SEVERITY OF NET BLOTCH (*Pyrenophora teres*) IN BARLEY: PHYTOHORMONE SIGNALING UNDER ALUMINIUM TOXICITY AND WATER DEFICIENCY

BY

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DECLARATION

Declaration by student

This thesis report is my original work and has not been presented for award of any certificate or degree in any other institution or University. No part of this work should be reproduced without prior permission from the authors and/or University of Eldoret.

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DEDICATION

To all the scientists, funders and relatives for their financial, professionalism and moral support together with encouragements which kept me more dedicated and focused throughout this work.

ABSTRACT

Aluminium toxicity, drought and net blotch coupled with their interactions, biochemical reactions and induction of hormonal signaling are responsible for low barley yields in Kenya. The studies aimed at identifying genotypes exhibiting multiple tolerances to the three stresses, assess interaction effect of drought and Al toxicity on net blotch severity; and assess hormonal signaling effect in the management of these three stress factors. Baseline screening for Al toxicity, drought and net blotch response was performed to group genotypes. Al tolerance was assessed on Magnavaca solution with 0 and 148 µM Al under 14 hour light intensity (340 µmoles $m^{-2}s^{-1}$) at 30 ^{o}C and 70% relative humidity alternated with dark conditions at 22 ^{o}C and 90% relative humidity inside a growth chamber. Data on net root length, relative net root growth, percent response and hematoxylin staining were recorded. For drought, electrical conductivities of leaf leachates were measured at 40 ^{o}C for 30 as C_1 and at 100 ^{o}C for 15 minutes as C_2 then used to determine the membrane stability index. Same genotypes were maintained at 20% (stressed) and 80% field capacities and height, tillering ability, number of grains per spike and 1000 seed weight were recorded. Net blotch screening was done under hot-spot field conditions in RCBD and scored on a 0 – 9 scale. Secondly, trait specific genotypes were inoculated with 5 x 10^3 spore concentration of *P. teres* at Zadoks growth stage 15 in a split – plot in CRD with 148 µM Al, 20% FC and 80% FC conditions as main plots and genotypes as sub plots. 0 – 7 severity scales was used. Thirdly, hormonal treatments with 50 µM SA, 20 µM ABA, SAxABA and a control were applied on barley affected by drought, Al toxicity and net blotch infection. Data were analyzed on Genstat statistical package version 14.1 and means separated using contrast, comparison. Genotypes differed significantly ($p < 0.05$) and only 4 out of 32 showed multiple tolerances to three stresses. The rest exhibited double and single tolerance. Also, initial exposure to drought and Al toxicity significantly reduced net blotch severity compared to unstressed set of the same genotypes. SA induced tolerance to disease while ABA signaled tolerance to drought and Al toxicity. Synergistic effect of SAxABA was minimal on net blotch but more significant for abiotic stresses. 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.

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CHAPTER ONE

INTRODUCTION

1.1 Background information

Barley (*Hordeum vulgare* L.), a member of the grass family, is a major cereal grain. Barley was ranked among cereal crops in the world and was fourth 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) 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 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 (Pietersea, *et al*., 2013; Raifa, Amira, Ahmed,

Mohamed, & Hanan, 2009) 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 (J. Wang, Raman, Zhang, Mendham, & Zhou, 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.

Abscisic acid (ABA), jasmonic acid (JA) and salicylic acid (SA) are the major phytohormones with key role in activating plants' defense mechanisms in response to diseases, drought and cation toxicity among other stresses (Cheong & Choi, 2003; Kaya, Kirnak, Higgs, & Saltali, 2002; Keskin, Sarikaya, Yuksel, & Memon, 2010). Under natural conditions, plants including barley are exposed to one or more of these stress factors at any given time. This implies that ABA, JA and SA are produced within the same plant especially when specific genes controlling their production in response to specific stress condition are present within a plant species or variety.

When produced within the same plant in response to stress, several studies with cereals and non-cereal crops indicates that JA and SA play antagonistic roles in some plant species such as tomato where SA induce acidic pathogen-related (PR) genes and inhibits basic PR genes. JA acts in a directly opposite manner (Pietersea, *et al*., 2013; Y. Wang,

Mopper, & Hasentein, 2001). In Kenya, no barley variety has been studied for its biochemical response to net blotch disease under the influence of exogenous phytohormones, drought and aluminium toxicity stresses. Furthermore, the roles of ABA, JA and SA in response to net blotch fungi, drought and aluminium toxicity stresses and their combinations are still unclear.

Therefore, this study aimed at: **1**) Assessing winter and spring barley genotypes for the response to *Pyrenophora teres*, drought and aluminium cation toxicity, **2**) Assessing the response of winter and spring barley genotypes to net blotch disease under the influence of drought and aluminium cation toxicity, **3**) Determining the synergistic and antagonistic effect of synthetic phytohormones (Salicylic and abscisic acids) on severity of net blotch, drought, aluminium cation toxicity in selected barley genotypes under screenhouse conditions. Such knowledge is useful in most crop variety improvement program that aims at not only conferring tolerance to drought, net blotch and aluminium toxicity in barley and other cereals but also take into consideration the biochemical responses, interactions among these stress factors and implications on actual yields.

1.2 Statement of the problem

The interactive effect of net blotch, drought and aluminium cation toxicity in barley is one of the major factors responsible for low yields and can cause up to 100% yield loss (Bekele, Shambel, & Abashamo, 2001; Demirevska, *et al*., 2008; Newton & Goodman, 2005). In Kenya, the actual yields still remain low in barley growing zones (EABL-UoE, 2010) which exhibit high frequencies of net blotch epidemics; unpredictable and intense droughts and soils that are increasingly acidic thus showing higher aluminium cation

concentrations which are highly toxic to plants. However, the complex interaction effect among net blotch fungi, drought and aluminium toxicity in major barley growing zones of Kenya (EABL-UoE, 2013) still remains unknown despite the fact that most soils in Kenya are acidic (Kisinyo, *et al*., 2013) and drought is unpredictable.

Other than genetic roles in tolerance, in response to these stresses, barley produce varying levels of ABA, JA and SA phytohormones (Pietersea, 2012; Sorooshzadeh, Javid, Moradi, Sanavy, & Allahdadi, 2011) whose effects can either be antagonistic or synergistic (Moons, Prinsen, Bauw, & Montaguasi, 1997; Y. Wang, *et al*., 2001) to the inherent response mechanisms (Aprile, *et al*., 2008; Keskin, *et al*., 2010). Once produced, these phytohormones influence the level of tolerance and/or susceptibility to biotic and abiotic stresses with each playing significant but distinct role in defense mechanism (Sorooshzadeh, *et al*., 2011). This scenario has not been properly studied and scientifically documented in barley despite the fact that the pathways leading to synthesis and catabolism of these hormones entirely depend on the genetic make-up of each variety, crop nutrition and environmental conditions (Owino, Ochuodho, Were, & Rop, 2014).

Under normal circumstances, the production of barley and most cereals take place in open fields where exposure to net blotch fungal infections, soil acidity thus aluminium cation toxicity and unpredictable water and nutrient deficiency is unavoidable. This implies that the quantities of ABA, SA and JA produced in response to these stresses also vary. The existence of winter and spring barley which require different growth conditions further complicates the entire scenario and may imply that in either of the two groups, a variety may produce different quantities of ABA, JA and SA in response to net blotch, water

stress and aluminium cation toxicity (J. Wang, *et al*., 2006) hence affecting the level of tolerance and susceptibility to these stresses.

1.3 Justification of the study

Proper understanding of the combined effects of biotic and abiotic stresses affecting the production of barley in Kenya require not only evaluation for the drought, aluminium toxicity and net blotch severity but also understanding the individual and combined roles of phytohormones and their physiological functions on the plant (Hashempour, Ghasemnezhad, Fotouhi Ghazvini, & Sohani, 2014). This is because when exposed to stress such as salt (Gupta & Huang, 2014) and water stress, 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, Parmar, Dave, Sudhir, & Panchal, 2013).

Other than understanding the phenotypic and biochemical responses due to stresses, plant breeders and protectionist must be well informed of the genetic implication of the observed responses especially when several factors are interacting that may cause yield loss. Expression of certain genes in plants take place when plants are stressed (Urwin, Atkinson, & Lilley, 2013) but this vary from one plant to another depending on the stress factor involved (Agrios, 2005; Aprile, *et al*., 2008; Balcke, *et al*., 2012; Lin, Cheng, Liao, & Kuo, 2013). On this basis, there are higher chances that tolerant varieties produce a combination of phytohormones whose effects are synergistic against the stress factor in question.

Therefore, for a variety improvement program aiming at tolerance to biotic and abiotic stress factors, studies involving screening for tolerance alongside the assessment of biochemical and genetic responses is vital. By using the commonly grown barley varieties in Kenya (spring and winter), this study focused on establishing the response pattern and phenotypic grouping against drought, net blotch disease and aluminium toxicity. Severity of net blotch fungi using trait-specific genotypes was considered important especially under the influence of water stress and aluminium cation toxicity to confirm whether there is any relationship between the three stresses. The roles of ABA and SA phytohormones and their combinations on the response to the three stresses were also studied to provide better understanding to the breeders and protectionists especially when net blotch infection, drought and aluminium toxicity are combined.

Answers to these knowledge gaps are important and key for accurate conferment of tolerance to net blotch disease in barley under the influence of drought and aluminium toxicity. Additionally, when properly understood, the information could help in singling out the most important phytohormones conferring tolerance to the three stresses affecting barley yields under field conditions (Mariey, *et al.*, 2013). The combination of phenotypic, biochemical and molecular studies are key in fine-tuning their research objectives aiming at multiple stress tolerance by selecting for specific genes coding for synthesis of specific phytohormones and other biochemical of main interest (Newton & Goodman, 2005).

1.4 Objectives

1.4.1 Broad objective

To improve net blotch disease management and productivity of barley through severity and interaction analysis under the influence of hormonal signaling and crosstalk, aluminium toxicity and water deficiency stresses.

1.4.2 Specific objectives

1. To evaluate the response patterns and phenotypic grouping of winter and spring barley genotypes to *Pyrenophora teres*, drought and aluminium toxicity under controlled conditions.

2. To investigate the response of trait-specific barley genotypes to net blotch severity under the influence of drought and aluminium toxicity in the screenhouse conditions.

3. To determine the effect of ABA and SA phytohormones and combinations on net blotch severity, drought, aluminium toxicity using trait-specific winter and spring barley genotypes under screenhouse.

1.4.3 Research questions

Objective 1:

- ✓ What are the effects of net blotch, drought and aluminium toxicity on growth parameters of winter and spring barley genotypes?

- ✓ Is there significant difference in the response to net blotch fungal disease, drought and aluminium cation toxicity between the spring and winter barley genotypes?

- ✓ Are the genotypes exhibiting tolerance to drought and aluminium toxicity also express tolerance to net blotch disease?

Objective 2:

- ✓ How do the winter and spring barley traits (tolerance and susceptibility to drought and aluminium toxicity) affect the severity levels to net blotch?
- ✓ How do the net blotch, drought and aluminium toxicity tolerant and susceptible barley genotypes respond to net blotch disease under the influence of induced water stress and aluminium cation toxicity?

Objective 3:

- ✓ What are the signal effects of exogenous application of ABA and SA phytohormones on drought, net blotch and aluminium toxicity tolerant and susceptible barley genotypes?
- ✓ Do the ABA, SA and ABA x SA interaction confer resistant/tolerance response to barley genotypes which previously showed susceptibility net blotch, drought and aluminium cation toxicity?

CHAPTER TWO

LITERATURE REVIEW

Under normal circumstances, most cereal crops including barley are grown in the field exhibiting different characteristics particularly in terms of temperature, water stress, soil mineral toxicities and disease severity. In response to such stresses, plants normally produce and magnify the expression of not only diverse but also stress - specific compounds including genes, biochemicals, phytohormones, pathogenesis proteins, enzymes, metabolites among others (Bach, Rodrigues, Antoniazzi, & Wadt, 2014; Kim, Lee, Lim, Baek, & Jung, 2015). Studies have indicated that once some of these compounds especially the phytohormones (jasmonic acid (JA), salicylic acid (SA) and Abscisic acid (ABA)) are produced within the same plant, their effects can be synergistic or antagonistic against the target stress factor, thus affecting the overall known resistance or susceptibility to the biotic and/or abiotic stress factors (Mauch-Mani, Rejeb, & Pastor, 2014; Wees, Vos, Moritz, & Pieterse, 2015).

For the hormones and other compounds to be produced as a response mechanism to the stress factor, specific genes must be expressed within the plant genome to signal their production in large quantities (Jiang, *et al*., 2013; Yoshida, *et al*., 2015). Such genes differ and perform specific roles from one plant species to another and this could imply that when cereal plants like barley is exposed to biotic and abiotic stress factors under field conditions, very specific defense phytohormones are produced under the influence of specific genes. This phenomenon is further complex especially with the existence of winter and spring adapted barley genotypes with diverse genetic makeup.

In Kenya, barley varieties are released annually as superior in terms of yield potential and disease tolerance. However, within a short period of time (usually 2-3 years), such

varieties become very susceptible to diseases like net blotch in barley growing zones and severe drought stress which is usually unpredictable. The existence of severe drought and net blotch disease coupled with increasing aluminium cation toxicity due to the increasing soil acidity in Kenya further reduce the yield recorded by farmers as huge losses (EABL-UoE, 2013; Kisinyo, *et al*., 2013; Owino, *et al*., 2014)

The complexity of the physiological processes and mechanisms involved in response to these stresses in barley calls for a proper study of these factors separately under controlled conditions. This would help to provide not only vital but also accurate information necessary in fine-tuning biotic and abiotic stress management strategies in barley, other cereals and non-cereal crops. This information is very important not only to pathologist but also plant breeders aiming at conferring multiple stress tolerance to cereal crops including winter and spring barley genotypes.

2.1 Response of barley to net blotch infection

When infected by one or more pathogenic organisms, barley just like other plants usually exhibit numerous changes in physiological processes due to the damage by the pathogens. The most common responses in plants include production of biochemicals, genetic, hormonal, pathogenesis related proteins, enzymatic and production of metabolites (Agrios, 2005). However, very little has been studied and documented in barley with focus on physiological and biochemical effects of drought, aluminium cation toxicity and net blotch disease on growth and development.

2.1.1 Pathogenesis proteins and enzyme production due to pathogen infection

Under incompatible host-pathogen interactions, damage caused by the pathogen remains restricted due to plant's defensive response and the most effective is the hypersensitive reaction where most cells around the infection site rapidly necroses (Agrios, 2005; Loon & Strien, 1999). When a plant is infected by pathogens, various novel proteins known as pathogenesis-related proteins (PRs) are induced and these PRs are coded for by the host plant but induced specifically in pathological or related situations. Once produced, the PRs do not only accumulate locally at the infected site, but are also induced systemically and are associated with the development of systemic acquired resistance (SAR) against further infection by fungi, bacteria and viruses. Induction of PRs has been found in many plant species belonging to various families, suggestive of a general role for these proteins in adaptation to biotic stress conditions (Bohlmann, *et al*., 1998) but this has not been practically tested in barley under infection by net blotch and other pathogenic organisms.

Some of the PRs identified include chitinases and β-1, 3-glucanases produced by tobacco, tomato and barley among other crops. These two exhibit potential antifungal activity and it has often been suggested that the collective set of PRs may be effective in inhibiting pathogen growth, multiplication and/or spread, and be responsible for the state of SAR in most plants through cell wall modification and/or tissue weakening (Ivarson & Leijman, 2009). Barley also produces some quantities of chitinases and β-1, 3-glucanases but at germination stage of growth (Bradford, Wu, Leubner-Metzger, & Meins.Jr, 2001) and this could be responsible for some of the seedling resistance to net blotch as observed under field conditions (Owino, *et al*., 2014).

In tomatoes, research indicates that accumulation of β-1, 3-glucanase mRNA, protein and enzyme activity can be reduced by application of 100 μM abscisic acid (ABA), which delays or prevents radical emergence but not endosperm cap weakening. In contrast, expression of chitinase mRNA, protein and enzyme activity is not affected by abscisic acid (Bradford, *et al*., 2001). With the existence of such knowledge, a number of plant breeders give less focus to such physiological processes which can have not only substantial but also unexplained causes on yield losses in barley and other crops. This finding further means that when exposed to stress, production of β-1, 3-glucanase and ABA can result into antagonistic effects on disease resistance or tolerance.

2.1.2 Genetic response to pathogen infection in barley

Studies have indicated that in plants, wounding alone cannot trigger the induction of pathogenesis related genes but it requires the presence of a growing fungus for such genes to be expressed (Boyd, Smith, Green, & Brown, 1994; Schulze-Lefert, Maekawa, Kracher, Vernaldi, & Themaat, 2012). This means that in every disease infection in barley or any other plants, certain defense-related genes must be produced. For cereal pathologists and breeders, this findings provides well defined genetics of gene-for-gene resistance in a host – pathogen system in which a number of important variables in the defense response can be examined in relation to pathogen development (Agrios, 2005).

Through genetic engineering, a number of barley genotypes have been made to exhibit resistance to a number of diseases including stem rust fungus by *Rpg1* gene (Rúa, McCulley, & Mitchell, 2013; Wettstein, *et al*., 2003) but constant co-existence has always resulted in breakdown of disease resistance and development of new pathogenic strains

through host-pathogen differentiation mechanisms (Agrios, 2005). This makes such approaches unreliable and short-lived hence the need for proper understanding of not only the phenotypic response but also the biochemical implications and changes within the plants as well as their synergistic and antagonistic relationships.

Plant disease resistance genes (R genes) encode proteins that detect pathogens and such genes have been used in resistance breeding programs for decades, with varying degrees of success. Also, numerous signal transduction components in the defense network have been defined, and several are being exploited as switches by which resistance can be activated against diverse fungal and viral pathogens both in barley and other plants (Andronic, Port, & Duca, 2015; McDowell & Woffenden, 2003).

However, not all the R gene transfers are beneficial to the plant and some have failed and instead resulted into a higher susceptibility to a number of biotic and abiotic stresses. Once these R genes are introduced into a plant, their expression tend to perform more than one role including signaling the production of compounds such as enzymes, proteins, hormones and other metabolites in a coordinated defense network (Aprile, *et al*., 2008; Wees, *et al.*, 2015; Y. Yang, Shah, & Klessig, 1997) and regulating the levels of such compounds. Lack of proper documentation on how these compounds particularly the phytohormones interact and affect the host plant physiology could be some of the reasons behind the unclear cases of susceptibilities to stresses instead of resistance by the transferred genes.

Further studies indicate that during infection of barley by *Rhynchosporium secalis*, the expression of genes encoding pathogenesis-related (PR) proteins PR-1, PR-5, and PR-9 are specifically expressed in the mesophyll of resistant barley genotypes. However, gene

expression in the mesophyll is likely to be triggered by an unknown signal that appears to originate in the epidermis and that is strongly amplified in the mesophyll (Knogge, *et al*., 2003). Such unclear circumstances still calls for the urgent need to understand the biochemical reactions within plants when infected by pathogens and this has not been properly studied in barley especially with net blotch as the most common disease in tropical and sub-tropical regions where the disease is most severe and damaging.

Other than expression of pathogenesis-related proteins during infection of barley, wild and mutant barley genotypes accumulates distinct sets of transcripts in response to pathogens of different trophic lifestyles such as *Puccinia hordei* and *Cochliobolus sativus*, causal agents of leaf rust and spot blotch respectively (Keisa, Rostoks, Kanberga-Silina, Nakurte, & Kunga, 2011; Muehlbauer, Millett, Xiong, Dahl, & Steffenson, 2009). In addition, the quantities of differentially accumulated gene transcripts in response to *P. hordei* are evenly distributed immediately after infection while for *C. sativus*; the differentially accumulating gene transcripts in response to infection have been identified at 24 hours after infection, the approximate time when the pathogen changes trophic lifestyles. Further findings indicate that resistant wild barley exhibits a different host response to biotrophic and hemibiotrophic pathogens, with genes related to oxidative stress having a particularly important role in defense against hemibiotrophs (Muehlbauer, *et al*., 2009).

2.2 Response of barley to drought stress

Prolonged period of water stress in barley and other cereals has resulted in major yield losses not only in Kenya but other parts of the world experiencing unpredictable drought when the crop is already in the field (Abdullah, Hareri, Naaesan, Ammar, &

ZuherKanbar, 2011; EABL-UoE, 2013). Such losses are due to the effect of water stress on the physiological processes within the plants including translocation and partitioning of photosynthates needed by plants for proper growth and development (Ashoub, Jedmowski, Momtaz, & Brüggemann, 2015). Previous research indicates that when barley is exposed to drought stress, the quantum yield of light reaction, water-use-efficiency, photosynthetic rates and leaf osmotic potential significantly reduces (Ashoub, *et al*., 2015) and this interferes with other biochemical processes such as hormonal balance and signal transduction as well as the gene expression within such stressed plant. In response to the water stress, various phytohormones and enzymes are produced. Once produced, their roles may be to synergize or antagonize the effect of each other and thus need to be well understood within the drought stress context to ascertain the exact roles of different phytohormones produced in mitigating drought tolerance in plants such as barley.

2.2.1 Biochemical responses of barley to drought stress

Plants including barley accumulate different organic and inorganic solutes in the cytosol to lower osmotic potential and maintain cell turgor. Under drought, maintaining leaf turgor is also achieved by osmotic adjustment in response to the accumulation of proline, sucrose, soluble carbohydrates, glycinebetaine, and other solutes in cytoplasm improving water uptake from dry soil (Xie, *et al*., 2011).

In wheat as one of the cereals, proline has been observed to enhance tolerance to water stress (Nayyar & Walia, 2003). Proline accumulation is the first response of plants exposed to water-deficit stress in order to reduce injury to cells. However, the quantity of proline within a stressed plant is affected by the level of available calcium and abscisic

acid (ABA) (Xie, *et al*., 2011). In other cereals like maize, progressive drought stress has been indicated to induce a considerable accumulation of proline in water stressed and the proline content increased as the drought stress progressed and reached a peak after 10 days of stress, and then decreased under severe water stress as observed after 15 days of stress (Anjum, Wang, Farooq, Khan, & Xue, 2011). These findings confirms that biochemical reactions take place within plants when stressed and calls for more research on how the produced biochemicals within a stressed plant interact to affect the level of each stress factor especially drought, aluminium cation toxicity and net blotch disease severity in barley.

Once produced, proline acts as a signaling molecule to modulate mitochondrial functions, influence cell proliferation or cell death and trigger specific gene expression, which is essential for plant recovery from stress (Szabados & Savoure, 2009). Accumulation of proline under stress in many plant species as well as barley has been correlated with stress tolerance, and its concentration has been shown to be generally higher in stress-tolerant than in stress-sensitive plants. After synthesis due to stress, proline influences protein to dissolve and this maintains membrane integrity under dehydration stresses and reduces oxidation of lipid membranes or photoinhibition. Furthermore, it also contributes to stabilizing sub-cellular structures, scavenging free radicals, and buffering cellular redox potential under stress conditions (Xie, *et al*., 2011).

Other than synthesis of proline in response to water stress, the second crucial step in plant defense is the timely perception of the stress in order to respond in a rapid and efficient manner. In most cases, after recognition, the plants' constitutive basal defense

mechanisms (Andreasson & Ellis, 2010) lead to an activation of complex signaling cascades of defense varying from one stress factor to another (Abou, Luo, Laluk, Mickelbart, & Mengiste, 2009).

When a plant is exposed to abiotic and/or biotic stress factors, specific ion channels and kinase cascades (Fraire-Velázquez, Rodríguez-Guerra, & Sánchez-Calderón, 2011) are activated, reactive oxygen species (ROS) (Laloi, Appel, & Danon, 2004), phytohormones like abscisic acid (ABA), salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) (Spoel & Dong, 2008) accumulate, and a reprogramming of the genetic machinery results in adequate defense reactions and an increase in plant tolerance in order to minimize the biological damage caused by the stress (Fujita, Fijita, Noutoshi, Takahashi, Narusaka, Yamaguchi-Shinozaki, *et al*., 2006).

The existence of such knowledge has not been exploited especially under a complex interaction among water stress, aluminium toxicity and net blotch disease in winter and spring adapted barley genotypes grown in tropics and sub-tropics. When studied in details, plant scientists especially those dealing with cereal crops could be in a better position to explain why numerous varieties of barley and other cereals cannot sustain their desirable trait for a long period of time after release for commercial production. This could also provide a baseline platform for most plant breeders to consider the biochemical responses in their breeding objectives to ensure that the varieties developed are able to synthesis such important biochemicals in response to biotic and abiotic stresses without compromising the yield aspects.

2.2.2 Gene expression in barley due to drought stress

Under normal growth conditions plants produce reactive oxygen species (ROS) which are always kept in balance with different anti- oxidants of the cell redox homeostasis (Kar, 2011). However, under stressful conditions, ROS are produced at a high rate and if they are not removed by the different antioxidants, they usually accumulate in plant cells. The accumulation of the ROS is harmful to the different macromolecules in the cell, since they cause degradation of proteins, DNA, and lipids and increase the permeability of membranes (deCarvalho, 2008; Gill & Tuteja, 2010). If the plant is not able to scavenge the ROS in a reasonable time, it will die eventually as a result of the continual accumulation of these ROS and the detrimental effects of their accumulation (Gill & Tuteja, 2010).

Recent studies using barley under different watering regimes discovered over expression and under expression of genes responsible for production of superoxide dismutase (SOD), Catalase (CAT) and ascorbate peroxide (APX) enzymes with key roles in drought tolerance in barley and other cereals (Harb, Awad, & Samarah, 2015). For instance, unique pattern of the activity of SOD, CAT, and APX under drought stress was revealed for all barley genotypes used but in some genotypes, the activity of SOD was high and that of CAT was low at the early stage of drought treatment. At the intermediate stage of water stress, the activity of APX was low, and no change in enzyme activity was shown at the late stage of drought. Gene expression of SOD, CAT2, and APX showed distinct profile in each genotype. In some genotypes under drought stress, CAT2 was downregulated while SOD and APX were not expressed at the early stage of drought. However, at the intermediate stage of drought, CAT2 was upregulated and APX was

downregulated in the drought treated plants, whereas the expression of SOD was not changed by drought treatment. At the late stage of drought, CAT2 was not expressed and SOD and APX were upregulated (Harb, *et al*., 2015).

The variation in the types of genes expressed at different stages of drought treatment together with the existence of winter and spring adapted barley genotypes further complicates the scenario. This also indicates the possibility of different genotypes to produce different genes under varying degree of water stress thus different responses. In Kenya, numerous barley genotypes are grown but no study has been performed to understand how they would respond to drought, the biochemical synthesis and the genes expressed under water stress; more so now due to climate change environments where temperatures are rising and rainfall is unpredictable. This may need urgent attention to ensure their economic sustainability in commercial production.

2.3 Response of barley to aluminium toxicity

For most plants including barley, aluminum interferes with cell division in root tips and lateral roots, increased cell wall rigidity by cross-linking pectins, and reduced DNA replication by increasing the rigidity of the double helix (Rout, Samantaray, & Das, 2001). Further research reveals that aluminium treatment results in a severe inhibition of DNA synthesis within 16 h–24 h and this could be due to the binding of Al to DNA (Rout, *et al*., 2001). These findings suggest that just like drought stress, aluminium toxicity affects plant growth and physiological functions not only at cellular level but also at the genetic level. With the increasing soil acidity in Kenya and other parts of the world, such

physiological dynamics and the complex interaction with other biotic and abiotic stress factors calls for proper understanding.

2.3.1 Symptoms of aluminium toxicity in plants

Inhibition of root and shoot growth is the visible symptom of Al toxicity but earliest symptoms are seen on roots. Root stunting is a consequence of Al induced inhibition of root elongation. When exposed to Al toxicity, plant roots are usually stubby and brittle and root tips and lateral roots become thick and may turn brown. This causes inefficiency in nutrient and water absorption. Young seedlings are more susceptible than older plants and Al apparently does not interfere with seed germination, but impairs the growth of new roots and seedling establishment (Mossor-Pietraszewska, 2001).

2.3.2 Aluminium uptake, transport and tolerance mechanisms in plants

Aluminium-tolerant plants may be grouped according to Al accumulation within their tissues. In one group (wheat, barley, soybean and pea), Al concentrations in the shoots are not different from those of Al-sensitive plants, but in the root Al concentrations are lower in certain tolerant cultivars compared to the sensitive cultivars. In such cases, Al tolerance involves an exclusion mechanism. In a second group of plants (wheat, barley and potato and grass and cabbage), Al tolerance is associated with less Al in plant shoots, entrapment of more Al in roots or both. The mechanism of tolerance in this scenario is through root fixation. In a third group, Al tolerance is directly associated with Al accumulation by the tops; such plants have high internal tolerance to Al particularly pine trees, tea and mangroves (shoot fixation mechanism) (Das, Rout, & Samantaray, 2000).

2.3.3 Biochemical responses of plants to aluminiun toxicity

Aluminium is one of the most abundant elements in the earth's crust, and toxic to many plants when the concentration is greater than 2 - 3 ppm with a soil pH less than 5.5. A significant correlation between low pH and high Al concentration has also been shown in acidified freshwater, where this metal may reach levels of 0.3 - 1.6 mM and cause serious metabolic derangement in some hydrophytes. In general, young seedlings are more susceptible to Al than older plants (Das, *et al*., 2000).

When absorbed by plants, Al has been shown to interfere with all the physiological processes within plants as well as reduced nutrient uptake since it forms strong complexes to precipitate nucleic acids (Das, *et al*., 2000). In relation to these effects of Al, it is possible that higher concentrations of Al in plant tissues affects the physiological processes, some of which could be related to the defense mechanisms against biotic and abiotic stresses. This needs to be ascertained and confirmed under controlled conditions using specific crops like barley under the influence of Al toxicity, water stress and disease infections.

2.3.4 Gene expression in plants due to aluminium toxicity

The toxic effects of aluminium on plants first take place in the roots, and the mechanisms have been reported (Mossor-Pietraszewska, 2001). For instance, in certain barley populations, Al tolerance is controlled by one major dominant gene (Das, *et al*., 2000) which is located on chromosome-4 (Stolen & Anderson, 1978). Al tolerance in barley, however, is expressed at a much lower level of Al concentration in the medium as compared to wheat, and it might be that only one sub-cellular compartment is involved in

Al tolerance in barley (Das, *et al*., 2000). This may imply that barley is more sensitive to Al toxicity than wheat.

Aluminum interferes with cell division and viability in root tips and lateral roots, increased cell wall rigidity by cross-linking pectins, and reduced DNA replication by increasing the rigidity of the double helix structure of the DNA and cause a severe inhibition of DNA synthesis within 16–24 hours. The binding of Al to DNA has been indicated have the potential of causing inhibition of cell division thus interferes with the function of the Golgi apparatus in the peripheral cap cells of intact roots, the quiescent centre, mitotic activity, and DNA synthesis (Mossor-Pietraszewska, 2001). This could imply that when subjected to Al toxicity, plants can express certain characteristics of susceptibility to other stress factors like diseases due to failure to synthesize and express genes responsible for disease tolerance.

