REACTION OF KENYAN BARLEY (*Hordeum vulgare*) CULTIVARS TO SCALD (*Rhynchosporium secalis*) AND ITS EFFECT ON YIELD AND SEED QUALITY

BY:

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DECLARATION

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I hereby declare this thesis as my original work and has not been previously presented to any University for any degree or any other award.

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DEDICATION

To my loving parents Mr. Sudi Otukho and Mrs Truphena Nyawate, my lovely daughters Vivian, Violet and Valentine who have been very supportive.

ABSTRACT

Scald caused by *Rhynchosporium secalis* (Oudem), is an important disease in Kenya.Three experiments were conducted to determine the effect of scald on the yield, grain and seed quality of barley as well as the role of seed borne inoculums in the transmission of the disease. In the first experiment, 143 barley genotypes were planted in a nursery and evaluated for resistance or susceptibility to scald at Mau Narok and Timau during the 2012/2013 barley growing season. Data on disease reaction was taken during the crop growth stages and grain yield for each entry taken at maturity. The data was analyzed to relate the effect of scald infection on grain yield and quality of the grain. Approximately 36 percent of the test lines were resistant to moderately resistant to scald. Cultivar Nguzo, HKBL 1512-5, Steptoe and QSMO005 were among those evaluated as resistant to scald. The genotypes that were resistant to scald gave higher grain and thousand kernel weights per plot in comparison to genotypes that were susceptible. In experiment two,12 barley varieties with diverse field resistances to scald were investigated in a field trial at the two sites, each in small plot in a randomized complete block design in a split plot layout (sprayed with fungicide and unsprayed). Grain yield of each of the two sub plots of the twelve varieties was taken at maturity and analyzed to assess the yield losses due to scald. Susceptible varieties gave a reduced grain yield and a significant yield loss per plot when not protected with fungicide spray. Cultivar Sabini, evaluated as susceptible, recorded the highest yield loss of 18.1 percent. Scald resistant Cultivar Nguzo and HKBL 1512-5 had the lowest yield losses of 3.81 and 3.22 percent respectively when not protected with fungicide spray. Finally, a seed-seedling transmission experiment was carried out in a greenhouse at KALRO-Njoro to determine the potential role of seed borne *R. secalis* as primary inoculum in the transmission and spread of scald. Seed infected with *R.secalis* at four levels of seed borne infection (nil, 20, 50 and >75 percent) was planted in plastic pots on sterile soil in a completely randomized design in two sub samples: seed-treated and not seed-treated with fungicide each in two replicates. Data on seed-seedling transmission was taken between plant growth stages 21 and 31. The rate of seed borne-seedling infection increased as the level of seed borne infection increased for the untreated seed category. The >75 percent seed infection category gave the highest rate of seed-seedling transmission. Seed treatment resulted in reduced rates of seed-seedling transmission except for the > 75 percent seed infection category. On conclusion of these experiments, some scald resistant barley genotypes were identified. The amount of yield losses and seed and grain quality decline to barley due to scald depends on the resistance or the susceptibility of the variety. Scald resistant varieties suffer little yield losses when attacked by the disease. Seed borne inoculums of *R. secalis* may act as a major source of infection of scald in the field. Seed dressing with appropriate fungicide is only beneficial for moderately infected seed.

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LIST OF ACRONYMS

AUDPC	Area Under Disease Progress Curve
CIMMYT	International Center for Maize and Wheat Improvement
DAP	Di-ammonium Phosphate
DRRW	Durable Rust Resistance in Wheat
EABL	East Africa Breweries Limited
EPZ	Export Processing Zones Authority - Kenya
EC	Emulsifiable Concentrate
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
IT	Infection Type
KALRO	Kenya Agricultural and Livestock Research Organization
KML	Kenya Malting Limited
LH	Low Highland ecological zone
LSD	Least Significance Difference
MAP	Mono Ammonium Phosphate
masl	Metres Above Sea Level
MR	Moderately Resistant
MS	Moderately Susceptible
NPBC	National Plant Breeding Centre
P	Probability level
R	Resistant
RCBD	Randomized Complete Block Design
S	Susceptible

USDA	United States Department of Agriculture
WP	Wettable Powder

DEFINITION OF TERMS

Breeding line	A genetic group that has been selected and bred for its special combination of traits
Culture	A clone of urediniospore that is maintained in a laboratory.
Cultivar	A cultivated variety as opposed to a botanical (taxonomic) variety
Genotype	A group of plants with the same genetic makeup
Germplasm	A collection material of genotypes of a plant
Infection type	The visible symptoms of disease produced by the interaction of host and pathogen in a specific environment
Inoculum	The spores or other propagules of the pathogen to which plants are exposed, and from which infection can take place
Isolate	A sample of a pathogen that is stored alive or maintained in isolation on plants or in nutrient media.
Lesions	Discoloration of the host tissue around the pathogen's point of entry
Pathogen	A specific biological causation agent of disease in plants or animals
Race	A group of genotypes within a pathogen species that is distinguished by its virulence.
Resistance	Capacity of a plant to reduce or stop the growth, development and reproduction of the natural enemy after establishment of intimate contact
Susceptibility	Incapacity of a plant to reduce the growth, development and reproduction of the natural enemy

Tolerance	Capacity of the host plant to restrict the symptoms or the harmful effects per unit of pathogen otherwise than by restricting the amount of infection
Virulence	The capacity of a pathogen to infect a plant with one or more major genes for (hypersensitivity) resistance, because it does not possess any of the corresponding genes for avirulence

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CHAPTER ONE

INTRODUCTION

1.0 Barley Production in Kenya

Barley (*Hordeum vulgare L.*) is a common staple in human and animal diets and is cultivated worldwide. Total world production in 2010 was 123.4 Million tonnes of which Kenya contributed only 64,000 tonnes (FAOSTAT, 2011) which is only 75% of Kenya's production potential. In Kenya barley is grown in high altitude and medium altitude areas with an annual rainfall of over 700mm (EPZA, 2005). The barley grown in Kenya is mainly for malting because of the price premium. All of the produced barley is by contract to farmers by the Kenya Maltings Limited (KML), a subsidiary of the East African Breweries Limited (EABL). Kenya Maltings Limited offers farmers input based on recruited acreage by ensuring that the recruited farmers enter into a production contract with the company. Both two-row and six row barley are produced in Kenya. \Although barley is fairly adaptable and can be grown in many agro-ecological regions in Kenya, barley for malt is preferably grown in the higher altitude, cool and wet regions of the country namely Mau-escarpment, Mount Kenya region, and Nakuru District and Moiben region. This is so because in malt barley, malting quality is measured by the percentage malt extract, which usually increases with the altitude due to the favourable cool weather prevalent. Hence, malt barley is grown mainly in regions with altitude exceeding 2300 meters above sea level. In these high altitude areas, there also occur a host of foliar diseases which hinder the production of the crop. One such disease that is causing a serious constraint in the production of barley in these areas is the barley scald disease caused by a fungus *Rhynchosporium secalis* (OUDEMANS) J.J. Davis

1.1 Scald disease of Barley

Scald of barley caused by *Rhynchosporium secalis*, recently renamed *Rhynchosporium commune* (Zaffarano *et. al.*, 2011) is a serious disease in all of the major barley growing regions of the world, The fungus is a serious pathogen in Australia, East Africa, Europe, Middle East and South Africa (Carmona and Barreto, 2003; Zaffarano *et. al*., 2006) and it

is reported to be most severe in cool, humid areas of temperate zones (Robbertse *et al*., 2000; Yahyaoui *et. al*., 2006). Barley is the only important host, but the fungus can also attack rye and some grasses. Scald is common in cool and semi-humid barley growing areas of Kenya and appropriate methods of determining and selection of resistant barley varieties in breeding programs have not been studied in depth. Commercial scald-resistant cultivars have not been developed in Kenya. Scald can be effectively controlled using integrated approach encompassing resistant varieties, cultural practices, and time of sowing, seed treatment and foliar fungicides. Since applications of fungicides are expensive it is necessary to develop resistant cultivars with high yields.

This research was carried out to determine the response of barley cultivars and breeding lines to barley scald disease under the environmental conditions found in the Kenya highlands, and to determine the effects of the disease on the yield and seed quality of barley.

1.2 Statement of the Problem

Scald of barley caused by *Rhynchosporium secalis* is a fungus disease that is a serious production constraint in major malt barley growing regions of Kenya because it can lead to significant yield losses in a barley crop and a decline in both the seed and quality of grain (Zhan *et. al*. 2008), which may be downgraded by the market. Seed testing laboratories do not routinely test for this disease and the importance of seed borne infection is not known, but is believed to be minor. The knowledge on the diversity of scald resistance within the Kenyan barley germplasm is not available. The yield losses from scald are due to the formation of shrivelled kernels with light test weight. The disease not only causes yield and quality losses, but may also be seed borne and therefore may be transmitted from infected seeds to seedlings. Except for two cultivars released in 2004 (Nguzo and Karne), most of the others are susceptible to the disease (Ndeda, 2004). Such cultivars require large inputs of fungicides which have negative environmental consequence and can hardly be afforded by many of the small scale growers who account for over 30 percent of the total production in Kenya.

The yield losses associated with the disease varies with the host cultivar and growing environment. Yield losses of up to 40 percent (Xi *et. al*., 2000) have been reported in other parts of the world. Development of host resistance as the foundation integrated management of scald is considered a key element is keeping losses due to the disease at a minimum. The variability of the scald pathogen, the emergence of new strains of *Rhynchosporium secalis* (Newton *et. al*. 2001) and the fact that resistances break down with time (Zhan *et al*. 2008 and Arova & Knogge, 2012) and the need to continuously identify new resistant germplasm is real and is the only way that higher barley productivity can be sustained.

1.3 Justification

Scald can attack a barley crop at any time but levels of infection are usually most severe just before or during heading. Yield losses as high as 40 percent has been reported (Paulitz and Steffenson, 2011) as a result of the disease in temperate countries but yield losses of 5-19 percent are more common. Studies of the genotypic differences and the

extent of yield loss and grain quality deterioration; both malting and seed quality due to the disease caused by *Rhynchosporium secalis* have not been done in Kenya. It is necessary that studies are carried out to obtain clear information about the effect on growth and yield of barley attacked by *Rhynchosporium secalis* and consequently be able to assess yield losses and seed quality decline attributable to this fungal disease in Kenya.

1.4 Objectives

1.4.1 Broad objective

The broad objective was to determine the effect of the attack by the barley scald disease on the growth, yield and seed quality of some Kenyan barley cultivars and breeding lines in Kenya.

1.4.2 The specific objectives

i. To determine the reaction of barley genotypes to scald disease.

ii. To analyze the potential role of seed borne *Rhynchosporium secalis* as primary inoculum in the transmission and spread of scald.

iii. To determine the extent of yield loss and seed quality deterioration as a result of scald.

