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Research Article

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Alternative screening method for drought tolerance in barley genotypes

Javan Omondi Were[1]*, Julius Onyango Ochuodho[1], Nicholas Kipkemboi Rop[1] and Sanjaya Gyawali[2]

[1]University of Eldoret, School of Agriculture and Biotechnology, Department of Seed, Crop and Horticultural Sciences, P.O. Box, 1125 - 30100, Eldoret, Kenya, [2]International Centre for Agricultural Research in the Dry Areas (ICARDA), Biodiversity and Integrated Gene Management Program, Morocco

ABSTRACT

Lack of genetically stable and durable drought tolerant winter and spring barley genotypes is one of the main contributing to low and unpredictable yields in Kenya and other parts of the world despite annual release of new and high yielding varieties. Therefore, the study was set to identify genotypes exhibiting tolerance to drought through physiological and phenotypic approaches. A total of 32 genotypes were planted in split-plot arrangement in completely randomized design replicated thrice. Genotypes were maintained under 20% and 80% field capacities. Phenotypic and physiological data were collected, converted to ratios then analyzed on Genstat version 14.1 VSN International Ltd at 5% level of significance. Significant differences were observed in winter and spring barley in terms of growth, tillering ability, grains formed per spike, 1000 seed weight and MSI ($p < 0.05$). Spring barley expressed higher tolerance to drought than winter barley especially in terms of height, number of grains per spike and seed weight. Water deficiency in cells and tissues might have altered and inhibited physiological and biochemical processes. The phenotypic and physiological methods corresponded and confirmed tolerance to drought in most winter and spring genotypes grown in Kenya.

KEYWORDS: Rapid screening, drought, barley, physiological, phenotypic, approaches

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Corresponding author:
javanomondiwere@yahoo.com

INTRODUCTION

Barley (*Hordeum vulgare* L.), a member of the grass family, is a major cereal grain ranked fourth was among cereal crops in the world both in terms of quantity produced (136 million tons) and in area under cultivation (566,000 Km^2) (FAOSTAT, 2009). Important uses include industrial processing as alcoholic and non-alcoholic beverages such as beer, wines, spirits (Ogle, 2006), food for humans with eight essential amino acids, carbohydrates and several minerals (USDA, 2011), feed to livestock and medicinal uses as a component of various health foods to control urinary tract infections, remove toxic substances from kidney and reduce chances of Type II diabetes among others (Ayto, 1990; Reshmi, 2013).

Despite its role in the Kenyan economy, the annual barley yields remain very unpredictable and below 3.0 t/ha (EABL-UoE, 2016). Additionally, in the past two decades, the annual area under barley in Kenya has been on the decreasing trend (below 20,000 ha) since late 1990s and this has persisted to date (EABL-UoE, 2013). As a result, deficits have been experienced in Kenya since most farmers hardly attain potential yield recorded at 5.5 t/ha (EABL-UoE, 2013). A lot of work have been done on breeding for high yielding varieties with much efforts geared towards screening for resistance to net blotch, drought which occupies about 40% for the worlds' agricultural land (Demirevska *et al*., 2008) and aluminium toxicity (EABL-UoE, 2016) but low barley yields is still a major challenge in Kenya.

In Kenya, the actual yields still remain low in barley growing zones despite the existence of numerous breeding lines and new varieties that are released annually for commercial production (EABL-UoE, 2010; EABL-UoE, 2016). This could be due to not only unpredictable and intense drought stresses but also the lack of durable and stable winter and spring adapted barley genotypes the can tolerate drought. This calls for a very rapid and reliable method of screening for drought tolerance among the advanced and genetically stable genotypes that can act as potential germplasm for variety improvement that aims at conferring tolerance to drought.

Lack of rapid and reliable selection approaches for drought tolerance is one of the main bottlenecks for many barley breeders not only in Kenya but also other parts of the world. The precision in such selections is further complicated by the interaction effects of drought and other stress factors such as diseases and

soil mineral toxicities which collectively can cause yield losses up to 100% (Bekele *et al*., 2001; Demirevska *et al*., 2008; Newton and Goodman, 2005). This compromises the accuracy and reliability of the results. In addition to the interferences by such interactions, most of the methods used to screen for tolerance to drought takes a long time and this could be responsible for the underperformances against drought tolerant barley and other cereals across the globe. This is because by the time a variety is released for commercial production, the environmental conditions could have greatly changed. The study therefore aimed at identifying drought tolerant winter and spring barley genotypes in Kenya through phenotypic and physiological approaches.

