

Research Application Summary

Severity expression of net blotch foliar infection as induced by initial exposure to aluminium toxicity and drought stress in barley

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Abstract

The interactions between aluminium toxicity, drought and net blotch may be the reasons for decreasing barley yields in Kenya despite availability of improved varieties. Such interactions induce diverse hormonal signaling and biochemical responses whose final impact on disease severity remains less understood and undocumented in barley. This research was set to identify the interaction effect of water deficiency and aluminium cation toxicity on net blotch severity. Trait-specific winter and spring barley were inoculated with 5×10^3 spore concentration of *Pyrenophora teres* at Zadoks growth stage 15 in a split – plot arrangement in completely randomized design with 148 μM Al, 20% Field capacity (FC) and 80% FC conditions as main plots and genotypes as sub plots. Assessment was based on 0 – 7 severity scale. Severity data was analyzed on Genstat statistical package version 16.0. Initial exposure to drought and Al toxicity significantly reduced net blotch severity compared to unstressed set of the same genotypes. In conclusion, tolerance to stress in barley involves a complex system of inherent traits, interaction of stress factors and activation of signal pathways induced by phytohormones.

Keywords: Aluminium toxicity, drought stress, interactions, net blotch, trait-specific barley

Résumé

Les interactions entre la toxicité de l'aluminium, la sécheresse et la tache nette peuvent être les raisons de la baisse des rendements d'orge au Kenya malgré la disponibilité de variétés améliorées. De telles interactions induisent diverses réponses hormonales de signalisation et biochimiques dont l'impact final sur la gravité de la maladie reste moins compris et non documenté dans l'orge. Cette recherche visait à identifier l'effet d'interaction de la carence en eau et de la toxicité des cations d'aluminium sur la gravité nette de la tâche. Des orges d'hiver et de printemps spécifiques au caractère ont été inoculées avec une concentration de 5×10^3 spores de *Pyrenophora teres* au stade de croissance 15 de Zadoks dans un arrangement de parcelles divisées dans une conception entièrement randomisée avec 148 μM Al, 20% et 80% de capacité de champs comme conditions principales et génotypes comme sous-parcelles. L'évaluation était basée sur une échelle de gravité de 0 à 7. Les données de gravité ont été analysées sur le logiciel statistique Genstat version 16.0.

L'exposition initiale à la sécheresse et à la toxicité en Al a considérablement réduit la gravité de la tache nette par rapport à un ensemble non stressé des mêmes génotypes. En conclusion, la tolérance au stress dans l'orge implique un système complexe de traits inhérents, l'interaction des facteurs de stress et l'activation des voies de signal induites par les phytohormones.

Mots-clés: toxicité de l'aluminium, stress dû à la sécheresse, interactions, tache nette, orge spécifique à un trait

Introduction

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 and now, screening for resistance to net blotch, drought and aluminium toxicity (EABL-UoE, 2016) but low barley yields is still a major challenge in Kenya. It is possible that the complexity in hormonal signaling and crosstalk (Raifa *et al.*, 2009; Pietersea *et al.*, 2013) due to response to interacting effects of pathogenic strains of net blotch, drought and aluminium toxicity play a major role such that even with new high yielding varieties, the actual yields are still suppressed.

Currently, there is increased incidence and severity of *P. teres* in Kenya (EABL-UoE, 2016) coupled with increasing scenario of aluminium cation toxicity (Wang *et al.*, 2006) and drought (Demirevska *et al.*, 2008) which occupies about 26% and 40% of worlds' agricultural land respectively. The results of such complex interactions call for proper understanding of not only the genetic roles but also the biochemical responses that are triggered by phytohormones. If not properly studied, the gap between potential and actual yield of barley may widen further. This is because when exposed to stresses like salt and water deficiency (Gupta and Huang, 2014), most plants usually exhibit morphological, physiological and biochemical and genetic responses whose effects entirely dependent on the severity of the stress factor (Keskin *et al.*, 2010; Subramanian *et al.*, 2013). Therefore, this study aimed at assessing the response of winter and spring adapted barley genotypes to net blotch disease under the influence of drought and aluminium cation toxicity.