1.5 Null Hypotheses

1. All Kenyan barley cultivars and breeding lines are susceptible to scald disease

2. Scald epidemics do not lead to significant yield losses and seed quality decline to barley cultivars and breeding lines grown in Kenya.

3. Seed borne *Rhynchosporium secalis* has no role in the transmission and spread of scald.

1.6 Expected outputs

1. Scald resistant genotypes evaluated may be used in the breeding program to improve the already existing superior commercial varieties that are susceptible or possible release of these as new varieties.
2. Documented information on the effects of scald pathogen *(Rhynchosporium secalis)* on the grain quality and seed health quality of barley.
3. Knowledge on the diversity of scald resistance available within Kenyan barley germplasm and this will be useful in planning future variety improvement schemes.

CHAPTER TWO

LITERATURE REVIEW

2.1 History of Barley

Barley (*Hordeum vulgare* L.), a member of the grass family, is a major cereal grain. It was one of the first cultivated grains and is now grown widely. Barley grain is a staple in Tibetan cuisine and was eaten widely by peasants in Medieval Europe. Barley has also been used as animal fodder, as a source of fermentable material for beer and certain distilled beverages, and as a component of various health foods. It is used in soups and stews, and in barley bread of various cultures. Barley grains are commonly made into malt in a traditional and ancient method of preparation. In a 2007 ranking of cereal crops in the world, barley was fourth both in terms of quantity produced (136 million tons) and in area of cultivation (566,000 square kilometres or 219,000 square miles) (FAO, 2009). Barley was one of the first domesticated grains in the Fertile Crescent, an area of relatively abundant water in Western Asia, and near the Nile river of northeast Africa (Badr *et. al*., 2000). The grain appeared at the same time as einkorn and emmer wheat. Wild barley (*H. vulgare* ssp. *spontaneum*) ranges from North Africa and Crete in the west, to Tibet in the east (Zohary and Maria, 2000). The earliest evidence of wild barley in an archaeological context comes from the Epipaleolithic at Ohalo II at the southern end of the Sea of Galilee. The remains were dated to about 8500 BC (Zohary and Maria, 2000). The earliest domesticated barley occurs at Aceramic Neolithic sites, in the Near East such as the Pre-Pottery Neolithic B layers of Tell Abu Hureyra, in Syria. By 4200 BC domesticated barley occurred as far as in Eastern Finland. Barley has been grown in the Korean Peninsula since the Early Mumun Pottery Period (*circa* 1500–850 BC) along with other crops such as millet, wheat, and legumes (Crawford and Gyoung, 2003).

In the Pulitzer Prize-winning book *Guns, Germs, and Steel*, (Diamond and Jared,1997),argue that the availability of barley, along with other domesticable crops and animals, in south-western Eurasia significantly contributed to the broad historical patterns that human history has followed for the last 13,000 years; *i.e.*, why Eurasian civilizations, as a whole, have survived and conquered others.

Barley beer was probably one of the first alcoholic drinks developed by Neolithic humans (Pellechia, 2006).Later barley was used as a currency (Pellechia, 2006). Alongside emmer wheat, barley was a staple cereal of ancient Egypt, where it was used to make bread and beer. The general name for barley is *jt* (hypothetically pronounced "eat"); *šma* (hypothetically pronounced "SHE-ma") refers to Upper Egyptian barley and is a symbol of Upper Egypt. The Sumerian term is *akiti*. According to Deuteronomy 8:8, barley is one of the "Seven Species" of crops that characterize the fertility of the Promised Land of Canaan, and it has a prominent role in the Israelite sacrifices described in the Pentateuch (see e.g. Numbers 5:15). A religious importance extended into the middle Ages in Europe, and saw barley's use in justice, via alphitomancy and the corsned.

2.1.1 Taxonomy of barley

Barley is a member of the grass family. It is a self-pollinating, diploid species with 14 chromosomes. The wild ancestor of domesticated barley, *Hordeum vulgare* subsp. *spontaneum*, is abundant in grasslands and woodlands throughout the Fertile Crescent area of Western Asia and northeast Africa, and is abundant in disturbed habitats, roadsides and orchards. Outside this region, the wild barley is less common and is usually found in disturbed habitats (Zohary and Maria, 2000). However, in a study of genome-wide diversity markers, Tibet was found to be an additional center of domestication of cultivated barley (Dai *et al*., 2012).

Wild barley has a brittle spike; upon maturity, the spikelets separate, facilitating seed dispersal. Domesticated barley has nonshattering spikes, making it much easier to harvest the mature ears (Zohary and Maria, 2000). The nonshattering condition is caused by a mutation in one of two tightly linked genes known as Bt_1 and Bt_2; many cultivars possess both mutations. The nonshattering condition is recessive, so varieties of barley that exhibit this condition are homozygous for the mutant allele (Zohary and Maria, 2000).

Barley is a widely adaptable crop. It is currently popular in temperate areas where it is grown as a summer crop and tropical areas where it is sown as a winter crop. Its

germination time is one to three days. Barley grows under cool conditions, but is not particularly winter hardy and is more tolerant of soil salinity than wheat, which might explain the increase of barley cultivation in Mesopotamia from the second millennium BC onwards. Barley is not as cold tolerant as the winter wheats (*Triticum aestivum*), fall rye (*Secale cereale*) or winter triticale (× *Triticosecale* Wittm. ex A. Camus.), but may be sown as a winter crop in warmer areas of Australia and Great Britain. Barley has a short growing season and is also relatively drought tolerant (Fernandez, 2000).

Barley was grown in about 100 countries worldwide in 2007. The world production in 1974 was 148,818,870 tonnes. Since then, there has been a slight decline in the amount of barley produced worldwide. (FAO, 2012). Based on barley production data of 2011, Russia was the world leader in barley production (Table 1)

Table 1: Top Barley Producers in the world in Million metric tonnes (Source: FAO, 2012)

Rank	Country	2009	2010	2011
1.	Russia	17.8	8.3	16.9
2.	Ukraine	11.8	8.4	9.1
3.	France	12.8	10.1	8.8
4.	Germany	12.2	10.4	8.7
5.	Australia	7.9	7.2	7.9
6.	Canada	9.5	7.6	7.7
7.	Turkey	7.3	7.2	7.6
8.	United Kingdom	6.6	5.2	5.4
9.	Argentina	1.3	2.9	4.0
10.	United States	4.9	3.9	3.3
-	**World Total**	**151.7**	**123.7**	**134.3**

2.2 Two-row and six-row barley

Spikelets are arranged in triplets which alternate along the rachis. In wild barley (and other Old World species of *Hordeum*), only the central spikelet is fertile, while the other two are reduced. This condition is retained in certain cultivars known as two-row barleys. A pair of mutations (one dominant, the other recessive) result in fertile lateral spikelets to produce six-row barleys (Zohary and Maria, 2000). Recent genetic studies have

revealed that a mutation in one gene, *vrs1*, is responsible for the transition from two-row to six-row barley.

Two-row barley has lower protein content than six-row barley, thus more fermentable sugar content. High protein barley is best suited for animal feed. Malting barley is usually lower protein ('low grain nitrogen', usually produced without a late fertilizer application) which shows more uniform germination, needs shorter steeping, and has less protein in the extract that can make beer cloudy. Two-row barley is traditionally used in English ale-style beers. Six-row barley is common in some American lager style beers, especially when adjuncts such as corn and rice are used, whereas two-row malted summer barley is preferred for traditional German beers (Komatsuda *et al.*, 2006).

Hulless or "naked" barley (*Hordeum vulgare* L. var. *nudum* Hook. f.) is a form of domesticated barley with an easier-to-remove hull. Naked barley is an ancient food crop, but a new industry has developed around uses of selected hulless barley to increase the digestible energy of the grain, especially for swine and poultry. Hulless barley has been investigated for several potential new applications as whole grain, and for its value-added products. These include bran and flour for multiple food applications (Bhatty, 2011).The genome of barley was sequenced in 2012 by Mayer and her team. The genome is composed of seven pairs of nuclear chromosomes (recommended designations: lH, 2H, 3H, 4H, 5H, 6H and 7H), and one mitochondrial and one chloroplastic chromosome, with a total of 5000 Mbp (Mayer *et. al.*, 2012).

2.3 Barley Farming in Kenya

Barley farming in Kenya was introduced by the colonial regime as animal food until 1929 when it was commercialised into beer making. It is now one of the principal raw materials used to process barley malt, a vital ingredient for beer brewing. Kenya’s barley growing area is estimated to be 85,000 hectares according to production estimates. However only 20,000 hectares is under barley production thus 65,000 hectares has not been utilised. Barley crop is grown in large-scale farms with relatively flat landscape to allow use of farm machineries from ploughing to harvesting. This method ensures high

efficiency in crop production and guarantees economies of scale in order to provide for local and export markets (EPZ, 2005).Barley does well in high and medium altitude with consistent annual rainfall of more than 635mm. It is commonly grown in the Rift Valley and Central provinces (Kenya's granary), owing to high productivity of foods and cash crops. Barley is grown in areas within the Mau escarpment especially in MauNarok and Narok districts. Other prominent barley producing districts in Kenya include Uasin Gishu and Timau in the Rift Valley and Central provinces, respectively.

East Africa Malting Ltd (EAML), a subsidiary of EABL, solely produces, processes and markets barley seeds and barley malt in Kenya. The company contracts two categories of farmers who have a minimum of 125 acreage of land to grow either seed barley or grain barley for processing (EPZ, 2005). The first categories of farmers grow seed barley and sell it to the company for production of three cultivars of barley namely Karne, Sabini, and Bima. Currently the company is only offering Sabini and Bima since Karne has become susceptible to net blotch, a fungal infection and gives lower yields. Kenya Plant Health Inspectorate Service (KEPHIS), an independent government seed quality regulatory agency, certifies seed quality standards of the seed barley before it is offered for sale to the farmers concerned with the barley crop for malt production. Seed barley is packaged in 50kg bags and cost between KShs 2,000 to 2,500 (US$ 25-31.25) (EPZ, 2005) .The second categories of farmers grow barley for processing malt for beer brewing. Regardless of the market or weather patterns in future, the company and farmers agree on the sale price of barley when signing the contract. The year 2004 sale price agreed upon was KShs. 1,520 (US$19) for a bag of 80kg (EPZ, 2005).

Courtesy of EABL, farmers are offered guaranteed loans aggregating KShs 450 million ($5.7 million) annually from banks such as Kenya Commercial Bank, Standard Chartered Bank of Kenya and Barclays Bank of Kenya, to finance 50% of crop production and repayment is done through individual accounts after the company pays farmers for their produce. Kenya barley malting infrastructure is currently worth over KShs.1.9 billion

($238 million) which includes malting industry in Nairobi, research facilities in Moiben, Uasin Gishu district and a barley filtration and storage facility in Molo (EPZ, 2005).