MATERIALS AND METHODS

A total of 32 barley genotypes, 16 adapted to winter growth conditions and 16 adapted to spring growth conditions were sourced from University of Eldoret – East African Breweries Limited Collaborative Barely Research and Variety Improvement program. These included both commercially produced varieties and genetically stable lines.

Phenotypic Approach to Determine Drought Tolerance in Barley

The barley seeds were sown in plastic containers filled with forest soil with pH measured at 6.2 (Were and Ochuodho, 2014) to reduce the stress due to acidity under greenhouse conditions. Three seeds of each genotype were planted per pot and at two leaf stage, two watering regimes of approximately 20% and 80% of the soil field capacity was adopted and maintained up to physiological maturity. This means that pots maintained at 20% field capacity (Stressed) received about 1,550 ml (22 ml per pot per day for 70 days) while those at 80% field capacity (Unstressed) were supplied with 6,500 ml (93 ml per pot per day for 70 days) of water through irrigation for the whole experimental period (Pauk *et al*., 2012).

Due to the obvious genetic differences between the winter and spring genotypes, screening for drought tolerance was done separately using split – plot arrangement in completely randomized design with each genotype replicated thrice. The two water regimes were used as main plots while genotypes were considered to be the sub-plot. Data on agronomic traits including height (cm), number of tillers, number of grains per main spike, and 1000 grain weight (g) were scored at physiological maturity growth stage.

Physiological Approach to Determine Drought Tolerance in Barley

The physiological approach was selected to act as a confirmatory test to the pot experiment. This was performed at the seed lab in School of Agriculture and Biotechnology, University of Eldoret. Membrane stability index (MSI) was determined by recording the electrical conductivity in mS (microSiemens) of leaf leachates on EC meter (HI 991301, HANNA Instruments – Woonsocket RI USA, ROMANIA) using double distilled water at 40 and 100 °C (Almeselmani *et al*., 2011).

The leaf samples for each barley genotype were obtained from the greenhouse experiment at 20% and 80% field capacities treatment in three replicates. For each sample, 0.25 g of leaf samples was cut into discs of uniform size and placed inside test tubes containing 25 ml of double distilled water in two sets. The first set was kept at 40 °C for 30 minutes while the second set at 100 °C in water bath for 15 minutes and their respective electrical conductivities C1 and C2 were measured by Conductivity meter. Membrane stability index (MSI) was calculated using the formula: $MSI = 1 - C1/C2 \times 100$

The higher the MSI, the more the tolerant a genotype was to drought. For effective comparison on samples subjected to 20% field capacity (Stressed) with those maintained at 80% field capacity (Unstressed), tolerance ratios for plant height, number of tillers, number of grains per main spike, 1000 seed weight and MSI were derived for each genotype using the formula: Tolerance ratio = Variable at 20% field capacity/ Variable at 80% field capacity

Genotypes with ratios equal to or closer to 1.0 were considered tolerant to drought while those closer to or equal to 0.0 were considered to be sensitive to drought stress.

Statistical Data Analysis

Data on ratios were subjected to analysis of variance on Genstat statistical software release 14.1 VSN International Ltd at 5% level of significance. The significant mean differences were tested using Duncan Multiple Range Test and results presented in table of ratios and Figures.

RESULTS

Winter and spring adapted barley genotypes expressed significant differences in their response to drought in terms of tillering ability, growth in terms of height, number of grains per main spike, thousand seed weight and membrane stability index ($p < 0.05$). The additive effects of genotype and field capacities was also significant hence a significant interaction ($p < 0.05$). The differences in ratios in terms of tillering ability, height, number of grains per main spike, thousand seed weight and membrane stability index among the spring and winter adapted barley was observed.

Among the spring barley, FANAKA and HKBL 1805-6 were the most tolerant to drought with reference to tillering ability. This implies that there was no much difference in the number of tillers when these genotypes were subjected to 20% (stressed) and 80% (unstressed) growth conditions. However, HKBL 1805-3 and HKBL 1629-14 expressed high sensitivity to drought which significantly affected their tillering ability hence lowest ratio among the spring barley. In terms of height, all the spring adapted barley expressed tolerance to water deficiency and scored above 0.8, an indication of 80% similarity between stressed and unstressed. In this regard, HKBL 1805-6 and HKBL 1774-3 were the most tolerant to drought with perfect similarity under stressed and unstressed conditions (Table 1).

