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Research Application Summary

Response of winter and spring barley genotypes to biotic and abiotic stresses in Kenya

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Abstract

Mixed response to net blotch disease, drought and aluminium toxicity in barley under different growing regions is one of the key problems leading to low average yield of barley in Kenya. The study aimed at determining whether spring and winter barley genotypes grown in Kenya would respond differently to the three stresses and establish a baseline for subsequent studies. A total of 16 spring and 16 winter genotypes were screened. Aluminium screening was done using 148 µM Al and 0 µM Al treatments in a completely randomized design (CRD) in laboratory and screenhouse. Data on relative net root growth, percent response, hematoxylin stain intensity, apical root length were collected. Drought screening was conducted under 20% and 80% field capacities alongside the membrane stability index (MSI) using EC meter. Data on MSI, height, tillers, heading, maturity, and 1000 seed weight were recorded. Net blotch screening was done in three sites identified as hot-spots for natural inoculation and confirmed in a screenhouse experiment. Field experiments were planted in a randomized complete block design while screenhouse in a CRD. Disease severity was assessed on a 0-9 scale. Significant differences were noted (p < 0.001) among the genotypes in their response to drought, net blotch and aluminium toxicity. Malt 1, HKBL 1663-3, Grace and Aliciana exhibited tolerance to the three stresses but other genotypes expressed mixed reactions to the same stresses. Mixed responses could mean that phenotypic expression of tolerance, resistance or susceptibility to stress is dependent on other external factors other than genetic make-up. In conclusion, winter and spring barley respond differently to biotic and abiotic stresses but the mechanism of response is dependent on other external factors.

Key words: Aluminium toxicity, drought, net blotch, response, yield

Résumé

La réponse mixte des orges à la maladie de la tâche réticulée, à la sécheresse et à la toxicité du sol en aluminium dans les différentes régions de culture est l'un des problèmes majeurs

de faible rendement de l'orge au Kenya. L'étude vise à déterminer si les génotypes d'orge d'hiver et de printemps cultivés au Kenya réagiraient différemment aux trois contraintes et d'établir une base de référence pour des études ultérieures. Un total de 16 génotypes de printemps et 16 génotypes d'hiver ont été évaluées. L'évaluation de la réponse à la toxicité du sol en aluminium a été faite en utilisant des taux de 148 mM et 0 mM d'aluminium dans un dispositif complètement aléatoire (CRD) au laboratoire et dans une serre. Les données ont été recueillies sur la croissance relative nette des racines, pourcentage de réponse, intensité de tâche hématoxyline, longueur des racines apicales. L'évaluation de la réponse à la sécheresse a été menée sous les sols de 20% et 80% de capacités en eau aux côtés de l'indice de stabilité de la membrane (MSI) en utilisant un mètre de mesure de la CE. Les données ont été collectées sur les paramètres tels que la hauteur, l'indice de stabilité de la membrane, l'enracinement, la maturité et le poids de 1000 grains. L'évaluation de la réaction à la maladie de la tâche réticulée a été faite à travers une inoculation naturelle sur trois sites identifiés comme zones à forte pression de la maladie, ce qui a été ensuite confirmé par une expérimentation sous serre. Les essais au champ ont été installés dans un dispositif de bloc aléatoire complet tandis qu'un dispositif aléatoire simple a été utilisé au cours de l'évaluation sous serre. La sévérité de la maladie a été évaluée sur une échelle de 0-9. Des différences significatives ont été notées (p < 0,001) entre les réponses des génotypes à la sécheresse, la tâche réticulée et leur sensibilité à la toxicité du sol en aluminium. Les génotypes Malt 1, HKBL 1663-3, Grace et Aliciana ont montrés une tolérance aux trois contraintes, mais d'autres génotypes ont exprimé des réactions mixtes par rapport aux mêmes contraintes. Les réponses mixtes pourraient signifier que l'expression phénotypique de la tolérance, de la résistance ou de la sensibilité au stress dépend des facteurs externes, autres que la constitution génétique. En conclusion, les orges d'hiver et de printemps réagissent différemment aux stress biotiques et abiotiques, mais le mécanisme de la réponse dépend d'autres facteurs externes.