EAML has also contracted transporters who deliver barley from farmers to various storage and processing destinations at an average cost of Kshs. 6.5 per tonne per kilometre. Production capacity of barley is unlimited owing to good weather, and on average one hectare of land can yield at least 2.6 tonnes of barley. According to EABL's financial results of 2003, the company processed 20,744 tonnes of net sale volume for KShs.734 million as opposed to 17,067 tonnes of net sale volume for Kshs. 590 million in 2002, which was an increase of 22% (EPZ, 2005).

2.3.1. Contribution of beer to Kenyan Economy

Beer and barley can be considered as one of economic sub-sectors inherited from the colonial era. The idea was noble as the industry evolved to a giant brewery across the East African region under the name East African Breweries Ltd (EABL). Currently the company controls about 95% of bottled beer marketed in Kenya, about 30% share in Tanzania and around 60% market share in Uganda. The other major player in the bottled beer market in the region (Uganda and Tanzania) is SAB Miller of South Africa, which controls over 60% of the Tanzanian market and about 30% of the Ugandan market (EPZ, 2005).SAB came with a bang in the Kenyan beer market but were out-competed by EABL and left the market. The market prospects for EABL within East Africa region is getting better as the sector focuses on innovation in the businesses and working with the respective governments in trying to reduce excise duty that is currently considered very high. The sub-sector has undergone tremendous changes and currently Kenya is one of the world's leading producers of quality beer having won various international award competitions on various brands of locally produced beer. The only major market player in the sector has been EABL though Castle Breweries Ltd of South Africa had ventured into the Kenyan market but opted out after sometime, citing problems in sourcing barley locally and the import duty charged by the government. Beer market growth is flat in all the three states due to economic hardships that have continued to affect beer industry,

coupled with high taxes, stiff competition from other beverage sub sectors and low consumer spending (EPZ, 2016).

Kenya is self-sufficient in beer and barley and has remarkably invested in all the East African countries commanding the highest market share within the region. High excise duties charged on beer makes the sub-sector one of the main revenue earners for the government. EABL is currently one of the highest corporate taxpayers with annual turnover of Kshs. 28.9 billion and employs more than 1600 people across the region (EPZ, 2016).

EABL currently enjoys trade monopoly in formal sector beer. Currently, branded beer accounts for 40% of alcohol market though it faces stiff competition from cheap spirits and illicit / traditional brews. There has been a reduction in beer sales volumes by more than one million hectoliters in the past decade. According to EABL half-year results, beer sales volumes went down by 4% while spirit sales volumes showed some gains during 2003, which indicates beer market shift to spirit or other cheap alcoholic beverages principally because of sale price considerations. Among key brands of beer available in the Kenyan market are Tusker Lager, Pilsner Lager, Tusker Export, Tusker Malt, Pilsner Ice, Pilsner Ice Light, Allsopps, White Cap, Citizen, and Guinness Stout (EPZ, 2005).

2.4 Scald Disease of Barley

Scald caused by *Rhynchosporium secalis* (Oudem), (renamed *Rhynchosporium commune*) is an important disease of barley, rye and others wild grasses, particularly species of Bromus (Ishkova *et al.,* 2002). It is a serious disease in all of the major barley growing regions of the world (Zaffarano *et. al*., 2006; Zhan *et al.,* 2008) and it is reported to be most severe on barley in cool, humid areas of temperate zones (Yahyaoui *et al.*, 2003). In the field, yield losses by *Rhynchosporium secalis* have been determined by various researchers and the current reported average yield losses vary from1 to 19 percent (Xi *et. al.*, 2000) but could be as high as 40 percent (Williams *et. al.*, 2003; Yahyaoui, 2003) or greater in highly susceptible cultivars (Paulitz and Steffenson, 2011). In Kenya this disease is currently one of the major constraints to barley production as a result of the

predominant environmental conditions that are conducive to the development of this disease. The pathogen is characterized by a high level of pathogenic variability as has been demonstrated in different regions of the world where the disease is a problem. The highly variable nature of *Rhynchosporium secalis* may result in new pathotypes that can overcome host plant resistance gene(s) after they are deployed. Diagnosis of scald is done by examining the crop at the milky ripe stage (Feekes scale). Assessment of scald is done on more than 25 main tillers that are selected at random along two diagonals from one corner to the opposite corner of the field. Assessment of scald infection is done by observing the percentage of the first and the second leaves of the tillers that are infected with the disease. The average of the first leaf and the second leaf is then taken and applied to the following formula to give a reasonable estimate of expected crop loss from scald.

$$\%\ yield\ loss = \frac{\left(\frac{2}{3} * \%\ area\ of\ flag\ leaf\ infected\right) + \left(\frac{1}{2} * \%\ area\ of\ second\ leaf\ infected\right)}{2}$$

2.4.1 Morphology, biology and epidemiology of scald

Rhynchosporium secalis fungus attacks the leaves and heads of barley plants and may cause significant losses if it spreads to upper parts of the plant. The pathogen is spread from plant to plant primarily by water-splash, dispersion of spores, and can persist in crop residues. The high pathogenic variability of the pathogen in natural populations as it has been repeatedly demonstrated in different regions of the world where the disease is a problem is a source of big concern.Scald on barley presents oval to lens-shaped or elongated spots (lesions) 0.5-2 x 0.1-0.5 up to 1-2 x 5-7 cm, surrounded by straw-coloured borders which develop mostly on the leaves and leaf sheaths (Lee *et al.,* 2001).

a) b)

Figure 1; Symptoms of scald attack on barley; a) Typical scald lesions on barley leaves - straw coloured areas surrounded by definite brown borders. b) Severe scald infected flag leaves. (Source: Practical guide to identification of selected diseases of wheat and barley; (CIMMYT, 1983).

The lesions at first, appear water-soaked, with dark green to pale grayish green color ; after which they dry out and the centers become light tan to straw brown to pale grayish green white, and are surrounded by prominent, dark brown to reddish brown borders. The lesions enlarge, merge and form elongated, irregular blotches of various sizes and shapes on the leaves. Older leaves may have a 'zonate' appearance (Figure 1). If the attack is severe the plant may completely dry up.

A fungus develops a superficial, loose stroma on which transparent conidias are produced from short cells. The conidium has one or two unequally sized cells of 12-20 x 2.3-5.4 um. The terminal cell hooks into a beak. The fungus over-seasons as mycelia on dead or living leaves of infected plants and on other crop debris. The scald fungi may infect seeds and can be carried on the seed (Lee *et al.*, 2001). Asexual stage has not been observed in nature. During prolonged periods of cool, moist weather in the spring, the scald fungi

resumes growth and produce large amounts of conidia. Spores produced on infected leaves are transported to other plants by rain drops and wind (Yahyaoui *et. al*., 2003). Under favourable conditions disease develops in 14-15 days (Nazarova *et al.*, 1998).

2.4.2 Life cycle of scald and yield losses to barley

Rhynchosporuim secalis is an obligate parasite hence cannot complete its life cycle in absence of a living plant host. *Rhynchosporium* fungi over-season as mycelia on dead or living leaves of infected plants and on other crop debris.during the prolonged periods of cool, moist weather in spring, the fungi resume growth on infected tissue and produce a large number of colorless, two-celled, microscopic spore: conidia. The conidia are carried by rain splashes and or air currents to new growth, where the leaves, leaf sheaths, and seedlings become infected. The scald fungi can also be carried on seed which result into seed-to- seedling transmission of the disease (Figure 2).

Scald is an important fungus in cool, humid, temperate climates. Disease develops only when the weather is cool (optimal at 10^0 C to 21^0 C) and the leaves are wet for a long time. Spore production and infection occurs repeatedly during cool, moist, humid periods that last at least 12 hours in the spring and early summer, and continue until the crop ripens. Scald is checked during hot, dry, summer weather. New infections occur in the fall when cool, damp weather returns. Infection of winter crops may be carried out in autumn by spores from debris of wild plants (Nazarova *et al*., 1998).

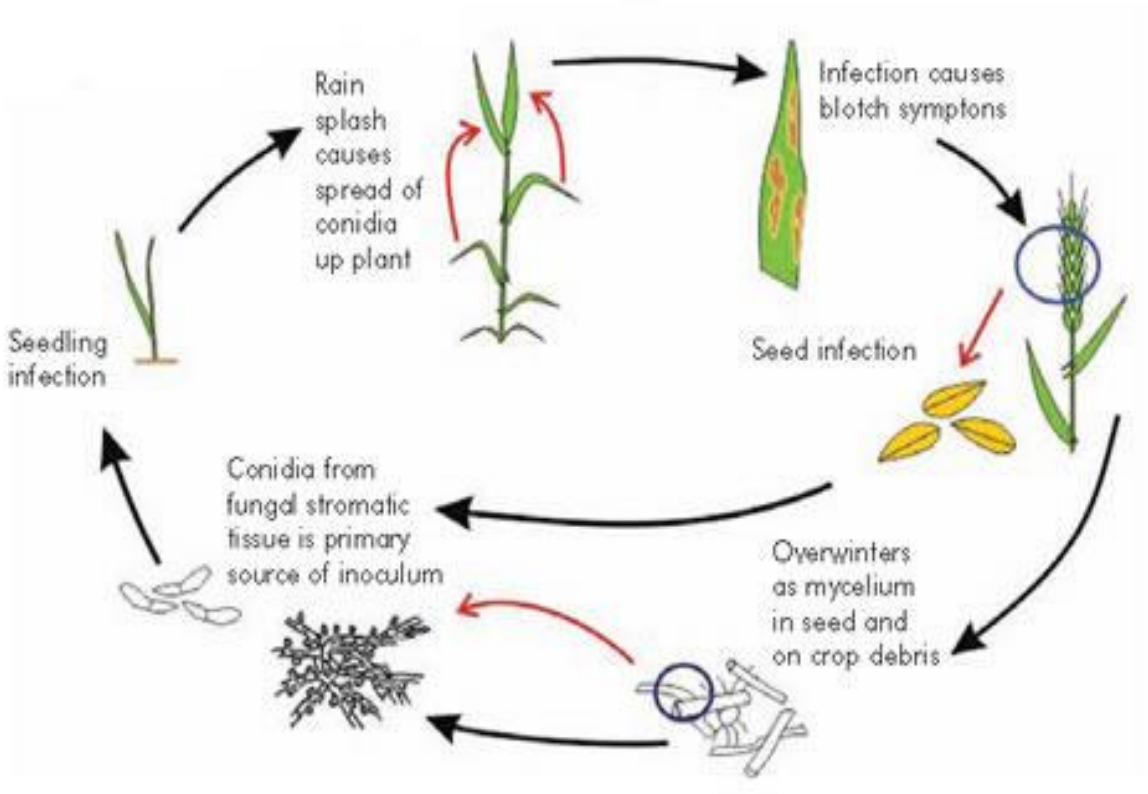

Figure 2; A typical life cycle of *Rhynchosporium secalis* on barley (Source: Practical guide to the identification of selected diseases of wheat and barley, CIMMYT, 1983).