Table 1: Table of ratios on the response of SPRING adapted barley to drought under screenhouse conditions

GENOTYPE	Number of tillers		Plant height		Grains per spike		1000 SWT		MSI	
FANAKA	0.9	cd	0.9	bcde	0.8	d	0.9	f	0.9	d
HKBL 1629-14	0.5	ab	0.8	abcd	0.3	a	0.6	ab	0.9	cd
HKBL 1629-5	0.7	bcd	0.8	ab	0.2	a	0.6	ab	0.9	cd
HKBL 1663-3	0.6	bc	0.8	abc	0.6	bc	0.9	ef	0.8	bc
HKBL 1674-4	0.8	bcd	0.9	bcde	0.5	b	0.7	bcd	0.9	bcd
HKBL 1719-4	0.7	bcd	0.9	bcde	0.5	bc	0.8	def	1.0	d
HKBL 1774-3	0.7	bcd	1.0	de	0.6	bc	0.6	bc	1.0	d
HKBL 1805-3	0.3	a	0.8	a	0.3	a	0.6	ab	0.6	a
HKBL 1805-6	0.9	d	1.0	e	0.8	d	0.9	ef	0.8	bcd
HKBL 1861-1	0.8	bcd	0.9	cde	0.6	bc	0.6	b	0.9	bcd
HKBL 1862-5	0.6	bc	0.8	abc	0.6	bc	0.5	a	0.9	bcd
KARNE	0.8	cd	0.9	cde	0.6	bc	0.9	ef	0.9	bcd
MALT 1	0.8	cd	0.9	cde	0.8	d	0.9	f	0.9	d
NGAO	0.7	bcd	0.9	bcde	0.7	cd	0.9	ef	0.8	b
NGUZO	0.8	bcd	0.9	bcde	0.7	cd	0.8	ef	0.9	bcd
SABINI	0.8	cd	0.9	bcde	0.7	cd	0.8	cde	0.9	cd
MEAN	0.7		0.9		0.6		0.7		0.9	
Probability	0.003		0.007		<0.001		<0.001		<0.001	
S.E	0.0775		0.0323		0.0517		0.0439		0.0359	
S.E.D	0.1096		0.0457		0.0732		0.0620		0.0507	
% CV	18.5		6.3		15.2		10.3		7.1	

While the growth in terms of height was less affected among the spring barley, yield parameters explained by the number of grains per main spike and thousand seed weight (TSW) were

significantly reduced under drought stress compared to unstressed conditions as reflected by the ratios. For instance, HKBL 1629-14, HKBL 1629-5 and HKBL 1805-3 had significant reduction in the number of grains per spike and this strongly corresponded to the thousand seed weight results. Physiologically (MSI), genotypes such as FANAKA, HKBL 1805-6, MALT 1, NGAO, NGUZO and SABINI expressed higher tolerance to drought and this was in agreement with majority of the scores for tillering ability, height, number of grains per spike and TSW (Table 1).

The use of phenotypic expression and physiological assessment to determine drought tolerance in spring adapted barley showed strong correspondence to each other. Specifically, at 20% field capacity, FANAKA and HKBL 1861-1 genotypes recorded MSI of 73 and 59 respectively. However, at 80% field capacity, higher membrane stability indices were recorded but still, FANAKA had higher MSI than HKBL 1861-1, an indication that FANAKA was better than HKBL 1861-1 in terms of tolerance to water deficiency though the ratios for MSI were the same. The phenotypic assessment confirms similar results when these two genotypes were subjected to different water deficiency conditions (Figure 1).

Unlike the spring adapted barley, majority of the winter genotypes were more sensitive to drought with significant differences in terms of tillering ability, plant height, number of grains per main spike, TSW and MSI under the influence of genotypes, field capacity and the interaction between genotype and field capacity ($p < 0.05$). In particular, only GRACE, TITOUAN and SY BATYK and PHILADEPHIA expressed significant tolerance in terms of tillering ability. Additionally, the effect of drought on height was more serious in QUENCH and NFC TIPPLE genotypes. With reference to all variables