Material and Methods

The research was carried out under controlled condition at the University of Eldoret, School of Agriculture and Biotechnology. Twelve (12) winter and twelve (12) spring barley previously screened, selected and coded for their response to drought, aluminium toxicity and net blotch disease (2 tolerant/resistant and 2 sensitive/susceptible for each stress factor) were used. The codes used were per the stress factor as follows: Net blotch – NBT1 and NBT2 (tolerant) and NBS1 and NBS2 (susceptible); Drought – DRT1 and DRT2 (tolerant) and DRS1 and DRS2 (sensitive); and Aluminium toxicity – ALT1 and ALT2 (tolerant) and ALS1 and ALS2 (sensitive). Numbers 1 and 2 represent the most tolerant/sensitive and the second most tolerant/sensitive for each stress factor. The winter and spring barley were planted separately using split

– plot arrangement in completely randomized design with each genotype replicated thrice. The drought (20% FC), aluminium toxicity (148 μ M) and control experiment (No stress) were considered as main plots while 12 trait-specific winter and 12 trait-specific spring barley were sub – plots. Planting was done using forest soil previously solarized for three months and mixed with phosphate fertilizer at planting.

Single spore isolate of *P. teres* was obtained from infected leaf samples (Owino *et al.*, 2013) and used to prepare conidial suspensions whose concentration was adjusted to 5×10^3 conidia/ ml (Xue and Burnett, 1995) using Buker - Turk haemocytometer (Mathur *et al.*, 1989). Barley seedlings were inoculated at Zadoks growth stage 15, usually 16 – 18 days after seeding (Xue and Burnett, 1995) inside inoculation chamber with approximate temperature of 27 °C and 70% relative humidity. These conditions were maintained by misting after every three hours using sterile distilled water and left under normal light regimes (Than *et al.*, 2008) for seven days to ensure successful infections.

Disease severity for each genotype was assessed from 7th to 35th day after inoculation at seven days interval on a 0 to 7 leaf symptom severity rating scale (Xue and Burnett, 1995) where 0 = 0 % and 7 = 76 -100% foliar severity expression. The 0 rating was considered the most resistant while 7 as the most susceptible to net blotch disease. The severity data was subjected to cluster analysis on Genstat statistical package version 16.0, VSN International Ltd at 5% level of significance. Euclidean test was performed using genotype means to generate dendrogram for winter and spring barley separately. Cluster comparisons were performed at 0.98 similarity coefficient matrix.

Results and Discussion

Both spring and winter barley with special traits responded differently to net blotch foliar infection under the influence of aluminium cation toxicity and drought. These results confirms that other than inherent traits such as tolerance and/or susceptibility to drought, aluminium toxicity and net blotch disease, initial exposure of barley to drought or aluminium toxicity had significant effect on disease severity (Plate 1).

Trait-specific spring barley clustered differently with varying levels of net blotch foliar severity under the influence of aluminium toxicity, water deficiency and unstressed conditions. In particular, four clusters were formed after initial exposure to aluminium toxicity where DRT1, DRT2, NBS1, NBT2 and ALS2 expressed resistant (immune) response to net blotch disease. Similarly, ALT1, ALT2, ALS1, NBT1, NBS2 and DRS1 exhibited tolerant response under the influence of aluminium toxicity. However, only DRS2 showed higher susceptibility to net blotch disease under aluminium toxicity. Exposing similar genotypes to drought stress gave a totally different severity levels which recorded higher disease levels than when subjected to aluminium toxicity. For instance, after initial exposure to drought stress, only DRS2 genotype which was the most susceptible to net blotch foliar infection expressed resistant response to the disease. In contrast, DRT1 which was the most resistant to disease under aluminium toxicity recorded the highest level of severity under drought stress. The rest of the genotypes showed tolerance under drought

stress. Inoculation of initially unstressed spring barley recorded higher net blotch severity irrespective of the inherent traits compared to those exposed to aluminium toxicity and drought stress before inoculation. For example, only four genotypes namely ALS1, ALS2, ALT1 and NBT1 were tolerant to the disease under unstressed conditions. The rest of the genotypes were susceptible to foliar infection (Figure 1).

The winter genotypes expressed completely different but higher disease severity and clusters under stress and unstressed conditions compared to the spring barley. In addition, none of the winter barley expressed immune (resistant) response to foliar infection under the aluminium toxicity, drought and unstressed conditions. Instead, the severity ratings were higher compared to the spring barley. However, the disease severities were lower after initial exposure to aluminium toxicity and drought stress compared to the unstressed conditions. This observation is similar to that of spring barley (Figure 2).