Mots clés: toxicité en aluminium, sécheresse, maladie de la tâche réticulée, réponse, rendement

Background

Annual global production of barley has been very unpredictable and remains below 3.0 t/ha (FAOSTAT, 2009) and in Kenya, annual area under barley has been decreasing (EABL, 2013; USDA, 2011) resulting in significant deficits experienced not only in Kenya but also worldwide (GWA, 2005). Despite a yield potential of over 5 t/ha, yields remain low at below 3.5 t/ha. Net blotch disease (Owino *et al.*, 2014), drought and aluminium toxicity (EABL, 2010) are the major biotic and abiotic challenges affecting commercial winter and spring barley production in Kenya.

However, research remains limited on varietal response to the three constraints. Moreover, a wide gap exist as to whether tolerance to one stress would influence responses to the other stresses. Such information is critical to enhance productivity of barley on-farm in Kenya and other countries. Therefore, this study aimed at assessing winter and spring

Fifth RUFORUM Biennial Regional Conference 17 - 21 October 2016, Cape Town, South Africa 689 barley genotypes for the response to net blotch *Pyrenophora tere*, drought and aluminium cation toxicity.

Materials and methods

Response to aluminium cation toxicity. Thirty two barley genotypes (16 winter and 16 spring) were screened for their response to aluminium toxicity, drought and net blotch disease. Aluminium screening was done in two stages. In the first stage, laboratory screening was done using nutrient solution containing 0 iM Al and 148 iM Al at a pH 0f 4.0 (Bal and Alkus, 2011; Magnavaca *et al.*, 1987; Maxim and Duþã, 1996; Ouma *et al.*, 2011). Hydroponic screening approach was used in the second stage and the genotypes were planted in the screenhouse using forest soil whose pH was tested at 6.2 in CRD with three replicates. Two aluminium treatments were supplied through irrigation. Data on aluminium cation tolerant and sensitive barley genotypes were assessed based on relative net root growth, percent response and hematoxylin staining intensity (1-5 scale) as a confirmatory test for nutrient solution. Under hydroponics, data on apical root length, number of fibrous roots, shoot length and total biomass accumulated were collected and subjected to analysis of variance (ANOVA) on Genstat statistical software release 14.1 VSN International Ltd at 5% level of significance. The staining intensity data on a 1 - 5 scale was first transformed (Log₁₀ + 1) before performing an ANOVA and means separated using Duncan Multiple Range Test (DMRT).

Screening for response to drought. The pot experiment (Pauk *et al.*, 2012) and membrane stability index (Abdullah *et al.*, 2011) approaches were used. Pot experiment was done under screenhouse conditions at 20% and 80% field capacities throughout the growing period. Membrane stability index (MSI) was determined by recording the electrical conductivity of leaf leachates in double distilled water at 40 for 30 minutes and $100 \,^{\circ}\text{C}$ for 15 minutes (Abdullah *et al.*, 2011). Electrical conductivities C1 and C2 were measured by Conductivity meter and membrane stability index (MSI) was calculated using the formula MSI = $[1 - (C_1/C_2)] \times 100$. The higher the MSI, the more the tolerant a genotype was to drought. Data on spike length (cm) per main spike, number of tillers per plant, number of grains per spike, heading time (in terms of days after sowing – DAS), plant height (cm), number of grains per spike, 1000 seed weight and membrane stability index (MSI) were subjected to analysis of variance (ANOVA) on Genstat statistical software release 14.1 VSN International Ltd at 5% level of significance. Mean differences for winter and spring genotypes were separated using DMRT.