Stress recognition activates signal transduction pathways that transmit information within individual cells and throughout the plant. These pathways lead to the expression of genes and resultant modification of molecular and cellular processes. Experimental data suggest the existence of a cascade pathway under Al stress. An increase in cytoplasmic Ca^{2+} level in wheat root apexes may be related to the expression of Al toxicity (Mossor-Pietraszewska, 2001).

Protein phosphorylation plays an important role in the regulation of various biological activities in plants and provides a signal transduction pathway for mediating extracellular stimuli into cells. The mitogen-activated protein kinase (MAPK) cascade is one of the major pathways for transmitting signals such as light, temperature stress, mechanical

stress, wounding, pathogen elicitors, drought, salt, hormone signaling, nutrient deprivation and Al stress (Ligterink & Hirt, 2001; Osawa & Matsumoto, 2001).

2.3.5 Changes in gene expression during aluminium stress in plants

Plants have both a constitutive (present in most phenotypes) and an adaptive (present only in tolerant phenotypes) mechanism for coping with elevated metal concentrations, both under genetic control. Over 20 genes induced by Al stress have been isolated from a range of plant species, including wheat, rye, rice, soybean, tobacco, and Arabidopsis (Mossor-Pietraszewska, 2001). Very little genetic studies have been done in barley especially with focus on genes regulating the combined effects of drought, Al toxicity and net blotch fungal disease infection. Most of the Al-induced genes seem to be general stress genes that are induced by a range of different plant stresses. It has been proposed that there are common mechanisms for gene induction by Al and oxidative stress. By analogy with other stress genes, these genes may play a role in protecting cells against Al stress and genetic variation in the response to Al toxicity has been found not only among plant species but also among cultivars within species (Mossor-Pietraszewska, 2001).

In hexaploid wheat, major genes influencing tolerance to Al are located on the short arm of chromosome 5A and the long arms of chromosomes 2D and 4D. In rye these genes are located on chromosomes 3R, 4R, and the short arm of 6R and Al tolerance is controlled by at least two major dominant and independent alleles i.e. Alt1 and Alt3, located on chromosomes 4R and 6R (Mossor-Pietraszewska, 2001). This information is not properly documented in winter and spring adapted barley genotypes which are commonly grown in arid and semi-arid areas despite the need for such information to fine-tune the breeding

objectives aiming at multiple tolerances against drought, Al toxicity and net blotch disease.

2.4 Phytohormones and roles in stress response

Growth and response to environmental cues are largely governed by phytohormones. The plant hormones ethylene, jasmonic acid (JA), and salicylic acid (SA) play a central role in the regulation of plant immune responses. In addition, other plant hormones, such as auxins, abscisic acid (ABA), cytokinins, gibberellins, and brassinosteroids, that have been thoroughly described to regulate plant development and growth, have recently emerged as key regulators of plant immunity to a number of biotic and abiotic stresses (Denance, Sanchez-Vallet, Goffner, & Molina, 2013).

Therefore, to develop hormone-based breeding strategies aiming to improve crop resistance to pathogens and other abiotic stresses like drought and Al toxicity, there is need to understand the processes involved in the regulation of hormone homeostasis during plant – pathogen – environment interactions, and how the pathogen and environment related stresses interfere with the hormone regulation within a stressed plant. Indeed, manipulation of a plant hormone pathway can result in enhanced resistance to a particular pathogen or abiotic stress, but it could also have a strong negative effect on plant growth and resistance to a distinct type of pathogen with a different life style and other stresses (Holeski, Jander, & Agrawal, 2012).

2.4.1 Jasmonic acid (JA) and plant stress responses

Since discovered in the 1960s as secondary metabolites from the oils of jasmine flowers (Demole, Lederer, & Mercier, 1962), the biological roles of JA have received increased

attention of researchers in the past decades. Jasmonates have gradually become realized as a defense and fertility hormone, and as such, it modulates several processes relating to development and stress responses. In *Arabidopsis* and tomato, JAs are directly involved in stamen and trichome development, vegetative growth, cell cycle regulation, senescence, anthocyanin biosynthesis regulation, and responses to various biotic and abiotic stresses (Avanci, Luche, Goldman, & Goldman, 2010; Browse, 2009; Pauwels & Goossens, 2011). These roles have not been researched and documented in a number of cereals including barley which faces several challenges of biotic and abiotic stresses under field conditions.

In monocots like barley, much less is known about the role of JAs in relation to its role in response to diseases like net blotch, drought stress and tolerance to aluminium cation toxicity. However, it has been shown JAs are required for sex determination, reproductive bud initiation and elongation, leaf senescence, pigmentation of tissues and responses to the infection and infestation by pathogens and insects respectively (Acosta, *et al*., 2009; Engelberth, Alborn, Schmelz, & Tumlinson, 2004; Tani, *et al*., 2008; Yan, *et al*., 2012).

In addition, jasmonic acid or its methyl ester can induce synthesis of a number of proteins of mostly unknown functions in barley and a spray application of 30 μg of jasmonic acid per plant has effectively protected barley against subsequent infection by *Erysiphe graminis* f.sp. *hordei* (Schweizer, Cees, & Mosinger, 1993). This observation has not been tested in barley using net blotch fungus which is the major barley disease in Kenya and other parts of the world (Owino, *et al.*, 2014). Similar study concluded that application of jasmonic acid on *E. graminis* simultaneously resulted in independent extracellular accumulation of both jasmonic acid-induced proteins and of pathogenesis-related proteins.

Moreover, jasmonic acid directly inhibits appressoria differentiation of the fungus and is not involved in the signal transduction mechanism leading to induction of pathogenesis-related proteins (Schweizer, *et al*., 1993). This means that under the normal field conditions, barley can produce its own jasmonic acid in response to infection by pathogens and this can be one of the mechanisms utilized by some barley genotypes to resist infection by *P. teres*.

Considering only the role of JA, it has been frequently demonstrated to be an indispensible phytohormone signal for resistance/susceptibility to several diseases caused by fungal, bacterial, and viral pathogens. Some of the researched and documented findings include: mediation of resistance of plants to necrotrophic pathogens such as *Botrytis cenerea* and *Alternaria brassicicola* and *Fusarium oxysporium* (Rowe, *et al.*, 2010; Thatcher, Manners, & Kazan, 2009); plays a positive role in resistance against viruses (Shang, *et al*., 2011), parasitic plants (Runyon, Mescher, & DeMoraes, 2010) root knot nematodes (Bhattarai, *et al*., 2008) and abiotic stress alleviation such as salinity and heat stress (Ismail, Riemann, & Nick, 2012; Rao, Lee, Creelman, Mullet, & Davis, 2000). Despite the many positive roles in plant protection, JA can also lead to susceptibility to certain pathogenic organisms such as *Pythium* species (Yan, *et al*., 2012). None of these has been done in barley in relation to major diseases like net blotch and abiotic stresses like drought and aluminium cation toxicity.

2.4.1.1 The roles of jasmonic acid in induced systemic resistance against pathogens

Jasmonic acid is an essential phytohormone for defense response against a wide spectrum of pathogens, alone or in combination with other hormones, such as ET, SA, and ABA

(Adie, *et al*., 2007; Browse, 2009). Although all plant hormones including GA, auxin (IAA), and brassinosteroids (BR) may be involved in plant defense responses against pathogens (Smith, De Moraes, & Mescher, 2009), numerous studies have shown that SA, JA, and ET are the major players in induced resistance of plants (Kunkel & Brooks, 2002).

The SA-mediated pathway is typically activated in response to pathogens and mediates the initiation of a hypersensitive response (HR) and induction of pathogenesis-related proteins (PRs) that confer systemic acquired resistance (SAR) against a broad array of pathogens (Smith, *et al*., 2009). Regarding the relationship of JA with ET, the widely held belief is that ET acts synergistically with JA in the activation of responses to pathogens (Lorenzo & Solano, 2005). Several defense-related genes including *PR1*, *PR3*, *PR4*, *PR5*, and *PDF1.2* are synergistically induced by JA and ET (Lorenzo, Piqueras, Sanchez-Serrano, & Solano, 2003) and exogenous application of JA and ET can activate expression of genes in both JA biosynthesis and signaling pathway (Chung, *et al*., 2008).

2.4.2 Salicylic acid (SA) and plant stress responses

Salicylic acid (SA) is an important endogenous immune signal in the induction of disease resistance response in plants (Denance, *et al*., 2013; D. L. Yang, Yang, & He, 2013). An increase in endogenous concentration of SA after an infection has been reported in many plant pathogen interactions, and this increase is correlated to the activation of defense mechanisms (Garcion, *et al*., 2008; Iwai, Seo, Mitsuhara, & Ohashi, 2007). SA signaling system activates not only local resistance but also systemic acquired resistance (SAR) observed in distal (systemic) tissues. SAR is an SA-dependent heightened defense to a

broad spectrum of pathogens that is activated throughout a plant following local infection (Liu, von Dahl, & Klessig, 2011).

Infection of plants by necrotizing pathogens, which induce the accumulation of SA, or treatment of plants with synthetic compounds, which are able to trigger SA signaling, causes the induction of a unique physiological state called "priming" (Po-Wen, Singh, & Zimmerli, 2013). SAR is associated with priming of defense (Luna, Bruce, Roberts, Flors, & Ton, 2012), and the priming results in a faster and stronger induction of defense mechanisms after pathogen attack (Conrath, *et al*., 2006; Po-Wen, *et al*., 2013) The priming can be inherited epigenetically from disease-exposed plants (Pastor, Luna, Mauch-Mani, Ton, & Flors, 2013), and descendants of primed plants exhibit next-generation systemic acquired resistance (Luna, *et al*., 2012). Moreover, transgenerational SA-induced SAR has also been reported (Pietersea, 2012). These studies show that SA is an important immune signal in plants triggering local, systemic, and also transgenerational systemic disease resistance.

2.4.3 Abscisic acid (ABA) and plant stress responses

Plants are sessile organisms and therefore they constantly encounter diverse biotic and abiotic stresses, including various pathogens, drought, and high salinity. These stresses affect plant growth and development and can severely impair crop production. The plant hormone abscisic acid (ABA) functions as a chemical signal in response to environmental stresses. Stress signals are converted to ABA and this triggers the activation of a number of plant physiological and developmental processes, thereby inducing adaptation to the stress conditions (Robert-Seilaniantz, Navarro, Bari, & Jones, 2007; Ton, Flors, & Mauch-Mani, 2009).

Under drought conditions, plants produce and accumulate increased amounts of ABA in the guard cells, and this induces stomatal closure to conserve water. The cellular and molecular mechanisms underlying ABA-induced stomatal closure have been extensively investigated (Lim, Luan, & Lee, 2014; Popko, Hansch, Mendel, Polle, & Teichmann, 2010; Wilkinson & Davies, 2010). ABA biosynthesis and catabolism are known to be major determinants of endogenous ABA levels in plant cells (Nilson & Assmann, 2007). The 9-*cis*-epoxycarotenoid dioxygenase (*NCED*) genes and cytochrome P450 *CYP707A* genes encode key enzymes for ABA biosynthesis and ABA catabolism, respectively. The *NCED3* gene is induced by drought stress and it up regulates endogenous ABA levels in over expressed transgenic plants, thereby leading to lower transpiration rates (Schwartz, Qin, & Zeevaart, 2003). Under conditions of biotic and abiotic stresses, ABA functions as a chemical messenger that induces stomatal closure through the activation and inactivation of ion channels by protein kinases and phosphatases (Ward, Maser, & Schroeder, 2009).

Recently, several studies have demonstrated that ABA plays a crucial role in pathogen response and that ABA signaling overlaps considerably between biotic stress resistance and abiotic stress tolerance. Plants possess physical and biochemical defense barriers that effectively protect them from diverse pathogens. Various foliar pathogens such as bacteria, fungi, and viruses are known to disrupt stomatal movement in order to successfully infect plants (Arnaud & Hwang, 2015; McLachlan, Kopischke, & Robatzek, 2014).

The first line of defense is the recognition of the evolutionary conserved pathogen materials called the pathogen-associated molecular pattern (PAMP) by plant pattern

recognition receptors (PRRS), thereby leading to PAMP-triggered immunity (PTI) (Monaghan & Zipfel, 2012; Schwessinger & Zipfel, 2008). The second line of defense is the recognition of effectors through plant resistance (R) proteins, thereby leading to effect triggered immunity (ETI) (Chisholm, Coaker, Day, & Staskawicz, 2006; Jones & Dangl, 2006). However, most pathogens have evolved mechanisms that allow them to overcome or circumvent plant physical barriers, including stomatal closure, thereby enabling them to successfully infect plants (Jones & Dangl, 2006).

The phytotoxin coronatine (COR) is a virulence factor produced by *P. syringae* and it can compromise PAMP-induced stomatal defense by suppressing PAMP-induced ABA signaling and promoting stomatal reopening (Brooks, Bender, & Kunkel, 2005; Lim, *et al*., 2014). In contrast, ABA signaling plays an antagonistic role in post-invasive defense response. *Pseudomonas syringae* type III secreted effector (T3SE) proteins upregulate ABA biosynthesis and also the signaling pathways, thereby inhibiting the plant defense response. In this process, ABA signaling antagonizes salicylic acid (SA)-mediated pathogenesis-related (PR) gene expression and callose deposition (Lim, *et al*., 2014).

2.4.4 Salicylic acid and abscisic acid interaction and plant stress responses

Abscisic acid plays a crucial role in adaptation to abiotic stress. However, its role in biotic stress responses is less understood (Mou & An, 2011). Generally, ABA is considered as a negative regulator of disease resistance (Bari & Jones, 2009). Application of exogenous ABA prevents SA accumulation and suppresses resistance to *P. syringae* in *Arabidopsis* (Mohr & Cahill, 2003) and similar results have been found in other plant species (Koga, Dohi, & Mori, 2004). Further reports indicated that ABA treatment suppresses systemic acquired resistance (SAR) induction, indicating an antagonistic interaction between SA

and ABA signaling (Yasuda, *et al*., 2008). Likewise, mutants impaired in ABA biosynthesis or sensitivity are more resistant to different pathogens compared to wild-type plants in both *Arabidopsis* and tomato (Mou & An, 2011).

Furthermore, increased ABA production and activation of ABA-responsive genes have been described during the interaction of plants with invading pathogens (Mou & An, 2011). Therefore, ABA is a negative regulator of plant defense signaling pathways mainly mediated by SA. It has been shown that ABA regulates defense response through its effects on callose deposition (Flors, *et al*., 2008), production of reactive oxygen intermediates (Xing, Jia, & Zhang, 2008), and regulation of defense gene expression (Adie, *et al*., 2007). It also could be possible that ABA-SA antagonism results from the indirect effect of ABA-JA/ET interactions (Adie, *et al*., 2007; Anderson, *et al*., 2004). However, the exact molecular mechanism of ABA action on plant defense responses to diverse pathogens remains unclear and detecting regulatory factors involved in the crosstalk of ABA with other phytohormones in plant defense warrants extensive future study.

2.5 Hormonal signaling, interactions and gene expression

As an important signal for plant development and defense, JA (jasmonic acid) does not act independently but cooperatively with other phytohormonal signaling pathways including SA (salicylic acid), Ethylene, and ABA. A number of studies have already attracted attention to plant hormone cross-talk as it relates to biotic and abiotic defense responses. In *Arabidopsis*, JA interacts synergistically with ethylene (Xu, *et al*., 2001) depending on particular stress, both synergistically and antagonistically with salicylic acid (Beckers & Spoel, 2006) and abscisic acid (ABA) (Anderson, *et al*., 2004) in plant-pathogen or -insect

interactions. In maize, JA positively regulates ABA and ET biosynthesis in senescing leaves (Yan, *et al*., 2012). In summary, it is clear that JA signaling exert its functions via interaction with multiple plant hormones but the crossroads of these interactions still remain to be explored.

2.5.1 Jasmonic acid – Salicylic acid signaling, interactions and gene expression

The mutually antagonistic interactions between SA and JA pathways has been shown by analysis of SA- and JA-marker gene expression in SA and JA signaling mutants of *Arabidopsis* (Mur, Kenton, Atzorn, Miersch, & Wasternack, 2006). Interestingly, exogenous SA promotes JA-dependent induction of defense gene when applied at low concentrations. However, at higher SA concentrations, JA-induced induction of defense gene (*PDF1.2*) is suppressed, suggesting the interaction between these pathways may be dose dependent (Mur, *et al*., 2006). The antagonistic interaction between SA and JA is mediated by the central regulator of SA signaling (i.e. WRKY70) (Spoel, *et al.*, 2003), which is a versatile transcription factor with roles in multiple signaling pathways and physiological processes. This central regulator regulates the antagonistic interactions between SA and JA pathways where over-expression of WRKY70 leads to the constitutive expression of the SA-responsive *PR* genes and increased resistance to SA-sensitive pathogens but reduces resistance to JA-sensitive pathogens. In contrast, suppression of WRKY70 leads to increased expression of JA-responsive genes and increased resistance to a pathogen sensitive to JA-dependent defenses (Li, Brader, & Palva, 2004).

2.5.2 Jasmonic acid -Ethylene signaling, interaction and gene expression

A number of studies provide evidence for positive interactions between the JA and ET signaling pathways. For example, both JA and ET signaling are required for the expression of the defense-related gene *PDF1.2* in response to infection by *Alternaria brassicicola*. Evidence that JA and ET coordinatively regulate many other defense-related genes has been obtained in an *A. thaliana* microarray experiment, which showed nearly half of the genes that are induced by ET are also induced by JA treatment (Schenk, *et al*., 2000).

Some evidence suggest also antagonistic interactions between the JA and ET defense pathways although a number of JA-specific or ET-specific genes were found in wounding and defense responses (Lorenzo, *et al*., 2003). For instance, research has revealed that the key signaling players for JA signaling downstream are also required for ET signaling (Lorenzo, *et al*., 2003), providing a reasonable explanation for the synergy in many ET/JA-regulated processes (Zhu, *et al*., 2011). On the other hand, Over-expression of ET-responsive transcription factors (ERF1 and ORA59) significantly activates JA responses (Lorenzo & Solano, 2005).

2.5.3 Jasmonic acid – abscisic acid signaling, interaction and gene expression

Very limited information for the interaction between JA and ABA is available so far. ABA and Methyl-Jasmonates (MeJA) have been reported to induce stomatal closure, most likely by triggering the production of reactive oxygen species (ROS) in stomatal guard cells (Munemasa, *et al*., 2007). Anderson *et al*. (2004) showed that interaction between ABA and ethylene signaling is mutually antagonistic in vegetative tissues. Exogenous

ABA suppressed both basal and JA/ethylene–activated transcription of defense genes. By contrast, ABA deficiency as conditioned by mutations in the *ABA1* and *ABA2* genes, which encode enzymes involved in ABA biosynthesis, resulted in up-regulation of basal and induced transcription from JA-ethylene responsive defense genes (Anderson, *et al*., 2004).

2.6 Research gaps and way forward

Numerous literatures clearly indicate that the response to biotic and abiotic stresses by the plants can vary from not only one species to another but even within the same species. Two main response mechanisms usually utilized by the plants in response to such stress factors include genes and phytohormones whose roles can be antagonistic and/or synergistic depending on the genetic constitution of the plant and the initial stress factor. In addition, phytohormones play major role in the response mechanisms to biotic and abiotic stress.

In regard to the synergistic and antagonistic hormonal cross-talk and existence of specific but unclear signal pathways in response to specific stress factors, a lot of research needs to be done with focus on whether there is link between tolerance or susceptibility to biotic and abiotic stresses in plants (barley in this case); whether initial exposure of barley with specific traits to drought and aluminium toxicity can affect the level of disease severity in relation to the biochemical response to the abiotic stresses; whether the exogenous application of phytohormones could induce tolerance and/or susceptibility to drought, Al toxicity and net blotch disease in barley. This information is vital especially in determining whether plant breeders or protectionists should refocus on gene and/or

hormonal regulated plant responses to biotic and abiotic stresses to ensure durable and long-lasting solution to combined stresses under field conditions.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Barley grouping and genotype selection

Two main groups of barley were used in this study, that is winter and spring adapted genetically stable genotypes. These included advanced breeding lines and commercially grown varieties which were sourced from East African Breweries Limited – University of Eldoret (EABL-UoE) Collaborative Barley Research Program. A total of 32 genotypes grouped as 16 spring and 16 winter adapted genotypes (Table 1) were subjected to initial screening against net blotch, drought and aluminium toxicity under field, screenhouse and laboratory conditions.

Table 1: Grouping, names and codes of barley genotypes screened for biotic and abiotic stresses.

NO.	SPRING ADAPTED GENOTYPES		NO.	WINTER ADAPTED GENOTYPES	
	NAME/CODE	SOURCE		NAME/CODE	SOURCE
1	HKBL 1629-14	EABL	**1**	GRACE	GMS
2	HKBL 1629-5	EABL	**2**	ALICIANA	GMS
3	HKBL 1663-3	EABL	**3**	PHILLADEPHIA	GMS
4	HKBL 1674-4	EABL	**4**	BEATRIX	GMS
5	HKBL 1719-4	EABL	**5**	NFC TIPPLE	GMS
6	HKBL 1774-3	EABL	**6**	MARTHE	GMS
7	HKBL 1805-3	EABL	**7**	ANNABEL	GMS
8	HKBL 1805-6	EABL	**8**	XANADU	GMS
9	HKBL 1861-1	EABL	**9**	PUBLICAN	SYNGENTA
10	HKBL 1862-5	EABL	**10**	SHUFFLE	SYNGENTA
11	MALT 1	EABL	**11**	TITOUAN	SYNGENTA
12	SABINI	EABL	**12**	SY-BATYK	SYNGENTA
13	NGUZO	EABL	**13**	SY-409-228	SYNGENTA
14	KARNE	EABL	**14**	SCRABBLE	SYNGENTA
15	NGAO	EABL	**15**	COCKTAIL	SYNGENTA
16	FANAKA	EABL	**16**	QUENCH	SYNGENTA

GMS means Global Malting Services while **EABL** means East African Breweries Limited

3.2 Screening barley for responses to net blotch, drought and aluminium toxicity

This objective consisted of three sub-experiments i.e. screening for aluminium toxicity, drought and net blotch disease severity. The baseline assessment was done to provide the characteristics of each genotype in terms of the response to the three stresses. Initial screenings for drought and aluminium response were done under laboratory and screenhouse conditions at the University of Eldoret, School of Agriculture and Biotechnology (SAB).

3.2.1 Laboratory screening of barley for response to aluminium toxicity

The 16 winter and 16 spring barley groups were assessed and analyzed separately for their response to aluminium toxicity as described by Magnavaca, modified by Ouma *et al*., (2011) using 148 µM Al treatment from $AlCl_3.6H_2O$ (Bal & Alkus, 2011; Maxim & Duþã, 1996).

3.2.1.1 Germination of barley seed

Barley seeds for each genotype were soaked in sterile distilled water for five minutes then surface sterilized with 1% sodium hypochlorite for 5 minutes as described by Ouma *et al*. (2011) to reduce the chances of contamination during germination. The seeds were then rinsed 8 times using sterile distilled water to remove traces of chloride. Clean seeds were then pre-germinated on paper rolls moistened with aerated distilled water then placed inside germination plastic trays. Trays were then incubated in a growth chamber at 27 ^{o}C under dark conditions for three days (Ouma, Gudu, Were, Ligeyo, & Kiplagat, 2011).

3.2.1.2 Experimental design for aluminium screening in the laboratory

Barley genotypes were arranged in a completely randomized design (CRD) consisting of two Al treatments (0 µM and 148 µM) and barley genotypes as experimental units replicated five times. The 8-litre trays were used to hold nutrient solution under continuous aeration using a 50-litre air compressor pump. The three day-old uniform-sized barley seedlings with no visible injury or root damage were placed inside plastic cups that are mounted on a perforated Styrofoam sheet, 35 seedlings per sheet (7 genotypes replicated 5 times). The transferred seedlings were then stabilized in a nutrient solution (Magnavaca, Gardner, & Clark, 1987; Ouma, *et al*., 2011) (Appendix 1) at a pH of 4.0 for 24 hours. Thereafter, the seedlings were transferred to a freshly prepared nutrient solution containing Al as $AlCl_3$ adjusted to a final concentration of 0 µM (control) and 148 µM Al (treatment). Each seedling was placed individually on cups (3 cm diameter) with small holes (0.5 cm) at the bottom of each cup to allow roots reach the solution inside a tray. The pH of the nutrient solution was maintained at 4.0 and monitored daily throughout the experiment.

After transfer into nutrient solution with and without Al, the seedlings were allowed to grow in a growth chamber for a photoperiod of 14 hours of light and 10 hours of darkness on daily basis. The photoperiod growth conditions were set at approximately 340 µmoles $m^{-2}s^{-1}$ of light intensity, temperature at 30 ^{o}C and relative humidity at 70%. The dark growth conditions were set at approximately 22 ^{o}C and 90% relative humidity inside the growth chamber. Experimental layout (Figure 1) and statistical linear model is shown below.

148 µM	V12	V6	V3	V1	V8	V9	V5	V13
	V12	V6	V3	V1	V8	V9	V5	V13
	V12	V6	V3	V1	V8	V9	V5	V13
	V2	V4	V10	V14	V11	V16	V7	V15
	V2	V4	V10	V14	V11	V16	V7	V15
	V2	V4	V10	V14	V11	V16	V7	V15
0 µM	V1	V6	V12	V18	V8	V9	V5	V13
	V1	V6	V12	V18	V8	V9	V5	V13
	V1	V6	V12	V18	V8	V9	V5	V13
	V2	V4	V10	V14	V11	V16	V7	V15
	V2	V4	V10	V14	V11	V16	V7	V15
	V2	V4	V10	V14	V11	V16	V7	V15

Figure 1: Randomization of SPRING and WINTER barley screened for response to aluminium toxicity under laboratory conditions. V1 – V16 are the genotype numbers

Linear model: $\mathbf{Y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ijk}}$ where:

$\mathbf{Y_{ijk}}$ = Total observation

$\boldsymbol{\mu}$ = Overall mean

$\boldsymbol{\alpha_i}$ = i^{th} effect of aluminium treatment

$\boldsymbol{\beta_j}$ = j^{th} effect of barley genotype

$\boldsymbol{\varepsilon_{ijk}}$ = Error term/residual effect

Hematoxylin staining was used as a confirmatory test for tolerance to Al toxicity. All barley genotypes per group was subjected to hematoxylin staining (Cancado, *et al*., 1999) and 0.2% hematoxylin solution containing 0.02% potassium iodide was prepared in distilled water. The five-day old seedlings grown under Al solution were gently shaken in 200 ml distilled water for 15 minutes on a mechanical shaker at 20 rpm to remove Al cation from the root surface. The water was then be replaced by 200 ml of aqueous hematoxylin stain [0.2% hematoxylin and 0.02% potassium iodide, w/v] and left at the

same low agitation for 20 minutes. The visual scores on stain colour intensity were done on a 1 – 5 scale as follows: **1** – Non-stained roots, classified as very tolerant; **2** – Faintly stained roots, classified as tolerant; **3** – Moderately stained roots, classified as moderately tolerant; **4** – Well stained roots, classified as sensitive; **5** – Deeply stained roots, classified as very sensitive genotypes.

3.2.1.3 Data on response to aluminium toxicity

The aluminium tolerant and sensitive barley genotypes were assessed based on net root length (NRL), relative net root growth (RNRG), degree of hematoxylin staining and percent response as described by Ouma *et al*., (2011). The root length measurements were performed using a calibrated ruler and the initial root length (IRL) was measured at the time of transferring the seedlings to the nutrient solution. Five days after transfer to the Al treatments (0 µM and 148 µM), the final root length (FRL) was measured. The NRL was obtained from the difference between FRL and IRL (NRL = FRL – IRL). The relative net root growth (RNRG) was obtained by dividing the corresponding NRL in Al treatment by NRL in control (RNRG = NRL (Al) ÷ NRL (Control). The greater the RNRG, the more resistant a genotype is to Al toxicity. The percent response to Al toxicity was also calculated using the formula: % Response = (Root length (Al treated) – Root length (Control) ÷ Root length (control)) x 100.

3.2.1.4 Statistical data analysis

Data on NRL, RNRG, percent response, hematoxylin staining intensity were 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 (Log_{10} + 1) before performing an ANOVA to ensure the data set assumes the normal distribution thus obeying the central limit theorem (CLT). Mean differences among the barley genotypes in each group (winter and spring) was separated using Duncan Multiple Range Test to ensure that only larger differences are justified to confirm true differences.

3.2.2 Screening barley genotypes for tolerance to drought

All the winter and spring genotypes initially screened for tolerance to aluminium toxicity were screened for drought resistance. The drought tolerance experiment was carried out as pot experiment in the greenhouse (Pauk, *et al*., 2012) and as laboratory experiments that uses membrane stability index (Abdullah, *et al*., 2011) to determine the level of tolerance to drought by different barley genotypes.

3.2.2.1 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 & Ochuodho, 2014) to reduce the stress due to acidity. 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 every watering regime received about 1,550 ml (22 ml per pot per day for 70 days) and 6,500 ml (93 ml per pot per day for 70 days) of water supplied through irrigation for the whole experimental period (Pauk, *et al*., 2012).

3.2.2.2 Planting in the greenhouse and experimental design

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 (CRD) with each genotype replicated thrice. The two water regimes (20% and 80%) were used as main plots while genotypes were considered to be the sub-plot as shown in the experimental layout and linear model below (Figure 2).

		SUB PLOTS							
MAIN PLOTS	20% FC	V12	V6	V3	V1	V8	V9	V5	V13
		V12	V6	V3	V1	V8	V9	V5	V13
		V12	V6	V3	V1	V8	V9	V5	V13
		V2	V4	V10	V14	V11	V16	V7	V15
		V2	V4	V10	V14	V11	V16	V7	V15
		V2	V4	V10	V14	V11	V16	V7	V15
	80% FC	V1	V6	V12	V18	V8	V9	V5	V13
		V1	V6	V12	V18	V8	V9	V5	V13
		V1	V6	V12	V18	V8	V9	V5	V13
		V2	V4	V10	V14	V11	V16	V7	V15
		V2	V4	V10	V14	V11	V16	V7	V15
		V2	V4	V10	V14	V11	V16	V7	V15

Figure 2: Randomization of SPRING and WINTER barley genotypes screened for tolerance to drought under greenhouse conditions. V1 – V16 are the genotypes

Linear model: $\mathbf{Y_{ij} = \mu + \alpha_i + \varepsilon_{j(i)} + \beta_k + \alpha\beta_{ik} + \varepsilon_{ijkl}}$ where:

$\mathbf{Y_{ijk}}$ = Total observation

$\boldsymbol{\mu}$ = Overall mean

$\boldsymbol{\alpha_i}$ = i^{th} effect of main plot (water stress)

$\boldsymbol{\varepsilon_{j(i)}}$ = j^{th} effect of main plot error

$\boldsymbol{\beta_k}$ = k^{th} effect of sub plot (barley genotype)

$\boldsymbol{\alpha\beta_{ij}}$ = i^{th} and k^{th} effect of water stress and genotype interaction respectively

$\boldsymbol{\varepsilon_{ijkl}}$ = l^{th} effect of total error/residual effect

3.2.2.3 Data on phenotypic response to water deficiency

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.