Yield losses to barley as a result of scald attack can be estimated in the field through experiments (James *et. al*. 1988) or by using non plot methods (Richardson, 1981) involving half field or single tillers. In field experiments, yield losses are measured in experiments involving fungicide protection and use of isogenic cultivars or lines. An example of the approach where use of isogenic lines is employed is the work of Schaller (1951), who compared yield differences between isogenic lines differing only for scald resistance. By this method he quantified yield loss at 22.3 percent.

2.4.3 Control of Scald Disease

Scald of barley caused by *Rhynchosporium secalis* is a polycyclic disease and can be transmitted through inoculation of conidia that is produced that is produced on crop debris and infected seeds. Secondary infection of the disease is by the dispersal of conidia on infected leaves. The best control strategy for the disease is the integrated approach combining plant host resistance for the disease by growing of resistant cultivars, the use of appropriate fungicides in the form of foliar sprays and seed dressers in addition to cultural practices such as the soil the crop rotation and the ploughing under the soil the stubble and other plant litter that my act as sources of inoculum of the disease. The use of resistant barley cultivars is the most effective and sustainable strategy to control the disease; however the *Rhynchosporium* fungus is a highly variable pathogen and is able to overcome resistances very quickly (Xi *et. al*., 2002; Zhan *et. al*., 2008: Avrora and Knogge, 2012).The pathogenic variability in *Rhynchosporium* has been studied by several researchers worldwide (Yahyahoui *et. al*, 2003); for example, Takauz (1991) used a set of 10 differential cultivars and identified 52 pathotypes using 256 isolates from Alberta. Fukuyama *et. al.* (1998) classified 36 different pathotypes according to their virulence on 14 differentials. These findings indicate that it is important to have good knowledge of the degree of pathogenic and genetic variability of the pathogen when breeding for resistance to scald. There is therefore demand for efficient breeding programs to come up with suitable molecular markers and gene pyramiding strategies (Looseley *et.al*., 2012). In Kenya most of the commercial cultivars are susceptible to scald except for Karne and Nguzo which were released in 2004 (Ndeda, 2004).

Chemical control of scald is the main management strategy of scald, however the variable nature of *Rhynchosporum* enables it to overcome new fungicides very fast (Avrova & Knogge, 2012; Nichola *et. al.*, 2014).additionally, the increasing bans on pesticides in EU reduce the opportunities to achieve good control of pathogens in cereals exclusively through application of fungicides (Hillocks, 2012). Foliar spray applications with recommended fungicides are useful when there can occur a sudden outbreak of scald disease particularly in the higher altitude areas where barley is grown especially during

the cool and humid climate. The foliar fungicide application is only profitable if done from the time when the crop's last leaf appears and the start of heading of the crop. Fungicide application is done when the first symptoms of scald appear or as a preventative measure for the disease.

The foliar fungicide application is recommended to link together with other disease treatment, by using a fungicide with required efficiency scale. Seed treatment is effective against diseases carried on or in the seed. Some of the better chemical treatments also protect the germinating seed to some extent against injurious soil-borne organisms. These treatments control the seed borne phase of several other barley diseases including, net blotch, and spot spores from neighbouring fields. Other diseases such as scald rusts, mildew, and some virus diseases are not controlled by seed treatments. Chemical fungicides are applied to seed in several forms and by several methods. They are marketed either as dusts or liquids. Fungicidal dusts may be applied to seed either directly or in a thick water suspension called slurry. Liquids are sprayed or misted directly on the seed in various types of treating machines (Shipton *et. al*., 1974).

Cultural practices such as rotation with other crops, burning stubble, ploughing under stubble and other plant litter that may enable disease organisms to live from one season to the next, late planting, proper land preparation, and time of seedling also may be important factors in disease control. Destruction of volunteer barley plants and grasses may reduce sources of primary inoculum (Shipton *et.al*. 1974). Wide row spacing of barley and use of fertilizers without excess nitrogen may reduce scald incidence by developing a less favourable microclimate for disease development. Barley scald can also be controlled by using variety rotation using barley varieties with different genetic resistance (Tekauz 2003; Turkington *et.al.* 2005)

CHAPTER THREE

MATERIALS AND METHODS

3.1.0 To determine the reaction of some Kenyan barley genotypes to scald disease

3.1.1 Description of the site

The experiment was carried out in an open field in two sites: at Timau in Ngushishi location and at Purko ranch in Mau Narok in Narok County. Ngushishi is located along the Nanyuki-Meru road in Meru County. The altitude is about 2771 meters above sea level and it lies between 0^o 20'S and 35^o 56'E. The area receives an average rainfall of 939mm per annum, with a mean temperature of 14.9 ^oC. The site is classified as Lower Highland 2 to 3 (LH2 – LH3) agro ecological zones and has a sub humid modified tropical climate, with a relative humidity of 95 percent. Soil type is predominantly *mollic Andosols* (Jaetzold & Schmidt, 2007).

Purko ranch is situated about 200m from Tipis town along Mau Narok – Narok road in Narok County. The altitude is about 2900 meters above sea level and it lies between 0^o 39' S and 35^o 57' E. The area receives an annual rainfall of between 1200 to 1500mm, with a mean temperature of 12.8 ^oC. The relative humidity ranges between 90 and 95 percent.

3.1.2 Plant materials and Methodology

One hundred and forty three barley breeding lines and cultivars used in this experiment were obtained from the East African Maltings Limited and the USDA barley program. The breeding lines/cultivars from the USDA barley program are selections from the durable resistance in rust (DRRW) testing project with good malting characteristics and also had shown some resistance to wheat stem rust disease caused by *Puccinia graminis*. These were investigated in field trials for their reaction to scald disease of barley caused by *Rhynchosporium secalis* and were planted in a disease nursery at two sites: Mau

Narok and Timau. The one hundred and forty three genotypes are summarized in the Table 2 that follows:

Table 2: A summary of the 143 cultivars and breeding lines used in the experiment

No.	Kenyan varieties	Varieties from USDA	Category	Important Attribute
1.	HKBL1629-14	-	Breeding line	Malt barley
2.	HKBL1595-5	-	Breeding line	Malt barley
3.	HKBL1675-3	-	Breeding line	Malt barley
4.	HKBL1591-8	-	Breeding line	Malt barley
5.	HKBL1629-5	-	Breeding line	Malt barley
6.	HKBL1629-12	-	Breeding line	Malt barley
7.	HKBL1629-4	-	Breeding line	Malt barley
8.	HKBL1629-19	-	Breeding line	Malt barley
9.	HKBL1621-15	-	Breeding line	Malt barley
10.	HKBL1622-6	-	Breeding line	Malt barley
11.	HKBL1673-9	-	Breeding line	Malt barley
12.	HKBL1642-9	-	Breeding line	Malt barley
13.	HKBL1675-8	-	Breeding line	Malt barley
14.	HKBL1674-4	-	Breeding line	Malt barley
15.	HKBL1629-10	-	Breeding line	Malt barley
16.	HKBL1595-1	-	Breeding line	Malt barley
17.	HKBL1512-5	-	Breeding line	Malt barley
18.	-	QSMO93	Breeding line	Malt barley
19.	-	Chevron01	Commercial cultivar	Stem rust resistance
20.	-	USDA344	Breeding line	Malt barley
21.	-	QSMO89	Breeding line	Malt barley
22.	-	Steptoe01	Commercial cultivar	Stem rust resistance
23.	-	Steptoe02	Commercial cultivar	Stem rust resistance
24.	-	09N6-08	Breeding line	Malt barley

25.	-	QSMO08	Breeding line	Malt barley
26.	-	Steptoe03	Commercial cultivar	Stem rust resistance
27.	-	QSMO94	Breeding line	Malt barley
28.	-	Steptoe04	Commercial cultivar	Stem rust resistance
29.	-	21481	Breeding line	Stem rust resistance
30.	-	QSMO97	Breeding line	Malt barley
31.	-	Steptoe05	Commercial cultivar	Stem rust resistance
32.	-	Steptoe06	Commercial cultivar	Stem rust resistance
33.	-	USDA620	Breeding line	Malt barley
34.	-	USDA386	Breeding line	Malt barley
35.	-	USDA384	Breeding line	Malt barley
36.	-	QSMO86	Breeding line	Malt barley
37.	-	USDA261	Breeding line	Malt barley
38.	-	USDA709	Breeding line	Malt barley
39.	-	USDA1771	Breeding line	Malt barley
40.	-	Steptoe07	Commercial cultivar	Stem rust resistance
41.	-	USDA341	Breeding line	Malt barley
42.	-	USDA708	Breeding line	Malt barley
43.	-	USDA143	Breeding line	Malt barley
44.	-	USDA622	Breeding line	Malt barley
45.	-	USDA1811	Breeding line	Malt barley
46.	-	USDA396	Breeding line	Malt barley
47.	-	USDA382	Breeding line	Malt barley
48.	-	USDA345	Breeding line	Malt barley
49.	-	07MB-405	Breeding line	Malt barley
50.	-	USDA1472	Breeding line	Malt barley
51.	-	Q218619	Breeding line	Stem rust resistance
52.		UT03B1953-64	Breeding line	Malt barley

53.	-	USDA623	Breeding line	Malt barley
54.	-	USDA380	Breeding line	Malt barley
55.	-	USDA1648 (Sebastian)	Breeding line	Stem rust susceptible
56.	-	08-MN-49	Breeding line	Malt barley
57.	-	Q21861DHP	Breeding line	Stem rust resistance
58.	-	USDA383	Breeding line	Malt barley
59.	-	08-WA-01	Breeding line	Malt barley
60.	-	Steptoe08	Commercial cultivar	Stem rust resistance
61.	-	USDA340	Breeding line	Malt barley
62.	-	USDA399	Breeding line	Malt barley
63.	-	Steptoe09	Commercial cultivar	Stem rust resistance
64.	-	Nguzo	Commercial cultivar	Malt barley/scald resistance
65.	-	QSMO55	Breeding line	Malt barley
66.	-	QSMO42	Breeding line	Malt barley
67.	-	QSMO33	Breeding line	Malt barley
68.	-	Steptoe10	Commercial cultivar	Stem rust resistance
69.	-	QSMO70	Breeding line	Malt barley
70.	-	09N2-52	Breeding line	Malt barley
71.	-	09N2-64	Breeding line	Malt barley
72.	-	09MT-38	Breeding line	Malt barley
73.	-	Q21861	Breeding line	Stem rust resistance
74.	-	QSMO15	Breeding line	Malt barley
75.	-	Steptoe11	Commercial cultivar	Stem rust resistance
76.	-	QSMO19	Breeding line	Malt barley
77.	-	QSMO18	Breeding line	Malt barley
78.	-	QSMO29	Breeding line	Malt barley
79.	-	Chevron02	Commercial	Stem rust resistance

			cultivar	
80.	-	15517	Breeding line	Malt barley
81.	-	14934	Breeding line	Malt barley
82.	-	08-UT-47	Breeding line	Malt barley
83.	-	SWISSHV65	Breeding line	Malt barley
84.	-	21486	Breeding line	Stem rust resistance
85.	-	CLHO14977	Breeding line	Malt barley
86.	-	08-AB-16	Breeding line	Malt barley
87.	-	SHECH/HAR	Breeding line	Malt barley
88.	-	Chevron03	Commercial cultivar	Stem rust resistance
89.	-	P1347245	Breeding line	Malt barley
90.	-	24767	Breeding line	Malt barley
91.	-	Chevron04	Commercial cultivar	Stem rust resistance
92.	-	Steptoe12	Breeding line	Stem rust resistance
93.	-	SWISSHV67	Breeding line	Malt barley
94.	-	05WA-328.8	Breeding line	Malt barley
95.	-	Chevron05	Commercial cultivar	Stem rust resistance
96.	-	08-AB-17	Breeding line	Malt barley
97.	-	06WA-466.6	Breeding line	Malt barley
98.	-	SWISSHV63	Breeding line	Malt barley
99.	-	ND25161	Breeding line	Malt barley
100.	-	08-UT-91	Breeding line	Malt barley
101.	-	Chevron06	Commercial cultivar	Stem rust resistance
102.	-	14938	Breeding line	Malt barley
103.	-	08-BA-69	Breeding line	Malt barley
104.	-	QSMO37	Breeding line	Malt barley
105.	-	25030	Breeding line	Malt barley
106.	-	08-WA-64	Breeding line	Malt barley