Screening for response to net blotch disease. Screening for net blotch disease was done under field and screenhouse conditions. Field screening was conducted in three sites with known hot-spot for net blotch (EABL, 2010) as described in previous studies (Owino *et al.*, 2014). For screenhouse experiment, one pathotype of *P. teres* was isolated and purified (Owino *et al.*, 2013). The conidial suspensions was made and adjusted to a concentration of 5×10^3 conidial ml (Xue and Burnett, 1995) after counting on Buker - Turk haemocytometer (Mathur *et al.*, 1989). Inoculation was done at Zadoks growth stage 15 (Xue and Burnett, 1995). Severity data was collected on a 0-9 scale and subjected to log transformation to ensure normalcy in the distribution curve followed by an analysis of variance

on Genstat release 14.1, VSN International Ltd at 5% level of significance. Mean response to net blotch was separated using DMRT.

Results

Response of winter and spring barley genotypes to aluminium cation toxicity. Among the spring barley genotypes, significant differences were observed in terms of percent response, relative net root growth (RNRG) and degree of hematoxylin (p < 0.001). For instance, HKBL 1663-3 genotype was the most tolerant but it did not differ significantly from HKBL 1808-3, Karne and Nguzo genotypes in terms of hematoxylin stain intensity. The HKBL 1674-4, Ngao and HKBL exhibited highest sensitivity to aluminium toxicity (Table 1 and Plate 1).

In terms of apical root length and formation of fibrous roots, the sensitive genotypes had the shortest apical root length which also expressed higher intensity of hematoxylin stain, an indication of much absorption of aluminium cations into the roots. The less stained roots were longer and formed fibrous roots in solution. These results corresponded well with those of the screenhouse (Plate 1)

The winter genotypes also differed significantly in terms of percent response, RNRG and intensity of hematoxylin stain (p < 0.001). However, based on averages and on genotype responses, the results indicate that winter genotypes are more sensitive to aluminium toxicity than spring genotypes. In terms of percent response, Beatrix and NFC Tipple genotypes

Table 1. Response of SPRING barley genotypes to aluminium toxicity at $148\,\mathrm{iM}$ Al solution under growth chamber conditions. The tolerant and sensitive grouping was done using hematoxylin staining intensity

GENOTYPE	% RESPONSE	DMRT	RNRG (RATIO)	DMRT	HEMATOXYLIN (1-5)	DMRT	AL CLASS
HKBL 1663-3	16.0	b	0.9	fg	1.7	а	TOTALANT
HKBL 1805-3	23.0	С	0.5	ab	1.7	а	ask.
KARNE	25.0	С	0.6	abc	1.7	а	Tr.
NGUZO	16.0	b	0.7	cdef	1.7	а	1
FANAKA	18.3	bc	0.8	efg	1.8	ab	
HKBL 1805-6	32.3	d	0.6	abc	1.8	ab	
HKBL 1862-5	39.0	d	0.6	abod	2.0	abc	
HKBL 1861-1	49.3	e	0.4	а	2.3	bod	
MALT 1	23.7	С	0.7	bode	2.3	bod	
HKBL 1719-4	5.0	а	1.0	g	2.5	cde	
HKBL 1629-14	34.3	d	0.7	bodef	2.8	def	
HKBL 1774-3	14.0	b	0.9	g	2.8	def	
SABINI	6.7	а	0.8	efg	2.8	def	A
HKBL 1629-5	15.3	b	0.7	def	3.0	ef	No.
NGAO	38.0	d	0.5	а	3.0	ef	A-9*
HKBL 1674-4	15.3	b	0.8	efg	3.3	f	SEESTITE
MEAN	23		0.7		2.3		
Probability	< 0.001		< 0.001		< 0.001		
S.E	2.188		0.0531		0.1764		
S.E.D	3.094		0.0751		0.2495		
% C.V	16.4		132		13.1		



Plate 1. Varying intensity of hematoxylin stain among the SPRING barley genotypes. Roots with low staining intensity were more tolerant than those showing high stain absorption.

recorded 56% and 50% respectively despite the low hematoxylin stain absorption by NFC Tipple. Among the winter genotypes, Grace and Publican were the most tolerant and sensitive to aluminium toxicity respectively (Table 2).