3.2.2.4 Physiological approach to determine drought tolerance in barley

The physiological approach was selected to act as a confirmatory test to the pot experiment under laboratory. 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 ^{o}C (Abdullah, *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 ^{o}C for 30 minutes while the second set at 100 ^{o}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- (C_1/C_2)] x 100. The higher the MSI, the more the tolerant a genotype was to drought.

3.2.2.5 Data analysis for drought tolerance

Data on spike length (cm) per main spike, number of tillers per plant, heading and physiological maturity, 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 Duncan Multiple Range Test. The results were presented using table of rations (20% FC/80% FC), table of means and plates.

3.2.3 Screening barley for tolerance to net blotch under field conditions

The response of winter and spring barley genotypes to net blotch was assessed under field conditions in three sites at Mau, Chepkoilel and Njoro which represents high, medium and low altitude zones respectively where barley is grown in Kenya.

3.2.3.1 Study sites and characteristics

Screening for net blotch disease severity was done under field conditions at Chepkoilel, Njoro and Mau sites which are known to be hotspot zones for the disease and they also exhibit high (Mau), medium (Chepkoilel) and low (Njoro) altitude characteristics where barley is mostly grown in Kenya (EABL-UoE, 2010).

Mau-Narok site is located at an altitude of 2900 m above the sea level and lies between latitudes 0°36’S and longitude 36°0’E. The area receives an average annual rainfall of 1200 - 1400 mm. The minimum temperatures of 6 - 14 °C and maximum of 22 - 26 °C (Wanyera, Macharia, & Kilonzo, 2010) have been reported. Chepkoilel site is located between longitude 35°18’ E and latitude 0° 30’N and at an altitude of 2140 m above the sea level. Rainfall ranges from 900 to 1300 mm with an annual average of 1124 mm. The average annual temperature is 23 °C with a minimum of 10 °C (Okalebo, *et al*., 1999). Njoro site stands at an altitude of 1,800 m above sea level with average temperature ranges between 17 – 22 °C, while the average annual rainfall is in the region of 1,000 mm (Walubengo, 2000).

3.2.3.2 Planting and experimental design

The 16 winter and 16 spring barley genotypes were planted separately in a in randomized complete block design (RCBD) consisting of 4 replications in all the three sites (Owino, *et al*., 2014). Planting Moiben, Njoro and Mau Narok was done in the months of May, June and August, 2015 respectively. The variation in time of planting is due to the differences in the onset of rains in these agro-ecological zones thus seasons.

The plot size for each genotype measured 1.5 m by 7 m (10.5 m^2). A seed rate of 84 kg/ha and phosphate source fertilizer was applied at 175 kg/ha during planting using self-propelled experimental plot seeder that drills eight rows per plot per genotype. Other agronomic practices were put in place to prevent weed and insect pest damage using herbicides (Pendimethalin + Chlorsulfuron) and insecticide (Dimethoate + Alpha-cypermethrin). In each site, the guard rows were planted using a known net blotch susceptible variety (Sabini) to act as a spreader and increase disease pressure on the other genotypes. Site layout for winter and spring genotypes and linear model is shown (Figure 3).

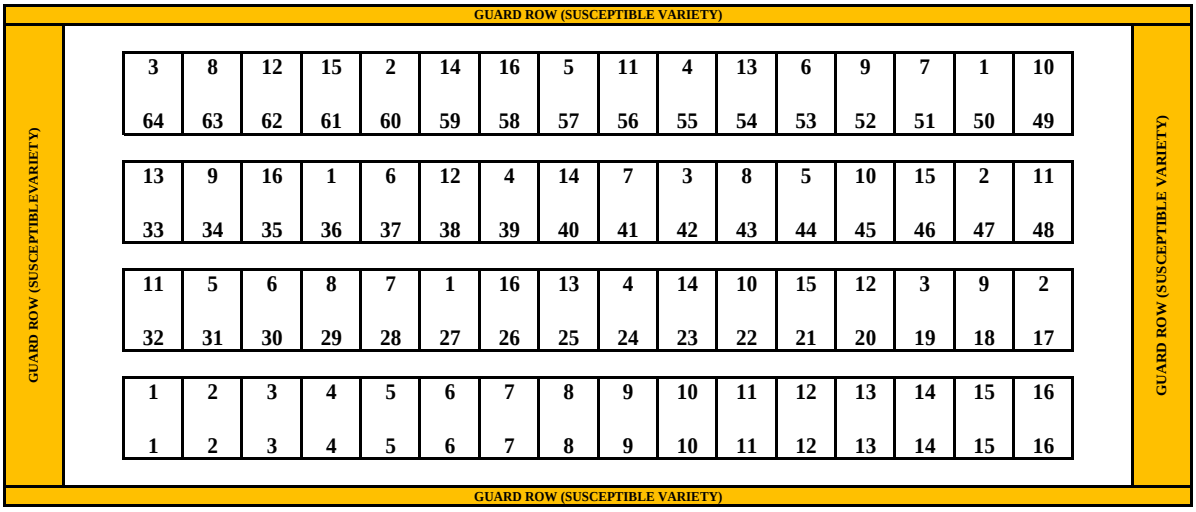


Figure 3: Randomization plan for WINTER and SPRING barley genotypes screened for their response to net blotch disease under field conditions. Lower numbers 1-60 (PLOT NUMBERS) while Upper numbers 1-16 (GENOTYPES)

Linear model: $\mathbf{Y_{ij} = \mu + S_i + \beta_{j(i)} + \alpha_k + S\alpha_{ik} + \varepsilon_{ijkl}}$ where:

$\mathbf{Y_{ijkl}}$ = Total observation

$\boldsymbol{\mu}$ = Overall mean

$\mathbf{S_i}$ = i^{th} effect of site

$\boldsymbol{\beta_{j(i)}}$ = j^{th} effect of block in every i^{th} site

$\boldsymbol{\alpha_k}$ = k^{th} effect of barley genotypes

$\mathbf{S\alpha_{ik}}$ = j^{th} and k^{th} interaction effect of site and barley genotypes respectively

$\boldsymbol{\varepsilon_{ijkl}}$ = Error term/residual effect

3.2.3.3 Source of inoculum and disease severity assessment

Natural inoculation approach was used under field conditions since these areas had been previously identified as hot-spot for net blotch inoculum (EABL-UoE, 2010). Adult plant

disease resistance approach was used to assess the response to net blotch in barley genotypes. Severity scale of 0 – 9 was used in determining the most resistant/tolerant and the most susceptible genotypes to net blotch disease (Owino, *et al*., 2014). Based on a 0 - 9 severity scale, winter and spring barley genotypes were grouped separately as follows: **0 – 3** = resistant to net blotch disease; **4 – 5** = tolerant to net blotch disease; **6 – 7** = susceptible to net blotch disease and **8 – 9** = highly susceptible to net blotch disease.

3.2.3.4 Disease severity analysis

The severity data were subjected to log transformation (Log_{10} x + 2) 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 differences for the genotypes in each of the barley groups were separated using Duncan Multiple Range Test. The severity means were used to perform Euclidean test in a Multivariate analysis to generate clusters for the winter and spring barley and determine the number of unique groups formed at a 0.98 similarity coefficient matrix.

3.3 Effect of drought and aluminium toxicity on net blotch severity

This experiment was conducted under screenhouse condition at the University of Eldoret, School of Agriculture and Biotechnology to determine how aluminium cation toxicity and drought stress conditions affect the level of net blotch disease.

3.3.1 Selection of barley genotypes

Twenty four (24) barley genotypes with specific traits (Table 2) were selected from the first experiment and used in subsequent experiments II and III. Genotypes with tolerance

or susceptibility to more than one stress factor were used repeatedly due to the limited number of available genotypes.

Table 2: Trait specific winter and spring barley genotypes selected for subsequent studies. Selection criteria: Aluminium –> ***degree of hematoxylin staining;*** **Net blotch –>** ***0 – 9 severity scale;*** **Drought - >** ***MSI plus two additional variables used in the assessment***

GENOTYPE GROUP	GENOTYPE NAME	TRAIT CODE	TRAIT DESCRIPTION
SPRING BARLEY	FANAKA	DRT1	Drought tolerant
	NGUZO	DRT2	Drought tolerant
	HKBL 1805-3	DRS1	Drought sensitive
	HKBL 1862-5	DRS2	Drought sensitive
	NGAO	NBT1	Net blotch tolerant
	MALT 1	NBT2	Net blotch tolerant
	SABINI	NBS1	Net blotch susceptible
	KARNE	NBS2	Net blotch susceptible
	HKBL 1663-3	ALT1	Aluminium tolerant
	HKBL 1805-3	ALT2	Aluminium tolerant
	HKBL 1674-4	ALS1	Aluminium sensitive
	NGAO	ALS2	Aluminium sensitive
WINTER BARLEY	GRACE	DRT1	Drought tolerant
	TITOUAN	DRT2	Drought tolerant
	BEATRIX	DRS1	Drought sensitive
	MARTHE	DRS2	Drought sensitive
	SHUFFLE	NBT1	Net blotch tolerant
	TITOUAN	NBT2	Net blotch tolerant
	QUENCH	NBS1	Net blotch susceptible
	BEATRIX	NBS2	Net blotch susceptible
	GRACE	ALT1	Aluminium tolerant
	ALICIANA	ALT2	Aluminium tolerant
	PUBLICAN	ALS1	Aluminium sensitive
	ANNABEL	ALS2	Aluminium sensitive

3.3.2 Planting and experimental design

The twelve winter and twelve spring barley genotypes were planted separately in a split – plot arrangement in completely randomized design with three replications per genotype (Figure 4). The drought (20% FC), aluminium toxicity (148 μM) and control experiment (No stress) were considered as main plots while the 24 trait-specific genotypes as sub – plots. Planting was done using plastic pots filled with forest soil previously solarized for three months and mixed with phosphate fertilizer at planting. Three seeds were planted

per pot for each genotype. These three treatments commenced immediately after seedling emergence to ensure maximum effect of the two abiotic stresses before inoculation with the net blotch fungus. The experimental layout and model for spring and winter genotypes (separately executed) is shown below.

REP 1	**148 µM Al** DRT1 DRT2 DRS1 DRS2 NBT1 NBT2 NBS1 NBS2 ALT1 ALT2 ALS1 ALS2	**20% FC** ALS1 ALS2 ALT1 ALT2 DRS1 DRS2 DRT1 DRT2 NBS1 NBS2 NBT1 NBT2	**80% FC** NBT2 NBT1 NBS2 NBS1 DRT2 DRT1 DRS2 DRS1 ALT2 ALT1 ALS2 ALS1
REP 2	**20% FC** ALS1 ALS2 ALT1 ALT2 DRS1 DRS2 DRT1 DRT2 NBS1 NBS2 NBT1 NBT2	**80% FC** NBT2 NBT1 NBS2 NBS1 DRT2 DRT1 DRS2 DRS1 ALT2 ALT1 ALS2 ALS1	**148 µM Al** DRT1 DRT2 DRS1 DRS2 NBT1 NBT2 NBS1 NBS2 ALT1 ALT2 ALS1 ALS2
REP 3	**80% FC** NBT2 NBT1 NBS2 NBS1 DRT2 DRT1 DRS2 DRS1 ALT2 ALT1 ALS2 ALS1	**148 µM Al** DRT1 DRT2 DRS1 DRS2 NBT1 NBT2 NBS1 NBS2 ALT1 ALT2 ALS1 ALS2	**20% FC** ALS1 ALS2 ALT1 ALT2 DRS1 DRS2 DRT1 DRT2 NBS1 NBS2 NBT1 NBT2

Figure 4: Randomization of trait-specific barley genotypes inoculated with net blotch fungus under the influence of drought and aluminium toxicity. DRT (drought tolerant); DRS (drought sensitive); NBT (net blotch tolerant); NBS (net blotch susceptible); ALT (aluminium tolerant); ALS (aluminium sensitive).

$\mathbf{Y_{ijkl} = \mu + \alpha_i + \varepsilon_{j(i)} + \beta_k + \alpha\beta_{ik} + \varepsilon_{ijkl}}$ where:

$\mathbf{Y_{ijkl}}$ = Total observation

$\boldsymbol{\mu}$ = Overall mean

$\boldsymbol{\alpha_i}$ = i^{th} effect of main plot (abiotic stress)

$\boldsymbol{\varepsilon_{j(i)}}$ = j^{th} effect of main plot error

$\boldsymbol{\beta_k}$ = k^{th} effect of sub-plot (barley genotypes)

$\boldsymbol{\alpha\beta_{ik}}$ = i^{th} and k^{th} interaction effect between genotypes and abiotic stress

$\boldsymbol{\varepsilon_{ijkl}}$ = split-plot error/residual effect

3.3.3 Isolation of *Pyrenophora teres*

Net blotch infected barley leaf tissues were sourced from Chepkoilel site and the fungus isolated as outlined by Owino *et al*., (2013) where the infected leaf samples were cut into 1 cm^2 pieces and surface-sterilized with 1% sodium hypochlorite solution for 30 sec, then rinsed three times in sterile distilled water for 10 sec. Leaf pieces were blot dried to remove excess water and aseptically transferred to freshly prepared full strength potato dextrose agar (PDA Oxoid, 3.9 %) in petri dishes. Petri dishes were incubated at 15-20 ºC for 12hr/12hr light/dark period inside a Gallenkamp incubator to induce sporulation. After five days of incubation, a single conidium was transferred on freshly prepared potato dextrose agar medium and incubated for two weeks to induce sporulation until pure culture of pathogen was obtained (Owino, Ochuodho, & Were, 2013).

3.3.4 Inoculum preparation and inoculation

Pure culture of one *P. teres* isolate was grown on full-strength PDA plates at 25°C under alternating 12 hours white fluorescent light and 12 hours dark cycle for 14 days to induce sporulation (Than, *et al*., 2008). Inoculum was then prepared by flooding each petri dish containing the single spore isolate with 5 ml of sterile distilled water, gently swirled and scrapped with a scalpel to harvest macro conidia. The conidial suspensions were passed through a double layer of cheesecloth and the inoculum adjusted to a concentration of 5 ×

10^3 conidia/ ml (Xue & Burnett, 1995) after counting on Buker - Turk haemocytometer (Mathur, Singh, & Hansen, 1989).

Barley seedlings were inoculated at Zadoks growth stage 15, usually attained 16 – 18 days after seeding (Xue & Burnett, 1995) inside inoculation chamber with relatively warmer temperatures above 27 ^oC and relative humidity above 70%. The seedlings were pre-moistened with sterile distilled water and sprayed immediately with about 0.5 ml of the inoculum per plant. The inoculation chamber was closed immediately to maintain high relative humidity.

After inoculation, high relative humidity (>70%) was maintained by misting after every three hours using sterile distilled water and left under normal light regimes (Than, *et al*., 2008) for about seven days to ensure successful infections. On the eighth day, the infected plants were returned to the normal greenhouse conditions.

3.3.5 Assessment of net blotch severity under drought and aluminium toxicity stresses

The response of individual barley genotypes belonging to winter and spring groups to *P. teres* was assessed from the 7^{th} to 35^{th} day after inoculation at seven days interval on a 0 to 7 leaf symptom severity rating scale (Xue & Burnett, 1995) where 0 = 0 %, 1 = 1-5 %, 2 = 6-10 %, 3= 11-20%, 4 = 21-30%, 5 = 31-50%, 6 = 51-75%, 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.

3.3.6 Severity analysis of net blotch as influenced by drought and aluminium toxicity

Similar to the field assessment, the severity data was log transformed 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. Contrast, comparison option was used to separate means with focus on how the previously observed inherent traits as well as drought and aluminium toxicity affect net blotch severity in barley. Cluster analysis was performed to determine whether the previously observed inherent traits clustered together in terms of the response to net blotch disease under controlled conditions.

3.4 Hormonal signaling effects on net blotch, drought and aluminium toxicity

In this experiment, the barley genotypes previously selected for their response to drought, aluminium toxicity and net blotch disease were planted separately under drought, aluminium toxicity and net blotch respectively then treated with the four phytohormones in the screenhouse. This means that a total of eight genotypes (four winter and four spring genotypes) with two tolerant and two susceptible for each stress factor in each of the winter and spring groups were used.

The 24 barley genotypes (Table 2) were subjected to drought, net blotch and aluminium cation toxicity stresses under the greenhouse conditions then supplied with three phytohormone treatments as soil drench: 50 µM salicylic acid (RANKEM SALICYLIC ACID $C_7H_6O_3$, RFCL Ltd, New Delhi, INDIA) (Fayez & Bazaid, 2014), 20 µM abscisic acid (TRANS-ABSCISIC ACID $C_{15}H_{20}O_4$ Duchefa, The Netherland) (Moons, *et al*., 1997), SA x ABA combination and distilled water as a control experiment.

3.4.1Effect of exogenous phytohormones on the response of barley to drought

3.4.1.1 Planting and experimental design

The eight barley genotypes (four winter and four spring) that had been previously selected for their response to drought (Table 2) were raised in 2-litre plastic pots filled with solarized forest soils (pH > 6.0) to reduce the effects of mineral toxicity and net blotch fungus infection. The phytohormones treatments were randomized as main plots and trait-specific genotypes as sub-plots in a split-plot arrangement in completely randomized design with three repeated observations. The experimental layout and statistical linear model is given below (Figure 5).

	WDRT = WINTER DROUGHT TOLERANT	**WDRS** = WINTER DROUGHT SENSITIVE	**SDRT** = SPRING DROUGHT TOLERANT	**SDRS** = SPRING DROUGHT SENSITIVE
REP 1	WDRT1 WDRT2 WDRS1 WDRS2 SDRT1 SDRT2 SDRS1 SDRS2	WDRT2 WDRT1 WDRS2 WDRS1 SDRT2 SDRT1 SDRS2 SDRS1	SDRS1 SDRS2 SDRT1 SDRT2 WDRS1 WDRS2 WDRT1 WDRT2	WDRT2 WDRT1 WDRS2 WDRS1 SDRT2 SDRT1 SDRS2 SDRS1
REP 2	WDRT2 WDRT1 WDRS2 WDRS1 SDRT2 SDRT1 SDRS2 SDRS1	SDRS1 SDRS2 SDRT1 SDRT2 WDRS1 WDRS2 WDRT1 WDRT2	WDRT1 WDRT2 WDRS1 WDRS2 SDRT1 SDRT2 SDRS1 SDRS2	WDRT2 WDRT1 WDRS2 WDRS1 SDRT2 SDRT1 SDRS2 SDRS1
REP 3	WDRT2 WDRT1 WDRS2 WDRS1 SDRT2 SDRT1 SDRS2 SDRS1	WDRT1 WDRT2 WDRS1 WDRS2 SDRT1 SDRT2 SDRS1 SDRS2	WDRT2 WDRT1 WDRS2 WDRS1 SDRT2 SDRT1 SDRS2 SDRS1	SDRS1 SDRS2 SDRT1 SDRT2 WDRS1 WDRS2 WDRT1 WDRT2
KEY	**50 µM SA**	**20 µM ABA**	**50 µM SA x 20 µM ABA**	**Distilled water**

Figure 5: Randomization plan for assessing the effects of application of exogenous phytohormones on drought tolerance among the identified barley genotypes exhibiting tolerant and sensitive traits

$\mathbf{Y_{ijkl}} = \mu + \alpha_i + \varepsilon_{j(i)} + \beta_k + \alpha\beta_{ik} + \varepsilon_{ijkl}$ where:

$\mathbf{Y_{ijkl}}$ = Total observation

μ = Overall mean

α_i = i^{th} effect of main plot (phytohormones)

$\varepsilon_{j(i)}$ = j^{th} effect of main plot error

β_k = k^{th} effect of sub-plot (trait-specific barley genotypes)

$\alpha\beta_{ik}$ = i^{th} and k^{th} interaction effect between phytohormones and barley genotypes

ε_{ijkl} = split-plot error/residual effect

3.4.1.2 Phytohormone preparation and treatment under drought stress

The pots were maintained at 80% field capacity after planting but immediately after emergence, water supply was reduced and maintained at 20% field capacity till the end of the experiment. The three phytohormone treatments of 50 µM SA (Fayez & Bazaid, 2014), 20 µM ABA (Moons, *et al*., 1997), 20 µM ABA + 50 µM SA combination and double distilled water as control were supplied twice as soil drench immediately after seedling emergence and two weeks after the first hormone treatment. The ABA was first dissolved in 5 ml of 0.2 M KOH and pH adjusted to 5.0 to ensure complete mixing with water. For SA, the powder was first dissolved Et-OH (Ethanol) followed by KOH to form salt which then dissolved in water. The barley seedling were subjected to water stress and maintained at 20 %FC immediately after the first hormonal treatment till the end of the experiment under greenhouse conditions.

3.4.1.3 Data on response to drought under the influence of hormones and analysis

Data on agronomic traits including tillering ability and plant height, total dry weight and membrane stability index (MSI) were scored and subjected to analysis of variance

(ANOVA) on Genstat statistical software release 14.1 VSN International Ltd at 5% level of significance. Mean separations for specific traits and phytohormones was done using contrast comparison to answer specific questions concerning the grouped genotypes and the effect of three phytohormone treatments on drought tolerance.

3.4.2 Effect of exogenous phytohormones on response of barley to aluminium toxicity

3.4.2.1 Planting and experimental design

Eight selected barley genotypes (Table 2) previously screened and selected for their tolerance and susceptible responses to aluminium toxicity were used. Similar to the drought experiment, the eight genotypes were planted in a split-plot arrangement in CRD with three repeated observations using solarized forest soil mixed with phosphate fertilizer. The three phytohormone treatments and distilled water as a control experiment were randomized first as main plots and the eight genotypes as sub-plots.

The experimental layout and linear model are similar to that in figure 9 but drought tolerant and susceptible genotypes from winter and spring are replaced by WALT 1 & 2, WALS 1 & 2, SALT 1 & 2 and SALS 1 & 2 (winter aluminium tolerant; winter aluminium sensitive; spring aluminium tolerant and spring aluminium sensitive).

3.4.2.2 Phytohormone treatment under aluminium cation toxicity

The 50 µM SA (Fayez & Bazaid, 2014), 20 µM ABA (Moons, *et al*., 1997), ABA x SA combination and double distilled water as control were applied as hormonal treatments. Each genotype was supplied with the four phytohormones twice i.e. immediately after emergence and two weeks after the first hormonal treatment. The supply of 148 µM aluminium cation contained in a nutrient solution commenced one day after the first

hormonal treatment and this was used to maintain the genotypes at 80 %FC up to physiological maturity.

3.4.2.3 Data on response to aluminiun toxicity under hormones and analysis

Data on height (cm), apical root length (cm), number of fibrous roots, root dry weight (g) and shoot dry weight (g) were recorded for all the trait-specific genotypes under different hormonal treatment. The data were subjected to analysis of variance (ANOVA) on Genstat statistical software release 14.1 VSN International Ltd at 5% level of significance and means separated using contrast comparison.

3.4.3 Effect of exogenous phytohormones on the response of barley net blotch

3.4.3.1 Planting and experimental design

Eight barley genotypes previously selected for their tolerant and susceptible response to net blotch were subjected to phytohormone treatments under greenhouse conditions. Throughout this study, 80% water capacity was maintained. Planting was done using solarized forest soil (pH > 6.0) to reduce aluminium toxicity. The four phytohormones were randomized first as main plot while the genotypes randomized afterwards as sub-plots in a split-plot arrangement in completely randomized design with three repeated observations per genotype.

The experimental layout and linear model are similar to that in figure 9 but drought tolerant and sensitive genotypes from winter and spring are replaced by WNBT 1 & 2, WNBS 1 & 2, SNBT 1 & 2 and SNBS 1 & 2 (winter net blotch tolerant; winter net blotch susceptible; spring net blotch tolerant and spring net blotch susceptible).

3.4.3.2 Phytohormone treatment and disease inoculation

The four treatments (50 µM SA (Fayez & Bazaid, 2014), 20 µM ABA (Moons, *et al*., 1997), ABA x SA combination and double distilled water as control) were supplied as soil drench immediately after seedling emergence and repeated two weeks after the first application. The 16-18 day old barley seedlings (Zadoks growth stage) initially treated with different phytohormones and their combinations were transferred to inoculation chamber with modified temperatures and relative humidity then inoculated with 0.5 ml $plant^{-1}$ of 5 x 10^3 conidia/ml spore concentration (Xue & Burnett, 1995). The phytohormone treatments was synchronized such that on the 9^{th} day after the first phytohormone treatment (Moons, *et al*., 1997), the seedlings were pre-moistened and immediately inoculated with one isolate of *P. teres* (Were & Ochuodho, 2012b) at Zadoks growth stage 15.

3.4.3.3 Net blotch foliar severity evaluation under hormones and analysis

Disease severity data was collected on a 0 – 7 scale at 7 days interval after inoculation up to 35^{th} days after inoculation and subjected to analysis of variance (ANOVA) on Genstat statistical software release 14.1 VSN International Ltd at 5% level of significance. The severity data was first transformed (Log_{10} (x + 2) before analysis to adhere to the central limit theorem rules on qualitative ranked data. Mean differences for grouped genotypes and phytohormone treatments were separated using contrast comparison.

CHAPTER FOUR

RESULTS

4.1 Response of barley to net blotch disease, aluminium toxicity and drought

4.1.1 Response of winter and spring barley genotypes to aluminium toxicity

Winter and spring adapted genotypes showed mixed reactions to aluminium cation toxicity. In particular, both winter and spring barley differed significantly in terms of net root length (NRL), percent response, relative net root growth (RNRG) and degree of hematoxylin staining ($p < 0.05$) (Appendix iv and v). Mixed reaction to aluminium toxicity was evident across a number of genotypes irrespective of whether they are winter or spring adapted.

Among the spring adapted barley, with reference to NRL, SABINI, NGUZO and HKBL 1674-4 genotypes were the most tolerant to Al toxicity and scored 7.0 cm, 5.6 cm and 5.3 cm respectively. In contrast to tolerance, HKBL 1861-1, NGAO and HKBL 1805-3 were the most sensitive to Al toxicity at 1.8 cm, 2.7 cm and 2.8 cm in the same order. The relative net root growth (RNRG) ratio ranked genotype HKBL 1719-4 to be the most tolerant to Al toxicity with a score of 1.0, indicating that the root length under 148 μM and 0 μM did not differ in length. In addition, majority of the spring barley were tolerant to Al and scored above 0.7 in terms of RNRG but HKBL 1861-1 consistently remained the most sensitive to Al toxicity just as observed earlier in terms of NRL (Table 3, Appendix iv).

Table 3: Response of SPRING barley to aluminium toxicity in nutrient solution containing 148 µM Al^{3+} under laboratory conditions.

GENOTYPE	NRL (cm)	DMRT	RNRG (Ratio)	DMRT	% RESPONSE	DMRT	HEMATOXYLIN (1-5)	DMRT
HKBL 1663-3	4.6	bc	0.9	ab	17.7	cde	1.7	f
HKBL 1805-3	2.8	cd	0.5	fg	23.0	bcde	1.7	f
KARNE	4.4	bc	0.6	fg	27.3	bcd	1.7	f
NGUZO	5.6	ab	0.7	bcde	15.0	cde	1.7	f
FANAKA	3.8	bcd	0.8	abc	15.7	cde	1.8	ef
HKBL 1805-6	4.5	bc	0.6	efg	32.3	bc	1.8	ef
HKBL 1862-5	3.5	bcd	0.6	defg	39.3	ab	2.0	def
HKBL 1861-1	1.8	d	0.4	g	49.3	a	2.3	cde
MALT 1	4.8	bc	0.7	cdef	29.7	bcd	2.3	cde
HKBL 1719-4	3.6	bcd	1.0	a	12.7	de	2.5	bcd
HKBL 1629-14	3.6	bcd	0.7	cdef	28.7	bcd	2.8	abc
HKBL 1774-3	3.7	bcd	0.9	a	14.0	de	2.8	abc
SABINI	7.0	a	0.8	abc	6.7	e	2.8	abc
HKBL 1629-5	3.0	cd	0.7	bcd	15.3	cde	3.0	ab
NGAO	2.7	cd	0.5	g	35.7	ab	3.0	ab
HKBL 1674-4	5.3	ab	0.8	abc	15.3	cde	3.3	a
MEAN	**4.0**		**0.7**		**23.6**		**2.3**	
Probability	0.001		<0.001		<0.001		<0.001	
S.E	0.664		0.051		5.160		0.177	
S.E.D	0.938		0.073		7.300		0.250	
% CV	24.6		12.8		23.8		13.1	

The percent response measurements further confirmed SABINI to be most tolerant genotype to Al toxicity by recording the lowest percentage. In this respect, other genotypes including HKBL 1719-4, HKBL 1774-3, NGUZO and those with percent response below 20% were tolerant to Al cation toxicity. Similarly, HKBL 1861-1, NGAO and HKBL 1805-6 expressed higher sensitivity to Al toxicity and most of the observations in terms of percent response correspond to NRL and RNRG scores.

In terms of the degree of hematoxylin staining, it is evident that different spring adapted barley genotypes utilized different mechanisms of responses to Al toxicity. For instance, HKBL 1663-3, HKBL 1805-3, KARNE and NGUZO exhibited the highest tolerance to Al cation toxicity by recording the least degree of staining. This shows that the roots did

not contain much Al cations hence less staining capacity. In comparison to NRL, RNRG and percent response, it is clear that KARNE and HKBL 1805-6 genotypes did not absorb much Al cations into the roots but still remained sensitive even to the low concentration of Al within the roots (Table 3 and Plate 1).