107.	-	QSMO16	Breeding line	Malt barley
108.	-	14942	Breeding line	Malt barley
109.	-	Steptoe13	Commercial cultivar	Stem rust resistance
110.	-	Chevron07	Commercial cultivar	Stem rust resistance
111.	-	QSMO54	Breeding line	Malt barley
112.	-	SWISSHV67	Breeding line	Malt barley
113.	-	SWISSHV50	Breeding line	Malt barley
114.	-	Chevron08	Commercial cultivar	Stem rust resistance
115.	-	SWISSHV68	Breeding line	Malt barley
116.	-	08-UT-73	Breeding line	Malt barley
117.	-	QSMO-49	Breeding line	Malt barley
118.	-	QSMO002	Breeding line	Malt barley
119.	-	QSMO005	Breeding line	Malt barley
120.	-	08-N2-22	Breeding line	Malt barley
121.	-	Q21861	Breeding line	Stem rust resistance
122.	-	QSMO059	Breeding line	Malt barley
123.	-	QSMO057	Breeding line	Malt barley
124.	-	QSMO061	Breeding line	Malt barley
125.	-	14942	Breeding line	Malt barley
126.	-	14905	Breeding line	Malt barley
127.	-	ND25882	Breeding line	Malt barley
128.	-	08-WA-42	Breeding line	Malt barley
129.	-	23027	Breeding line	Malt barley
130.	-	Chevron09	Commercial cultivar	Stem rust resistance
131.	-	QSMO79	Breeding line	Malt barley
132.	-	Chevron10	Commercial cultivar	Stem rust resistance
133.	-	Rawson	Commercial cultivar	Feed barley

134.	-	04WA-122.9	Breeding line	Malt barley
135.	-	08-N2-47	Breeding line	Malt barley
136.	-	08-N2-87	Breeding line	Malt barley
137.	-	08-N2-48	Breeding line	Malt barley
138.	-	Diamora	Commercial cultivar	Malt barley
139.	-	P1386458	Breeding line	Malt barley
140.	-	FEG192-1	Breeding line	Malt barley
141.	-	QSMO90	Breeding line	Malt barley
142.	-	SWISSHV66	Breeding line	Malt barley
143.	-	Q21861	Breeding line	Stem rust resistance

The one hundred and forty three cultivars and breeding lines were planted in Timau and Mau-Narok each for one season, on 3rd March 2012 and 22nd September 2012 respectively, in a randomized complete block design (RCBD) with three replications. Test plots consisted of double two-meter long rows with an inter-row spacing of 20 centimetres. Each plot was adjacent to a scald spreader row, which consisted of a highly susceptible cultivar (Sabini). The spreader rows were inoculated with scald isolates where necessary.

The inoculum was prepared by grinding diseased barley leaves from a barley crop that was severely infected with scald in a food chopper, filtering the juice through cheese cloth and diluting 1/5 with distilled water. The inoculum was then applied to plants in the field by spraying or injection at tillering and booting stages of growth. Sowing was done by hand at a seed rate of 80 Kg/Ha. Compound fertilizer 11:52:0 (MAP) was applied at the rate of 120 Kg/Ha. Broad leaved weeds were controlled by manual weeding.

3.1.3 Data collection and analysis

3.1.3.1 Scald disease response and severity score

Assessment of disease severity (per cent leaf area affected on leaf capable of expressing disease) was made on two dates, between growth stages 30 and 77 (Zadocks *et al.*, 1974). Plant infection response to scald infection based on size of necrotic leaf blotches and the associated necrosis was classified into four discrete categories, R= Resistant, MR= Moderate Resistant to MS= Moderately Susceptible and S= Susceptible. The visual scale (Aoki *et. al*., 2011) in Table 3 was used to score for damage. After scoring the data was extrapolated to get percentage disease severity.

Table 3: A scale used for rating plant reaction to scald in a field test (Aoki et al., 2011)

Severity of Infection	**Host response**	**Disease symptoms**
0	Immune	No visible spots or lesions.
1	Highly resistant	Few visible small spots.
2	Resistant	Lesions occupy about ¼ of all leaves.
3	Moderately resistant – Moderately susceptible	Lesions occupy about ½ of all leaves.
4	Moderately Susceptible	Lesions occupy about ¾ of all
5	Susceptible	leaves.
		Most leaves weathered.

3.1.3.2 Determination of Grain Yield

All the one hundred and forty plots were harvested using sickles and then threshed mechanically. The grain weight from each subplot was obtained after seed cleaning. Moisture content of grains was obtained using a digital computerized moisture meter model 700. The yield figures were adjusted to standard 12.5 percent moisture content

then converted to t/ha. Data collected on final disease score was used to develop the Area Under Disease Progress Curve (AUDPC) and Final Disease Score (FDS) for each treatment. These data were used to classify and ordinate the genotypes on their responses to *Rhynchosporium secalis*. Grain yield data was used to culculate the average grain yield per treatment per site.

3.2.0 To analyze the potential role of seed borne R*hynchosporium secalis* as primary inoculum in the transmission and spread of scald.

3.2.1 Condition in the Greenhouse

The experiment was carried out in a greenhouse at Kenya Agricultural Research Institute (KARI) in Njoro. KARI – Njoro, in a chamber where the temperature fluctuated between 14^0 C and 23^0 C with about 90 percent humidity.

3.2.2 Plant materials and Methodology

Four categories of seed samples; clean (0 infection), low infection (20-25%), moderate infection (50%), and severe infection (>75%) of this pathogen were planted for this test. The four categories of seed infection were achieved by mixing clean (scald-free) barley seeds with those that were infected with scald and obtained from a barley crop that was severely infected with scald symptoms. The isolation and identification of *Rhynchosporium secalis*, the causal agent of scald was done by incubating barley seeds from the infected crop on Lima Bean Agar (LBA) in Petri plates at 18-20^0 C. Schein and Karelo (1956) for 14 days. The scald infection symptoms on the plant were then correlated to the seed- borne infection. The seed health testing results indicated that this particular seed sample had an average of 20% seed borne infection with *Rhynchosporium secalis*. To come up with the four categories of seed infection, clean scald-free seeds were mixed with the scald-infected seeds in the following proportions 1:0, 2:1, 1:2 and 0:1 clean: infected seeds respectively. For every barley sample half of the sub-sample was pre-treated with iprodione + thiram (50 +150 grams per 100 kilograms seed) before planting. Half sample was not treated. Plastic planting pots of twelve centimeter diameter

and having drainage holes in the bottom were used as planting containers. A mixture of sterilized forest soil and clean sand in a ratio of 1:1 was mixed with five grams of fertilizer (MAP) and packed three-quarter full per pot for each sub-sample of the four categories. About 15 seeds from each sub-sample of the four categories of seed were planted per pot, making sure that they are well spread from one another. The planting depth was about 3 centimeters and each sub-sample was replicated twice. Each pot was labeled with seed category, sub-sample, date of planting and replicate number. Frequent watering was done to make sure that there was adequate moisture for germination and growth of the potted plants. The pots were placed in the greenhouse in a completely randomized design (CRD) in a chamber where the temperature fluctuated between 14^0C and 23^0C.After emergence, the seedlings were misted with water and covered using polyethylene bags. Observation of symptom development on the seedlings was noted and recorded 28 days after planting and every ten days thereafter.

3.2.3 Data collection and analysis

3.2.3.1 Assessment of rate of seed borne infection to seedling transmission

Observation of infection on seedlings was noted 28 days after planting and thereafter every ten days. Seedlings with lesions were noted per category of seed infection and the rate of transmission to seedlings for both the treated and untreated seed was also noted. The infection type (IT) of the disease was scored on two lower leaves 28 days after sowing using the following 0-4 scale according to Ali (1974), (Table 4).

Table 4: A scale used for rating plant reaction 28 days post-inoculation (Ali, 1974)

Scale	Symptom description
0	No visible symptoms
1	Small lesions at the tip or on the margin and base of leaf blades.
2	Narrow band of lesion extending over the blades.
3	Broad well developed lesions covering large areas.
4	Leaves wilted no evidence of discrete lesions.

Data collected was used to plot a bar graph showing the four categories of seed sample (i.e. No Infection, Low Infection, Moderate Infection and High Infection) versus the scald infection severity. This graph also compared the seedling reaction that emerged from the fungicide treated seeds and untreated seeds. The data was analyzed by analysis of variance (ANOVA) procedure.

3.3.0 To determine the extent of yield loss and seed quality deterioration as a result of scald.

3.3.1 Description of site

The experiment was carried out in an open field in two sites: at Timau in Ngushishi location and at Purko ranch in Mau Narok County (**Refer to section 3.1.0**).

3.3.2 Plant materials and Methodology

Barley samples that were analyzed in this experiment were obtained from the East Africa Maltings Limited. To assess yield losses caused by scald under natural field infection, twelve genotypes comprising of commercial cultivars and breeding lines of barley were selected from four disease categories (susceptible, moderately susceptible, moderately resistant and resistant). Two genotypes were selected from each category, plus three checks (two resistant and one susceptible). The twelve genotypes are summarized in Table 5.

