeding programmes has occurred naturally and exists in the germplasm collections of new and old cultivars, land races and genotypes. This variation through crosses is recombined to produce new, desired gene recombinations. When existing germplasm fails to provide the desired recombinants it is necessary to resort to other sources of variation. Mutation induction techniques provide tools for the rapid creation and increase of variability in crop species. Induced mutations contributed significantly to plant improvement worldwide, and in some cases have made an outstanding impact on the productivity of many crops (Maluszynski, 1990).

There are over 2252 mutant varieties officially released and recorded in the FAO/International Atomic Energy Agency (IAEA) mutant varieties Database (Maluszynski *et al,* 2000). These varieties were developed either directly after mutagenic treatment or through crosses involving mutant varieties or mutant lines. The cumulative number of officially released mutant cultivars indicates that more than 50% of these varieties were released during the last 10-20 years (Maluszynski *et al*, 1995) contrary to popular belief, this figure clearly indicates that the tendency to apply mutation technique has increased, and year by year this approach is being used more widely to generate desired genetic sources of particular plant characteristics. The impact of induced mutations on the income of farmers and national economies was assessed at the FAO/International Atomic Energy Agency (IAEA)

International Symposium of plant mutation breeding for crop improvement held in Vienna in 1990. Some of the outstanding examples of economic impact ranged from rice in China, Japan and USA to barley in the former Czechoslovakia and other central European countries. Crop mutant cultivars were mainly developed in seed propagated plant species (Maluszynski *et al*, 1995). In cereals, mutation techniques were most successfully applied for improving rice, barley, bread wheat, durum wheat and maize. In Africa, Kenya included, very little has been done hence there is a need to adopt this breeding technique. However this technique has been very successful in the few areas that it has been applied e.g. in Cowpea (Pathak, 1986). The above evidence shows that mutation technique form a good means of creating and selecting for variability. This study was therefore undertaken to use mutation to create and select for variability for drought tolerance in bread wheat in Kenya. It was hypothesized that there is a possibility of identifying mutant wheat lines that are tolerant to drought and suitable for adoption in the marginal areas of Kenya.

2.0 MATERIALS AND METHODS

2.1 Mass Selection of the Mutants

Irradiated wheat seeds of Kenyan variety 'Pasa' were planted in the cage in blocks in 1996. The blocks consisted of different doses of irradiation, which was 140gy, 160gy and 200gy (KARI, 1997). M_1 plants were bulked and advanced to M_2. The M_2 plants were planted in 3 blocks according to the level of irradiation in 1997. Screening was done during the crop development period and at maturity ears were harvested from the selected plants. The ears were planted in rows and observed at M3 for physical characteristics in late 1997 for confirmation of the performance. From the rows 21 lines were advanced to M4. The selection was based on performance after exposure to drought conditions, on a scale of 1-5, where 1=very poor performance where there was almost no seed set, 2=poor performance with few seeds, 3=fair with some seeds, 4=good performance and 5 was very good performance where there was hardly any sign of drought stress through crop development to maturity, and the seed set was optimum.

2.2 Field Evaluation of the Mutants

Ten selections were made at M_4. The seed harvested at M_4 were bulked in rows and were planted in 3 sites in 1998 and screened for performance and drought tolerance. The scoring was on phenotypic characteristics at a scale of 1-5, where 1 was the poorest and 5 the best. The 3 sites were Katumani and Naivasha, which represent the ASALS of Kenya and Njoro under the rainshelter.

Six of the lines were selected and included in a National Dryland Wheat Performance Trial (NDLWPT) in 1999 and 2000. The six mutants (coded KM10, KM14, KM15, KM18, KM20 and KM21) and two checks were included in the study. The checks included one drought tolerant line 'Duma' and a susceptible variety 'Pasa', origin of the mutant collections. The entries were planted in 4 sites namely Mogotio, Lanet, Katumani and Naivasha in two seasons, 1999 and 2000. Mogotio lies in Agro-Ecozone UM4, with low agricultural potential (1890 m a.s.l., latitude 1^0 35'S, longitude 36^0 66'E). Average annual rainfall is 767mm, with mean annual minimum and maximum temperatures of 7.9 and 24.8, respectively. Soils are *Vitric Andosols* with moderate to high soil fertility, well-drained deep loam to sandy loam soil (Jaetzold and Schimdt, 1983). National Plant Breeding Research Centre, Njoro (latitude 0^0 20'S, longitude 35^0 56'E, altitude 2160 m). Njoro receives average annual rainfall of 931-mm. Mean maximum and minimum temperature is 22.7^0C and 7.9^0C, respectively. The soils are well drained Mollic Andosols with sandy loam. Katumani (National Dryland Research Research Centre) lies in semi-arid zone low potential dryland area within Agro-Ecozone UM 4 (1560m a.s.l., latitude $1^0$35' S, longitude $37^0$14'E). The average annual rainfall is about 716mm, with mean minimum and maximum temperatures of 13.9^0C and 24.7^0C, respectively. Water loss through evaporation is about 1800 mm per year, creating an annual water deficit of about 1048 mm (ICRAF, 1988). The soils are Ferral Chromic Luvisols, which are well-drained, deep sandy loam to clay loam. Naivasha lies in Agro-Ecozone LH5 with low agricultural potential (1829m a.s.l, latitude $0^0$31' S, longitude $36^0$15'E). Average annual rainfall is 729mm while mean minimum and maximum temperatures are 7.7^0C and 26.0^0C, respectively. Soils are *Solenetz* with undifferentiated saline phase and sodic, saline silt loam to clay. Lanet has average annual rainfall of 779 mm with a range of 588-1089 mm per annum. The soils are well drained Mollic Andosols with sandy light loam (Jaetzold and Schimdt, 1983).