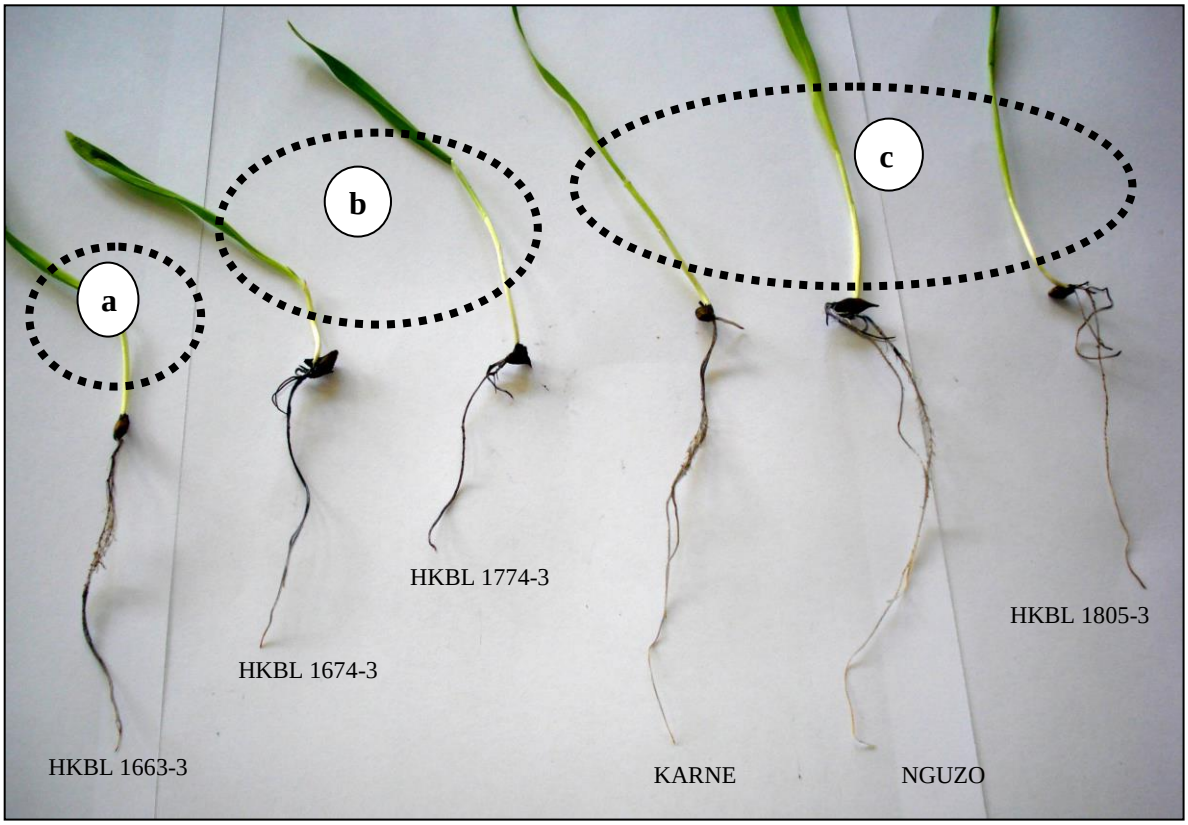


Plate 1: Grouping of SPRING barley based on degree of hematoxylin staining. The 'a', 'b' and 'c' groupings represent the major categories in terms of response to aluminium toxicity. 'a' – tolerant by fixation, 'b' – sensitive and 'c' – tolerant by exclusion. (Source: Author, 2017)

Group 'a' and 'c' were considered tolerant to aluminium toxicity. However, in group 'a', much aluminium cations were absorbed hence much staining but in group 'c', the aluminium was excluded from the roots thus low degree of staining. Group 'b' absorbed more aluminium cations which significantly affected root growth and development thus sensitive (Plate1)

The same scenario was observed in SABINI and HKBL 1719-4 genotypes which despite absorbing much Al cations into their root systems, they still expressed tolerance in terms of NRL, RNRG and percent response. The roots of such genotypes are less affected when exposed to higher concentration of Al cation, an indication of a totally different tolerance mechanism compared to those that exclude Al cations from their roots (Table 3).

The winter adapted barley expressed similar patterns of response to Al cation toxicity in terms of NRL, RNRG, percent response and degree of hematoxylin staining. However, majority of the genotypes were more tolerant to Al toxicity compared to the spring adapted ones. For example, QUENCH, TITOUAN and SY BATYK were the most tolerant to Al toxicity not only in reference to NRL but also in terms of RNRG, percent response and hematoxylin staining except that SY BATYK used a different mechanism of tolerance compared to QUENCH and TITOUAN. GRACE was more tolerant to cation toxicity than ALICIANA but the two genotypes were the best in terms of excluding aluminium from their roots (Table 4, Appendix v).

Table 4: Response of WINTER barley to aluminium toxicity in nutrient solution containing 148 µM Al^{3+} under laboratory conditions.

GENOTYPE	NRL (cm)	DMRT	RNRG (Ratio)	DMRT	% RESPONSE	DMRT	HEMATOXYLIN (1-5)	DMRT
GRACE	3.9	cd	0.8	abc	10.7	ef	1.3	f
ALICIANA	2.7	d	0.5	ef	28.0	bcd	1.7	ef
PHILLADEPHIA	5.6	abc	0.7	bcd	21.7	cde	1.7	ef
NFC TIPPLE	2.7	d	0.4	f	50.0	a	1.8	ef
SHUFFLE	5.9	abc	0.8	abc	16.3	def	1.8	ef
SY 409-228	5.2	abc	0.7	bcd	19.7	cde	1.8	ef
COCKTAIL	5.1	abc	0.8	abc	20.3	cde	2.0	de
SY BATYK	6.1	ab	0.9	a	1.3	f	2.3	de

Other than tolerance, NFC TIPPLE, BEATRIX and SCRABBLE genotypes were more sensitive to Al toxicity with reference to their performance in terms of NRL, RNRG and % response. However, despite their nature of being sensitive to mineral toxicity, SCRABBLE and BEATRIX absorbed much Al cations into their roots as indicated by the degree of hematoxylin staining. On the other hand, NFC TIPPLE excluded much cation from the root system but still remained very sensitive to low doses of Al cation especially in terms of percent response which corresponds to RNRG and NRL scores (Table 4).

The degree of hematoxylin staining and root morphologies formed three major groups among the winter adapted barley genotypes screened against Al cation toxicity. These include groups ‘d’, ‘e’ and ‘f’ just like the spring barley genotypes. Group‘d’ were highly sensitive to aluminium toxicity while groups ‘e’ and ‘f’ were tolerant but with different response mechanisms of tolerance. However, when the spring and winter genotypes are

compared with reference to effects of Al cations on roots and the degree of staining, it is evident that the winter genotypes are more sensitive than spring genotypes (Plate 2).

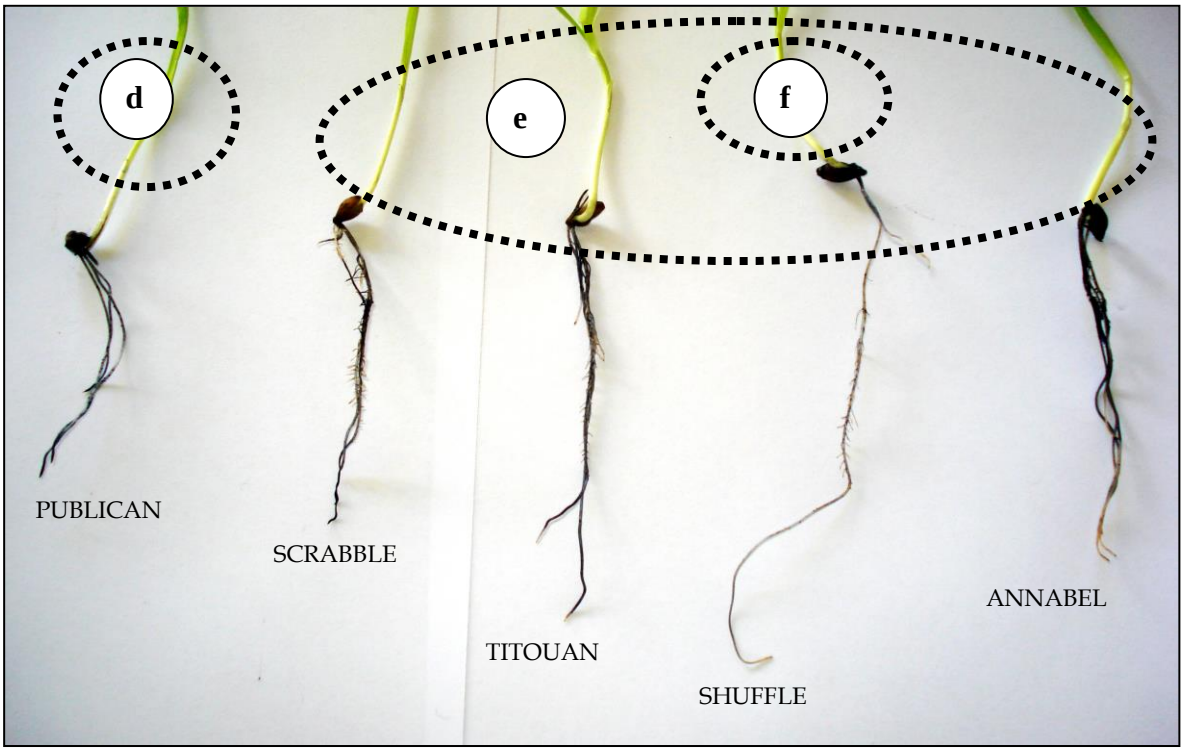


Plate 2: Grouping of WINTER barley based on degree of hematoxylin staining. The 'd', 'e' and 'f' represents major groups formed by genotypes under aluminium toxicity. 'd' - sensitive, 'e' – tolerant by fixation and 'f' tolerant by exclusion (Source: Author, 2017)

4.1.2 Response of winter and spring barley to drought

Both winter and spring barley expressed significant differences in terms of tillering ability, growth in terms of height, number of grains per main spike, thousand seed weight and membrane stability index in their response to drought ($p < 0.05$). Moreover, most of the interactions between these genotypes and field capacities were significant ($p < 0.05$) (Appendix vi, vii, viii and ix).

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. For instance, among the spring barley, FANAKA and HKBL 1805-6 were the most tolerant to drought in terms of tillering ability. This implies that there was no much difference in the number of tillers when these same genotypes are subjected to 20% (stressed) and 80% (unstressed) growth conditions. However, HKBL 1805-3 and HKBL 1629-14 were highly sensitive to drought which significantly affected their tillering ability hence lowest ratio among the spring barley. Additionally, in reference to height, all the spring barley proved tolerant to drought and was less affected as the genotypes scored above 0.8. In this regard, HKBL 1805-6 and HKBL 1774-3 were the most tolerant to drought and expressed perfect similarity under stressed and unstressed conditions (Table 5, Appendices vi and vii).

Even though the growth in terms of height was less affected among the spring barley, the number of grains per main spike and thousand seed weight (TSW) were significantly

reduced under drought stress compared to unstressed condition. For example, HKBL 1629-14, HKBL 1629-5 and HKBL 1805-3 expressed significant reduction in the number of grains per spike and this strongly corresponded to the thousand seed weight results. However, most genotypes such as FANAKA, HKBL 1805-6, MALT 1, NGAO, NGUZO and SABINI exhibited consistent tolerance and stability in their response to drought in terms of tillering ability, height, number of grains per spike, TSW and MSI (Table 5).

The use of phenotypic expression and physiological assessment to determine drought tolerance in barley proved to be highly corresponding to each other. In particular, FANAKA and HKBL 1861-1 genotypes recorded MSI of 73 and 59 respectively at 20% field capacity (Appendix 7). Higher membrane stability indices were recorded at 80% field capacity but still, FANAKA had higher MSI than HKBL 1861-1, an indication that FANAKA was better than HKBL 1861-1 in terms of drought tolerance. Phenotypic assessment confirms similar results when these two genotypes were subjected to different field capacities under greenhouse conditions (Plate 3).

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 ($p < 0.05$) (Appendix viii and ix).

Specifically, only four genotypes namely GRACE, TITOUAN and SY BATYK and PHILADEPHIA expressed significant tolerance in terms of tillering ability. The rest of the genotypes were sensitive to drought and their tillering ability greatly affected and reduced under drought stress conditions. Additionally, the effect of drought on height was more serious especially in QUENCH and NFC TIPPLE genotypes. With reference to all variables assessed, only GRACE maintained stable tolerance to drought stress.

Unlike spring genotypes, majority of the winter barley showed mixed reactions to drought stress. For instance, the sensitivity of winter barley to drought was more expressed in compared to the spring barley especially with reference to effect on 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 their grain development and seed weight. Most of the phenotypic observation under drought stress corresponded to that of physiological results of MSI (Table 6).

Table 6: Response of winter adapted barley to drought under screenhouse conditions. The rations were by dividing the variables under 20% FC by same variables under 80% FC i.e. 20%FC/80%FC. Ratios above 0.7 are considered tolerant to drought

GENOTYPE	Number of tillers	DMRT	Plant height	DMRT	Grains per spike	DMRT	1000 SWT	DMRT	MSI	DMRT
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		0.03054		0.0481		0.0669		0.02499	
S.E.D	0.0657		0.0432		0.068		0.0946		0.03535	
% CV	14.4		7.0		24.4		15.9		5.5	

Phenotypically with respect to MSI and the field capacities, the results confirmed that ALICIANA whose MSI was approximately 74 expressed more tolerance than TITOUAN whose MSI was 37 at 20% FC. Further, among the winter barley, the sensitive genotypes

like TITOUAN exhibited delayed heading and growth in terms of height that was highly affected at 20% FC. Other tolerant genotypes including ALICIANA, ANNABEL, GRACE and COCKTAIL did not exhibit significant difference both in their MSI and a number of growth parameters assessed (Plate 4).

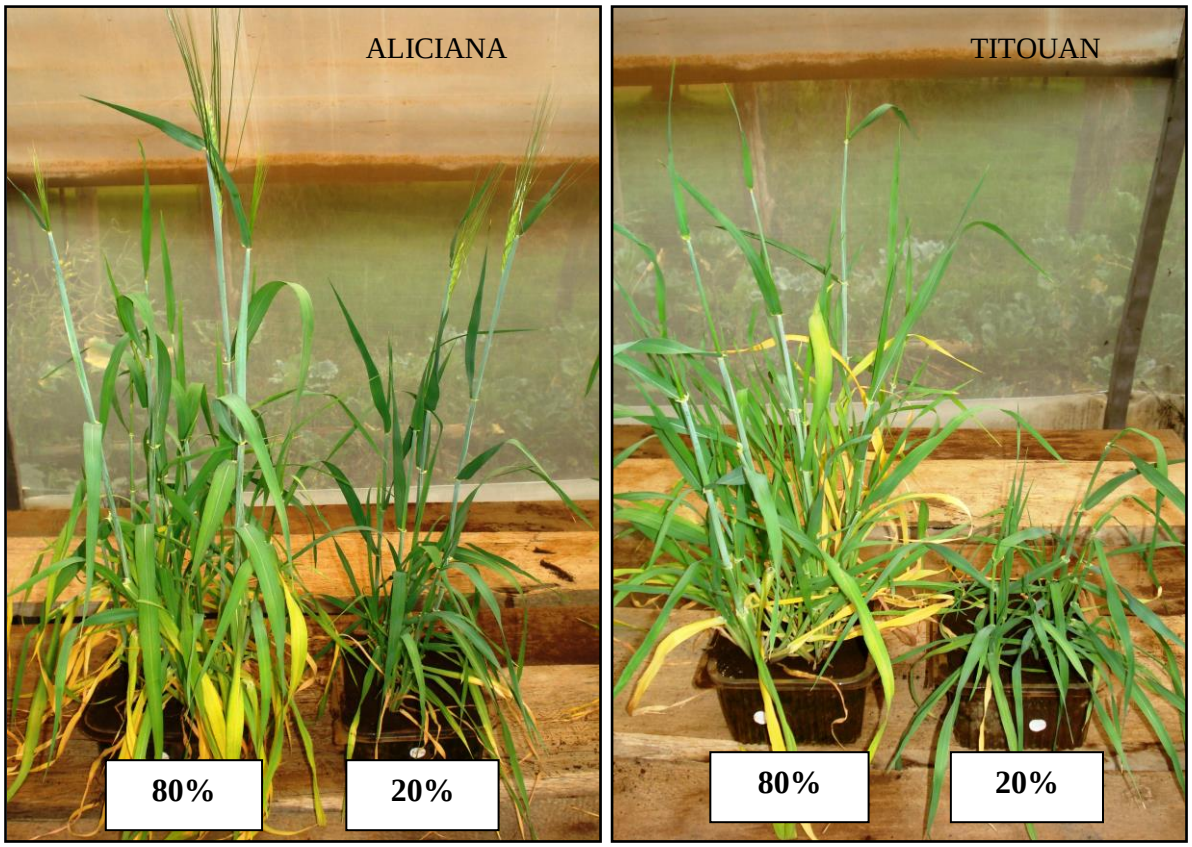


Plate 4: Growth of WINTER barley at 80% (unstressed) and 20% (stressed) field capacities. ALICIANA genotype exhibits more tolerance to water deficiency compared to TITOUAN (Source: Author, 2017)

4.1.3 Response of winter and spring barley to net blotch disease

Significant differences were expressed by both spring and winter barley in terms of net blotch disease severity under the influence of genotypes and genotype x site interactions ($p < 0.05$) (Appendix x). For spring barley, mixed responses to net blotch foliar infection were common and this varied not only from one variety to another but also from one site

to the other. However, some genotypes had stable resistance or susceptible responses to the disease across all the sites. For example, NGAO and MALT 1 proved to be the most tolerant (severity scale below 3.0) to *P. teres* across the high, medium and low altitude growing zones represented by Mau, Chep and Njoro sites respectively.

On the other hand, SABINI and KARNE genotypes were the most susceptible to net blotch fungus (severity scale above 7.5). Moreover, some genotypes expressed significantly different responses in their response to *P. teres* under different environmental conditions and in reference to this, HKBL 1774-3, HKBL 1719-4 and HKBL 1629-14 expressed high disease severity under high altitude zones (Mau) but the same genotypes recorded low disease severity in medium altitude zones (Figure 6).

On average, only three genotypes including NGAO, MALT 1 and HKBL 1663-3 recorded the least disease severity while NGUZO, SABINI and KARNE were the most susceptible to fungal infection under field conditions. However, majority of the genotypes scored between 3.0 and 4.5 on a 0 – 9 severity scale hence the formation of three main groups in terms of disease severity levels among the spring barley screened (Figure 6).

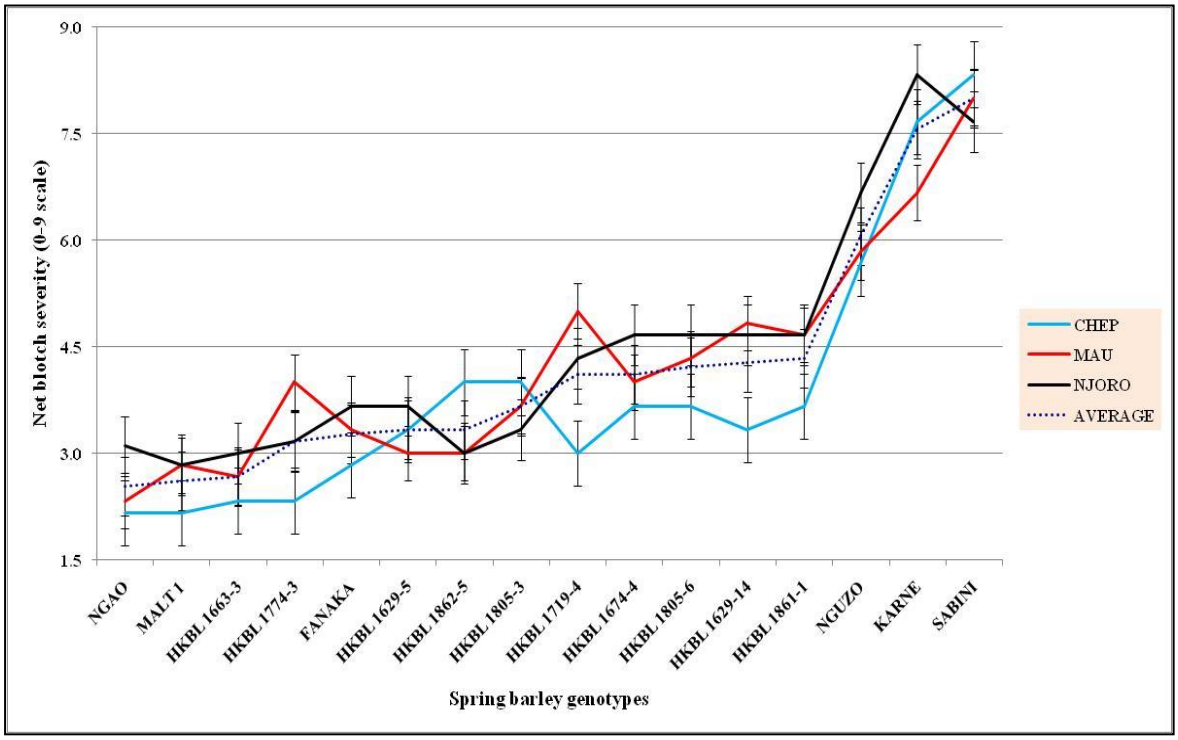


Figure 6: Response of SPRING barley to net blotch foliar infection under field condition. The assessment was done on a 0 – 9 severity scale. Error bars represent standard error

Additional results indicates that in highly susceptible genotypes, net blotch disease can cause up to 100% foliar damage while in resistant genotypes, minimal damage was seen on lower leaves only. For example, under medium altitude conditions at Chepkoilel site, SABINI leaves were all infected at grain filling stage but MALT 1 showed higher tolerance to the disease under the same inoculum load (Plate 5).

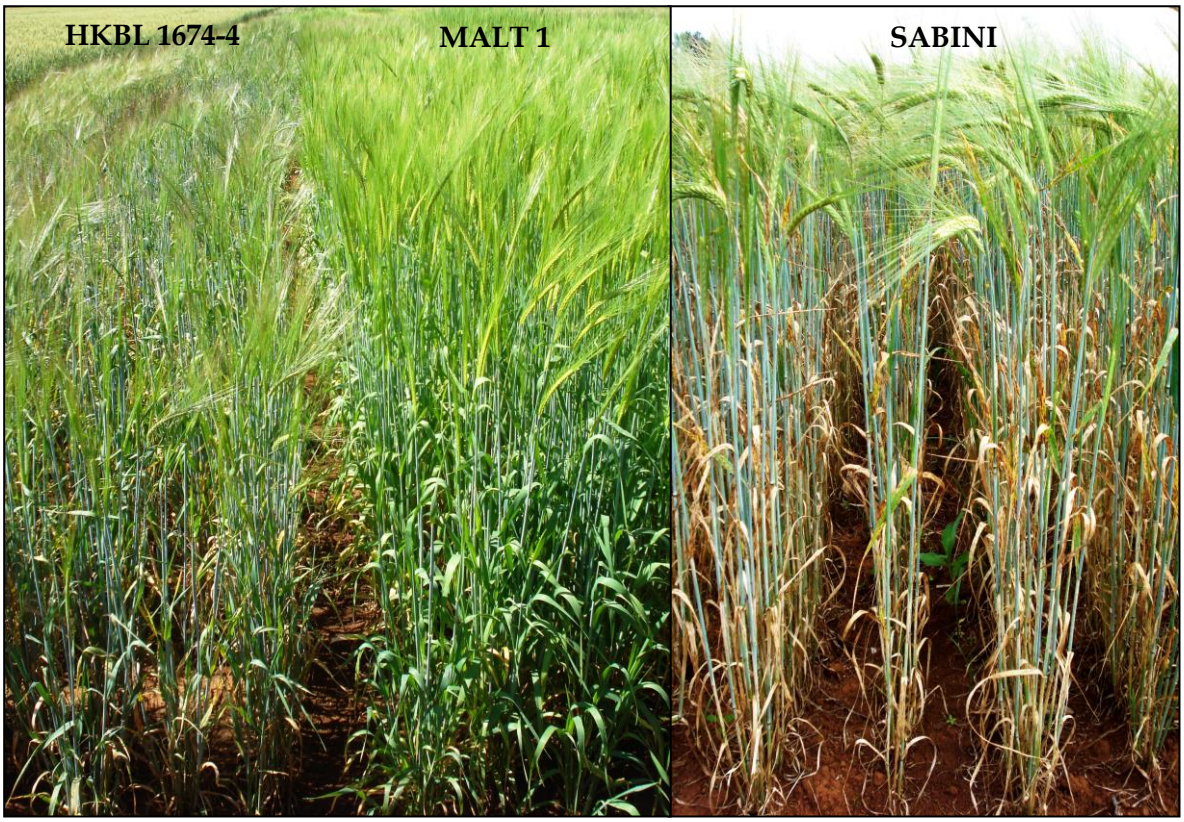


Plate 5: Response of spring barley to net blotch foliar infection under field condition. MALT 1 shows more tolerance while SABINI exhibits highest susceptibility at Chepkoilel site (Source: Author, 2017)

Similar pattern of mixed reactions to foliar infection by net blotch fungus was evident among the winter barley genotypes. However, majority of these genotypes exhibited low disease levels compared to the spring genotypes. For instance, SHUFFLE and TITOUAN were the most tolerant while QUENCH and BEATRIX were the most susceptible to the disease. Moreover, mixed response to foliar infection was common in a number of genotypes with others showing low disease in one site but highly susceptible to foliar infection under different environmental conditions just like the spring barley (Figure 7).

For example, among the winter genotypes, SCRABBLE and SY BATYK expressed net blotch disease tolerance at Chepkoilel site (Medium altitude) but the same genotypes were

highly susceptible to disease at Mau Narok site (High altitude). Also, PHILADEPHIA showed disease tolerance at low altitude zone (Njoro site) but the same genotype exhibited high disease symptoms at Mau Narok site. Focusing on winter and spring genotypes, the spring barley expressed higher disease levels across the low, medium and high altitude zones compared to the winter genotypes which exhibited low disease levels especially under low and medium altitude zones (Figure 6 and 7).

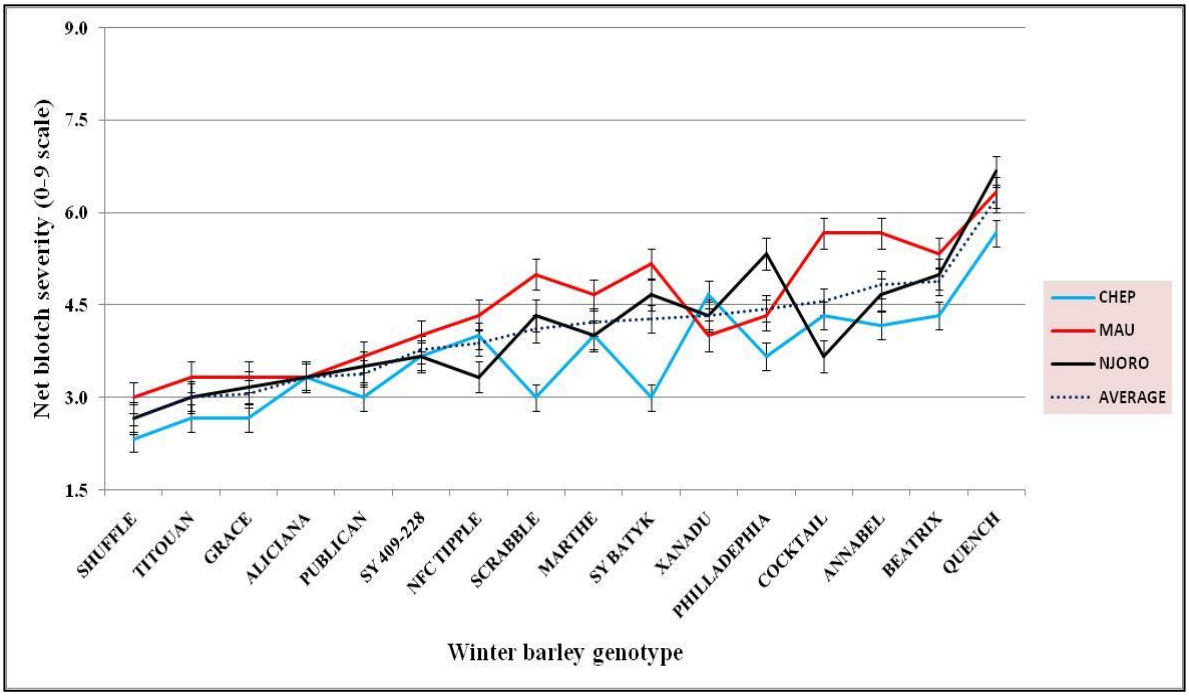


Figure 7: Response of WINTER barley to net blotch foliar infection under field condition. The assessment was done on a 0 – 9 severity scale. Error bars represent standard error

Three main clusters were formed at 0.98 similarity matrix by the spring barley genotypes in terms of their response to net blotch fungal infection under field conditions. For instance, KARNE and SABINI genotypes which were the most susceptible to net blotch grouped together 'III' while NGUZO formed its own unique cluster 'II'. However, apart from these three genotypes, the rest of the spring genotypes did not differ thus hence

formed one large group 'I' with several other sub-groups which expressed dissimilarity towards similarity matrix closer to 1.00 (Figure 8).

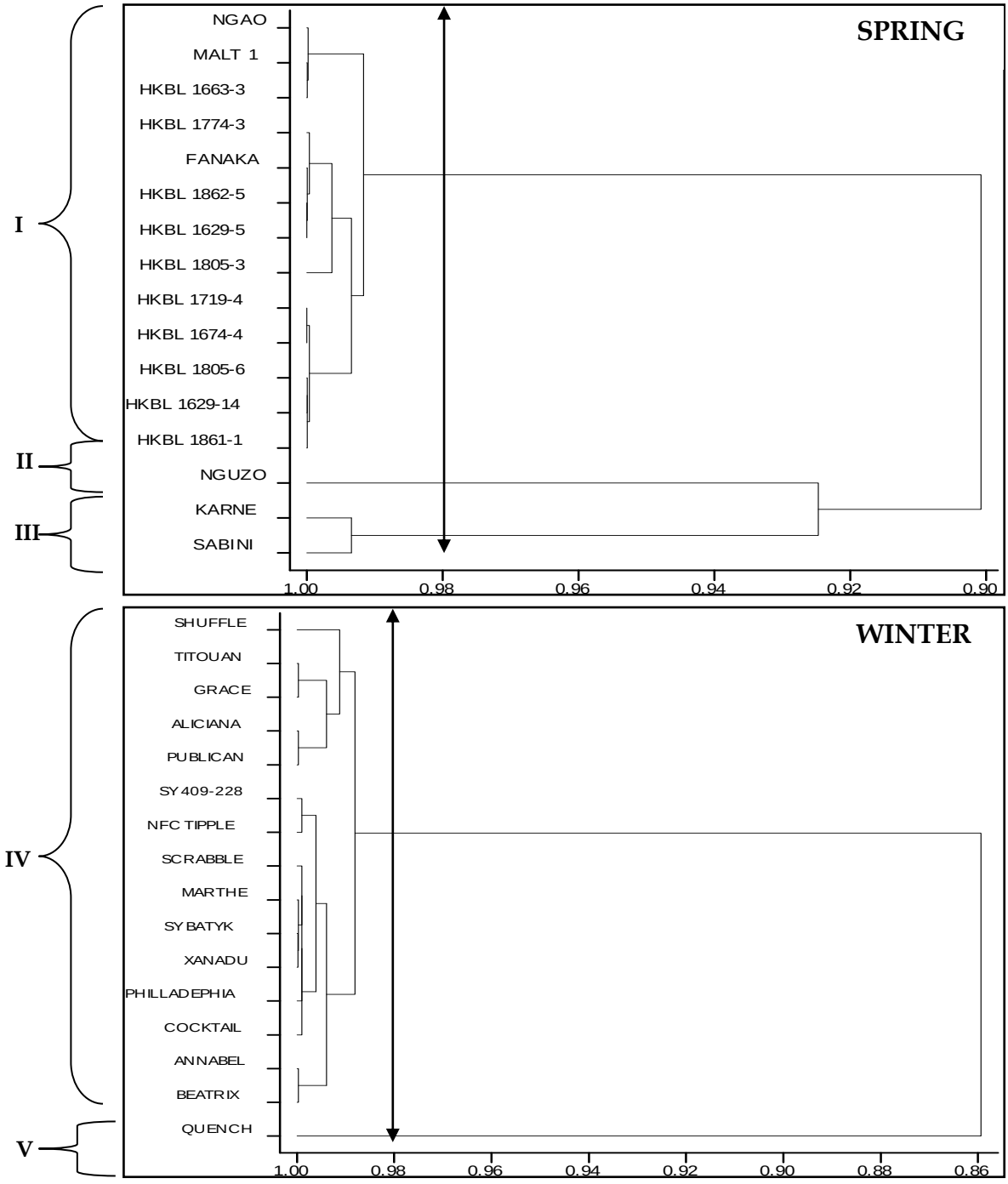


Figure 8: Dendrogram grouping of winter and spring barley genotypes in terms of their response to net blotch disease under field conditions. Five major groups '**I – V**' were formed at 0.98 similarity matrix

Contrary to the spring barley, the winter genotypes formed two main groups in terms of their response to net blotch fungal infection under field conditions thus less diverse. For instance, the largest group 'IV' showed two other sub-groups consisting of the most tolerant (SHUFFLE, TITOUAN, GRACE, ALICIANA and PUBLICAN) and susceptible genotypes which further differed in terms of the level of susceptibility to net blotch. QUENCH which expressed the highest disease level on average formed its own group 'V' as it expressed the highest foliar infection under field conditions across all the agro ecological zones (Figure 8). Therefore, the spring barley expressed more diversity than winter barley in terms of foliar infection by the net blotch fungus.