The growth of apical root length and formation of fibrous roots were significantly reduced in winter genotypes due to aluminium toxicity compared to the spring genotypes. This is presented in Plate 2 where Publican (most sensitive) had the shortest and most stained roots compared to Grace and Philadephia genotypes (Plate 2).

Mixed response to drought was recorded in relation to the previous response to aluminium cation toxicity but all the genotypes belonging to winter and spring groups differed significantly in their response to drought (p < 0.001). In spring genotypes, Sabini and HKBL 1774-3 genotypes showed tolerance to drought but initially, showed sensitive reaction to aluminium toxicity. In contrast, HKBL 1805-3 which showed tolerance to aluminium toxicity proved to be the most susceptible to drought. However, some genotypes that were tolerant to aluminium also expressed tolerance to drought including Fanaka, Malt 1, Nguzo, Karne and HKBL 1663-3 (Table 3).

For the winter genotypes, Scrabble and Annabel which showed initial sensitivity to aluminium toxicity proved to be tolerant to drought in terms of MSI. However, Grace and Aliciana were tolerant to both aluminium and drought but SY 409-228 which was tolerant to aluminium toxicity expressed the highest sensitivity to water stress (Table 3).

Table 2. Response of WINTER barley genotypes to aluminium toxicity at $148\,\mathrm{iM}\,\mathrm{Al}$ solution under growth chamber conditions. The tolerant and sensitive grouping was done using hematoxylin staining intensity

GENOTYPE	% RESPONSE	DMRT	RNRG (RATIO)	DMRT	HEMATOXYLIN (1-5)	DMRT	AL CLASS
GRACE	10.7	b	0.8	fgh	1.3	а	2
ALICIANA	28.0	f	0.5	Ь	1.7	ab	JON SHANS
PHILLADEPHIA	21.7	de	0.7	cde	1.7	ab	The state of
NFC TIPPLE	50.0	h	0.4	а	1.8	bo	1
SHUFFLE	16.3	cd	0.8	fgh	1.8	bc	
SY 409-228	19.7	œ	0.7	cde	1.8	bc	
COCKTAIL	20.3	d	0.8	efg	2.0	bc	
SY BATYK	1.3	а	0.9	h	2.2	cd	
SCRABBLE	36.0	g	0.5	ab	2.5	de	
BEATRIX	56.0	i	0.6	C	2.8	e	
QUENCH	14.3	bc	0.8	fgh	2.8	e	
MARTHE	27.7	f	0.7	od	3.3	f	A
TITOUAN	19.0	od	0.9	gh	3.3	f	A Section 1
A NNA BEL	28.0	ef	0.7	def	3.7	fg	A-9"
PUBLICAN	29.7	f	0.6	C	3.8	g	SERSITIVE
MEAN	25		0.7		2.4		
Probability	< 0.001		< 0.001		< 0.001		
S.E	1.7860		0.0298		0.1200		
S.E.D	2.5250		0.0421		0.1698		
% C.V	17.4		10.3		12		



Plate 2. Varying intensity of hematoxylin stain among the WINTER barley genotypes. Roots with low staining intensity were more tolerant than those showing high stain absorption

Table 3. Response of WINTER and SPRING barley genotypes to drought. The higher the membrane stability index (MSI), the more the tolerant a genotype is to drought