The trial was laid out in a randomized complete block design replicated three times. Each plot was 6m x 0.8 rows. The seeds were hand drilled in rows 20cm apart. The parameters that were taken included; grain yield in tons per hectare, Hectolitre weight and plant height (height of plant from soil level to tip of the spike) at physiological maturity.

2.3 Statistical Analysis

Analysis of variance was used to evaluate the genotypes using general linear model (GLM) SAS (1996) package. Fisher's least significance test (LSD) at $P<0.05$ was used to separate the differences among genotype means. Data from each site was analysed individually for the two seasons and also combined over the years, sites and seasons. Yield stability analysis was done by determining the regression coefficients (b_i) and deviation means squares using SAS (SAS, 1996) according to method of Eberhart and Russell (1966).

3.0 RESULTS

3.1 Yield Performance

The results show that there were significant differences in yield within some sites and over the two years (Table 1). The presence of significant genotype x environment interaction suggests that genotypes responded differently to the different environments and varied in their ranking, which may obscure selection for drought tolerance.

There was also variation in yield between the mutant lines and the check varieties within the different sites. In 1999 the mutant line KM14 performed well in all the sites and was ranked highest amongst mutants in Naivasha with a mean yield of 0.61 th-1, after Duma (0.64 th-1) (Table 2). Mutant lines KM14, KM21 and KM15 yielded better than the Parent variety Pasa in Lanet though yield difference was not significant (Table 2). In Mogotio, 'Pasa' had the highest yield followed by KM21, KM14 and KM10 respectively (Table 2). KM21 had the lowest yield. Overall genotypes produced the lowest yield in Naivasha in 1999 followed by Katumani while Mogotio was ranked first (Table 2).

Tablel: Mean squares from ANOVA on yield, for the eight wheat lines over the 4 sites in the two years

Source of variation	df	Mean squares
Years	1	18.8**
Sites	3	1.9**
Blocks	16	0.38**
Wheat lines	7	0.08
Wheat line x year	7	0.23
Wheat line x site	21	0.4*
Year x site	3	6.07**
Wheat line x site x years	21	0.08

*, ** Significant at 0.05 and 0.01, respectively

Table 2: Mean grain yield (tons/ ha) for the six mutants for the two years in different sites as compared to the check variety 'Duma ' and the original parent 'Pasa' for the years 1999 and 2000 in different sites.

Year	Yield in tons/ha 1999				2000				
Genotype	Naivasha	Lanet	Mogotio	Katumani	Naivasha	Lanet	Mogotio	Katumani	Mean
KM10	0.35c	0.41a	1.07ab	0.42a	1.37a	1.36a	0.97ab	1.79a	0.97
KM14	0.61ab	1.34a	1.08ab	0.5a	0.93a	1.21ab	0.98ab	1.8a	1.06
KM15	0.60a	1.26a	0.91ab	0.58a	1.06a	1.18ab	0.82ab	2.0a	1.05
KM20	0.44abc	0.81a	0.79ab	0.43a	1.7a	0.99bc	0.71ab	1.97a	0.98
KM21	0.45abc	0.49a	1.29ab	0.55a	1.27a	0.75d	1.16ab	2.13a	1.01
KM18	0.4bc	0.43a	0.78ab	0.53a	1.26a	0.92bcd	0.70ab	2.41a	0.93
DUMA	0.62a	0.48a	0.61b	0.47a	1.74a	0.89bcd	0.54b	2.33a	0.96
PASA	0.36c	0.38a	1.47a	0.48a	0.64a	0.58d	1.33a	1.95a	0.90
Mean	0.48	0.7	1.0	0.50	1.25	0.98	0.90	2.04	
LSD	0.216	-	0.85	-	-	0.373	0.77	-	
P(F-ratio)	<0.05	ns	<0.05	Ns	ns	<0.05	<0.05	Ns	
SE	0.12	0.84	0.49	0.14	0.67	0.22	0.44	0.40	