4.1.4 Multiple tolerance to biotic and abiotic stresses in winter and spring barley

Triple, dual and single tolerances were observed among the spring and winter barley in terms of net blotch disease, aluminium toxicity (hematoxylin) and drought (MSI) stresses. Multiple tolerances were expressed by only two genotypes out of 16 spring barley genotypes and these were FANAKA and MALT 1. Dual tolerance to net blotch foliar infection and aluminium toxicity was observed in another two genotypes namely HKBL 1663-3 and HKBL 1862-5. In all the commercially grown spring barley, single tolerance was the common feature. For example, KARNE and NGUZO were only tolerant to aluminium toxicity but expressed high susceptibility to net blotch and drought stress. Similarly, NGAO together with HKBL1629-5 and SABINI showed tolerance to net blotch disease and drought stress respectively but were susceptible to other stress factors (Table 7).

Table 7: Multiple tolerances to net blotch foliar infection, drought and Al toxicity stresses among the genetically stable winter and spring barley genotypes in Kenya. The (+) are susceptible or sensitive while the (-) are tolerant to stress factors

MULTIPLE TOLERANCE BY SPRING ADAPTED BARLEY				MULTIPLE TOLERANCE BY WINTER ADAPTED BARLEY			
Genotype	**Net blotch (0-9 scale)**	**Al (Hematoxylin)**	**Drought (MSI)**	**Genotype**	**Net blotch (0-9 scale)**	**Al (Hematoxylin)**	**Drought (MSI)**
FANAKA	-	-	-	ALICIANA	-	-	-
HKBL 1629-14	+	+	+	ANNABEL	+	+	-
HKBL 1629-5	-	+	+	BEATRIX	+	+	+
HKBL 1663-3	-	-	+	COCKTAIL	+	-	+
HKBL 1674-4	+	+	+	GRACE	-	-	-
HKBL 1719-4	+	+	+	MARTHE	+	+	+
HKBL 1774-3	-	+	+	NFC TIPPLE	+	-	+
HKBL 1805-3	+	-	+	PHILLADEPHIA	+	-	+
HKBL 1805-6	+	-	+	PUBLICAN	-	+	+
HKBL 1861-1	+	-	+	QUENCH	+	+	+
HKBL 1862-5	-	-	+	SCRABBLE	+	+	-
KARNE	+	-	+	SHUFFLE	-	-	+
MALT 1	-	-	-	SY 409-228	+	-	+
NGAO	-	+	+	SY BATYK	+	-	+
NGUZO	+	-	+	TITOUAN	-	+	+
SABINI	+	+	-	XANADU	+	+	-

KEY					
	Net blotch	+	Above 3.5 on 0 -9 severity scale	-	Below 3.5 on 0 - 9 severity scale
	Aluminium	+	Above 2.5 on 1 - 5 staining scale	-	Below 2.5 on 1 - 5 staining scale
	Drought	+	Below 65 on % MSI	-	Above 65 on % MSI

Among winter barley, only GRACE and ALICIANA expressed triple tolerance to net botch, drought and Al cation toxicity. Dual tolerance to net blotch and aluminium toxicity was noted in SHUFFLE only. PUBLICAN and TITOUAN expressed single tolerance to net blotch foliar infection while COCKTAIL, NFC TIPPLE, PHILLADEPHIA, SY 409-228 and SY BATYK showed single tolerance to aluminium toxicity. ANNABEL, SCRABBLE and XANADU were only tolerant to drought but susceptible to the other stress factors. Similar to the spring barley, none of the commercially produced winter barley expressed either triple or dual tolerance. Instead, QUENCH exhibited triple susceptibility to the three stresses while COCKTAIL was only tolerant to aluminium toxicity (Table 7).

4.2 Interaction effect of drought and Al toxicity on net blotch severity in barley

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.

4.2.1 Effect of inherent traits on net blotch under the influence of abiotic stress

Under the significant influence of drought and aluminium toxicity as the abiotic stresses ($p < 0.05$) in spring barley, the inherent barley traits did not have significant effect on net blotch foliar infection ($p < 0.05$). However, through contrast comparison, only drought tolerant spring barley differed significantly from those genotypes with known tolerance to aluminium toxicity (Appendix xi). Similar contrast questions proved to be very different under the significant interaction ($p < 0.05$) effect between abiotic stresses and inherent barley traits. For instance, when inherent traits interact with abiotic stresses, the aluminium tolerant and sensitive genotypes; net blotch tolerant and susceptible genotypes; drought tolerant and sensitive genotypes and aluminium tolerant and drought tolerant genotypes comparisons differed significantly in terms of net blotch foliar infection ($p < 0.05$) (Appendix xi).

With respect to the significant interaction effect between inherent trait and time, only drought tolerant and drought sensitive as well as the aluminium tolerant and net blotch tolerant comparisons differed significantly ($p < 0.05$). The rest of the other comparisons did not differ significantly ($p > 0.05$). Under the complex and significant interaction effect

of abiotic stresses, inherent barley traits and time interval post inoculation, all the contrast comparisons between each of the barley traits had significant effects on net blotch foliar infection ($p < 0.05$) (Appendix xi).

The contrast comparison for the three stress factors including drought, aluminium toxicity and unstressed conditions for the spring barley revealed that there were significant differences in net blotch disease severity from one stress factor to the other ($p < 0.05$) (Appendix xii).

For example, in terms of mean scores, spring barley genotypes had progressive disease increase in leaves observed from 7 to 35 days after inoculation. After initial exposure to aluminium stress, genotypes exhibiting tolerance to drought (DRT1) and susceptibility to net blotch (NBS1) expressed low disease levels and both recorded 1.7 on a 0 – 7 severity scale 35 days after inoculation. However, under initial exposure to drought stress, the DRT1 and NBS1 genotypes became highly susceptible and recorded high disease severity at 3.8 and 2.3 respectively 35 days after inoculation (Table 8).

Table 8: Net blotch disease severity among the SPRING barley genotypes exhibiting specific traits under the influence of initial exposure to aluminium cation toxicity and drought stress in controlled conditions

STRESS FACTOR	TRAIT	DISEASE SEVERITY (1-5 SCALE) - DAYS AFTER INOCULATION (DAI)					MEAN	
		7 DAI	14 DAI	21 DAI	28 DAI	35 DAI	TRAIT	STRESS
ALUMINIUM TOXICITY	ALS1	0.3	0.8	0.8	1.5	2.5	**1.2**	**1.4**
	ALS2	0.3	0.3	1.0	1.8	1.8	**1.1**	
	ALT1	0.3	0.7	1.0	1.8	2.8	**1.3**	
	ALT2	0.5	1.0	1.7	2.2	2.5	**1.6**	
	DRS1	0.3	1.0	1.5	1.5	2.5	**1.4**	
	DRS2	0.5	0.7	1.2	2.8	3.5	**1.7**	
	DRT1	0.7	0.8	1.2	1.3	1.7	**1.1**	
	DRT2	0.7	0.8	1.5	1.7	2.0	**1.3**	
	NBS1	1.0	1.2	1.5	1.5	1.7	**1.4**	
	NBS2	0.3	0.8	1.7	2.0	2.3	**1.4**	
	NBT1	0.5	0.8	1.8	1.8	2.3	**1.5**	
	NBT2	0.5	0.8	1.3	1.7	1.8	**1.2**	
AV.		**0.5**	**0.8**	**1.3**	**1.8**	**2.3**	**1.4**	
DROUGHT STRESS	ALS1	0.3	1.5	1.7	2.5	3.0	**1.8**	**1.6**
	ALS2	0.2	0.3	0.8	1.2	2.5	**1.0**	
	ALT1	0.7	1.0	1.3	2.2	3.0	**1.6**	
	ALT2	1.0	1.2	1.8	1.8	2.3	**1.6**	
	DRS1	0.5	1.0	1.5	1.7	2.7	**1.5**	
	DRS2	0.3	0.8	1.0	1.7	1.8	**1.1**	
	DRT1	0.8	1.3	1.5	3.2	3.8	**2.1**	
	DRT2	1.0	1.5	1.7	1.8	2.7	**1.7**	
	NBS1	0.3	0.8	0.8	1.8	2.3	**1.2**	
	NBS2	0.8	1.2	1.3	1.8	2.5	**1.5**	
	NBT1	0.3	1.5	1.5	1.8	2.5	**1.5**	
	NBT2	0.8	1.2	1.5	2.5	3.2	**1.8**	
AV.		**0.6**	**1.1**	**1.4**	**2.0**	**2.7**	**1.6**	
UNSTRESSED	ALS1	0.7	1.0	1.5	1.5	2.5	**1.4**	**1.8**
	ALS2	1.0	1.5	1.5	2.3	2.8	**1.8**	
	ALT1	0.8	1.0	1.2	1.8	2.8	**1.5**	
	ALT2	0.3	0.3	1.0	2.2	3.3	**1.4**	
	DRS1	0.3	0.5	1.0	3.0	4.0	**1.8**	
	DRS2	0.8	1.0	1.3	3.7	4.3	**2.2**	
	DRT1	1.0	1.2	1.2	2.3	3.3	**1.8**	
	DRT2	0.7	1.0	1.8	3.0	3.5	**2.0**	
	NBS1	0.8	1.2	1.3	3.0	4.0	**2.1**	
	NBS2	0.3	1.2	1.2	2.5	3.5	**1.7**	
	NBT1	1.0	1.7	1.7	1.8	2.3	**1.7**	
	NBT2	0.8	1.0	1.2	2.5	3.2	**1.7**	
AV.		**0.7**	**1.0**	**1.3**	**2.5**	**3.3**	**1.8**	
MEAN (DAI)		**0.6**	**1.0**	**1.3**	**2.1**	**2.8**	**1.6**	

	Plant stress (PS)	Plant trait (PT)	Infection time (IT)	PS x PT	PS x IT	PT x IT	PS x PT x IT
Probability	0.005	0.051	<0.001	<0.001	<0.001	<0.001	<0.001
S.E	0.0409	0.0793	0.0321	0.1376	0.0644	0.1272	0.2206
S.E.D	0.0578	0.1121	0.0454	0.1947	0.0911	0.1799	0.312
% CV	21.4						

Under unstressed conditions, all spring barley expressed higher disease levels irrespective of their inherent traits. Among the traits that recorded the highest disease levels when unstressed include all drought sensitive and net blotch susceptible genotypes (DRS1,

DRS2, NBS1, NBS2). The most sensitive genotype to aluminium toxicity (ALS1) and the most tolerant genotype to net blotch disease (NBT1) remained tolerant under unstressed conditions. The overall mean for unstressed condition remained higher at 1.8 on a 0 – 7 severity scale irrespective of the inherent trait and this further confirms the findings (Table 8).

Initial exposure of spring barley to aluminium cation toxicity and drought stress resulted into low disease severity at 1.4 and 1.6 respectively on a 0 – 7 severity scale under greenhouse conditions. In addition, most barley responded differently depending on their inherent trait. Based on traits, the second most drought tolerant genotype (DRT2) gave a more susceptible reaction to net blotch under unstressed condition ‘a’ but after being exposed to aluminium and drought stresses, this genotype became more tolerant to net blotch foliar infection. However, NGAO which is known to have NBT1 and ALS2 traits maintained tolerance response across the stressed and unstressed conditions. Irrespective of the inherent traits, the pathogenicity of *Pyrenophora teres* was slightly higher under unstressed conditions compared to those initially subjected drought and aluminium toxicity stresses before inoculation (Plate 6).

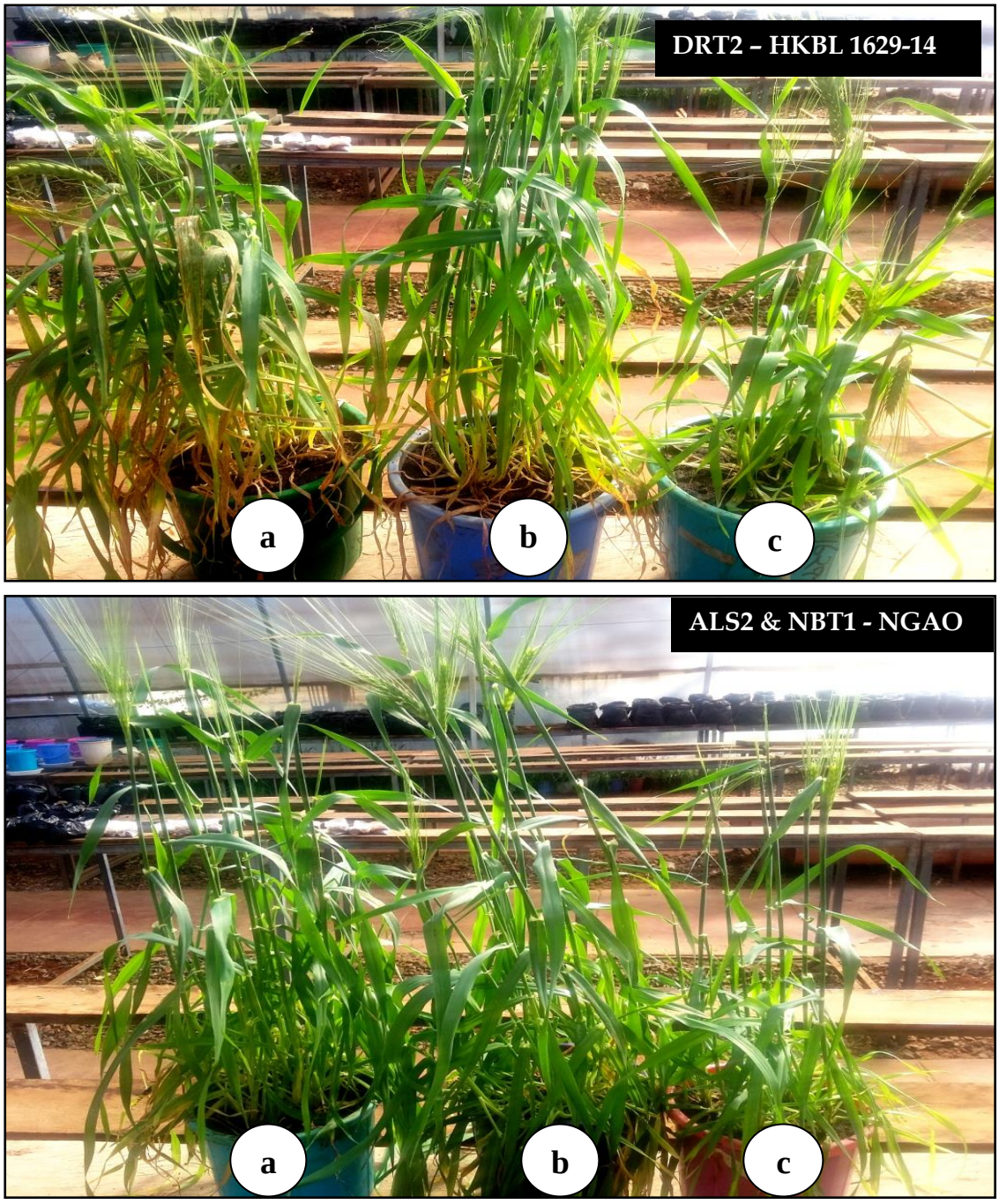


Plate 6: Net blotch disease severity as expressed by HKBL 1629-14 (Drought tolerant) and NGAO (Net blotch tolerant but sensitive to Al toxicity) genotypes under unstressed 'a', aluminium toxicity 'b' and drought stress 'c' conditions (Source: Author, 2017)

Among the trait specific winter adapted barley, initial exposure to aluminium toxicity, drought stress and unstressed conditions did not have significant effect on pathogenicity

of *P. teres* ($p > 0.05$). However, the inherent traits had significant effect in the disease severity ($p < 0.05$) and in this regard, the mean separation through contrast comparison confirmed that winter barley with known tolerance and known susceptibility to aluminium and drought differed significantly in their severity levels on net blotch disease ($p < 0.05$). Further, drought tolerant (DRT 1&2) and net blotch tolerant (NBT 1&2) winter barley differed significantly in their response to net blotch disease ($p < 0.05$) (Appendix xiii).

The two-way interaction between stress factors and inherent traits had significant effect on net blotch severity ($p < 0.05$). Additionally, only drought tolerant and sensitive (DRT1&2 Vs DRS1&2) as well as aluminium and net blotch tolerant (ALT 1&2 Vs NBT1&2) comparisons did not differ significantly ($p > 0.05$). The three-way interaction was significant ($p < 0.05$) just as earlier observed in spring barley. However, unlike the spring barley, not all contrast comparison questions were significant. Specifically, drought and aluminium tolerant (DRT1&2 Vs ALT1&2) as well as drought tolerant and sensitive (DRT1&2 Vs DRS1&2) comparisons did not differ significantly ($p > 0.05$) (Table 9, Appendix xiii).

Disease severity under the influence of drought and aluminium toxicity as well as drought and unstressed conditions did not differ significantly ($p > 0.05$). However, the disease severity under the influence of aluminium toxicity differed significantly from unstressed conditions ($p < 0.05$) (Table 9, Appendix xiv).

Table 9: Net blotch disease severity among the WINTER barley genotypes exhibiting specific traits under the influence of initial exposure to aluminium cation toxicity and drought stress in controlled conditions

STRESS FACTOR	TRAIT	DISEASE SEVERITY (1-5 SCALE) - DAYS AFTER INOCULATION (DAI)					MEAN	
		7 DAI	14 DAI	21 DAI	28 DAI	35 DAI	TRAIT	STRESS
ALUMINIUM TOXICITY	ALS1	1.0	1.5	2.0	2.5	2.8	**2.0**	
	ALS2	0.5	0.8	1.0	1.8	2.5	**1.3**	
	ALT1	0.7	1.5	1.7	2.3	2.5	**1.7**	
	ALT2	0.5	1.0	1.7	2.3	2.8	**1.7**	
	DRS1	1.0	1.5	2.0	2.5	2.8	**2.0**	
	DRS2	0.5	0.8	1.0	1.8	2.5	**1.3**	
	DRT1	0.7	1.5	1.7	2.3	2.5	**1.7**	
	DRT2	0.5	1.0	1.7	2.3	2.8	**1.7**	
	NBT1	1.0	1.5	2.0	2.5	2.8	**2.0**	
	NBT2	0.5	0.8	1.0	1.8	2.5	**1.3**	
	UNSTRESSED	0.7	1.5	1.7	2.3	2.5	**1.7**	
	UNSTRESSED	0.5	1.0	1.7	2.3	2.8	**1.7**	
DROUGHT	ALS1	1.0	1.5	2.0	2.5	2.8	**2.0**	
	ALS2	0.5	0.8	1.0	1.8	2.5	**1.3**	
	ALT1	0.7	1.5	1.7	2.3	2.5	**1.7**	
	ALT2	0.5	1.0	1.7	2.3	2.8	**1.7**	
	DRS1	1.0	1.5	2.0	2.5	2.8	**2.0**	
	DRS2	0.5	0.8	1.0	1.8	2.5	**1.3**	
	DRT1	0.7	1.5	1.7	2.3	2.5	**1.7**	
	DRT2	0.5	1.0	1.7	2.3	2.8	**1.7**	
	NBT1	1.0	1.5	2.0	2.5	2.8	**2.0**	
	NBT2	0.5	0.8	1.0	1.8	2.5	**1.3**	
	UNSTRESSED	0.7	1.5	1.7	2.3	2.5	**1.7**	
	UNSTRESSED	0.5	1.0	1.7	2.3	2.8	**1.7**	
UNSTRESSED	ALS1	1.0	1.5	2.0	2.5	2.8	**2.0**	
	ALS2	0.5	0.8	1.0	1.8	2.5	**1.3**	
	ALT1	0.7	1.5	1.7	2.3	2.5	**1.7**	
	ALT2	0.5	1.0	1.7	2.3	2.8	**1.7**	
	DRS1	1.0	1.5	2.0	2.5	2.8	**2.0**	
	DRS2	0.5	0.8	1.0	1.8	2.5	**1.3**	
	DRT1	0.7	1.5	1.7	2.3	2.5	**1.7**	
	DRT2	0.5	1.0	1.7	2.3	2.8	**1.7**	
	NBT1	1.0	1.5	2.0	2.5	2.8	**2.0**	
	NBT2	0.5	0.8	1.0	1.8	2.5	**1.3**	
	UNSTRESSED	0.7	1.5	1.7	2.3	2.5	**1.7**	
	UNSTRESSED	0.5	1.0	1.7	2.3	2.8	**1.7**	

In terms of inherent traits, majority of the winter barley responded differently to net blotch disease in respect to their previously observed traits. However, majority of the response pattern were almost similar to those of the spring genotypes. For example, under aluminium toxicity, the most tolerant genotype to net blotch (NBT1) proved to be the

most tolerant to disease 35 days after inoculation. In contrast, this same genotype expressed higher disease level under drought stress and again lower disease under unstressed conditions (Table 9).

Additional results indicate that genotypes known to be the most tolerant to aluminium (ALT1) showed lower disease levels under the influence of aluminium cation toxicity by scoring 2.8 on a 0 – 7 severities scale 35 DAI. However, under drought and unstressed conditions, the same genotype was highly susceptible to net blotch foliar infection at 3.7 and 4.5 severities rating respectively (Table 9).

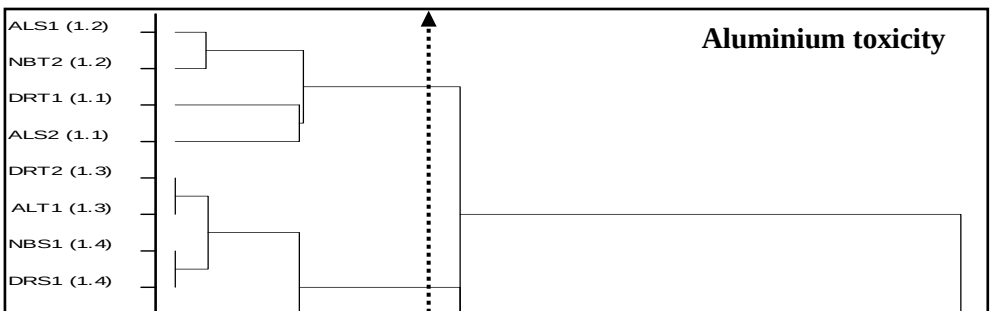
For seedling resistance to net blotch disease under the influence of stress factors, it was observed that majority of the genotypes under drought and aluminium toxicity recorded low disease levels except ALS1 which showed higher disease symptoms 7 DAI compared to the rest. However, under unstressed conditions, higher disease severity was observed in most of the genotypes compared to the observations under stressed conditions. To start with, ALS1 genotype expressed not only the highest disease under unstressed condition but also under drought and aluminium toxicity conditions thus low seedling resistance. However, DRT2 genotype exhibited seedling resistance across all stress and unstressed conditions thus higher seedling resistance (Table 9).

The Euclidean test in spring barley with special traits revealed the formation of four main clusters in barley exposed aluminium toxicity and unstressed conditions. The barley under drought formed only three main groups (Figure 9). For example, under the influence of aluminium toxicity, ALS1, NBT2, DRT1 and ALS2 grouped together as the most tolerant genotypes to net blotch foliar infection. Similarly, DRS2 genotype formed its own cluster

as the most sensitive to net blotch disease under the influence of aluminium toxicity (Figure 9).

Under drought stress, only ALS1 and NBT2 clustered together in terms of foliar infection by net blotch disease while ALS2 and DRT1 formed different clusters under the influence of drought stress. Under drought stress, DRT1 expressed the highest susceptibility but the same genotype showed high tolerance to net blotch under aluminium toxicity (Figure 9).

The unstressed condition further gave a more diverse grouping of spring barley compared to the groupings under aluminium toxicity and drought stress conditions. However, unlike under drought stress, the NBT1, DRT1 and ALS2 clustered together just like that observed under aluminium toxicity. Also, under unstressed condition, all the genotypes known to be tolerant to aluminium (ALT1 and ALT2) and net blotch (NBT1 and NBT2) expressed similar responses thus clustered together. Genotypes with tolerance to aluminium were the most tolerant to net blotch disease under unstressed conditions. Drought tolerant genotypes had mixed reactions to the disease and did not cluster together (Figure 9).



The Euclidean test on the winter genotypes gave completely different trait groupings under stress and unstressed conditions compared to the spring barley but all formed three clusters under drought, aluminium toxicity and unstressed conditions. For instance, ALS1 genotype clustered together with other genotypes under the influence of aluminium toxicity in spring barley but, in winter barley, ALS1 formed a unique cluster of its own and was very sensitive to net blotch foliar infection across other growth conditions (Figure 10).

Under drought condition, ALS1, NBT1 and NBS2 were the most sensitive to net blotch disease while DRT2, ALS2, NBS1 and DRT1 were the most tolerant and formed two separate groups. Moreover, the NBS1 and NBS2 genotypes which are known to be most susceptible to net blotch disease recorded lower disease levels under the influence of drought and aluminium toxicity compared to the higher disease level under unstressed conditions. Similar observation was made for the ALS1 genotype which is known to be the most sensitive to aluminium toxicity except for the DRS1 (most sensitive to drought) genotype which expressed tolerance to net blotch disease across the diverse conditions of stress factors (Figure 10).

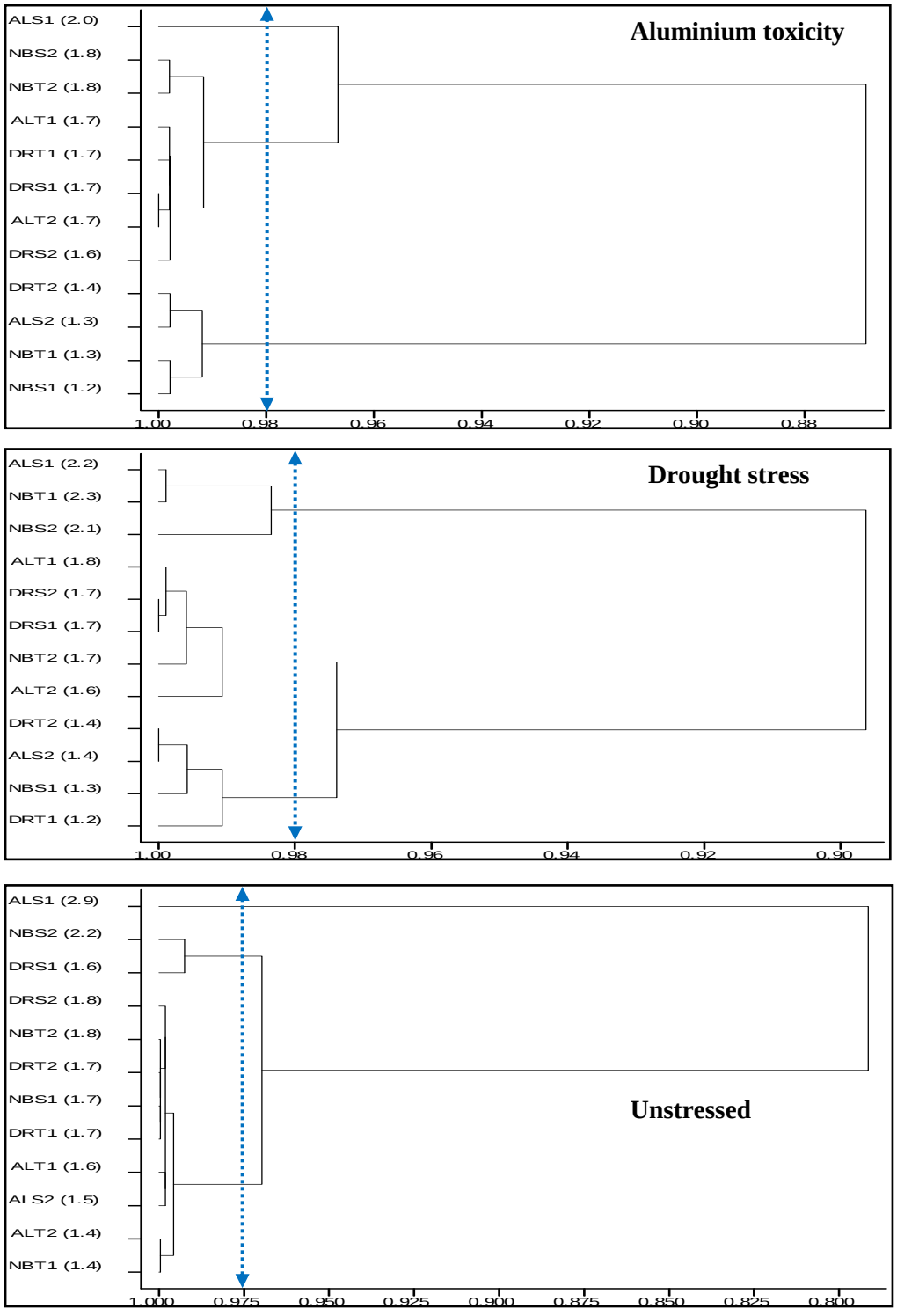


Figure 10: Grouping of trait-specific WINTER barley genotypes in terms of net blotch severity levels under the influence of aluminium toxicity, drought stress and unstressed conditions. Grouping was achieved through Euclidean test and nearest neighbor cluster analysis

4.3 Hormonal signaling effect on disease and abiotic stresses in barley

Trait-specific spring and winter barley responded differently to net blotch disease, aluminium cation toxicity and drought stresses under the influence of different phytohormones and their combinations.

4.3.1 Hormonal signaling effect on net blotch foliar infection in barley

Phytohormones as well as the two-way and three-way interactions among phytohormones, spring barley traits and time taken after inoculation had significant influence on net blotch severity under greenhouse conditions ($p < 0.05$). The contrast comparison on treatment means revealed the existence of significant differences between ABA and SA; ABA and CONTROL; ABA and SAxABA; and SA and CONTROL treatments on net blotch foliar infection. However, SA and SAxABA comparisons did not differ significantly in terms of net blotch disease severity (Appendix xv).

Irrespective of the inherent spring barley traits, barley treated with SA recorded the least disease levels hence were the most tolerant to net blotch disease. For example, when treated with SA, NBS2 genotype which is known to be susceptible to the disease was the most tolerant 35 DAI but NBS1 (known to be most susceptible), remained susceptible to net blotch. However, SAxABA combination proved to be not only inhibitory in disease progress but also synergistic in effect. In this regard, even the most susceptible genotypes expressed tolerance to net blotch foliar infection when treated with hormones (Table 10).