MSI FOR SPRING BARLEY GENOTYPES			MSI FOR WINTER BARLEY GENOTYPES				
GENOTYPE	MEAN	DMRT	GENOTYPE	ME AN	DMRT		
MALT 1	73.4	а	SCRABBLE	73.9	а		
FANAKA	71.9	a	GRACE	73.7	а		
SABINI	67.6	ab	ANNABEL	67.8	b		
NGUZO	64.4	bc	ALICIANA	66.6	b		
HKBL 1774-3	64.0	bc	XANADU	65.0	b		
KARNE	61.4	bc	NFCTIPPLE	57.6	c		
HKBL 1861-1	60.9	С	BEATRIX	56.7	C		
HKBL 1663-3	60.1	cd	SY BATYK	55.4	cd		
HKBL 1719-4	59.1	cde	COCKTAIL	54.9	cd		
NGAO	54.5	def	PHILLADEPHIA	54.0	cd		
HKBL 1862-5	54.3	def	TITOUAN	51.0	d		
HKBL 1629-5	53.4	efg	PUBLICAN	45.5	е		
HKBL 1674-4	51.0	fg	SHUFFLE	44.0	е		
HKBL 1805-6	47.4	gh	QUENCH	37.5	f		
HKBL 1629-14	43.2	h	MARTHE	36.9	f		
HKBL 1805-3	25.7	i	SY 409-228	33.0	f		
MEAN	57.0			55.0			
Probability	< 0.001			< 0.001			
S.E	2.030			1.542			
S.E.D	2.871			2.181			
% C.V	6.2			4.9			

In terms of the effect on growth and development, the MSI results correspond highly with the screenhouse results under different field capacities. For instance, Malt 1 and Sabini genotypes were the most tolerant at 20% FC with Malt 1 being the least affected. However, in winter genotypes, Titouan was much affected compared to Aliciana (Plate 3).

Response of winter and spring barley genotypes to net blotch disease. Barley genotypes responded differently to net blotch fungus in different sites. The interaction effect between site and genotype was also significant (p < 0.001). In the winter group, Ngao, Malt 1 and HKBL 1663-3 were the most tolerant in descending order while Sabini, Karne and Nguzo were the most susceptible to net blotch disease under field conditions. This confirms that Malt 1 and HKBL 1663-3 have multiple tolerances to aluminium toxicity, drought and net blotch disease (Table 4).

Among the winter genotypes, Grace and Aliciana proved to be tolerant to aluminium toxicity, drought and net blotch disease while Quench and Beatrix were the most susceptible to net blotch disease (Table 5).

The mixed reactions in response to aluminium toxicity, water stress and net blotch disease among the spring and winter barley genotypes could be an indication that these traits are controlled by different genes whose expression varies from one stress condition to another (Pietersea *et al.*, 2013). It is also possible that in response to biotic and abiotic stresses,

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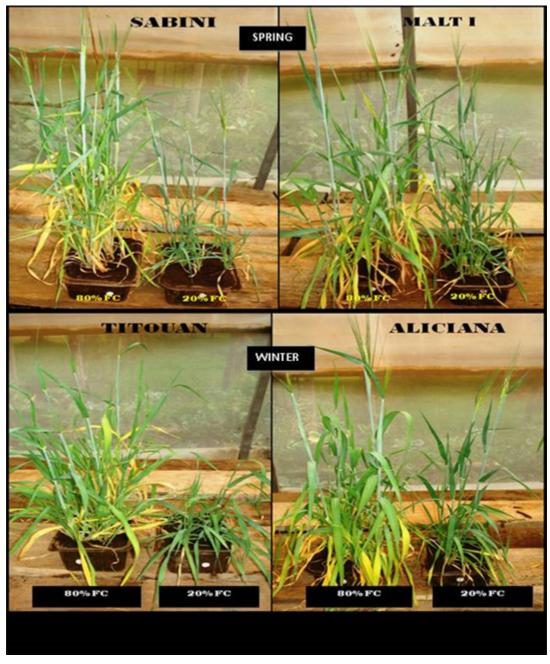


Plate 3. Effects of water stress (drought) on growth and development of WINTER and SPRING barley genotypes in the screenhouse. The 20% FC (stressed) while the 80% FC (not stressed)

Table 4. Response of SPRING barley genotypes to net blotch disease under field conditions in different sites. The scores were done on a 0-9 scale with 0-3 (tolerant), 3.5-4.0 (susceptible) while >5 very susceptible