In 2000, Katumani recorded the highest grain yield average of 2.04 t h^{-1}, followed by Naivasha (1.25 t h^{-1}) (Table 2). Mogotio and Lanet were ranked lowest. In Naivasha, 'Duma' and KM20 had the highest yields in this site at 1.74t h^{-1} and 1.7 t h^{-1} respectively, but not significantly so from the other entries. This consistency was not repeated in all the sites but all mutants showed stability of yield in all sites (Table 4). An example is KM14 and KM21, which were as good as the check variety in all the sites. 'Pasa' outyielded the mutants in Mogotio, but the yield difference was not significant. However, it performed poorly in all the other three sites (Table 2).

In Katumani, in year 2000, the check variety 'Duma', KM14 and KM15 had higher yields than other genotypes (Table 2). KM14 was the highest yielder in year 2000 best performer while in Naivasha it performed slightly poorly. In 1999 there were no significant differences in yield of the entries as shown in the ANOVA (Table 2) in Lanet and Katumani. KM14 had the highest average yield (1.06t h^{-1}) over the two years across sites although not significantly different from the other entries (Table 3). It was rated among the best in all the sites. In Lanet, it had the highest yield (1.28t h^{-1}) which was significantly better than the Parent variety 'Pasa' with 0.48t h^{-1}. The original parent 'Pasa' performed well in Mogotio only, but had significantly lower yields in all the other sites. Its average yield was 0.90t h^{-1}, which was 17% lower than KM14. This was also below the overall mean of 0.98t h^{-1} (Table 3).

Table 3: Average grain yield of the 8 wheat lines over the 2 years across the four sites

Variety	Sites				Overall mean
	Naivasha	Mogotio	Katumani	Lanet	
KM10	0.86ab	1.02abc	1.10b	0.88ab	0.97
KM14	0.77ab	1.03abc	1.15ab	1.28a	1.06
KM15	0.85ab	0.86bcd	1.29ab	1.22ab	1.05
KM18	0.83ab	0.74cd	1.47a	0.67ab	0.98
KM20	1.07a	0.75cd	1.2ab	0.9ab	0.93
KM21	0.86ab	1.23ab	1.34ab	0.62ab	1.01
DUMA	1.17a	0.58d	1.4ab	0.69ab	0.96
PASA	0.50b	1.40a	1.2ab	0.48b	0.90
Grand mean	0.87	0.95	1.27	0.84	0.98
Lsd	0.55	0.41	0.35	0.40	
SE	0.216	0.121	0.087	0.404	
P(F-ratio)	P<0.05	P<0.05	P<0.05	P<0.05	

The results indicate that there was G x E interaction in the yield of the test genotypes for both sites and the years (Table 4). All the lines however stood out as stable over environments. All the lines tested had a regression coefficient not significantly different from 1, which according to Eberhart and Russel (1966) is a good measure of stability.

Table 4: Yield regression coefficient and standard error for the 8 wheat lines over the two years and across the four sites.

Wheat line	Sites Coefficient of regression	Standard error	Years Coefficient of regression	Standard error
KM10	0.52	0.73	1.31	0.30
KM14	0.26	0.74	0.56	0.39
KM15	0.64	0.79	0.67	0.42
KM18	1.78	0.77	1.27	0.39
KM20	0.60	0.78	1.16	0.36
KM21	1.37	0.66	1.03	0.34
DUMA	1.28	0.94	1.34	0.46
PASA	1.55	0.80	0.73	0.45
P (F-ratio)	ns	ns	ns	ns

Hectolitre weight

There were significance difference hectoliter weight between in the mutants and the parent Pasa and the check variety in all the sites (Table 5). The mutant line KM14 had significantly lower weight in Mogotio and Lanet. No single line maintained its rank over the sites. The character therefore might not be a good one to select on as there is change of ranking for the lines making it difficult to make a choice (Table 4).