Table 10: Net blotch foliar disease severity under the influence of phytohormones and their combinations on trait-specific SPRING barley genotypes under greenhouse conditions

PHYTOHORMONE	BARLEY TRAIT	NET BLOTCH SEVERITY (0-7 SEVERITY SCALE) - SPRING BARLEY					MEAN (TRAIT)	MEAN (PHYTOHORMONE)
		7 DAI	14 DAI	21 DAI	28 DAI	35 DAI		
ABA	NBS1	1.8	2.8	5.8	6.0	6.5	**4.6**	**3.1**
	NBS2	1.7	2.7	3.2	3.5	3.8	**3.0**	
	NBT1	0.7	1.7	3.0	3.0	3.0	**2.3**	
	NBT2	1.0	1.8	2.7	2.7	3.2	**2.3**	
CONTROL	NBS1	2.2	3.5	5.3	5.7	6.0	**4.5**	**3.5**
	NBS2	1.7	3.3	5.5	5.5	5.7	**4.3**	
	NBT1	1.2	2.0	3.2	3.3	3.3	**2.6**	
	NBT2	1.7	2.7	3.0	3.0	3.2	**2.7**	
SA	NBS1	1.5	2.3	3.7	3.7	3.8	**3.0**	**2.2**
	NBS2	1.0	1.7	2.3	2.0	2.3	**1.9**	
	NBT1	0.7	1.0	1.8	2.0	2.5	**1.6**	
	NBT2	1.2	1.7	2.3	2.7	2.8	**2.1**	
SA x ABA	NBS1	0.8	1.3	2.2	2.5	3.0	**2.0**	**2.3**
	NBS2	1.5	2.0	2.5	2.7	3.0	**2.3**	
	NBT1	1.7	2.3	3.0	3.2	3.5	**2.7**	
	NBT2	1.3	2.0	2.5	2.5	2.8	**2.2**	
MEAN (DAI)		**1.3**	**2.2**	**3.3**	**3.4**	**3.7**	**2.8**	**2.8**

	PHYTOHORMONE (PH)	TRAIT (TR)	TIME (TI)	PH x TR	PH x TI	TR x TI	PH x TR x TI
Probability	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
S.E	0.1074	0.0876	0.0499	0.1859	0.1397	0.1251	0.2577
S.E.D	0.1519	0.1239	0.0706	0.2629	0.1975	0.1769	0.3645
% CV	12.5						

ABA on its own did not have much inhibitory effect in terms of disease severity compared to SA and SAxABA combinations. When treated with ABA, pathogenicity and disease severity were higher especially in those genotypes known to be susceptible to the disease (i.e. NBS1 and NBS2) and by 35 DAI, NBS1 rated as high as 6.5 on a 0 – 7 severity scale. Moreover, net blotch tolerant genotypes maintained their true traits and expressed low disease levels compared to those known to be susceptible. In control pots (no hormone), all spring barley had higher disease levels than those treated with hormones. The disease severity was also noted to be high from 7 DAI to 35 DAI (Table 10).

In reference to a specific genotype e.g. NBS2 know to be the second most susceptible to net blotch disease, the genotype expressed much tolerance to net blotch foliar infection under the influence of SA and SAxABA combination. However, when treated with ABA

alone, the level of infection was very high especially on the lower leaves as the disease progressed from 7 DAI to 35 DAI. Under no hormonal treatment, this genotype was very susceptible to net blotch which was not only expressed on symptoms but also through stunted growth (Plate 7).

Plate 7: Signaling effect of exogenous phytohormones on net blotch foliar infection and severity as expressed in KARNE genotype, previously found to be the second most susceptible to the disease (NBS2). Source: Author, 2017

For the winter barley, the phytohormones as well as the interactions among the hormones, time interval and traits had significant effects on net blotch severity ($p < 0.05$). However, only ABA and SAxABA mean comparison did not differ significantly ($p > 0.05$) in winter genotypes. This observation is different from that of the spring barley which expressed

significant differences between ABA and SAxABA treatments. The rest of the mean comparisons differed significantly from each other through contrast, comparison (Appendix xvi).

The winter barley genotypes also expressed similar mean response pattern just like the spring genotypes in terms of hormonal signaling effect. For instance, SA had the inhibitory effect on disease progress thus low severity at 2.1 on average. In addition, ABA alone recorded the second lowest disease in winter barley but this did not differ significantly from the mean score under SAxABA treatments which averagely expressed 2.6 and 3.0 for ABA and SAxABA respectively (Table 11).

Table 11: Net blotch foliar disease severity under the influence of phytohormones and their combinations on trait-specific WINTER barley genotypes under greenhouse conditions

PHYTOHORMONE	BARLEY TRAIT	NET BLOTCH SEVERITY (0-7 SEVERITY SCALE) - WINTER BARLEY					MEAN (TRAIT)	MEAN (PHYTOHORMONE)
		7 DAI	14 DAI	21 DAI	28 DAI	35 DAI		
ABA	NBS1	1.5	2.2	2.5	3.2	3.7	**2.6**	**2.6**
	NBS2	1.5	2.8	4.8	4.8	5.2	**3.8**	
	NBT1	1.3	2.0	2.5	2.5	2.8	**2.2**	
	NBT2	0.8	1.2	2.2	2.5	2.5	**1.8**	
CONTROL	NBS1	2.0	3.3	5.3	5.5	5.8	**4.4**	**3.7**
	NBS2	1.7	3.0	5.8	6.0	6.0	**4.5**	
	NBT1	1.2	2.5	3.2	3.3	3.8	**2.8**	
	NBT2	1.3	2.8	3.3	3.7	3.7	**3.0**	
SA	NBS1	1.7	2.0	2.5	2.7	3.0	**2.4**	**2.1**
	NBS2	1.3	2.0	2.7	3.0	3.3	**2.5**	
	NBT1	0.8	1.3	1.7	1.8	2.3	**1.6**	
	NBT2	1.3	1.7	2.0	2.2	2.5	**1.9**	
SA x ABA	NBS1	2.0	2.7	3.8	3.8	4.5	**3.4**	**3**
	NBS2	2.0	3.0	4.5	4.8	4.8	**3.8**	
	NBT1	1.7	2.2	2.7	2.7	3.2	**2.5**	
	NBT2	1.0	2.0	2.8	3.0	3.2	**2.4**	
MEAN (DAI)		**1.4**	**2.3**	**3.3**	**3.5**	**3.8**	**2.9**	**2.9**

	PHYTOHORMONE (PH)	TRAIT (TR)	TIME (TI)	PH x TR	PH x TI	TR x TI	PH x TR x TI
Probability	0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.003
S.E	0.1376	0.0997	0.0468	0.2208	0.1611	0.1301	0.2771
S.E.D	0.1946	0.1409	0.0662	0.3122	0.2278	0.1841	0.3918
% CV	11.4						

In reference to the individual traits of the winter barley, enhanced tolerance was observed across all the genotypes treated with phytohormones and disease severity levels differed from one hormone to the other compared to the CONTROL treatment. Moreover, it is apparent that even under the signaling effect of hormones, barley traits also play significant roles in disease levels and the net blotch susceptible winter barley are more likely to show tolerance after phytohormone treatments (Table 11).

4.3.2 Hormonal signaling effect on aluminium cation toxicity in barley

In spring barley, the apical root length, plant height, root dry weight, number of fibrous root and shoot dry weight differed significantly under the influence of phytohormone and aluminium cation toxicity ($p < 0.05$). However, the additive effect between phytohormone and inherent barley trait were not significant for all variables above ($p > 0.05$) except on number of fibrous roots ($p < 0.05$) (Appendix xvii).

Mean separation for plant height and number of fibrous roots showed significant differences in the ABA and CONTROL; ABA and SA; ABA and SAxABA; SA and CONTROL; and SA and SAxABA comparisons ($p < 0.05$). However, for apical root length, only ABA and SA mean comparison did not have significant difference ($p > 0.05$). Similarly, for shoot dry weight, ABA and SAxABA comparison did not differ significantly while for the root dry weight, SA and CONTROL as well as ABA and SAxABA mean comparisons did not differ significantly ($p > 0.05$).

In terms of means, trait-specific spring barley genotypes known to be sensitive (ALS1 & 2) and tolerant (ALT1 & 2) to aluminium toxicity responded differently to aluminium

toxicity in terms of height, number of fibrous roots, root dry weight and shoot dry weight under the influence of phytohormones (Figure 11).

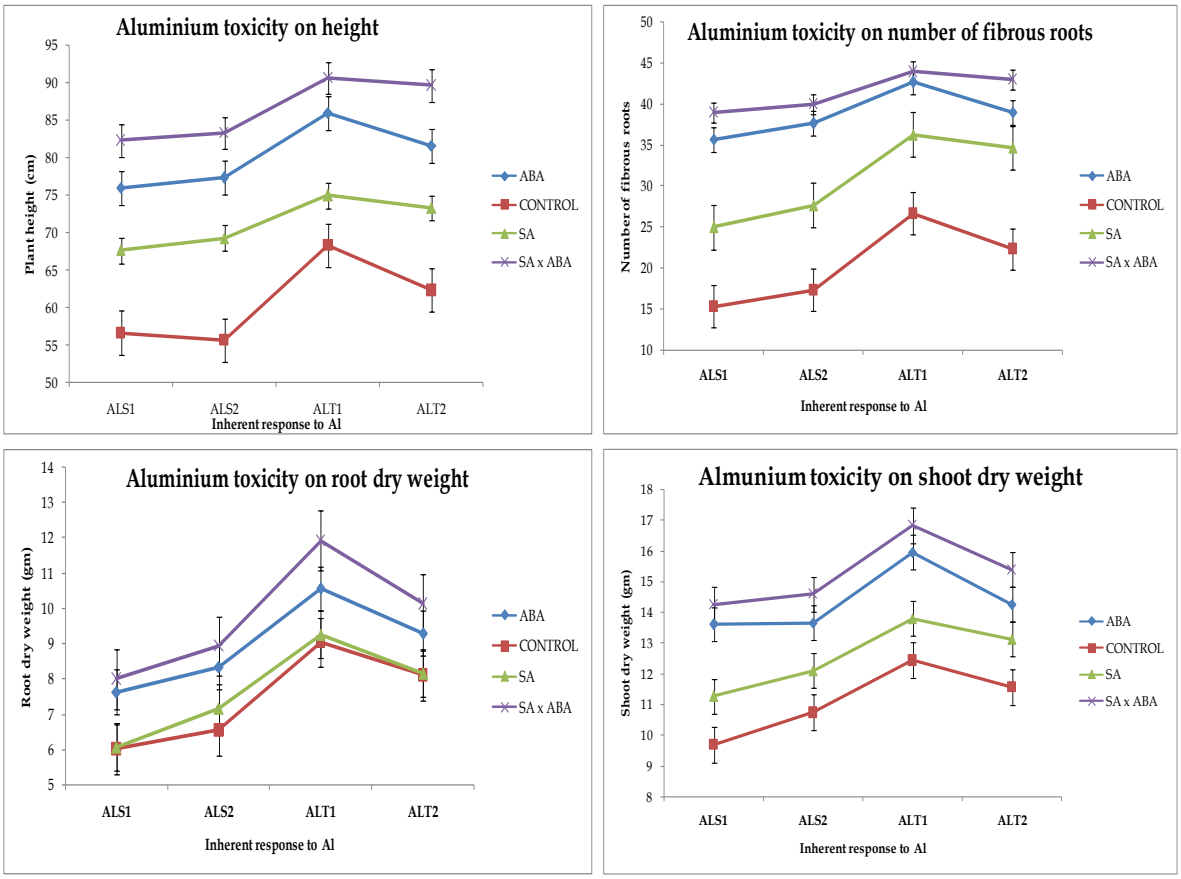


Figure 11: Response of SPRING barley genotypes to aluminium toxicity under the interaction effects of phytohormones and inherent traits

Therefore, SAxABA application on spring barley synergistically enhanced the highest tolerance level to aluminium toxicity through increased plant height, number of fibrous roots, shoot and root dry weights. Treatment with ABA alone also improved tolerance level better than SA but all the genotypes that were not treated with any phytohormone remained sensitive to aluminium toxicity (Figure 11).

Similar observations were made on winter barley with known response to aluminium toxicity in terms of significant effects of phytohormones ($p < 0.05$) and signaling effects to enhance tolerance to aluminium toxicity. However, the winter barley expressed low response to hormonal treatments compared to spring barley and a number of variables did not show significant difference under the influence of exogenous hormonal application through contrast comparison. For example, the enhancement effect of ABA on apical root length elongation under the influence of aluminium toxicity did not differ significantly from the effect of SA ($p > 0.05$). Similarly, for height and number of fibrous roots, ABA and SAxABA did not differ significantly in winter barley. The rest of the comparisons were significant ($p < 0.05$) (Figure 12, Appendix xviii).

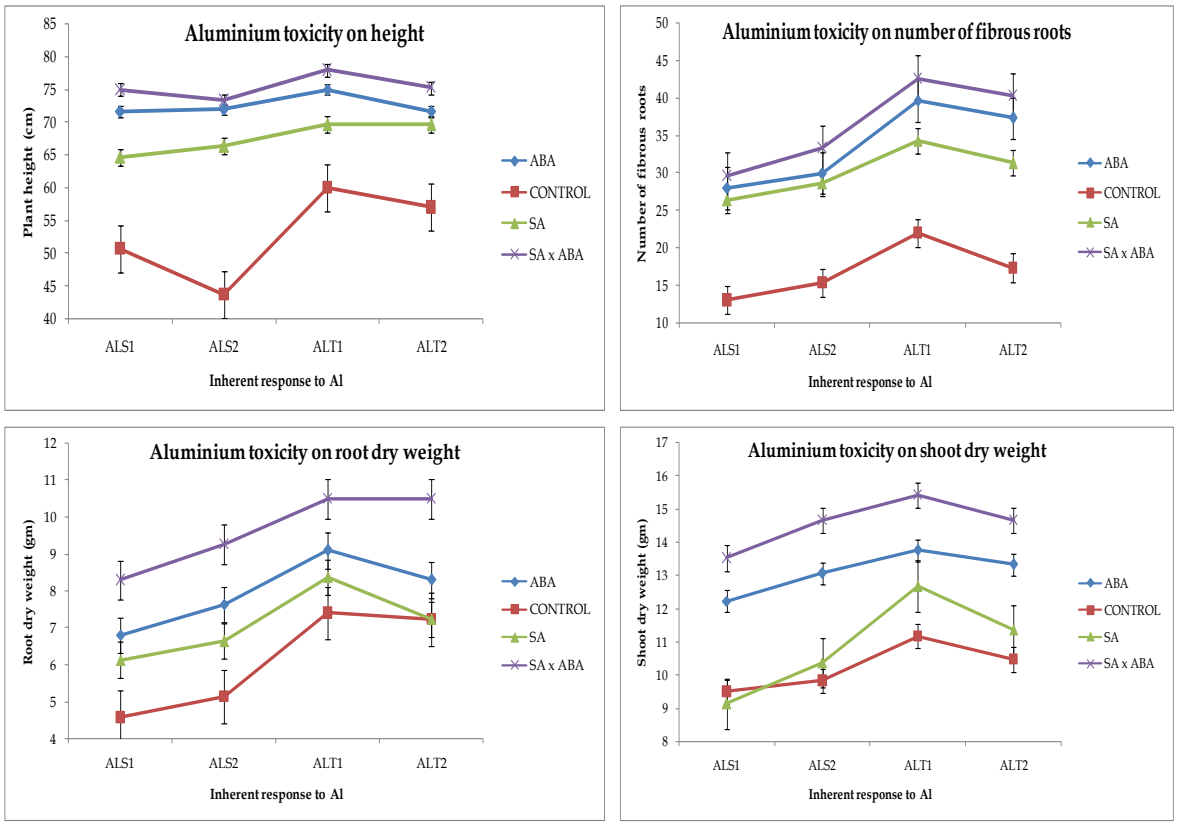


Figure 12: Response of WINTER barley genotypes to aluminium toxicity under the interaction effects of phytohormones and inherent traits

The level of tolerance to aluminium toxicity increased in winter barley known to be sensitive just like the tolerant genotypes under the influence of ABA, SA and SAxABA applications. Better growth in terms of height, more fibrous root formation and higher shoot and dry weights were common on genotypes treated with SAxABA which had more synergistic effect that when the two hormones are applied separately. However, ABA was better than SA in terms of enhancing tolerance to aluminium toxicity when applied alone on winter barley and this observation corresponds to the earlier observation on spring barley genotypes (Plate 8, Figure 13).

Plate 8: Root and shoot growth enhancement effect by phytohormones in aluminium sensitive winter and spring barley genotypes under 148 μM Al toxicity. Source: Author, 2017

Group behaviour in terms of apical root length elongation also confirmed that SAxABA had a synergistic effect in enhancing tolerant to aluminium toxicity in both winter and spring barley genotypes (Figure 13).

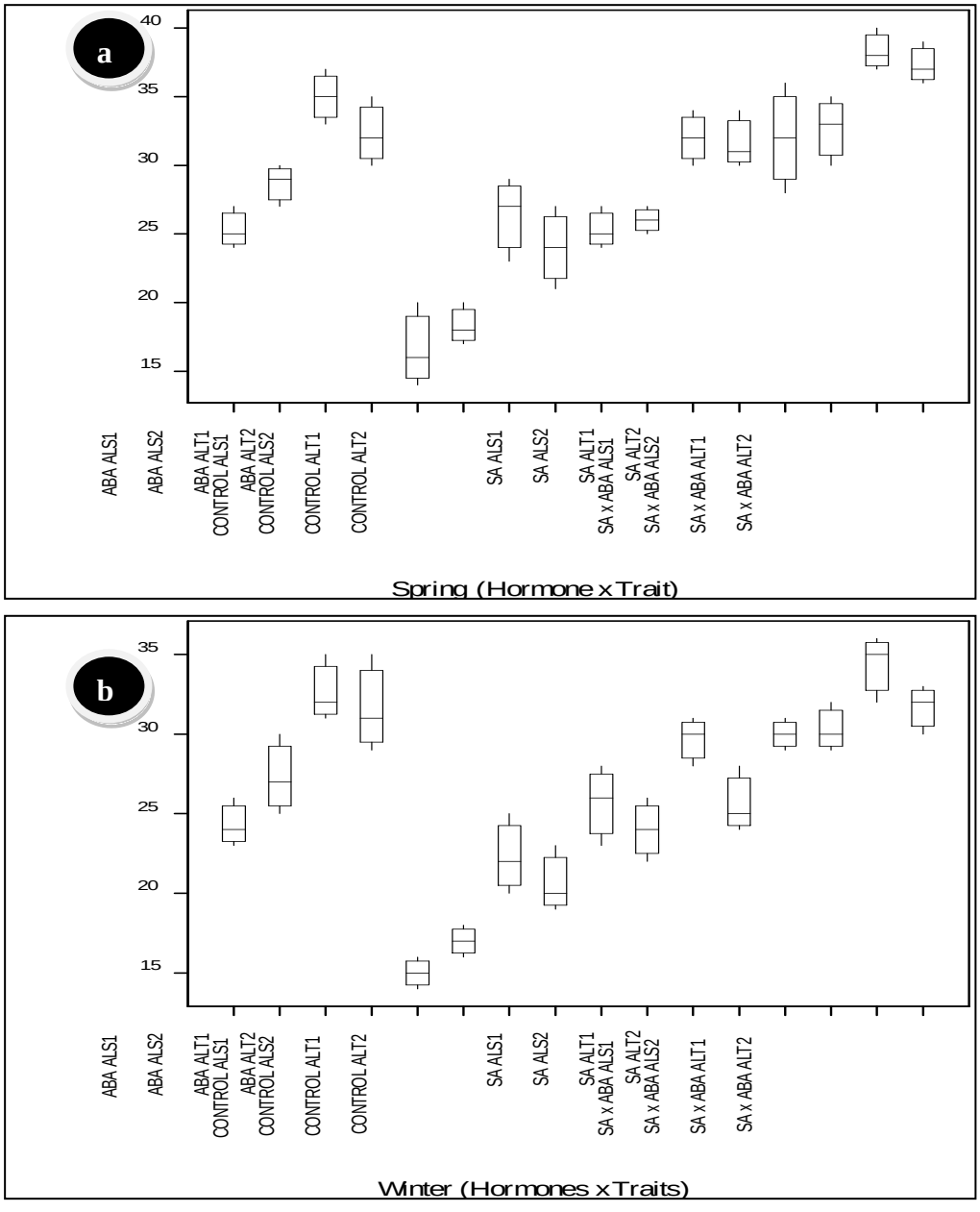


Figure 13: Hormonal signaling effect on response to aluminium toxicity in some trait-specific barley genotypes adapted to spring 'a' and winter 'b' growth conditions. S1 and T1 represent the most sensitive and tolerant genotypes while S2 and T2 represent the second most sensitive and tolerant respectively.

The tolerant genotypes in both winter and spring groups had higher apical root length when treated with ABA than when treated with SA as soil drench. The untreated genotypes were more inhibited by aluminium toxicity in their apical root length elongation than those that were treated with phytohormones (Figure 13).

4.3.3 Hormonal signaling effect on water stress (drought) in barley

Exogenous treatments with phytohormones significantly enhanced drought tolerance in barley under greenhouse conditions with reference to plant height, tillering ability, biomass accumulation (total dry weight) and membrane stability index. For spring barley, the interaction and additive effect between phytohormone and inherent barley traits significantly affected growth in terms of height and biomass accumulation ($p < 0.05$). However, the interaction between phytohormone and traits did not affect tillering ability and membrane stability index in spring barley ($p > 0.05$) (Appendix xix).

In winter barley, the phytohormones had significant effect on the same variables just like the spring barley ($p < 0.05$). Additionally, the additive effect of phytohormone and inherent traits were significant for growth in terms of height and tillering ability ($p < 0.05$) but such interactions did not have additive effects on biomass accumulation and membrane stability index ($p > 0.05$) (Appendix xx).

For height, the mean comparisons on the enhancement effect of phytohormones on height, tillering ability, total dry weight and membrane stability index revealed that SA, ABA, SAxABA and CONTROL differed significantly but this varied from one measurement to the other. Specifically, in spring barley, only ABA and SAxABA comparison did not

differ significantly in terms of the enhancement effect on height (Figure 14, Appendix xix and xx).

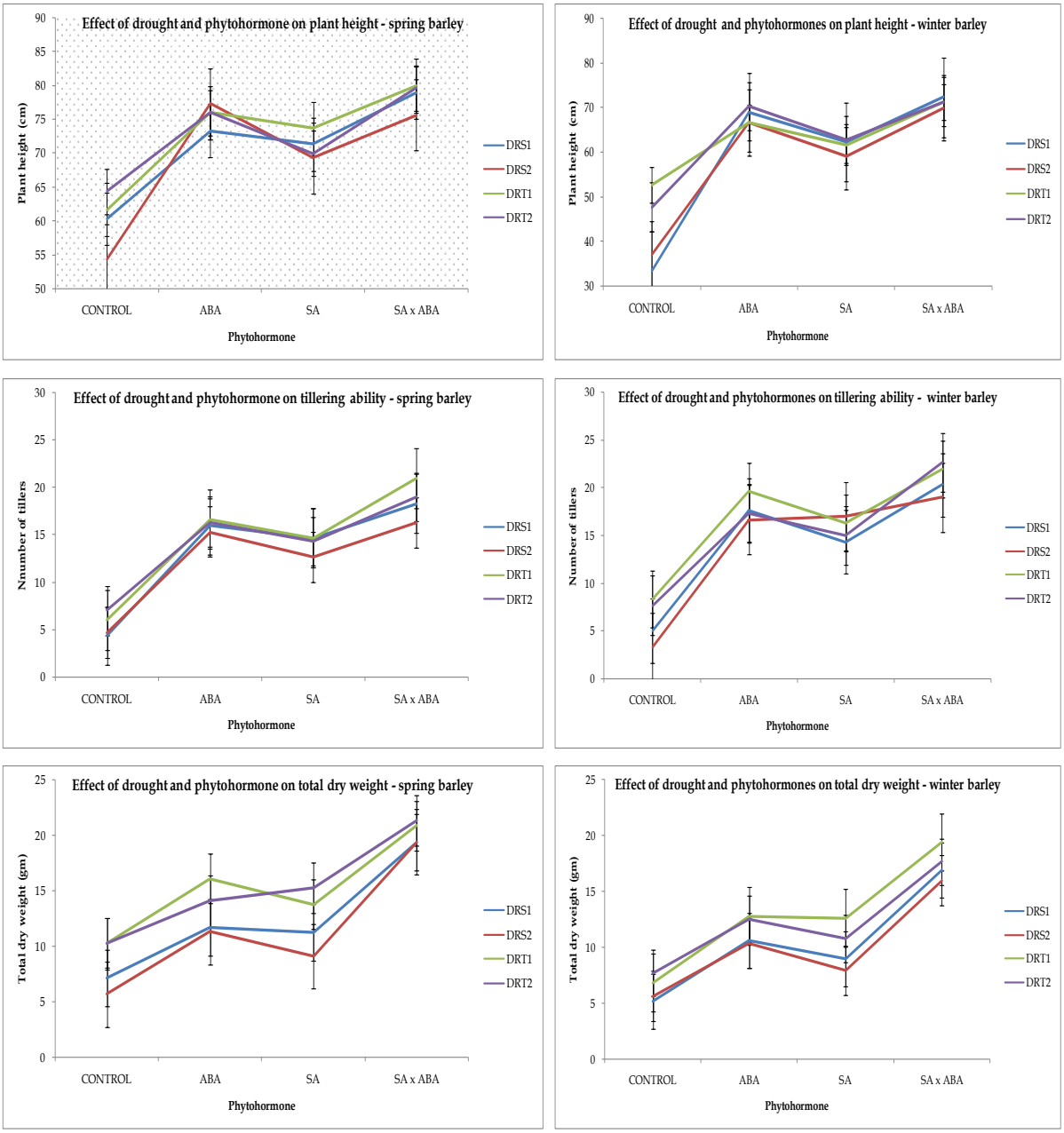


Figure 14: Hormonal signaling effect on growth characteristics of winter and spring barley exhibiting different tolerance (DRT1 & 2) and susceptibility (DRS1 & 2) levels to drought stress under controlled conditions of 20% field capacity

Other comparisons including ABA and CONTROL; ABA and SA; SA and CONTROL; and SA and SAxABA expressed significant differences in terms of hormonal effect on height under the influence of drought. Winter barley also expressed a similar scenario in terms of height just like the spring barley. However, when applied alone, SA performed poorly compared to ABA in enhancing drought tolerance in both winter and spring barley in terms of height, tillering ability, biomass accumulation and MSI (Figure 14, Plate 9).

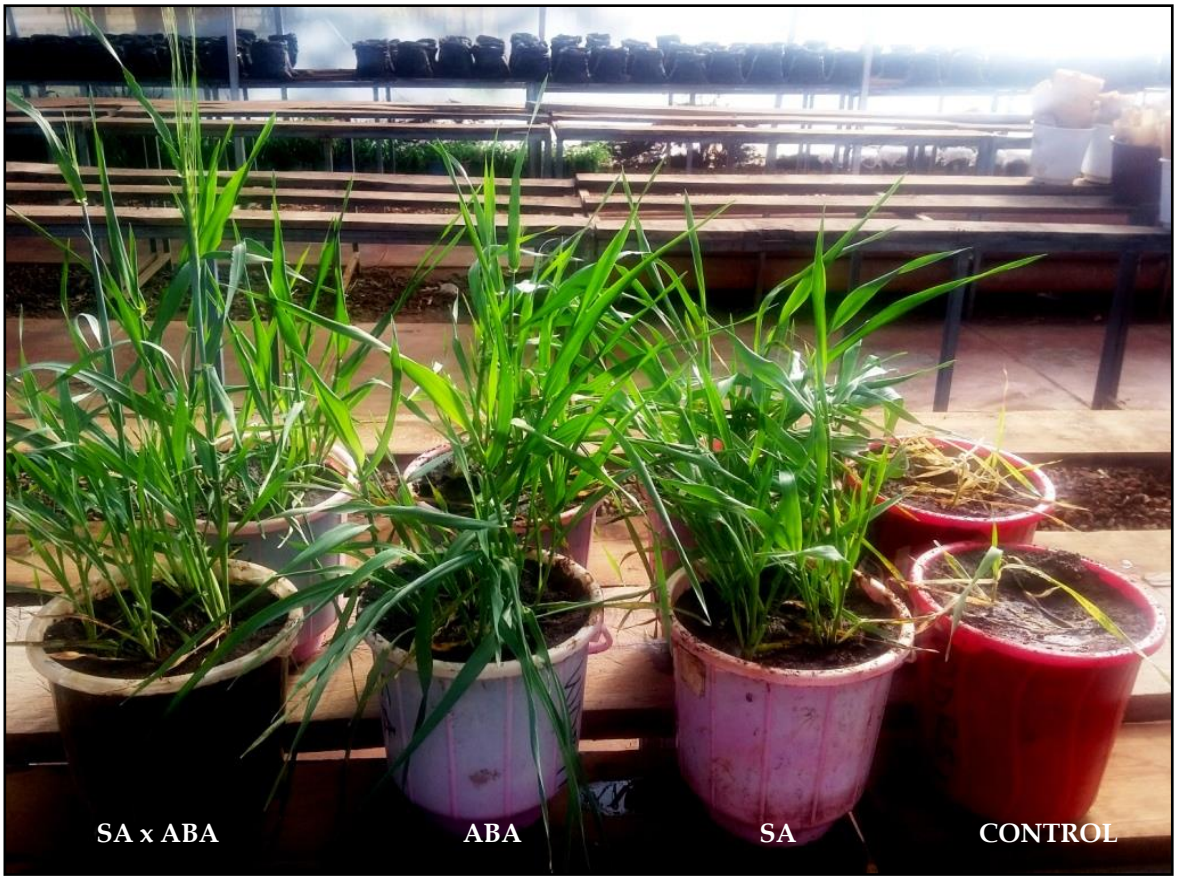


Plate 9: Hormonal signaling effect on growth parameters of barley (WDRT1 – SCRABBLE) under controlled condition (20% field capacity) in the greenhouse (Source: Author, 2017)

In tillering ability, only ABA and SA phytohormone comparison did not differ significantly under the influence of drought in both spring and winter genotypes. All other mean comparisons differed significantly. In terms of biomass accumulation, all

phytohormones and their combinations differed significantly through contrast mean comparisons and both winter and spring barley treated with phytohormones performed better that the untreated barley (Figure 14, Appendix xix and xx).

The physiological approach through membrane stability index revealed a similar trend of hormonal signaling effect as observed in height, tillering ability and biomass accumulation. Moreover, the group responses to drought under the influence of phytohormones confirms that there was a synergistic effect on drought tolerance enhancement when SA and ABA phytohormones are combined than when applied singly especially on winter and spring barley previously identified to be tolerant to drought. When applied singly on winter and spring barley, ABA expressed the highest tolerance enhancement effect than SA in terms of MSI with drought tolerant genotypes recording higher MSI than drought sensitive ones. Also, all the genotypes treated with hormones performed better than those untreated irrespective of their inherent traits and their adaptation (Figure 15).