Scientifically, low net blotch severity in spring and winter barley after initial exposure to drought and aluminium stress compared to unstressed barley could imply that exposure of barley to aluminium and drought stress induced the production of certain compounds that are antifungal in nature. These include proline whose accumulation has been proved to be highly correlated with stress tolerance and its concentration known to be higher in stress-tolerant but low in stress-sensitive plants (Szabados and Savoure, 2009). When produced within stressed plant, proline initiates timely perception and rapid response to stress in efficient manner and after recognition of stress, basal defense mechanisms are constituted (Andreasson and Ellis, 2010). These activates a complex signaling of defense varying from one stress factor to the other (Abou *et al.*, 2009).

Other than proline, initial exposure of plants to abiotic stress activates specific ion and enzyme channels (Fraire-Velázquez *et al.*, 2011) and when such occurs, the reactive oxygen species (ROS) (Laloi *et al.*, 2004) and several phytohormones accumulate within the stressed plant and immediately reprograms the genetic machinery to ensure adequate defense is achieved with minimum biological damage to the plant (Spoel and Dong, 2008; Fujita *et al.*, 2006). Such findings further explains why there were very high net blotch severities under unstressed conditions in both winter and spring barley compared to those initially exposed to drought and aluminium toxicity stress before inoculation. In addition, under aluminium and drought stress, some of the major signal transduction pathways such as mitogen-activated protein kinase (MAPK) (Ligterink and Hirt, 2001; Osawa and Matsumoto, 2001) are activated and these transports information not only within individual cells but also throughout the entire plants. Once activated, these pathways trigger the expression of genes and resultant modifications of cellular and molecular processes (Mossor-Pietraszewska, 2001) which lead to a strong defense mechanism against biotic and other abiotic stresses. These principles strongly correlate and support the observation made in barley genotypes with specific traits when infected by net blotch fungus under the influence of drought and aluminium toxicity.