Table 5: A summary of the twelve cultivars and breeding lines used

No.	Cultivar/Breeding Line	Category	Characteristics
1.	Sabini	Cultivar	Good malting quality, susceptible to scald
2.	Nguzo	Cultivar	High yielding, moderate resistance to scald
3.	Karne	Cultivar	Resistance to scald and net blotch
4.	HKBL 1629-14	Breeding line	Promising yielder
5.	HKBL 1629-4	Breeding line	Promising yielder/net blotch resistance

6.	HKBL 1674-4	Breeding line	Promising yielder
7.	HKBL 1621-15	Breeding line	Promising yielder
8.	HKBL 1629-19	Breeding line	Promising yielder
9.	HKBL 1642-9	Breeding line	Promising yielder
10.	HKBL 1512-5	Breeding line	Promising yielder/scald resistance
11.	HKBL 1622-6	Breeding line	Promising yielder
12.	HKBL 1675-8	Breeding line	Promising yielder

The twelve cultivars and breeding lines were planted in Timau and Mau-Narok each for one season, 3rd March 2012 and 22nd September 2012 respectively, in a paired arrangement split-plot layout in an RCBD with three replications. There were two treatments consisted of Treatment A (sprayed with fungicide Folicur (Tebuconazole) and Treatment B (unsprayed). The fungicide was applied at a rate of 1 Litre/ha, at early stems elongation i.e. at growth stage 32 (Zadocks *et al.,* 1974), 14 days and 28 days according to the recommendations from the manufacturer. Each of the entries (main plot) were planted in plots measuring 6 meters by 1.5 meters with an inter-row spacing of 20 centimetres and at the recommended commercial seed rate of 80 kg/ha and DAP fertilizer at a rate of 175 kg/ha. The evidence of scald infection was ascertained by use of a known susceptible cultivar as a check (Sabini). Among the twelve entries one susceptible check (Sabini) and two resistant checks (Karne and Nguzo) were included. Each plot was adjacent to a scald spreader row of a known very susceptible genotype (Sebastian). Weeding was done according to agronomic recommendations for barley production. At physiological maturity when the barley plants had dried and turned golden brown, each sub-plot was harvested manually using a sickle after which it was threshed individually and packed in a brown khaki paper bag. The actual weight of harvested grain per sub-plot and their respective moisture content were taken and recorded. Other data that were taken include the a thousand kernel weight at the adjusted weight of 12.5 percent moisture for each of the sub-plot harvest.

3.3.3 Data collection and analysis

3.3.3.1 Assessment of Scald severity

Assessment of disease severity (percent leaf area affected on leaf capable of expressing disease) was done on five dates, between growth stages 30 and 77 (Zadoks *et al.*, 1974). Plant infection response to scald infection based on size of necrotic leaf blotches and the associated necrosis was classified into four discrete categories, R= Resistant, MR= Moderate Resistant to MS= Moderately Susceptible and S= Susceptible. The visual scale in Table 3 was used to score for damage. After scoring the data was extrapolated to get percentage disease severity.

3.3.3.2 Assessment of yield and its components

Both the protected and the unprotected subplots were harvested by sickles and then threshed mechanically. The grain weight from each subplot was obtained after seed cleaning. Moisture content of grains was obtained using a digital computerized moisture meter model 700. The yield figures were adjusted to standard 12.5 per cent moisture content then converted to ton/ha. The harvested grain crop was cleaned by winnowing to remove chaff and other impurities.

One thousand unbroken grains from each entry were counted using an electronic grain counter (contador pfeuffer® model) and their weight in grams recorded as thousand kernel weights (TKW).

3.3.3.3 Determination of the percentage yield loss

The effects of scald disease on the yield of barley was determined by calculating the percentage reduction (percentage yield loss) in yield of each attacked (unprotected) subplot relative to its corresponding unattacked (protected) subplot. Percentage yield losses were calculated by the following formulae:

$$\%\text{Yield Loss} = \left(\frac{A-B}{A}\right) * 100$$

Where; **A**=Yield data recorded for unattacked crop.

B=Yield data recorded for attacked crop.

3.3.3.4 Determination of malting quality (Total crude proteins)

Using samples from a mixture of grains for corresponding replicated entries, grain quality analysis was done to determine whether the scald disease affected the malting quality and commercial value of the barley crop, in particular, the protein content. Malting quality basically depends upon on grain nitrogen and germination. The grain nitrogen should be as low as possible: ideally between 9 and 11.5 percent dry basis. (Briggs, 1978 and Anon, 1981). The Kjeldahl method was used to determine the amounts of nitrogen in the seed samples of barley from the protected and the unprotected subplots of each of the twelve cultivars/breeding lines to conclude whether there were differences in the protein contents of the samples between the subplots of each of the twelve genotypes, as a result of scald disease. The Kjeldahl method consists of three steps: digestion of the sample; distillation to separate the nitrogen from the digestion mixture and titration to calculate the amount of nitrogen found in the sample:

$$\%\ nitrogen\ in\ sample = \left(\frac{grams\ nitrogen}{grams\ sample}\right) * 100$$

The amount of crude protein (CP) was then found by multiplying the percent nitrogen factor by 6.25:

CP = % nitrogen in sample*6.25

The data were subjected to analysis of variance and means were separated using least significant difference (L.S.D.).

CHAPTER FOUR

RESULTS

4.1.1 Reaction and Severity of Cultivars to Scald

31 genotypes were evaluated as moderately susceptible (MS) to scald (Figure 3), and they showed a moderately susceptible (MS) reaction with plants exhibiting medium sized lesions occupying about three-quarter (¾) of all leaves. Among the moderately susceptible genotypes, the following breeding lines/cultivars recorded the highest disease severity: USDA396 (63.0% in Mau Narok and 65.5% in Timau), Q218619 (65.5% in Mau Narok and 63.3% in Timau), QSMO15 (60.4% in Mau Narok and 63.3% in Timau) and Diamora (63.3% in Mau Narok and 62.1% in Timau) (Appendix 2).

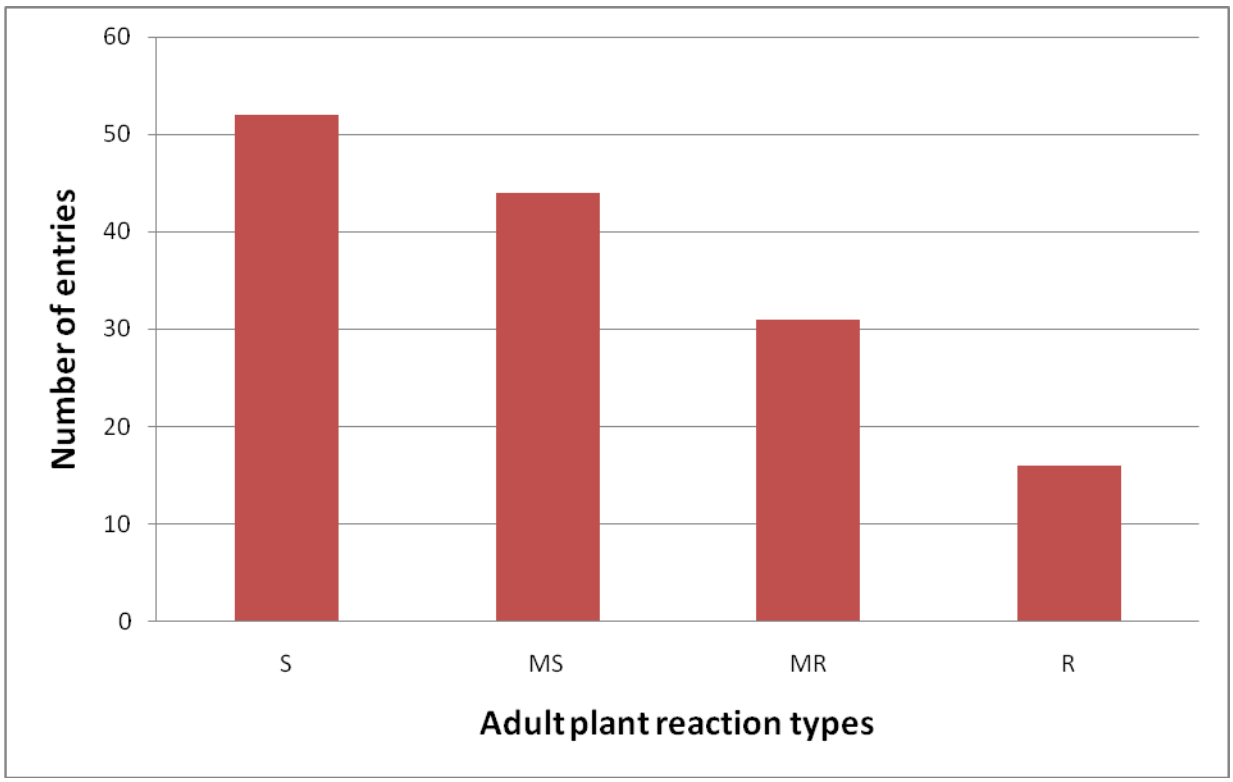

Figure 3: Frequency distribution of scald infection type (Susceptible, Moderately Susceptible, Moderately Resistant and Resistant) of 143 barley cultivars evaluated during 2013 in Timau.

52 genotypes out of a total of 143 barley lines tested were evaluated as susceptible to scald. The *Rhynchosporium secalis* susceptible varieties/breeding lines succumbed to high severities (Appendix 2). Breeding line/cultivar Steptoe04 and QSMO97 had the lowest severity among the susceptible group of 8.0% (Mau Narok) and 15.0% (Timau), and 6% (Mau Narok) and 17.5% (Timau). HKBL1622-6 (Plate 1), a breeding line introduced by East Africa Malting Ltd, suffered significantly the highest severity of 70.0% in Mau Narok and 73.0% in Timau among the susceptible group.

Scald susceptible genotypes were associated with clear susceptible (S) host response with huge lesions occupying more than three-quarters (¾) of all the leaves. In extreme cases of susceptible host response, the entire plants appeared withered by the scald disease.

Plate 1; Lesions on leaves of barley line HKBL 1622-6 as a result of scald attack during 2013 in Timau.(Source: Author, 2013)

44 genotypes out of the 143 breeding lines /cultivars were grouped as moderately resistant (MR) to scald. These included QSMO005 (20.0% in Mau Narok and 19.5% in Timau), QSMO16 (18.0% in Mau Narok and 12.0% in Timau), FEG192-1 19.0% in Mau Narok and 18.0% in Timau), SWISSHV66 (21.5% in Mau Narok and 23.3% in Timau), Chevron08 (25.5% in Mau Narok and 20.2% in Timau) and HKBL1512-5 (17.0% in Mau Narok and 19.5% in Timau) were among the genotypes that recorded significantly lower scores in disease severity. These plants exhibited small lesions occupying about half (½) of all leaves.

16 genotypes (Figure 3) which were grouped as resistant (R) showed few visible small spots while others had small lesions occupying about a quarter (¼) of all leaves. Breeding line/cultivars Nguzo (5.0% in Mau Narok and 6.0% in Timau), QSMO90 (6.0% in Mau Narok and 11.4% in Timau), 08-UT-73 (13.0% in Mau Narok and 16.1% in Timau), SWISSHV67 (14.2% in Mau Narok and 17.2% in Timau), 05WA-328.8 (18.0% in Mau Narok and 10.0% in Timau) and USDA386 (11.0% in Mau Narok and 10.2% in Timau) all recorded significantly the lowest disease severity.