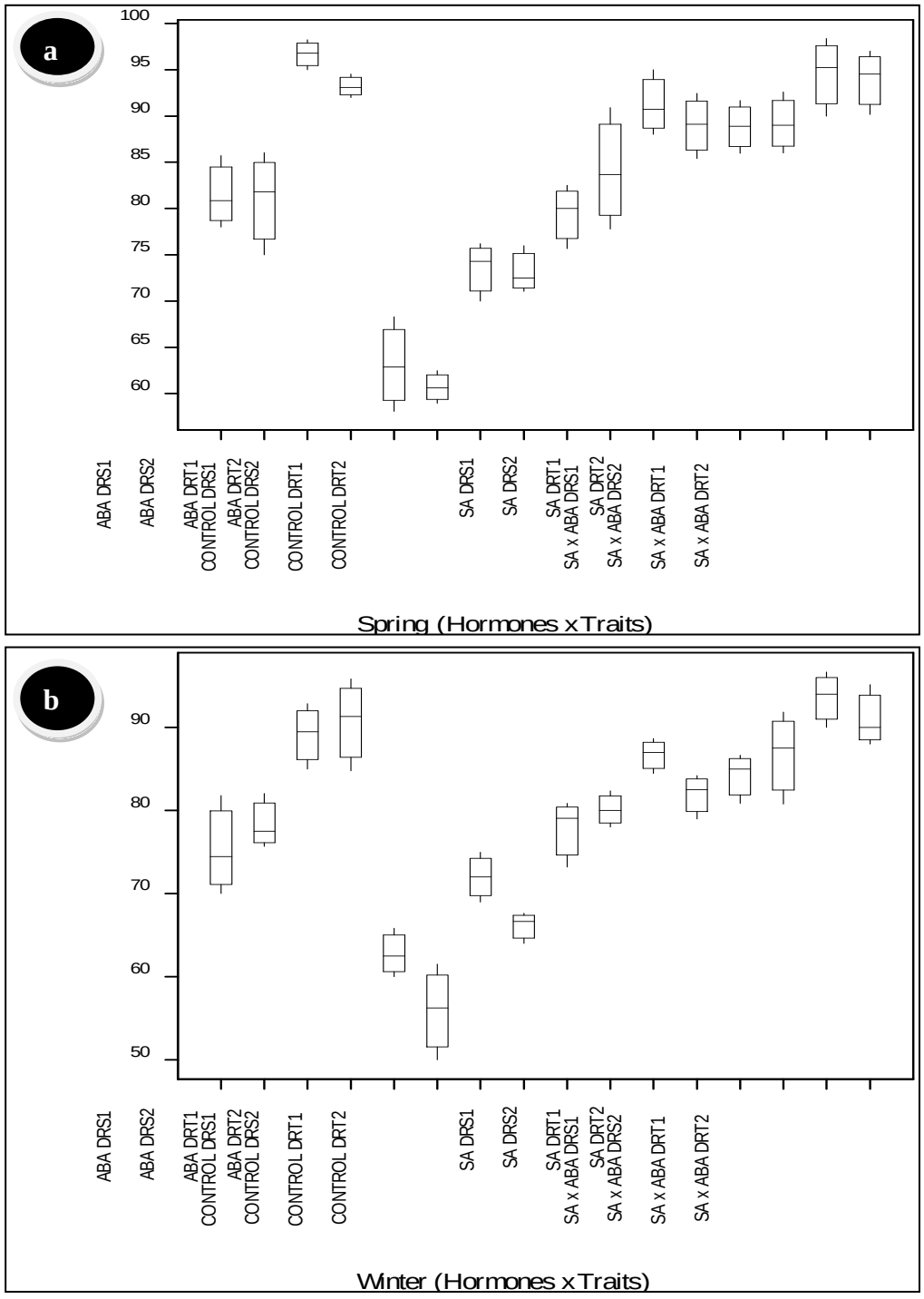


Figure 15: Hormonal signaling effect on response to drought in some trait-specific barley genotypes adapted to spring 'a' and winter 'b' growth conditions. S1 and T1 represent the most sensitive and tolerant genotypes while S2 and T2 represent the second most sensitive and tolerant respectively.

Additional results indicate that the spring barley were more responsive to exogenous application of phytohormones than the winter barley. This is reflected on the membrane stability index where spring barley recorded higher MSI compared to the winter barley. This observation is similar to that made when winter and spring barley was subjected to aluminium toxicity. Also, more findings show that when combined together, SA and ABA had synergistic and tolerance enhancement effect on net blotch foliar infection, aluminium toxicity and drought. However, ABA enhanced more tolerance to abiotic stresses including drought and aluminium toxicity than SA which proved to enhance more tolerance to biotic stress (net blotch foliar infection) in winter and spring barley.

CHAPTER FIVE

DISCUSSIONS

5.1 Response to aluminium toxicity, drought and net blotch disease

5.1.1 Response of winter and spring barley to aluminium toxicity

Mixed responses to aluminium toxicity as expressed by various winter and spring barley genotypes in terms of net root length, relative net root growth, percent response and degree of hematoxylin staining could be due varying genetic make of each genotype (Das, *et al*., 2000). Exposure of barley roots to aluminium cation led to low net root length and relative net root growth especially among the sensitive winter and spring genotypes. This could be due to interference with cell division at the root tips, increased cell wall rigidity by cross-linking pectins and reduced DNA replication through increased rigidity of the double helix structure (Rout, *et al*., 2001).

Varying degree of staining intensity indicates that the screened barley utilized different mechanisms of reducing the effect of aluminium cations in the roots. High staining intensity together with stunted roots among the sensitive genotype means that much aluminium absorption took place and the cations remained in its active and toxic state thus affecting the root development. Moreover, it is possible that some winter and spring genotypes utilized fixation and exclusion mechanisms to reduce the effects of cations on root growth and development. For instance, genotypes with low hematoxylin staining intensity could have utilized exclusion mechanism (Das, *et al*., 2000) hence did not absorb aluminium from the nutrient solution. This also means that the aluminium concentration in the roots of varieties that used exclusion method was low.

Other than the exclusion from the root tissues, some genotypes absorbed much aluminium into their root and shoot tissues and fixed hence the effect on root growth and development was minimal. This means that such genotypes used fixation mechanism of aluminium tolerance to reduce the effects of aluminium toxicity. It could also mean that such genotypes had high internal tolerance to aluminium cation and therefore, despite the accumulation of much cations in their tissues, the uptake and transport of essential nutrients such as Ca, Mg, K and P as well as the activity of many enzymes and metabolic pathway for repair mechanism was not affected (Das, *et al*., 2000).

Specifically, group ‘a’ consisted of the barley genotypes that utilized fixation mechanism to immobilize the Al cation within the root tips thus no interference with root growth and development despite absorption of substantial aluminium cation. The second group ‘b’ was genotypes that were very sensitive to Al cation. This group absorbed much Al cations which interfered with cell division and normal physiological functions (Mossor-Pietraszewska, 2001) thus root stunting was evidence. For KARNE, NGUZO and HKBL 1805-3 genotypes in group ‘c’, exclusion mechanism was evidently expresses and the roots were less stained, an indication of organized cell functions to exclude Al cation from being absorbed into the xylem and phloem tissues at the root tip.

With reference to RNRG and % response, some genotypes were tolerant to Al toxicity through fixation mechanism. This means that such genotypes could absorb and fix aluminium cations into their roots without interfering with the normal root growth and development. For example, HKBL 1719-4 genotype recorded the highest RNRG of 1.0, implying that there was no significant difference between the roots treated with 148 μM

and that with 0 μM Al despite absorbing much Al cations. Additionally, the same genotype had the least % response, a further indication that the effects of Al cation on roots were insignificant. This could mean that in such genotypes, the presence of Al cation does not affect the physiological functions such as the uptake and transport of essential nutrients, plasma membrane, membrane transport proteins and activities of enzymes among others (Das, *et al*., 2000; Mossor-Pietraszewska, 2001).

The results for the response to Al cation toxicity by the winter and spring barley in Kenya corresponds to the findings by other researchers (Das, *et al*., 2000; Mossor-Pietraszewska, 2001). In particular, the formation of three distinct groups irrespective of whether the genotype is winter or spring adapted clearly corresponds to the previous findings that tolerance to aluminium toxicity in barley is controlled by a single dominant gene, found to be located in chromosome 4 (Stolen & Anderson, 1978). This gene whose role is known to provide a signal transduction pathway such as mitogen-activated protein kinase (MAPK) which transmits signal for stresses including Al cation toxicity (Ligterink & Hirt, 2001; Osawa & Matsumoto, 2001) could be present in genotypes exhibiting exclusion and fixation mechanisms of tolerance. However, the same gene could be absent in Al sensitive genotypes such as PUBLICAN and HKBL 1674-4 thus higher concentration of Al cations in their root systems.

For the genotypes that showed mixed response to aluminium toxicity in terms of % response, RNRG and hematoxylin staining, it is possible that there were changes in gene expressions during aluminium toxicity stress (Mossor-Pietraszewska, 2001). Such mixed responses due to the varied location of the Al tolerance gene had been documented in

wheat and rye. In wheat for example, major genes influencing Al tolerance are located on the short arm of chromosome 5A and long arms of chromosomes 2D and 4D. Therefore, being that wheat and barley are closely related, it is possible that when subjected to Al stress, the response mechanisms and variations observed were influenced by the genes expressed and the chromosomes in which the Al tolerance gene is located for each barley genotype.

5.1.2 Response of winter and spring barley to drought

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. Under 20% FC (drought stress), 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 which expressed sensitive response to drought. This could mean that drought stress interfered with a number of physiological processes such as translocation and partitioning of photosynthates needed for proper growth and development (Ashoub, *et al*., 2015).

The winter genotypes proved to be more affected by drought compared to the spring barley. As indicated by the previous research, under drought stress, the quantum yield of light reaction, water-use-efficiency, photosynthetic rates and leaf osmotic potential significantly reduces. It is possible that these processes are more affected in winter barely than in spring barley 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.

Additionally, when subjected to drought stress, barley just like other plants experiences interference with vital biochemical processes including hormonal balance and signal transduction hence gene expression in response to water deficiency (Ashoub, *et al*., 2015). This could mean that among the drought tolerant genotypes such as FANAKA and GRACE, such biochemical processes were least affected but among the sensitive ones like HKBL 1805-3 and BEATRIX, the biochemical reactions were adversely affected.

Accumulation of organic and inorganic solutes in the cytosol is key in maintaining cell turgor for drought tolerance to be realized in most plants. Under drought stress, proline accumulation is the first response to water-deficit stress and once produced, the water uptake from the dry soil is improved. When produced, proline acts as signaling molecule modulate 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).

In this regard, it is possible that even among the drought tolerant winter and spring barley, the variation in the level of tolerance could have been influenced by the quantity of proline whose production depends on calcium and ABA levels. This could also mean that different genotypes signaled the expression of different genes for the production of varying levels of ABA but winter genotypes could have produced low levels of this hormone thus low proline hence more sensitivity to drought compared to spring barley.

5.1.3 Response of winter and winter barley to net blotch disease

Varied response to net blotch disease from one genotype to another as observed under field conditions could be due to the differences in the genetic constitution of the screened winter and spring barley. For instance, among the spring barley, NGAO and SABINI were the most tolerant and susceptible respectively but all screened barley expressed responses varied from one site to another. The varied response per site could be due to site differences in terms of inoculum load, favorable temperature and relative humidity which play important role in pathogenicity and virulence of various pathogenic strains (Owino, *et al*., 2014; Were & Ochuodho, 2012a).

Under the incompatible host-pathogen interaction, pathogenesis-related proteins (PRs) such as chitinases and β-1, 3-glucanases are produced when a plants including barley is infected by pathogens. Once produced, these PRs have antifungal activity and induce systemic acquired resistance (SAR) in barley (Agrios, 2005). Therefore, it is imperative for plant breeders and protectionists to utilize such knowledge in developing new barley varieties that express such self-defense mechanisms against net blotch fungal infection.

This is because among the screened winter and spring barley, most genotypes might have exhibited tolerance to net blotch through such mechanisms but the varied responses could have been due to different levels of PRs produced (Ivarson & Leijman, 2009). Also, incompatible host-pathogen interaction due to different environmental conditions could have restricted the entry of *P. teres* into the host plant thus leading to varied responses by the spring genotypes from one site to the other. Just like other crops, it is possible that the net blotch resistant barley genotypes like MALT 1 and NGAO produced pathogenesis-

related proteins (PRs) that has antifungal activities which inhibits the growth of *P. teres* fungus hence lower disease severity (Ivarson & Leijman, 2009).

Studies have also indicated that wounding caused by fungal pathogens alone cannot trigger the induction of resistant genes but also the presence of a growing fungus (Schulze-Lefert, *et al*., 2012). Therefore, it means that all the winter and spring barley which showed symptoms produced defense-related genes in response to infection but some could have been recessive or dominant or mixed hence the observed responses through a gene-for-gene resistance in a host-pathogen system (Agrios, 2005) under field conditions.

5.1.4 Multiple tolerance to biotic and abiotic stress in barley

Lack of multiple tolerance among the spring and winter barley except for the few out of the total screened could imply that tolerance to Al toxicity, drought and net blotch stress factors are regulated by different genes whose expressions to signal the resistance mechanisms highly depend on existing environmental conditions (Agrios, 2005). For instance, when subjected to drought, plants including barley experience significantly reduced quantum yield of light reaction, water-use-efficiency, photosynthetic rates and osmotic potential (Aprile, *et al*., 2008) and this interferes with key biochemical processes including hormonal balance, signal transduction and gene expression (Xie, *et al*., 2011). This could have played significant role in the mixed responses under drought, aluminium cation toxicity and net blotch disease.

5.2 Net blotch severity under the influence of drought and aluminium toxicity

Low net blotch severity in spring and winter barley after initial exposure to drought and aluminium stress compared to unstressed barley which expressed higher disease severity could mean that exposure of barley to abiotic stress induced the production of certain compounds that are antifungal in nature. For instance, in many plant species, proline 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 & Savoure, 2009). There are higher possibilities that under drought and aluminium toxicity stresses, the winter and spring barley genotypes known to be tolerant to these stress factors produced higher levels of proline thus higher tolerance to net blotch fungal infection and disease development.

Additionally, 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 & Ellis, 2010) and these activates a complex signaling of defense varying from one stress factor to the other (Abou, *et al*., 2009). Such mechanisms could be one of the reasons as to why all the spring and winter barley screened for net blotch foliar infection under the influence of drought and aluminium toxicity stresses expressed different levels of tolerance and susceptibility to the disease. For instance for both spring and winter barley, the defense mechanism in place was stronger than that under aluminium stress thus the low averages on disease.

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 such as abscisic acid (ABA), salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) accumulate within the stressed plant and immediately reprograms the genetic machinery to ensure adequate defense is achieved with minimum biological damage to the plant (Fujita, Fijita, Noutoshi, Takahashi, Narusaka, & Yamaguchi-Shinozaki, 2006; Spoel & Dong, 2008). 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.

Moreover, under aluminium and drought stress, some of the major signal transduction pathways such as mitogen-activated protein kinase (MAPK) (Ligterink & Hirt, 2001; Osawa & 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. The low disease could have been due to the activated MAPK pathway which might have been triggered by initial exposure of winter and spring barley to aluminium and drought stress factors before inoculation by the pathogen. This further explains the higher disease levels in unstressed winter and spring barley which might have not activated the MAPK pathway hence low defense mechanism against infection and disease development.

5.3 Effects of phytohormones on aluminium toxicity, drought and net blotch disease

Exogenous application of phytohormones such as SA, ABA and SAxABA expressed significant effects on stress factors but the best signaling effect varied from one stress factor to the other.

5.3.1 Effect of phytohormones on net blotch foliar infection and severity in barley

With reference to net blotch disease and hormonal signaling effect, SA, ABA and their combination led to low disease severity even among the spring and winter barley genotypes known to be susceptible to the disease and this could be due to induction of resistance mechanisms within the plants treated with hormones.

In the recent past, SA had been identified to play important role in the signaling and induction of immune system to diseases in plants (Denance, *et al*., 2013). The study therefore confirms the same principle in barley with reference to net blotch fungus where application of this hormone could have resulted into activation of not only local resistance but also systemic acquired resistance (SAR) to net blotch in barley as previously observed in the distal (systemic) tissues of other plants. It had also been documented that SAR is an SA-dependent defense mechanism that induces not resistance to a broad spectrum of pathogens (Liu, *et al*., 2011). This explains why even the genotypes known to be susceptible to net blotch recorded lower severity compared to those not treated with SA.

ABA application on barley followed by inoculation with net blotch fungus did not signal the induction of disease resistance compared to SA hence higher disease severity. This could mean that when spring and winter barley were treated with ABA then inoculated with net blotch fungus, there was higher disease severity possibly due to negative

regulation of disease resistance by ABA (Mou & An, 2011). Just like in *P. syringae* (Mohr & Cahill, 2003), the exogenous application of ABA also prevents SA accumulation and suppresses resistance to *P. teres* in barley thus responsible for the higher disease severity among the winter and spring barley.

Such negative regulations to disease tolerance have been attributed to suppression of SAR by ABA (Yasuda, *et al*., 2008), an indication that ABA has antagonistic interaction with SA in terms of disease tolerance. This further explains why there was no significant difference between SA and SAxABA combination in terms of induction of disease tolerance in barley. Moreover, the lack of significant difference in disease severity when SA is compared to the combination of SA and ABA could mean that the observed tolerance in the SAxABA combination was largely due to SA and not ABA and the antagonistic ability of ABA on SA was more strong in winter barley than in spring barley hence higher foliar infection in winter under SAxABA.

However, the disease severity when barley was treated with ABA was much lower than when there is no hormone at all. This could be due to previous reports that ABA regulates defense response through its effect on production of reactive oxygen (Xing, *et al*., 2008), cellulose deposition (Flors, *et al*., 2008) and regulation of defense gene expression (Adie, *et al*., 2007).

5.3.2 Effect of phytohormones on aluminium and drought stress in barley

Exogenous application of SA, ABA and SAxABA improved the level of tolerance to drought and aluminium cation toxicity in winter and spring barley compared to those not treated with phytohormones. This could mean that these hormones induced the activation

of biochemical mechanisms, signaling pathways and genes needed for abiotic stress tolerance to be expressed in plants (Anjum, *et al*., 2011; Xie, *et al*., 2011).

For both drought stress and aluminium toxicity, SAxABA, ABA and SA ranked 1st, 2nd and 3rd in terms of the level of tolerance, an indication of the synergistic effect of SAxABA combination on the two abiotic stresses. This contradicts the previous findings which showed antagonistic effect on biotic stress factors especially the fungal disease infection when SA and ABA are applied in combination (Mou & An, 2011). It is possible that the antagonistic effect between SA and ABA is stronger against biotic stress factors such as net blotch but under abiotic stresses like aluminium and drought stress, SA and ABA induce synergistic effect to reduce the effects of the two stresses.

For instance, application of ABA and SAxABA might have triggered the activation of numerous physiological processes which induced adaptation to drought stress conditions even among the genotypes known to be sensitive to drought (Robert-Seilaniantz, *et al*., 2007). This is because under drought stress, application of ABA may have induced stomatal closure to conserve water. The better performance by some of the drought tolerant winter and spring barley could have been due to induction of *NCED3* gene due to drought stress. Once produced within a drought tolerant plant, this gene may have upregulated the endogenous ABA levels whose effects could have been magnified by the exogenous ABA to lower transpiration rates (Schwartz, *et al*., 2003).

Similar physiological processes could have led to the observed tolerance to aluminium toxicity among the winter and spring barley genotypes. For example, under the influence of SAxABA, root elongation and formation of fibrous roots was realized than in the

genotypes treated with SA or no hormone at all. Though not clearly understood and documented, it is possible that application of ABA and SAxABA combination enhance proper root and shoot development even when barley is subjected to aluminium toxicity. The observed tolerances especially under the influence of SAxABA and ABA could also imply that exogenous application of these hormones may have triggered the expression of specific genes within the barley plant to signal their production in large quantities (Mauch-Mani, *et al*., 2014; Wees, *et al*., 2015) hence higher tolerance to aluminium than barley not treated with phytohormones.

CHAPTER SIX

CONCLUSIONS, RECOMMENDATIONS AND WAY FORWARD

6.1 Conclusions

1. Spring barley genotypes are more tolerant to aluminium toxicity, drought stress and net blotch foliar infection than the winter genotypes. FANAKA, MALT 1, ALICIANA and GRACE are the only winter and spring barley grown in Kenya exhibiting multiple tolerances to drought, aluminium toxicity and net blotch disease. Some barley genotypes also exhibit dual and single stress tolerances.

2. Initial exposure to drought and aluminium toxicity significantly triggered the induction of net blotch tolerance among the winter and spring barley genotypes irrespective of the inherent traits. All the winter and spring adapted barley genotypes initially exposed to aluminium toxicity stress expressed more tolerance than those under drought stress. When unstressed, all barley genotypes showed more susceptibility to net blotch foliar infection.

3. Salicylic acid (SA) is the key hormone in inducing tolerance to net blotch foliar infection among the winter and spring barley genotypes. However, Abscisic acid (ABA) is the best in inducing tolerance to abiotic stress especially in reference to drought stress and aluminium cation toxicity. The synergistic effect of SA when combined with ABA is more stronger in inducing tolerance to abiotic stresses but the same combination of hormones is weaker in ensuring net blotch disease tolerance.

6.2 Recommendations

1. In any disease management and variety improvement program, there is need to consider multiple tolerances to the complex interaction among drought, aluminium toxicity and net blotch disease in barley. This is because different genotypes do not respond in a similar manner to different stress factors hence may express a short-lived tolerance.

2. In reducing net blotch severity in barley or to minimize the use of chemicals in net blotch disease management, this study recommends dry-planting and/or planting barley in soils which are slightly acidic. This is because initial exposure to these stresses seems to initiate the activation of disease tolerance pathways, mechanisms and biochemicals hence low disease levels.

3. To ensure net blotch disease tolerance in barley, the study recommends selection of genotypes containing genes responsible for the synthesis of SA hormone. To ensure durable and long-lasting tolerance to drought and aluminium toxicity, genotypes with genes for ABA and SA hormones synthesis should be selected.

6.3 Way forward

1. Other than understanding the genetics and biochemical systems induced by the interaction effects of biotic and abiotic stresses, the realization and attainment of stable and long-lasting tolerance among the winter and spring barley require a detailed understanding of hormonal signaling pathways together with the inter-

relationships among the stress factors, phytohormones and genetic implications and roles.

2. For effective management of biotic and abiotic stresses in barley and other crops, researchers should focus on precision breeding with variety selection under complex interaction effects of drought, soil mineral toxicities and disease hot-spot zones.

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APPENDICES

Each step of this work was done under the guidance by the University of Eldoret supervisors (Prof. J.O Ochuodho and Dr. N.K. Rop) and Consultative Group for International Agricultural Research (CGIAR) supervisor Dr. S. Gyawali (ICARDA). The work plan is shown below (Table

Appendix I: Research work plan (2015 – 2017)

ACTIVITY	TIME-LINE								
	AUG, 2015	SEP-NOV, 2015	DEC, 2015 - JUN, 2016	JUL - SEPT, 2016	OCT-NOV, 2016	DEC, 2016	JAN-FEB, 2017	MAR, 2017	APR-DEC, 2017
PROPOSAL WRITING & REVIEW PROPOSAL PRESENTATION PLANNING FOR IMPLEMENTATION									
IMPLEMENTATION OF PHASE I (INITIAL SCREENING) ALUMINIUM DROUGHT NET BLOTCH									
IMPLEMENTATION OF PHASE II (PHYTOHORMONES) ALUMINIUM DROUGHT NET BLOTCH									
IMPLEMENTATION OF PHASE III (GENETIC DIVERSITY)									
DATA ANALYSIS THESIS WRITING									
THESIS PRE-DEFENSE & CORRECTIONS									
THESIS EXTERNAL EXAMINATION									
THESIS DEFENSE									
RESULT DISSEMINATION GRADUATION (PHD)									

Appendix II: Composition of nutrient solution used in screening barley genotypes for aluminium toxicity

	Element & Source		Stock Solution Recipes					Final Nutrient Solution Cation			
No.	**Elemer**	**Source**	**MW**	**g/L orig**	**g/L adj**	**(M)**	**(M) adj**	**mL stock/L**	**mg/L**	**mM**	
1	**Ca**	Ca(NO3)2.4H2O	238	270	166	1.14	0.7	5	Ca: 141.1	3.5	
		NH4NO3	80	33.8	20.8	0.42	0.26		NH4-N: 18.2	1.3	
2	**K**	KCl	74.6	18.6	8.59	0.25	0.12	5	K: 22.5	0.6	
		K2SO4	174	44	20.3	0.25	0.12		K: 45.6	1.2	
		KNO3	101	24.6	11.4	0.24	0.11		K: 22.5	0.6	
3	**Mg**	Mg(NO3)2. 6H2O	256	142	43.9	0.56	0.17	5	Mg: 20.8	0.9	
4	**P**	KH2PO4	136	17.6	1.23	0.13	0.01	5	K: 1.7	0	
5	**Fe**	Fe(NO3)3 .9H2O	404	10.1	6.22	0.03	0.02	5	Fe: 4.3	0.1	
		HEDTA	278	8.35	5.14	0.03	0.02				
6	**Micro**										
	Mn	MnCl2 .4H2O	198	2.34	1.8	0.01	0.01	1	Mn: .5	0	
	B	H3BO3	61.8	2.04	1.57	0.03	0.03				
	Zn	ZnSO4 .7H2O	287	0.88	0.68	0	0		Zn: .15	0	
	Cu	CuSO4 .5H2O	250	0.2	0.15	0	0		Cu: .04	0	
	Mo	Na2MoO4 .2H2O	242	0.26	0.2	0	0		Na: .04	0	
7	**Al**	AlCl3.6H2O	133.34	23 µM	12	0.12	0.025	5	Al: 3.996	0.07	

Source: Bal and Alkus, (2011); Magnavaca *et al*., (1987); Minella and Sorrells, (2002); Ouma *et al*., (2011)

Appendix III: Approximate research budget for a period of 24 months

RUFORUM Doctoral Research Grant (DRG) Budget - 2015

	Budget Item	Unit	Quantity (no. of months)	Cost (USD)	Total Cost (USD)
1	Net blotch screening equipment and chemicals	Approximate	3 months	1,000	**1,500**
2	Drought screening eqipment	Approximate	3 months	500	**500**
3	Aluminium screening chemicals and equipment renovation	Approximate	3 months	2,000	**2,000**
4	Experimental site hire, preparation and trials management	2 acres per 5 sites @ 200 USD per acre	8 months	3,000	**3,000**
5	Field, laboratory and screenhouse supervision allowances	University & ICARDA personnel	12 months	3,000	**3,000**
6	Molecular analysis chemicals, bench fee and technical support	Approximate	4 months	1,500	**1,500**
7	Disemmination of results (Workshops, Conferences, e.t.c)	Approximate	N/A	2,000	**2,000**
8	Publications and purchase of stetionaries	Approximate	N/A	1,500	**1,500**
	TOTAL				**15,000**

The grants are for a maximum of US$15,000 over a period of two years, and are meant to provide funds for student research, support supervision, produce the thesis and related publications, as well as report back to the communities they worked with. The students who apply for these grants are those who are self financed or are sponsored through grants which do not provide research funds.

The grants WILL NOT cover tuition fees or stipend for the students.