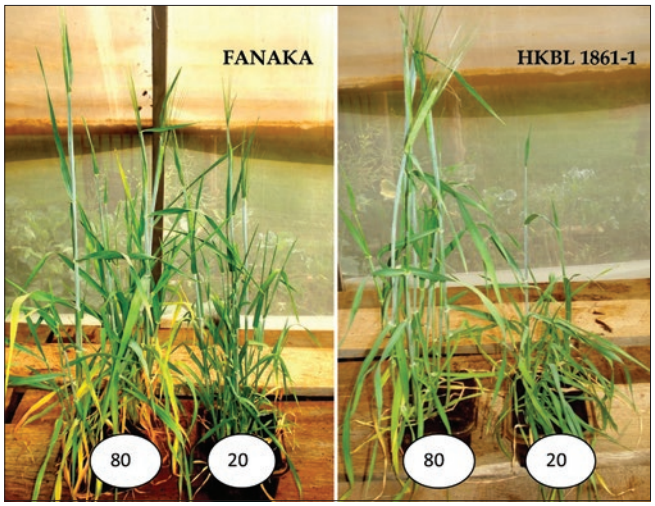

Figure 1: Growth differences of SPRING barley at 80% (unstressed) and 20% (stressed) field capacities under greenhouse conditions. FANAKA genotype exhibits more tolerance to water stress compared to HKBL 1861-1

assessed, only GRACE maintained stable tolerance to drought stress (Table 2).

Further, most of the winter barley showed mixed reactions to drought stress and the sensitivity to drought was more expressed compared to the spring barley specifically with reference to yield parameters – the number of grains formed per spike and TSW. For instance, BEATRIX and QUENCH expressed sensitivity across all the variables and this had significant effect on grain development and seed weight. Similarly, just like the spring adapted genotypes, most of the phenotypic observation under drought stress corresponded to that of physiological results of MSI (Table 2).

With reference to phenotypes under the influence of different field capacities, most of the winter adapted genotypes responded

Table 2: Table of ratios on the response of WINTER adapted barley to drought under screenhouse conditions

GENOTYPE	Number of tillers		Plant height		Grains per spike		1000 SWT		MSI	
ALICIANA	0.5	cd	0.8	cde	0.3	abcd	0.7	bcdef	0.7	bcdef
ANNABEL	0.6	cde	0.8	bcd	0.4	de	0.7	bcdefg	0.7	bcdefg
BEATRIX	0.5	bc	0.6	a	0.1	ab	0.4	a	0.4	a
COCKTAIL	0.3	a	0.8	bc	0.4	cde	0.9	fg	0.9	fg
GRACE	0.8	fg	0.8	bcd	0.7	g	0.8	fg	0.8	fg
MARTHE	0.6	cde	0.8	bcd	0.1	a	0.6	abcd	0.6	abcd
NFC TIPPLE	0.5	bc	0.5	a	0.4	cde	0.8	cdefg	0.8	cdefg
PHILLADEPHIA	0.7	def	0.7	b	0.3	bcd	0.6	abcde	0.6	abcde
PUBLICAN	0.4	ab	0.9	def	0.2	abc	0.8	defg	0.8	defg
QUENCH	0.5	bc	0.5	a	0.5	ef	0.6	ab	0.6	ab
SCRABBLE	0.5	cd	0.9	ef	0.3	cde	0.8	defg	0.8	defg
SHUFFLE	0.4	ab	0.9	ef	0.6	fg	0.8	fg	0.8	fg
SY 409-228	0.6	cde	0.6	a	0.4	cde	0.9	g	0.9	g
SY BATYK	0.7	efg	0.9	f	0.2	abcd	0.6	abc	0.6	abc
TITOUAN	0.8	g	0.9	ef	0.4	cde	0.7	bcdefg	0.7	bcdefg
XANADU	0.6	cde	0.8	bc	0.4	cde	0.8	efg	0.8	efg
MEAN	0.6		0.8		0.3		0.7		0.7	
Probability	<0.001		<0.001		<0.001		<0.001		<0.001	
S.E	0.0465		0.0305		0.0481		0.0669		0.0250	
S.E.D	0.0657		0.0432		0.0680		0.0946		0.0354	
% CV	14.4		7.0		24.4		15.9		5.5	

in a similar trend just like the spring genotypes. For instance, more tolerance characteristics were phenotypically expressed more in ALICIANA than TITOUAN which was more sensitive to water stress. Further, among the drought sensitive winter genotypes like TITOUAN, significant reduction on height and delayed heading and forced maturity was observed at 20% FC. The tolerant genotypes including ALICIANA, ANNABEL, GRACE and COCKTAIL did not record significant differences in their MSI in comparison to a number of growth parameters assessed (Figure 2).