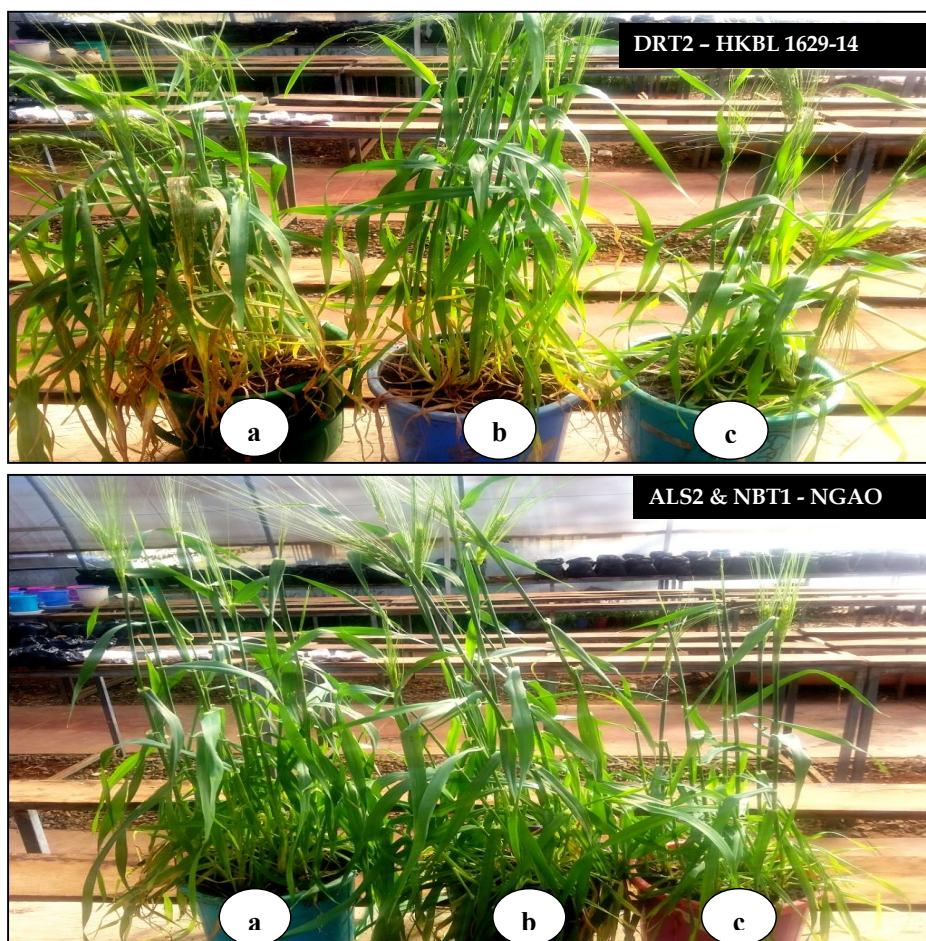


Plate 1: Net blotch disease severity as expressed by HKBL 1629-14 (Drought tolerant) and NGAO (Net blotch tolerant but sensitive to Al toxicity) genotypes under unstressed 'a', aluminium toxicity 'b' and drought stress 'c' conditions (Source: Author, 2017)

Conclusions

Initial exposure to drought and aluminium toxicity significantly triggered the induction of net blotch tolerance in winter and spring barley irrespective of the inherent traits. When initially exposed to aluminium toxicity and drought stresses, all genotypes expressed lower disease severity compared to those under unstressed conditions.

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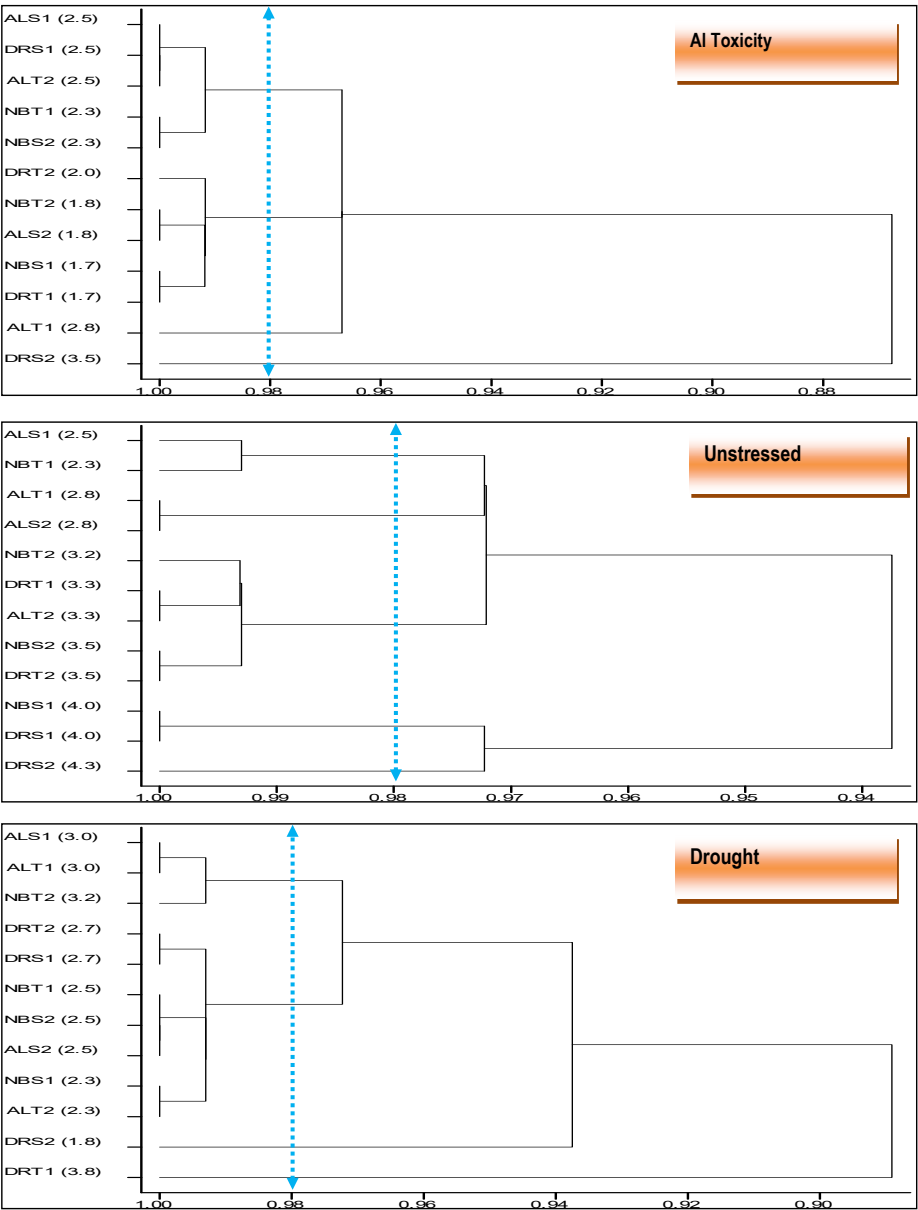