One genotype namely Steptoe08 showed immune reaction (0%) in Mau Narok but recorded a 5% disease severity in Timau. Steptoe08 showed no visible spots or lesions in Mau Narok but exhibited very few visible small spots in Timau.

4.1.2 Grain Yield

Results showing grain yield recorded on 143 genotypes that were studied in this experiment are summarized in Appendix 2. Generally all genotypes that were classified as susceptible (S) recorded a significantly ($P \leq 0.05$) low grain yield in both Mau Narok and Timau. Breeding lines/cultivars Streptoe01 (2.1 t/ha in Mau Narok and 1.9 t/ha in Timau), 08-WA-01 (2.1 t/ha in Mau Narok and 1.7 t/ha in Timau), HKBL1629-4 (1.8 t/ha in Mau Narok and 1.5 t/ha in Timau), HKBL1674-4 (1.8 t/ha in Mau Narok and 1.6 t/ha in Timau) and HKBL1629-10 (1.5 t/ha in Mau Narok and 1.6 t/ha in Timau) recorded the lowest grain yield. This low grain yield could be attributed to the fact that all these lines/cultivars had a high scald disease severity of above 50%.

All the genotypes that were grouped as moderately susceptible (MS) recorded a significantly ($P \leq 0.05$) higher yield compared to susceptible genotypes. An example of these genotypes include: QSMO70 (2.5 t/ha in Mau Narok and 2.2 t/ha in Timau), HKBL1675-8 (2.4 t/ha in Mau Narok and 2.3 t/ha in Timau), SHECH/HAR (2.3 t/ha in Mau Narok and 2.1 t/ha in Timau), SWISSHV67 (2.5 t/ha in Mau Narok and 2.3 t/ha in Timau), Rawson (2.5 t/ha in Mau Narok and 2.4 t/ha in Timau) and QSMO057 (2.5 t/ha in Mau Narok and 2.6 t/ha in Timau). All the above genotypes had a scald disease severity of between 40% - 50%.

The genotypes that were grouped as resistant (R) recorded a slightly higher grain yield than the moderately susceptible genotypes. These genotypes include: QSMO16 (3.3 t/ha in Mau Narok and 3.5 t/ha in Timau), 08-N2-87 (3.5 t/ha in Mau Narok and 3.1 t/ha in Timau), QSMO059 (3.8 t/ha in Mau Narok and 3.3 t/ha in Timau), Chevron09 (3.6 t/ha in Mau Narok and 3.2 t/ha in Timau) and CLHO14977 (3.8 t/ha in Mau Narok and 3.2 t/ha in Timau). All these genotypes recorded a scald disease severity of between 10% - 20% (Appendix 2).

Breeding lines/cultivars that were grouped as highly resistant (HR) recorded the highest grain yield which was significantly ($P \leq 0.05$) different from the rest of the genotypes. These breeding lines/cultivars include: Nguzo (4.5 t/ha in Mau Narok and 3.8 t/ha in Timau), QSMO97 (4.3 t/ha in Mau Narok and 3.8 t/ha in Timau), SWISSHV65 (4.1 t/ha in Mau Narok and 3.9 t/ha in Timau) and Chevron04 (4.4 t/ha in Mau Narok and 4.0 t/ha in Timau). This highest grain yield is probably attributed to the fact that all the above four genotypes had less than 8% scald disease severity (Appendix 2).

4.2.1 The severity of scald on seedlings from *R. secalis* infected seed

Results showing the levels of scald severity as a result of seed to seedling transmission are summarized graphically in Figure 5. In general barley seedlings that emerged from seed samples that were treated with fungicide recorded a low scald infection compared to those that emerged from untreated seed samples.

Seedlings that emerged from the category of seed sample with no disease infection (i.e. 0% infection) recorded the lowest mean scald infection which was significantly ($P \leq 0.05$) different from the rest of the categories. No infection was recorded in treated pots of nil (0%) infection.

The next category of low infection (i.e. 20 – 25% infection) seed sample gave rise to seedlings that had a slightly high *Rhynchosporium secalis* infection compared to the no infection (0% infection) category. These seedlings scored a mean of between 0 – 1.7 scald disease severity on a 0 – 4 scale (Figure 4), for the treated and the untreated samples respectively. As expected, seedlings that emerged from the seed sample that was treated with fungicide still recorded a lower scald infection than those that emerged from untreated seed sample.

The third category of medium infected barley seeds (50% infection), gave rise to seedlings that scored a mean of between 1 – 2 scald disease severity on the 0 – 4 scale. The untreated seeds in this category gave rise to seedlings that had a significantly ($P \leq 0.05$) high mean scald infection severity than the seedlings that emerged from the treated seed sample. The untreated scored a mean disease severity of 2 while the treated sample scored 1 on the 0 – 4 scale (Figure 4).

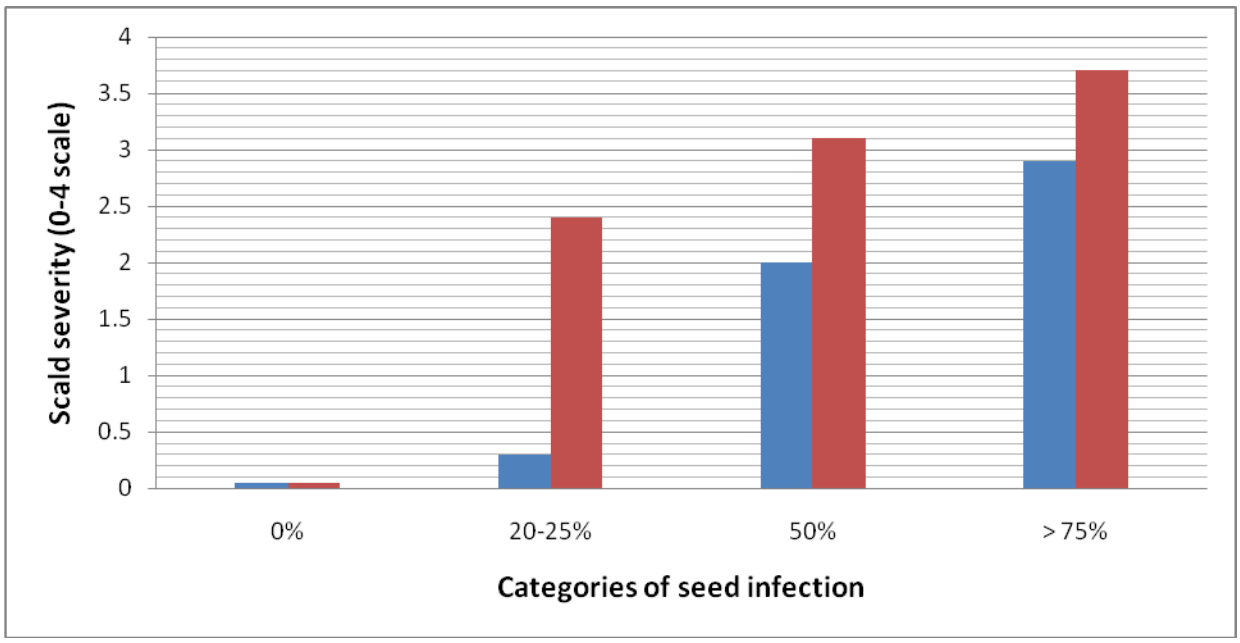

Figure 4; The severity of scald of seedling resultant from four categories of seed infection when the seed is treated and when not treated.

Plate 2: Photograph showing lesions developing on leaves of barley seedlings (over 75 percent seed infection category) as a result of seed-seedling transmission of scald (Source: Author, 2013)

This observation further confirms that seed treatment with appropriate fungicides to seed infected with *Rhynchosporium secalis* will significantly reduce its seed to seedling transmission.

The last group of barley seedlings emerged from a highly infected seed sample (>75% infection). This category of seedlings generally had the highest scald disease infection which was significantly ($P \leq 0.05$) different from the other three categories. However, in this category the untreated seeds gave rise to seedlings with a high scald disease severity than those ones that emerged from the treated seed sample. This observation indicates that when barley seed is highly infected with *Rhynchosporium secalis* , even when treated with fungicide will still transmit seed to seedling infection significantly. This makes it

necessary to carry out seed health testing on barley seed prior to planting to quantify the level of seed infection with *Rhynchosporium secalis*. Seed with high infection levels (>75%) even when treated may still act as a source of seed to seedling transmission and hence seed with such high levels of infection should not be used as 'seed' for planting a barley crop.

4.3.1 Severity of scald infection

There were significant ($P<0.05$) differences in scald disease severity among the twelve cultivars/breeding lines when left unsprayed (uncontrolled) in Mau Narok and Timau. Breeding line HKBL 1512-5 had the lowest scald disease severity (21.67%) making it the best performing line among the twelve (Table 6 and 7). Nguzo and Karne too performed well both recording a disease severity of 26.67%. This confirms previous reports by EAML that cultivars Nguzo and Karne are resistant to scald disease. Lines HKBL 1629-4, HKBL 1674-4, HKBL 1621-15, HKBL 1629-19, HKBL 1642-9, HKBL 1622-6 and HKBL 1675-8 were all moderately susceptible recording scald disease severity of between 31.67% and 36.67% (Table 8). Cultivar Sabini and line HKBL 1629-14 were highly susceptible to scald recording a disease severity of 55% and 50% respectively (Table 7 and 8). This affirms previous reports by EAML that Sabini is highly susceptible to *Rhynchosporium secalis* (Ndeda, 2004).

On the other hand, there was no significant ($P<0.05$) difference among the twelve cultivars/breeding lines in terms of scald disease response when sprayed with fungicide both in Timau and Mau Narok. However, among the twelve, Sabini had a slightly higher scald infection (3.67% and 2.27%) (Table 6 and 7). This is probably because of its nature of being more susceptible than the rest. Nevertheless, all the cultivars/breeding lines recorded less than 5% disease severity drawing a conclusion that *Rhynchosporium secalis* can be managed using proper suitable fungicides.

4.3.2 Yield of Commercial Cultivars

The results showing grain yield of the twelve lines/cultivars is summarized in Tables 6 and 7. Generally breeding lines/cultivars Nguzo, HKBL1512-5 and Karne recorded the

highest grain yield of 3.88 t/ha, 3.47 t/ha and 2.97 t/ha respectively which were not significantly different from each other but were significantly different from the other nine breeding lines. This is attributed to the fact that Nguzo, HKBL1512-5 and Karne were resistant to *Rhychosporium secalis* which did not significantly affect their grain yield. Furthermore grains from these three lines/cultivars appeared to be large, healthy and plump hence recording the highest weight.