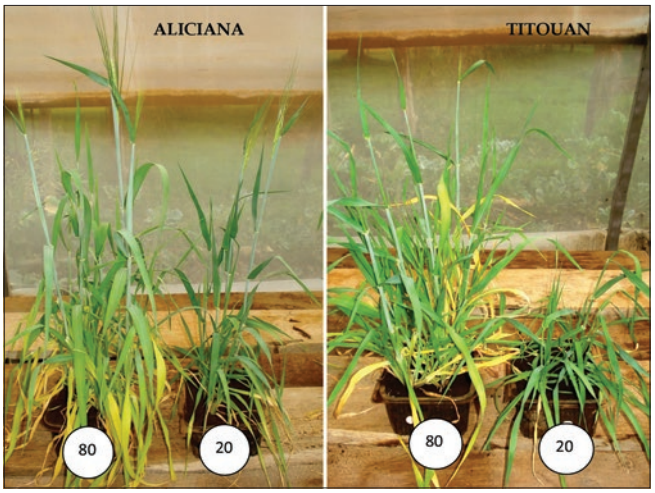

Figure 2: Growth of WINTER barley at 80% (unstressed) and 20% (stressed) field capacities under greenhouse conditions. ALICIANA genotype exhibits more tolerance to drought compared to TITOUAN

DISCUSSION

Diverse responses to drought among the winter and spring barley grown in Kenya confirms that these genotypes were different from each other and that the degree of tolerance differed from one genotype to the other. When subjected to water deficiency, low tillering ability, stunted growth, low number of grains per main spike and low thousand seed weight was common among the winter and spring barley and it could mean that inadequate supply of water interfered with a number of physiological processes such as translocation and partitioning of photosynthates needed for proper growth and development (Ashoub *et al*., 2015; Varga *et al*., 2014).

The higher sensitivity of winter genotypes compared to spring genotypes could further indicate that under drought stress, the quantum yield of light reaction, water-use-efficiency, photosynthetic rates and leaf osmotic potential (Siosemardeh *et al*., 2010)are significantly reduced hence leading to the higher sensitivity to drought (Ashoub *et al*., 2015) which is finally expressed by the integrity of the cell wall membrane as confirmed by the membrane stability index.

Among the drought tolerant genotypes such as FANAKA and GRACE, it is possible that a number of biochemical processes were least affected compared to sensitive genotypes such as HKBL 1805-3 and BEATRIX. This is because in most plants, other than interference with physiological processes (Wang *et al*., 2016), water deficiency in plant cells and tissues inhibits and/or alters vital biochemical processes such as hormonal balance, stress signal transduction pathways and gene expression (Ashoub *et al*., 2015). For the drought tolerant winter and spring barley genotypes, the variation in the level of tolerance could have been influenced by a number of factors including the quantity of proline whose production depends on calcium and ABA levels. This is in agreement with the previous findings that drought stress, proline accumulation is the first response to water-deficit stress (Siosemardeh *et al*., 2010) and once produced, the water uptake from the dry soil is greatly enhanced. When produced, proline acts as signaling molecule to moderate important mitochondrial functions which are needed for drought tolerance such as cell proliferation as well as triggering of specific gene expression such as those needed for ABA synthesis. However, the quantity of proline produced depends

on the level of available calcium and ABA (Xie *et al*., 2011). This could also mean that different genotypes signaled the expression of different genes for the production of varying levels of ABA. However, the winter genotypes could have produced low levels of this hormone thus low proline concentration in cells and tissues (Nascente *et al*., 2016) which resulted to higher sensitivity to drought compared to spring barley.

CONCLUSIONS AND RECOMMENDATION

Majority of the spring barley including FANAKA, NGUZO, NGAO, MALT 1, KARNE and HKBL 1805-6 were tolerant to drought stress across all parameters while for the winter genotypes, only GRACE was tolerant across all parameters. In addition, tillering ability, height and 1000 seed weight were the most affected variables in barley under drought stress in both winter and spring genotypes. Lastly, the greenhouse water stress experiment and MSI gave corresponding results on tolerance to drought by barley and this provides a cheaper and faster technique of screening barley for their response to water stress. The study recommends genetic confirmation on the observed physiological and phenotypic responses to drought by barley.

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