Figure 1: Grouping of trait-specific SPRING barley genotypes in terms of net blotch severity levels under the influence of aluminium toxicity, drought stress and unstressed conditions. Grouping was achieved through Euclidean test and nearest neighbour cluster analysis

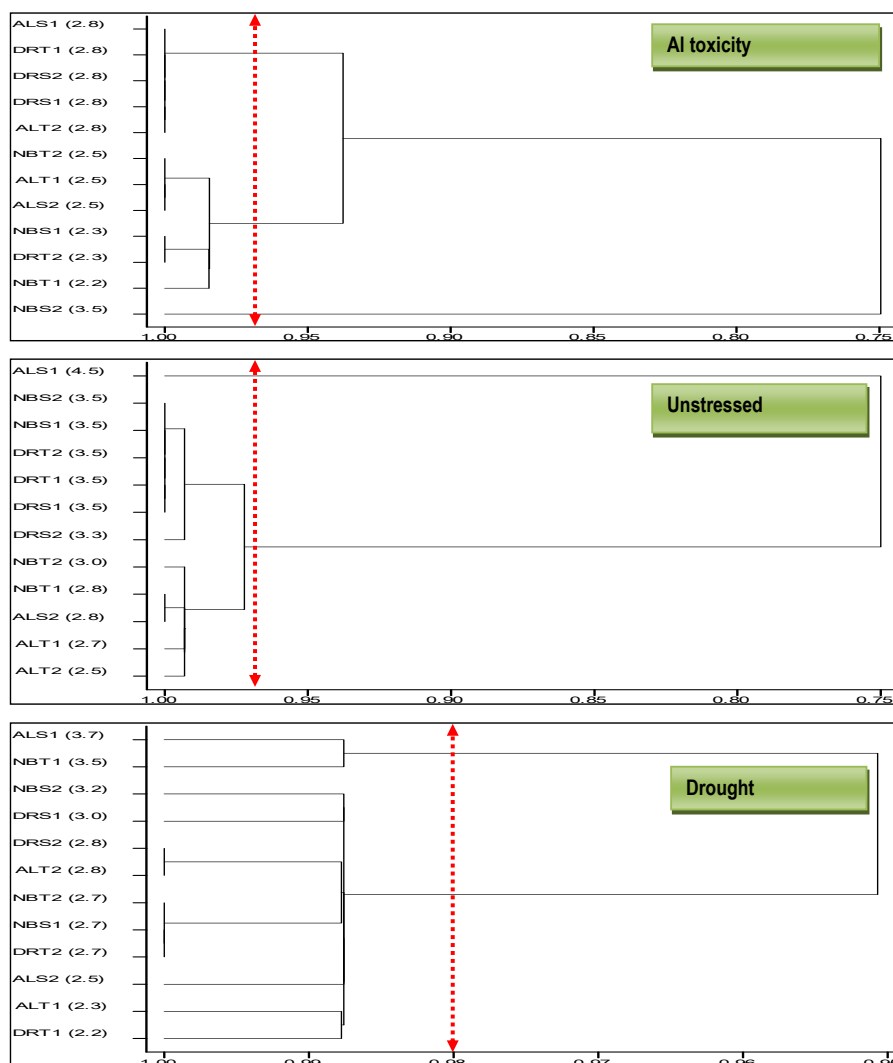


Figure 1: Grouping of trait-specific WINTER barley genotypes in terms of net blotch severity levels under the influence of aluminium toxicity, drought stress and unstressed conditions. Grouping was achieved through Euclidean test and nearest neighbour cluster analysis

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