Table 5: Test weight (Kg/hectolitre) for the six mutants as compared with the check variety, Duma, and the original parent, Pasa for the years 1999 and 2000 in different sites

Genotype	Hectolitre weight Mogotio	Katumani	Naivasha	Lanet
KM10	68.93	69.67ab	69.6c	67.33a
KM14	67.80	71.30a	71.07abc	61.47b
KM15	69.93	66.67c	72.27ab	62.87ab
KM20	69.07	67.67bc	71.47abc	63.33ab
KM21	67.53	71.3a	70.13abc	64.67ab
KM18	68.27	67.3bc	66.53d	65.67ab
DUMA	68.27	68.67abc	70.27abc	64.67ab
PASA	71.33	68.67abc	72.40a	64.00ab
Mean	69.36	68.52	71.12	63.58
Lsd	ns	2.75	2.415	4.9
P(F- ratio)	P<0.05	P<0.05	P< 0.05	P<0.05
SE	6.3	2.7	2.1	8.7

Plant Height

There was great variation in height over sites (Table 3). Three mutants (KM10, KM15 and KM20 were significantly taller than the Pasa parent but similar to the check variety. The other mutants KM14, KM21 and KM18 were as tall as the original parent Pasa in Naivasha (Table 6). This case was not repeated in Lanet and Naivasha. KM14 was as tall as Duma in these two sites but KM21 was shorter than even the parental genotype Pasa. The variation in height in the mutants indicates the usefulness in creating variability through mutation in wheat. The tallest line was KM14 in Lanet (66cm) while the shortest was KM15 (34cm) in Mogotio. It is interesting to note such great variation created from the same original parent. The mutants also varied amongst themselves in same site and also between sites. This indicates the high influence environment has on plant height.

Table 6: Plant height (Cm) of the six mutants as compared to the check variety, Duma, and the original parent, Pasa for the years 2000 in different sites

Genotype	Plant height (cm)			
	Mogotio	Katumani	Naivasha	Lanet
KM10	47.0b	61.0bc	51.0bc	48.0bc
KM14	60.0a	47.0c	46.0bcd	66.0a
KM15	40.0b	75.0a	46.0bcd	56.0abc
KM20	45.3b	50.0c	54.0bc	54.0bc
KM21	62.3a	58.0bc	51.0bcd	66.0a
KM18	62.3a	65.0bc	60.0ab	58.0abc
DUMA	62.3a	60.0bc	60.0ab	64.0ab
PASA	44.0b	70.0ab	53.0bc	48.0bc
P(F-ratio)	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$

4.0 DISCUSSION

The results show variation in yield and yield components measured amongst test genotype. The 6 mutants varied between themselves and their original parent. The best mutant (KM14) out yielded the original parent, 'Pasa', by 18% and the Check variety, 'Duma', by 10% from overall means. In most of the sites the mutants out yielded their original parent by over 0.8 t h^{-1} (more than half). For example, in Lanet mutant KM14 had a grain yield of 1.28t h^{-1} while Pasa had only 0.48t h^{-1}. Such results were also recorded in Naivasha where the difference between Pasa and KM20 was 0.57t h^{-1}. However, similar trend was not repeated in

Mogotio where 'Pasa' outyielded the lowest mutant by 47%. According to Blum (1996) definition of drought tolerance, in terms of yield, the mutant lines were able to overcome drought and produce some grains. The grain yield stability displayed by all the entries in the varying environment (time and space) is a good characteristic that can be exploited when any of these lines is commercialized. However, Eberhart and Rusell (1966) noted that an ideal variety is one that combines high yield with stability of performance. In this regard genotypes KM14 and KM15 were stable and highest yielding, hence their basis of recommendation for release as varieties for planting in different locations in the ASALS. This is in agreement with earlier observations (Allard and Bradshaw, 1964) that a variety that combines high yield and stability is acceptable over a wide range of environmental conditions.

Variation was also recorded in grain weight and plant height in most sites. Some of the mutants were significantly shorter than the Pasa parent e.g. KM14 and KM15 while others were taller than the Pasa parent e.g. KM21. This could be due to the easiness of mutation when it comes to semi-dwarfness (Maluszynski *et al*, 2001). This is an indication that fewer genes control height in wheat and also the effect of the environment is high. Results showed that that plant height was affected by mutation as earlier expressed (Maluszynski *et al,* 2001). Seed weight has been used in other studies (Kimurto *et al.*, 2003) and maybe a better character to use than hectolitre weight. Similarly, Omanya *et al.,* (1996) reported that in sorghum grain yield correlated positively with seed weight, while Odenyo *et al.*, (1996) noted that 1000-kernel weight was the most reliable predictor of yield as it is the most stable parameter. Therefore, these yield components may be used reliably as a selection criterion. Hectolitre weight is however a good indicator of better quality breed wheat.

5.0 CONCLUSION

Mutation technique was shown to create variability in wheat and can be used in breeding for complex characteristics such as drought tolerance. The rich variation expressed in the mutants is an indication of the effect on the positive changes that can be exploited in selection for various characters. It is also important to note that the mutation process did not alter most of the parent's characteristics. The time taken to select and release the variety was much shorter than in conventional breeding. From these results KM21 and KM14 were found to be superior in yield and shorter than the parent. These were recommended for release as varieties for the Dryland. KM14 was released in 2001 as Njoro BW1. The recommendations were based on stable high performance of the line over season and sites.

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