GENOTYPE		SITE		MEAN	DACDE	onoun.
GENOTYPE	CHEP	MAU	NJORO	MEAN	DMRT	GROUP
NGAO	2.2	2.3	3.1	2.5	a	
MALT 1	2.2	2.8	2.8	2.6	a	
HKBL 1663-3	2.3	2.7	3.0	2.7	ab	₹0,
HKBL 1774-3	2.3	4.0	3.2	3.2	bc	POLERANT
FANAKA	2.8	3.3	3.7	3.3	c	AN.
HKBL 1629-5	3.3	3.0	3.7	3.3	c	₹
HKBL 1862-5	4.0	3.0	3.0	3.3	c	
HKBL 1805-3	4.0	3.7	3.3	3.7	cd	
HKBL 1719-4	3.0	5.0	4.3	4.1	de	
HKBL 1674-4	3.7	4.0	4.7	4.1	de	4.
HKBL 1805-6	3.7	4.3	4.7	4.2	de	BLE
HKBL 1629-14	3.3	4.8	4.7	4.3	e	& LECEPTIBLE
HKBL 1861-1	3.7	4.7	4.7	4.3	e	.€ ^{Ob}
N GUZ O	5.7	5.8	6.7	6.1	f	÷,,
KARNE	7.7	6.7	8.3	7.6	g	
SABINI	8.3	8.0	7.7	8.0	g	
MEAN	3.9	4.3	4.5	4.2		

	Genotype	Site	Genotype x Site	
Probability	< 0.001	< 0.001	< 0.001	
S.E	0.1875	0.0812	0.3247	
S.E.D	0.2651	0.1148	0.4592	
% C.V	13.4			

Table 5. Response of WINTER barley genotypes to net blotch disease under field conditions in different sites. The scores were done on a 0-9 scale with 0-3 (tolerant), 3.5-4.0 (susceptible) while > 5 very susceptible

CENOTURE		SITE		MEAN	DAME	GROUP
GENOTYPE	CHEP	MAU	NJORO		DMRT	
HUFFLE	2.3	3.0	2.7	2.7	a	
IITOUAN	2.7	3.3	3.0	3.0	ab	20
GRACE	2.7	3.3	3.2	3.1	ab	E.E.
LICIANA	3.3	3.3	3.3	3.3	bc	TOTERANT.
UBLICAN	3.0	3.7	3.5	3.4	bc	
Y 409-228	3.7	4.0	3.7	3.8	cd	
FC TIPPLE	4.0	4.3	3.3	3.9	cde	
CRABBLE	3.0	5.0	4.3	4.1	def	
LARTHE	4.0	4.7	4.0	4.2	def	
Y BATYK	3.0	5.2	4.7	4.3	defg	TOLK
ANADU	4.7	4.0	4.3	4.3	defgh	ALL.
HILLADEPHIA	3.7	4.3	5.3	4.4	efgh	'ECE
OCKTAIL	4.3	5.7	3.7	4.6	fgh	SUSCEPTIBLE
NNABEL	4.2	5.7	4.7	4.8		
EATRIX	4.3	5.3	5.0	4.9	gh h	
UENCH	5.7	6.3	6.7	6.2	i	
ŒAN	3.7	4.4	4.1	4.1		

201 (0.000)	Genotype	Site	Genotype x Site	
Probability	< 0.001	< 0.001	0.002	
SE	0.1901	0.0823	0.3293	
SE.D	0.2688	0.1164	0.4656	
% C.V	14			

barley like other plants produce signal phytohormones in response whose effects can be synergistic or antagonistic under different stress conditions (Balcke *et al.*, 2012; Keskin *et al.*, 2010) and once produced, the hormones play very distinct roles in regulating the response to stress factors other than the genetic make-up of the plant (Sorooshzadeh *et al.*, 2011).

Conclusion and way forward

Winter and spring barley genotypes respond differently to biotic and abiotic stress factors and these response mechanisms seems to be regulated by other external factors which act alongside the genetic make-up. On the basis of previous findings, this work continues to establish the influence of drought and aluminium toxicity on net blotch severity, evaluate the role of phytohormones in regulating the three stresses and finally establish the gene expression in response to drought, aluminium toxicity and net blotch disease.

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