Appendix IV: Anova tables for response to aluminium toxicity among spring barley genotypes

Analysis of variance

Variate: NRL

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
GENOTYPE	15	72.373	4.825	3.65	0.001
Residual	32	42.267	1.321		
Total	47	114.639			

Analysis of variance

Variate: RNRG

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
GENOTYPE	15	1.13243	0.075495	9.51	<.001
Residual	32	0.254099	0.007941		
Total	47	1.386529			

Analysis of variance

Variate: %_RESPONSE

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
GENOTYPE	15	6046.81	403.12	5.05	<.001
Residual	32	2556.67	79.9		
Total	47	8603.48			

Analysis of variance

Variate: HEMATOXYLIN

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
GENOTYPE	15	15.16667	1.01111	10.79	<.001
Residual	32	3.0000	0.09375		
Total	47	18.16667			

Appendix V: Anova tables for response to aluminium toxicity among winter barley genotypes

Analysis of variance					
Variate: NRL					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
GENOTYPE	15	90.498	6.033	5.2	<.001
Residual	32	37.1	1.159		
Total	47	127.598			

Analysis of variance					
Variate: RNRG					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
GENOTYPE	15	0.940494	0.0627	9.16	<.001
Residual	32	0.21908	0.006846		
Total	47	1.159574			

Analysis of variance					
Variate: %_RESPONSE					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
GENOTYPE	15	6295.98	419.73	5.69	<.001
Residual	32	2362	73.81		
Total	47	8657.98			

Analysis of variance					
Variate: HEMATOXYLIN					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
GENOTYPE	15	27.6615	1.8441	17.7	<.001
Residual	32	3.3333	0.1042		
Total	47	30.9948			

Appendix VI: Anova tables for the response of spring barley genotypes to drought under greenhouse conditions

Analysis of variance
Variate: TILLERS

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REPLICATE stratum	2	9.771	4.885	12.68	
FIELD_CAPACITY	1	770.667	770.667	1999.57	<.001
Residual	2	0.771	0.385	0.09	
GENOTYPE	15	1643.958	109.597	26.72	<.001
FIELD_CAPACITY.GENOTYPE	15	169.667	11.311	2.76	0.003
Residual	60	246.125	4.102		
Total	95	2840.958			

Analysis of variance
Variate: HEIGHT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REPLICATE stratum	2	6.25	3.12	10.71	
FIELD_CAPACITY	1	1742.51	1742.51	5974.32	<.001
Residual	2	0.58	0.29	0.02	
GENOTYPE	15	2851.07	190.07	14.26	<.001
FIELD_CAPACITY.GENOTYPE	15	322.66	21.51	1.61	0.097
Residual	60	799.83	13.33		
Total	95	5722.91			

Analysis of variance
Variate: GRAINS_SPIKE

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REPLICATE stratum	2	14.812	7.406	2.55	
FIELD_CAPACITY	1	1863.844	1863.844	641.32	0.002
Residual	2	5.812	2.906	0.79	
GENOTYPE	15	3871.74	258.116	70.38	<.001
FIELD_CAPACITY.GENOTYPE	15	162.656	10.844	2.96	0.001
Residual	60	220.042	3.667		
Total	95	6138.906			

Analysis of variance
Variate: %1000_SWT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REPLICATE stratum	2	34.771	17.385	0.97	
FIELD_CAPACITY	1	1989.26	1989.26	110.84	0.009
Residual	2	35.896	17.948	3.51	
GENOTYPE	15	7010.99	467.399	91.45	<.001
FIELD_CAPACITY.GENOTYPE	15	391.906	26.127	5.11	<.001
Residual	60	306.667	5.111		
Total	95	9769.49			

Analysis of variance
Variate: MSI

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REPLICATE stratum	2	25.23	12.61	0.83	
FIELD_CAPACITY	1	1489.15	1489.15	98.38	0.01
Residual	2	30.27	15.14	1.43	
GENOTYPE	15	10340.85	689.39	65.29	<.001
FIELD_CAPACITY.GENOTYPE	15	478	31.87	3.02	0.001
Residual	60	633.54	10.56		
Total	95	12997.04			

Appendix VII: Table of means on the phenotypic and physiological response of SPRING barley to drought under greenhouse conditions

GENOTYPE	Number of tillers			Height (cm)			No. of grains per spike			Thousand seed weight (g)			MSI (% Ratio)		
	20% FC	80% FC	DMRT	20% FC	80% FC	DMRT	20% FC	80% FC	DMRT	20% FC	80% FC	DMRT	20% FC	80% FC	DMRT
FANAKA	19	23	**bcd**	72.7	78.0	**a**	26	32	**a**	45.0	47.7	**a**	73.4	77.3	**a**
HKBL 1629-14	22	26	**a**	74.7	82.7	**a**	25	30	**a**	43.3	48.3	**a**	71.9	77.0	**ab**
HKBL 1629-5	19	23	**bcde**	65.3	74.0	**bc**	20	28	**b**	30.3	40.7	**c**	67.6	74.7	**bc**
HKBL 1663-3	21	26	**ab**	71.3	80.3	**a**	23	32	**a**	36.7	43.7	**b**	64.4	72.0	**cd**
HKBL 1674-4	15	24	**def**	64.3	77.7	**b**	18	29	**b**	42.0	49.0	**a**	60.1	75.0	**cd**
HKBL 1719-4	16	19	**fgh**	66.0	72.3	**bcd**	15	25	**c**	30.3	35.7	**c**	61.4	70.0	**de**
HKBL 1774-3	17	24	**cde**	68.0	71.3	**bc**	11	20	**e**	21.7	33.7	**de**	64.0	67.3	**de**
HKBL 1805-3	16	21	**efg**	65.7	71.0	**bcd**	14	23	**cd**	18.7	31.0	**efgh**	60.9	68.0	**def**
HKBL 1805-6	19	26	**abc**	62.0	71.0	**bcde**	21	29	**b**	39.0	44.3	**b**	54.5	69.7	**ef**
HKBL 1861-1	14	20	**gh**	62.3	69.0	**cdef**	11	19	**e**	24.0	30.0	**def**	59.1	62.3	**fg**
HKBL 1862-5	12	20	**h**	56.0	66.7	**fgh**	12	19	**e**	13.7	30.0	**i**	54.3	60.3	**gh**
KARNE	10	15	**ij**	56.3	70.7	**efg**	4	18	**f**	17.7	32.0	**fg**	53.4	59.0	**h**
MALT 1	9	12	**j**	60.7	68.3	**defg**	10	21	**e**	22.7	33.0	**d**	51.0	57.3	**hi**
NGAO	17	18	**fgh**	68.7	71.3	**bc**	16	19	**de**	32.7	37.7	**c**	47.4	56.3	**i**
NGUZO	8	14	**j**	53.7	63.3	**h**	4	12	**g**	16.0	28.3	**gi**	43.2	47.3	**j**
SABINI	7	20	**i**	52.7	69.0	**gh**	4	17	**f**	17.7	32.0	**efg**	25.7	44.7	**k**
MEAN	**15**	**21**	**18**	**63.8**	**72.3**	**68**	**15**	**23**	**19**	**28.2**	**37.3**	**32.8**	**57.0**	**64.9**	**61.0**
	FC	**Gen.**	**FC x Gen.**	**FC**	**Gen.**	**FC x Gen.**	**FC**	**Gen.**	**FC x Gen.**	**FC**	**Gen.**	**FC x Gen.**	**FC**	**Gen.**	**FC x Ge**
Probability	<0.001	<0.001	0.003	<0.001	<0.001	0.097	0.002	<0.001	0.001	0.009	<0.001	<0.001	0.01	<0.001	0.001
S.E	0.090	0.827	1.136	0.078	1.491	2.043	0.246	0.782	1.098	0.611	0.923	1.404	0.562	1.327	1.901
S.E.D	0.127	1.169	1.606	0.11	2.108	2.889	0.348	1.106	1.553	0.865	1.305	1.986	0.794	1.876	2.689
% CV	11.3			5.4			10.1			6.9			5.3		

Appendix VIII: Anova tables for the response of winter barley genotypes to drought under greenhouse conditions

Analysis of variance
Variate: TILLERS

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	11.521	5.76	2	
FIELD_CAPACITY	1	2730.667	2730.667	946.37	0.001
Residual	2	5.771	2.885	1.06	
GENOTYPE	15	994.958	66.331	24.26	<.001
FIELD_CAPACITY.GENOTYPE	15	370	24.667	9.02	<.001
Residual	60	164.042	2.734		
Total	95	4276.958			

Analysis of variance
Variate: HEIGHT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	21.396	10.698	0.26	
FIELD_CAPACITY	1	6192.094	6192.094	149.32	0.007
Residual	2	82.937	41.469	4.27	
GENOTYPE	15	4564.823	304.322	31.32	<.001
FIELD_CAPACITY.GENOTYPE	15	1896.74	126.449	13.01	<.001
Residual	60	583	9.717		
Total	95	13340.99			

Analysis of variance
Variate: GRAINS_SPIKE

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	2.312	1.156	0.14	
FIELD_CAPACITY	1	4523.76	4523.76	560.36	0.002
Residual	2	16.146	8.073	4.22	
GENOTYPE	15	919.156	61.277	32.01	<.001
FIELD_CAPACITY.GENOTYPE	15	234.406	15.627	8.16	<.001
Residual	60	114.875	1.915		
Total	95	5810.656			

Analysis of variance
Variate: %1000_SWT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	38.021	19.01	4.57	
FIELD_CAPACITY	1	1134.375	1134.375	272.93	0.004
Residual	2	8.312	4.156	1.05	
GENOTYPE	15	6242.5	416.167	104.77	<.001
FIELD_CAPACITY.GENOTYPE	15	238.292	15.886	4	<.001
Residual	60	238.333	3.972		
Total	95	7899.833			

Analysis of variance
Variate: MSI

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	7.78	3.89	0.29	
FIELD_CAPACITY	1	5321.757	5321.757	400.89	0.002
Residual	2	26.55	13.275	1.84	
GENOTYPE	15	6793.114	452.874	62.8	<.001
FIELD_CAPACITY.GENOTYPE	15	2017.412	134.494	18.65	<.001
Residual	60	432.689	7.211		
Total	95	14599.301			

Appendix IX: Table of means on the phenotypic and physiological response of SPRING barley to drought under greenhouse conditions

GENOTYPE	Number of tillers			Height (cm)			No. of grains per spike			Thousand seed weight (g)			MSI (% Ratio)		
	20% FC	80% FC	DMRT	20% FC	80% FC	DMRT	20% FC	80% FC	DMRT	20% FC	80% FC	DMRT	20% FC	80% FC	DMRT
ALICIANA	15	28	b	64.0	72.3	ab	9	26	b	32.7	40.0	a	73.9	79.0	a
ANNABEL	22	29	a	58.3	74.7	ab	17	26	a	33.7	40.0	a	73.7	78.0	a
BEATRIX	16	28	b	47.0	61.0	cde	8	22	cd	23.0	31.7	c	67.8	72.3	b
COCKTAIL	15	29	b	62.3	75.7	a	7	29	b	26.7	40.0	b	66.6	71.7	b
GRACE	12	20	def	48.0	63.0	cd	7	19	efg	22.3	27.0	d	65.0	70.7	bc
MARTHE	11	22	def	35.0	68.3	def	7	21	de	20.7	26.3	de	57.6	73.0	cd
NFC TIPPLE	8	25	def	44.7	59.0	def	7	20	def	19.7	22.3	f	54.9	70.3	de
PHILLADEPHIA	13	20	def	45.7	63.7	cde	5	18	ghi	10.3	17.0	hi	54.0	69.7	ef
PUBLICAN	12	23	cde	40.0	70.3	cd	3	19	hij	9.0	22.7	gh	56.7	64.0	ef
QUENCH	15	21	cd	55.0	59.0	c	4	17	ij	11.3	19.7	h	55.4	65.0	ef
SCRABBLE	8	23	ef	53.3	61.7	c	4	21	efgh	19.3	23.7	ef	45.5	72.3	f
SHUFFLE	22	27	a	61.7	68.7	ab	8	22	d	15.3	21.0	g	51.0	66.7	f
SY 409-228	11	28	c	61.3	67.7	b	12	21	bc	30.0	36.0	b	44.0	73.3	f
SY BATYK	11	23	def	32.7	63.0	f	10	21	cd	11.3	20.3	gh	37.5	66.3	g
TITOUAN	11	19	f	44.3	57.0	ef	2	17	j	9.0	15.0	i	36.9	58.3	h
XANADU	13	22	de	35.3	60.7	f	6	18	fghi	15.3	17.0	gh	33.0	61.0	h
MEAN	**13**	**24**	**19**	**49.3**	**65.4**	**57.3**	**7**	**21**	**16**	**19.4**	**26.2**	**22.8**	**54.6**	**69.5**	**62.0**
	FC	**Gen.**	**FC x Gen.**	**FC**	**Gen.**	**FC x Gen.**	**FC**	**Gen.**	**FC x Gen.**	**FC**	**Gen.**	**FC x Gen.**	**FC**	**Gen.**	**FC x Gen.**
Probability	0.001	<0.001	<0.001	0.007	<0.001	<0.001	0.002	<0.001	<0.001	0.004	<0.001	<0.001	0.002	<0.001	<0.001
S.E	0.245	0.675	0.956	0.929	1.273	1.975	0.41	0.565	0.875	0.294	0.814	1.152	0.526	1.096	1.591
S.E.D	0.347	0.955	1.352	1.314	1.8	2.793	0.58	0.799	1.238	0.416	1.151	1.63	0.744	1.55	2.25
% CV	8.8			12.2			9.8			8.7			4.3		

Appendix X: Anova results for the response of spring and winter barley genotypes to net blotch disease under field conditions exhibiting different agro-ecological zones

Analysis of variance **Variate: NET_BLOTCH - SPRING** **Source of variation**	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
REPLICATE stratum	2	2.3318	1.1659	3.69	
SITE	2	8.2839	4.1419	13.1	<.001

Appendix XI: Anova results for net blotch severity in trait-specific SPRING barley genotypes under controlled conditions. CONTRAST COMPARISON for INHERENT TRAITS in barley

Analysis of variance **Variate: DISEASE SEVERITY - SPRING** **Source of variation**	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
REP stratum	2	45.0398	22.5199	74.95	
STRESS	2	15.8398	7.9199	26.36	0.005

Appendix XII: Anova results for net blotch severity in trait-specific spring barley genotypes under controlled conditions. CONTRAST COMPARISON for STRESS FACTORS in barley

Analysis of variance

Variate: DISEASE SEVERITY

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	45.0398	22.5199	74.95	
STRESS	2	15.8398	7.9199	26.36	0.005

Appendix XIII: Anova results for net blotch severity in trait-specific winter barley genotypes under controlled conditions. CONTRAST COMPARISON for INHERENT TRAITS in barley

Analysis of variance **Variate: DISEASE SEVERITY** **Source of variation**	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
REP stratum	2	19.73426	9.86713	22.18	
STRESS	2	4.68426	2.34213	5.26	0.076
Residual	4	1.77963	0.44491	1.58	

Appendix XIV: Anova results for net blotch severity in trait-specific winter barley genotypes under controlled conditions. CONTRAST COMPARISON for STRESS FACTORS in barley

Analysis of variance **Variate: DISEASE SEVERITY** **Source of variation**	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
REP stratum	2	19.73426	9.86713	22.18	
STRESS	2	4.68426	2.34213	5.26	0.076
Drought Vs Al toxicity	1	0.95069	0.95069	2.14	0.218

Appendix XV: Anova results for net blotch severity in trait-specific SPRING barley genotypes under controlled conditions. CONTRAST COMPARISON for PHYTOHORMONES in barley

Analysis of variance

Variate: NET BLOTCH - SPRING

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	5.1646	2.5823	3.73	
PHYTOHORMONE	3	74.9917	24.9972	36.12	<.001
ABA Vs CONTROL	1	8.0083	8.0083	11.57	0.014

Appendix XVI: Anova results for net blotch severity in trait-specific WINTER barley genotypes under controlled conditions. CONTRAST COMPARISON for PHYTOHORMONES in barley

Analysis of variance **Variate: NET BLOTCH - WINTER** **Source of variation**	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
REP stratum	2	7.3889	3.6944	3.25	
PHYTOHORMONE	3	79.274	26.4247	23.26	0.001
ABA Vs CONTROL	1	32.5833	32.5833	28.68	0.002

Appendix XVII: Anova results for effects of aluminium toxicity on apical root length, plant height, number of fibrous roots, root dry weight and shoot dry weight in trait-specific SPRING barley genotypes under the influence of phytohormones in controlled conditions

Analysis of variance					
Variate: A_ROOT_LENGTH_cm - SPRING					
Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
REP stratum	2	28.292	14.146	1.29	
PHYTOHORMONE	3	1087.417	362.472	33.1	<.001

Analysis of variance

Variate: ROOT_DWT_g - SPRING

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	5.6929	2.8465	5.47	
PHYTOHORMONE	3	43.5573	14.5191	27.9	<.001
ABA Vs CONTROL	1	14.2604	14.2604	27.41	0.002
ABA Vs SA	1	10.0104	10.0104	19.24	0.005
ABA Vs SAxABA	1	3.7604	3.7604	7.23	0.036
SA Vs CONTROL	1	0.375	0.375	0.72	0.428

Analysis of variance Variate: SHOOT_DWT_g - SPRING Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	1.7954	0.8977	0.59	
PHYTOHORMONE	3	124.57	41.5233	27.51	<.001
ABA Vs CONTROL	1	64.0267	64.0267	42.42	<.001
ABA Vs SA	1	19.44	19.44	12.88	0.012
ABA Vs SAxABA	1	4.86	4.86	3.22	0.123
SA Vs CONTROL	1	12.9067	12.9067	8.55	0.026
SA Vs SAxABA	1	43.74	43.74	28.98	0.002
Residual	6	9.0562	1.5094	5.89	
TRAIT	3	44.0967	14.6989	57.31	<.001
PHYTOHORMONE.TRAIT	9	2.1133	0.2348	0.92	0.528
ABA Vs CONTROL.TRAIT	3	1.3567	0.4522	1.76	0.181
ABA Vs SA.TRAIT	3	1.3767	0.4589	1.79	0.176
ABA Vs SAxABA.TRAIT	3	0.1667	0.0556	0.22	0.884
SA Vs CONTROL.TRAIT	3	0.06	0.02	0.08	0.971
SA Vs SAxABA.TRAIT	3	0.6433	0.2144	0.84	0.487
Residual	24	6.155	0.2565		
Total	47	187.7867			

Appendix XVIII: Anova results for effects of aluminium toxicity on apical root length, plant height, number of fibrous roots, root dry weight and shoot dry weight in trait-specific WINTER barley genotypes under the influence of phytohormones in controlled conditions

Analysis of variance Variate: A_ROOT_LENGTH_cm - WINTER Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	10.167	5.083	0.73	
PHYTOHORMONE	3	1095.833	365.278	52.6	<.001
ABA Vs CONTROL	1	610.042	610.042	87.85	<.001
ABA Vs SA	1	32.667	32.667	4.7	0.073
ABA Vs SAxABA	1	45.375	45.375	6.53	0.043
SA Vs CONTROL	1	360.375	360.375	51.89	<.001
SA Vs SAxABA	1	155.042	155.042	22.33	0.003
Residual	6	41.667	6.944	3.66	
TRAIT	3	244.833	81.611	43.05	<.001
PHYTOHORMONE.TRAIT	9	43.667	4.852	2.56	0.032
ABA Vs CONTROL.TRAIT	3	2.458	0.819	0.43	0.732
ABA Vs SA.TRAIT	3	28	9.333	4.92	0.008
ABA Vs SAxABA.TRAIT	3	19.458	6.486	3.42	0.033
SA Vs CONTROL.TRAIT	3	21.792	7.264	3.83	0.023
SA Vs SAxABA.TRAIT	3	2.125	0.708	0.37	0.773
Residual	24	45.5	1.896		
Total	47	1481.667			

Analysis of variance Variate: HEIGHT_cm - WINTER Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	20.167	10.083	0.47	
PHYTOHORMONE	3	3636.063	1212.021	56.37	<.001
ABA Vs CONTROL	1	2340.375	2340.375	108.85	<.001
ABA Vs SA	1	150	150	6.98	0.038
ABA Vs SAxABA	1	48.167	48.167	2.24	0.185
SA Vs CONTROL	1	1305.375	1305.375	60.72	<.001
SA Vs SAxABA	1	368.167	368.167	17.12	0.006
Residual	6	129	21.5	4.05	
TRAIT	3	332.229	110.743	20.85	<.001
PHYTOHORMONE.TRAIT	9	253.521	28.169	5.3	<.001
ABA Vs CONTROL.TRAIT	3	185.458	61.819	11.64	<.001
ABA Vs SA.TRAIT	3	20.333	6.778	1.28	0.305
ABA Vs SAxABA.TRAIT	3	4.833	1.611	0.3	0.823
SA Vs CONTROL.TRAIT	3	140.125	46.708	8.79	<.001
SA Vs SAxABA.TRAIT	3	17.833	5.944	1.12	0.361
Residual	24	127.5	5.312		
Total	47	4498.479			

Analysis of variance Variate: NO_FIB_ROOTS - WINTER Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	2.667	1.333	0.15	
PHYTOHORMONE	3	2708.833	902.944	102.87	<.001
ABA Vs CONTROL	1	1700.167	1700.167	193.69	<.001
ABA Vs SA	1	77.042	77.042	8.78	0.025
ABA Vs SAxABA	1	45.375	45.375	5.17	0.063
SA Vs CONTROL	1	1053.375	1053.375	120	<.001
SA Vs SAxABA	1	240.667	240.667	27.42	0.002
Residual	6	52.667	8.778	4	
TRAIT	3	787.167	262.389	119.57	<.001
PHYTOHORMONE.TRAIT	9	64.667	7.185	3.27	0.01
ABA Vs CONTROL.TRAIT	3	28.167	9.389	4.28	0.015
ABA Vs SA.TRAIT	3	26.458	8.819	4.02	0.019
ABA Vs SAxABA.TRAIT	3	2.458	0.819	0.37	0.773
SA Vs CONTROL.TRAIT	3	2.125	0.708	0.32	0.809
SA Vs SAxABA.TRAIT	3	34.333	11.444	5.22	0.006
Residual	24	52.667	2.194		
Total	47	3668.667			

Analysis of variance Variate: ROOT_DWT_g - WINTER Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	5.2087	2.6044	4.86	
PHYTOHORMONE	3	81.844	27.2813	50.87	<.001
ABA Vs CONTROL	1	21.0938	21.0938	39.33	<.001
ABA Vs SA	1	4.5067	4.5067	8.4	0.027
ABA Vs SAxABA	1	17.0017	17.0017	31.7	0.001
SA Vs CONTROL	1	6.1004	6.1004	11.37	0.015
SA Vs SAxABA	1	39.015	39.015	72.75	<.001
Residual	6	3.2179	0.5363	3.05	
TRAIT	3	42.3656	14.1219	80.44	<.001
PHYTOHORMONE.TRAIT	9	3.5785	0.3976	2.26	0.053
ABA Vs CONTROL.TRAIT	3	1.8046	0.6015	3.43	0.033
ABA Vs SA.TRAIT	3	0.1733	0.0578	0.33	0.804
ABA Vs SAxABA.TRAIT	3	0.575	0.1917	1.09	0.372
SA Vs CONTROL.TRAIT	3	2.3579	0.786	4.48	0.012
SA Vs SAxABA.TRAIT	3	1.2617	0.4206	2.4	0.093
Residual	24	4.2133	0.1756		
Total	47	140.4281			

Analysis of variance Variate: SHOOT_DWT_g - WINTER Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	9.7588	4.8794	8.89	
PHYTOHORMONE	3	144.2317	48.0772	87.56	<.001
ABA Vs CONTROL	1	49.0204	49.0204	89.27	<.001
ABA Vs SA	1	29.4817	29.4817	53.69	<.001
ABA Vs SAxABA	1	13.0537	13.0537	23.77	0.003
SA Vs CONTROL	1	2.4704	2.4704	4.5	0.078
SA Vs SAxABA	1	81.7704	81.7704	148.92	<.001
Residual	6	3.2946	0.5491	3.99	
TRAIT	3	29.325	9.775	70.95	<.001
PHYTOHORMONE.TRAIT	9	5.0433	0.5604	4.07	0.003
ABA Vs CONTROL.TRAIT	3	0.3346	0.1115	0.81	0.501
ABA Vs SA.TRAIT	3	3.485	1.1617	8.43	<.001
ABA Vs SAxABA.TRAIT	3	0.1546	0.0515	0.37	0.773
SA Vs CONTROL.TRAIT	3	2.7479	0.916	6.65	0.002
SA Vs SAxABA.TRAIT	3	2.8212	0.9404	6.83	0.002
Residual	24	3.3067	0.1378		
Total	47	194.96			

Appendix XIX: Anova results for effects of drought on plant height, tillering ability, total dry weight and membrane stability index in trait-specific SPRING barley genotypes under the influence of phytohormones in controlled conditions

Analysis of variance Variate: HEIGHT - SPRING Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	40.625	20.312	1.08	
PHYTOHORMONE	3	2353.083	784.361	41.82	<.001
ABA Vs CONTROL	1	1441.5	1441.5	76.85	<.001
ABA VS SA	1	126.042	126.042	6.72	0.041
ABA Vs SAxABA	1	51.042	51.042	2.72	0.15
SA Vs CONTROL	1	715.042	715.042	38.12	<.001
SA Vs SAxABA	1	337.5	337.5	17.99	0.005
Residual	6	112.542	18.757	3.51	
TRAIT	3	100.917	33.639	6.3	0.003
PHYTOHORMONE.TRAIT	9	153.917	17.102	3.2	0.011
ABA Vs CONTROL.TRAIT	3	117.833	39.278	7.36	0.001
ABA VS SA.TRAIT	3	38.125	12.708	2.38	0.095
ABA Vs SAxABA.TRAIT	3	45.458	15.153	2.84	0.059
SA Vs CONTROL.TRAIT	3	68.125	22.708	4.25	0.015
SA Vs SAxABA.TRAIT	3	11.167	3.722	0.7	0.563
Residual	24	128.167	5.34		
Total	47	2889.25			

Analysis of variance Variate: TILLERS - SPRING Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	27.542	13.771	3.25	
PHYTOHORMONE	3	1172.167	390.722	92.09	<.001
ABA Vs CONTROL	1	672.042	672.042	158.39	<.001
ABA VS SA	1	24	24	5.66	0.055
ABA Vs SAxABA	1	40.042	40.042	9.44	0.022
SA Vs CONTROL	1	442.042	442.042	104.18	<.001
SA Vs SAxABA	1	126.042	126.042	29.71	0.002
Residual	6	25.458	4.243	3.36	
TRAIT	3	38.167	12.722	10.07	<.001
PHYTOHORMONE.TRAIT	9	20	2.222	1.76	0.13
ABA Vs CONTROL.TRAIT	3	4.125	1.375	1.09	0.373
ABA VS SA.TRAIT	3	1.333	0.444	0.35	0.788
ABA Vs SAxABA.TRAIT	3	8.458	2.819	2.23	0.111
SA Vs CONTROL.TRAIT	3	7.458	2.486	1.97	0.146
SA Vs SAxABA.TRAIT	3	7.125	2.375	1.88	0.16
Residual	24	30.333	1.264		
Total	47	1313.667			

Analysis of variance Variate: TOTAL_DRY_WT - SPRING Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	2.0554	1.0277	3.13	
PHYTOHORMONE	3	887.0483	295.6828	901.51	<.001
ABA Vs CONTROL	1	148.0067	148.0067	451.26	<.001
ABA VS SA	1	5.3204	5.3204	16.22	0.007
ABA Vs SAxABA	1	291.9037	291.9037	889.99	<.001
SA Vs CONTROL	1	97.2038	97.2038	296.37	<.001
SA Vs SAxABA	1	376.0417	376.0417	1146.52	<.001
Residual	6	1.9679	0.328	0.87	
TRAIT	3	143.3783	47.7928	126.37	<.001
PHYTOHORMONE.TRAIT	9	26.44	2.9378	7.77	<.001
ABA Vs CONTROL.TRAIT	3	3.96	1.32	3.49	0.031
ABA VS SA.TRAIT	3	12.5479	4.1826	11.06	<.001
ABA Vs SAxABA.TRAIT	3	10.2746	3.4249	9.06	<.001
SA Vs CONTROL.TRAIT	3	2.4813	0.8271	2.19	0.116
SA Vs SAxABA.TRAIT	3	14.7817	4.9272	13.03	<.001
Residual	24	9.0767	0.3782		
Total	47	1069.9667			

Analysis of variance Variate: MSI - SPRING Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	2.83	1.42	0.08	
PHYTOHORMONE	3	4487.61	1495.87	83.95	<.001
ABA Vs CONTROL	1	2815.4	2815.4	158	<.001
ABA VS SA	1	37.61	37.61	2.11	0.197
ABA Vs SAxABA	1	57.2	57.2	3.21	0.123
SA Vs CONTROL	1	2202.23	2202.23	123.59	<.001
SA Vs SAxABA	1	187.57	187.57	10.53	0.018
Residual	6	106.91	17.82	1.53	
TRAIT	3	1083.34	361.11	30.95	<.001
PHYTOHORMONE.TRAIT	9	168.5	18.72	1.6	0.17
ABA Vs CONTROL.TRAIT	3	10.87	3.62	0.31	0.818
ABA VS SA.TRAIT	3	67.09	22.36	1.92	0.154
ABA Vs SAxABA.TRAIT	3	125.32	41.77	3.58	0.029
SA Vs CONTROL.TRAIT	3	39.61	13.2	1.13	0.356
SA Vs SAxABA.TRAIT	3	22.97	7.66	0.66	0.587
Residual	24	280.06	11.67		
Total	47	6129.25			

Appendix XX: Anova results for effects of drought on plant height, tillering ability, total dry weight and membrane stability index in trait-specific WINTER barley genotypes under the influence of phytohormones in controlled conditions

Analysis of variance Variate: HEIGHT - WINTER Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	15.125	7.562	0.58	
PHYTOHORMONE	3	5911.75	1970.583	149.9	<.001
ABA Vs CONTROL	1	3901.5	3901.5	296.79	<.001
ABA Vs SA	1	273.375	273.375	20.8	0.004
ABA Vs SAxABA	1	57.042	57.042	4.34	0.082
SA Vs CONTROL	1	2109.375	2109.375	160.46	<.001
SA Vs SAxABA	1	580.167	580.167	44.13	<.001
Residual	6	78.875	13.146	2.77	
TRAIT	3	232.417	77.472	16.31	<.001
PHYTOHORMONE.TRAIT	9	563.083	62.565	13.17	<.001
ABA Vs CONTROL.TRAIT	3	391.5	130.5	27.47	<.001
ABA Vs SA.TRAIT	3	7.125	2.375	0.5	0.686
ABA Vs SAxABA.TRAIT	3	10.458	3.486	0.73	0.542
SA Vs CONTROL.TRAIT	3	337.125	112.375	23.66	<.001
SA Vs SAxABA.TRAIT	3	4.167	1.389	0.29	0.83
Residual	24	114	4.75		
Total	47	6915.25			

Analysis of variance Variate: TOTAL_DRY_WT - WINTER Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	2.2754	1.1377	1.58	
PHYTOHORMONE	3	775.9675	258.6558	359.14	<.001
ABA Vs CONTROL	1	164.8504	164.8504	228.89	<.001
ABA Vs SA	1	13.5	13.5	18.74	0.005
ABA Vs SAxABA	1	210.6337	210.6337	292.46	<.001
SA Vs CONTROL	1	84.0004	84.0004	116.63	<.001
SA Vs SAxABA	1	330.7837	330.7837	459.29	<.001
Residual	6	4.3213	0.7202	0.87	
TRAIT	3	70.8042	23.6014	28.41	<.001
PHYTOHORMONE.TRAIT	9	13.2342	1.4705	1.77	0.127
ABA Vs CONTROL.TRAIT	3	1.4812	0.4937	0.59	0.625
ABA Vs SA.TRAIT	3	4.1067	1.3689	1.65	0.205
ABA Vs SAxABA.TRAIT	3	2.0313	0.6771	0.82	0.498
SA Vs CONTROL.TRAIT	3	10.1612	3.3871	4.08	0.018
SA Vs SAxABA.TRAIT	3	2.0046	0.6682	0.8	0.504
Residual	24	19.9367	0.8307		
Total	47	886.5392			

Analysis of variance Variate: TILLERS - WINTER Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	3.792	1.896	0.33	
PHYTOHORMONE	3	1486.729	495.576	86.92	<.001
ABA Vs CONTROL	1	828.375	828.375	145.29	<.001
ABA Vs SA	1	28.167	28.167	4.94	0.068
ABA Vs SAxABA	1	60.167	60.167	10.55	0.017
SA Vs CONTROL	1	551.042	551.042	96.65	<.001
SA Vs SAxABA	1	170.667	170.667	29.93	0.002
Residual	6	34.208	5.701	3.31	
TRAIT	3	51.729	17.243	10.01	<.001
PHYTOHORMONE.TRAIT	9	50.188	5.576	3.24	0.01
ABA Vs CONTROL.TRAIT	3	11.792	3.931	2.28	0.105
ABA Vs SA.TRAIT	3	13.5	4.5	2.61	0.075
ABA Vs SAxABA.TRAIT	3	9.5	3.167	1.84	0.167
SA Vs CONTROL.TRAIT	3	36.458	12.153	7.06	0.001
SA Vs SAxABA.TRAIT	3	25.667	8.556	4.97	0.008
Residual	24	41.333	1.722		
Total	47	1667.979			

Analysis of variance Variate: MSI - WINTER Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	2	25.43	12.72	0.8	
PHYTOHORMONE	3	4329.35	1443.12	90.83	<.001
ABA Vs CONTROL	1	2392.46	2392.46	150.59	<.001
ABA Vs SA	1	36.83	36.83	2.32	0.179
ABA Vs SAxABA	1	172.89	172.89	10.88	0.016
SA Vs CONTROL	1	1835.59	1835.59	115.54	<.001
SA Vs SAxABA	1	369.32	369.32	23.25	0.003
Residual	6	95.32	15.89	1.29	
TRAIT	3	1010.43	336.81	27.25	<.001
PHYTOHORMONE.TRAIT	9	239.02	26.56	2.15	0.065
ABA Vs CONTROL.TRAIT	3	90.64	30.21	2.44	0.089
ABA Vs SA.TRAIT	3	115.54	38.51	3.12	0.045
ABA Vs SAxABA.TRAIT	3	47.58	15.86	1.28	0.303
SA Vs CONTROL.TRAIT	3	126.44	42.15	3.41	0.034
SA Vs SAxABA.TRAIT	3	16.15	5.38	0.44	0.73
Residual	24	296.62	12.36		
Total	47	5996.18			