Sabini, HKBL1622-6, HKBL1642-9, HKBL1621-15, HKBL1675-8 and HKBL1629-4 recorded significantly ($P<0.05$) lower grain yield (2.90 t/ha, 2.76 t/ha, 2.35 t/ha, 2.07 t/ha, 2.07 t/ha and 2.05 t/ha respectively) than Nguzo, Karne and HKBL1512-5. From the data collected during this field trial it was evident that these six breeding lines were all moderately susceptible to *Rhychosporium secalis.* This explains why the grain yield from the six breeding lines/cultivars is slightly lower than that of Nguzo, Karne and HKBL1512-5 which were highly resistant to *Rhychosporium secalis*.

HKBL1674-4, HKBL1629-14 and HKBL1629-9 recorded the lowest grain yield (1.95 t/ha, 1.74 t/ha and 1.72 t/ha respectively) among the twelve breeding lines/cultivars. This grain yield was significantly ($P<0.05$) lower than that of Nguzo, Karne, HKBL1512-5, Sabini, HKBL1622-6, HKBL1642-9, HKBL1621-15, HKBL1675-8 and HKBL1629-4. This lowest grain yield could be due to the fact that HKBL1674-4, HKBL1629-14 and HKBL1629-9 were highly susceptible to *Rhychosporium secalis* which affected the physiological processes of these three breeding lines including their rate of photosynthesis during grain filling. The grains from the three breeding lines (HKBL1674-4, HKBL1629-14 and HKBL1629-9) appeared small, unhealthy and shrivelled. The results showing the 1000 kernel weight of the twelve breeding lines/cultivars is summarized in Tables 6 and 7. Generally there was no significant ($P<0.05$) difference in 1000 kernel weight between the sprayed (controlled) and unsprayed (uncontrolled) treatments of Nguzo, HKBL1512-5, Karne, HKBL1622-6 and HKBL1674-4. This is probably attributed to the fact that the five lines/cultivars appeared to be moderate to highly resistant to scald infection hence, even if left unsprayed their kernel weight would not reduce significantly.

On the other hand there was a significant ($P<0.005$) difference between the sprayed (controlled) and unsprayed (uncontrolled) treatments of Sabini, HKBL1675-8, HKBL1629-4, HKBL1629-14, HKBL1642-9, HKBL1629-19 and HKBL1621-15. This is probably because the seven lines were highly susceptible to scald, so when left unsprayed their kernel weight is significantly reduced. The grains of unsprayed treatments appeared unhealthy and shrivelled because of scald infection which is known to reduce the photosynthetic surface area hence reducing the rate of photosynthesis during grain filling.

There was significant ($P<0.05$) difference in terms of 1000 kernel weight among the twelve breeding lines/cultivars when left unsprayed (uncontrolled). Nguzo and HKBL1512-5 recorded the highest kernel weights of 40.67g and 34.33g respectively which were significantly higher than kernel weights recorded by the rest of the breeding lines/cultivars. This is probably because the two lines are resistant to scald. The grains from the two breeding lines/cultivars appeared to be large, healthy and plump, hence recording the highest weight. HKBL1622-6, Karne and HKBL1674-4 recorded kernel weights of 30.67g, 30.33g and 30.33g respectively which was significantly different from kernel weights of Nguzo and HKBL1512-5. Breeding lines/cultivars HKBL1675-8, HKBL1629-4, Sabini, HKBL1629-14, HKBL1642-9, HKBL1629-19 and HKBL1621-15 recorded the lowest kernel weights 27.33g, 26.67g, 26.00g, 25.67g, 24.67g, 23.00g and 22.67g respectively significantly different from Nguzo, HKBL1512-5, HKBL1622-6, Karne and HKBL1674-4. Grains from these seven lines appeared small, unhealthy and shrivelled due to the high severity of scald which probably affected their photosynthetic rate during grain filling.

4.3.3 Percentage Yield loss of Commercial Cultivars due to scald

The results showing the percentage yield loss is summarized in Table 6 and 7 and graphically in Figure 6. Breeding line HKBL1512-5 and cultivar Nguzo recorded the lowest percentage yield loss (3.22% and 4.08%) which was significantly different from the rest of the breeding lines/cultivars. This lowest percentage yield loss can be attributed to the fact that HKBL1512-5 and Nguzo are highly resistant to *Rhychosporium secalis*,

hence was not greatly affected. Karne, HKBL1674-4, HKBL1622-6, HKBL1629-19, HKBL1675-8, HKBL1642-9, HKBL1629-4 and HKBL1621-15 recorded a slightly higher percentage yield loss significantly different from that of HKBL1512-5 and Nguzo. This is probably because these eight breeding lines/cultivars are moderately susceptible to *Rhychosporium secalis*.

HKBL1629-14 and Sabini recorded the highest percentage yield losses of 13.43% and 17.11% respectively, which was significantly different from the other ten breeding lines/cultivars. This is probably because HKBL1629-14 and Sabini are highly susceptible to *Rhychosporium secalis* which led to this highest yield loss.

Table 6: Means of scald severity, yield and percentage yield loss for sprayed and unsprayed plots assessed on twelve cultivars/breeding lines of barley in Mau Narok (Purko Ranch)

	SPRAYED				UNSPRAYED				
TREATMENT	**Scald (% severity infection)**	**1000kernel weight (g)**	**Protein content (%)**	**Yield (t/ha)**	**Scald (% severity infection)**	**1000kernel weight (g)**	**Protein content (%)**	**Yield (t/ha)**	**YIELD LOSS (%)**
Sabini	3.67*a*	39.67*b*	9.95*cd*	3.59*ab*	55.00*d*	26.00*def*	9.95*c*	2.97*abc*	17.16*d*
Nguzo	0.67*a*	45.00*a*	8.78*ab*	4.05*a*	26.67*ab*	40.67*a*	8.31a	3.88*a*	4.08*ab*
Karne	0.67*a*	33.67*cd*	8.46*a*	3.08*abc*	26.67*ab*	30.33*bc*	8.47*ab*	2.90*abc*	5.85*abc*
HKBL 1629-14	2.33*a*	31.33*de*	8.73*a*	2.01*de*	50.00*d*	25.67*def*	8.54*ab*	1.74*e*	13.43*d*
HKBL 1629-4	1.00*a*	31.00*de*	10.16*cd*	2.19*de*	31.67*bc*	26.67*cde*	10.27*c*	2.05*de*	6.51*bc*
HKBL 1674-4	2.33*a*	35.33*c*	10.16*cd*	2.06*de*	31.67*bc*	30.33*bc*	10.42*c*	1.95*de*	5.33*abc*
HKBL 1621-15	1.00*a*	27.33*f*	10.53*d*	2.23*de*	33.33*c*	22.67*f*	9.11*abc*	2.07*de*	7.29*c*
HKBL 1629-19	2.33*a*	27.00*f*	10.27*cd*	1.81*e*	31.67*bc*	23.00*ef*	9.89*c*	1.72*e*	5.38*abc*
HKBL 1642-9	2.33*a*	27.33*f*	10.21*cd*	2.51*cde*	36.67*c*	24.67*def*	10.43*c*	2.35*de*	6.31*bc*
HKBL 1512-5	0.67*a*	39.33*b*	9.84*bcd*	3.58*ab*	21.67*a*	34.33*a*	9.90*c*	3.47*ab*	3.22*a*
HKBL 1622-6	2.33*a*	35.33*c*	9.42*abc*	2.93*abc*	36.67*c*	30.67*b*	9.21*abc*	2.76*cde*	5.84*abc*
HKBL 1675-8	2.33*a*	29.67*ef*	9.53*abcd*	2.20*de*	31.67*bc*	27.33*bcd*	9.69*bc*	2.07*de*	5.92*abc*
L.S.D.	3.15	3.26	1.10	1.06	5.49	3.56	1.35	0.99	2.54
C.V. (%)	13.33	5.70	6.7	23.40	9.40	7.40	8.40	23.50	10.11

Means within columns followed by the same letter are not significantly different (P<0.05)

Table 7: Means of scald severity, yield and percentage yield loss for sprayed and unsprayed plots assessed on twelve cultivars/breeding lines of barley in Timau (Ngushishi)

	SPRAYED				UNSPRAYED				YIELD LOSS (%)
TREATMENT	**Scald (% severity infection)**	**1000kernel weight (g)**	**Protein content (%)**	**Yield (t/ha)**	**Scald (% severity infection)**	**1000kernel weight (g)**	**Protein content (%)**	**Yield (t/ha)**	
Sabini	2.27*a*	38.77*b*	9.90*cd*	3.59*ab*	55.00*d*	26.00*def*	9.50*c*	2.97*abc*	18.16*d*
Nguzo	0.37*a*	45.20*a*	8.70*ab*	4.05*a*	26.67*ab*	40.67*a*	8.07a	3.88*a*	3.81*ab*
Karne	0.47*a*	33.67*cd*	8.63*a*	3.08*abc*	26.67*ab*	30.33*bc*	8.47*ab*	2.90*abc*	4.52*abc*
HKBL 1629-14	1.33*a*	31.33*de*	8.73*a*	2.01*de*	50.00*d*	25.67*def*	8.50*ab*	1.74*e*	11.19*d*
HKBL 1629-4	1.00*a*	31.00*de*	10.13*cd*	2.19*de*	31.67*bc*	26.67*cde*	10.67*c*	2.05*de*	6.14*bc*
HKBL 1674-4	2.33*a*	35.33*c*	10.15*cd*	2.06*de*	31.67*bc*	30.33*bc*	10.23*c*	1.95*de*	5.33*abc*
HKBL 1621-15	1.00*a*	27.33*f*	10.30*d*	2.23*de*	33.33*c*	22.67*f*	9.17*abc*	2.07*de*	8.18*c*
HKBL 1629-19	2.13*a*	27.00*f*	10.67*cd*	1.81*e*	31.67*bc*	23.00*ef*	9.83*c*	1.72*e*	5.381*abc*
HKBL 1642-9	1.33*a*	27.33*f*	10.13*cd*	2.51*cde*	36.67*c*	24.67*def*	10.47*c*	2.35*de*	6.311*bc*
HKBL 1512-5	0.57*a*	39.33*b*	9.80*bcd*	3.58*ab*	21.67*a*	34.33*a*	9.97*c*	3.47*ab*	3.221*a*
HKBL 1622-6	2.33*a*	35.33*c*	9.40*abc*	2.93*abc*	36.67*c*	30.67*b*	9.27*abc*	2.76*cde*	5.843*abc*
HKBL 1675-8	2.33*a*	29.67*ef*	9.57*abcd*	2.20*de*	31.67*bc*	27.33*bcd*	9.87*bc*	2.07*de*	5.915*abc*
L.S.D.	3.11	3.26	1.12	1.06	5.49	3.56	1.16	0.99	2.33
C.V. (%)	12.34	5.70	6.90	23.40	9.40	7.40	7.30	23.50	10.11

Means within columns followed by the same letter are not significantly different (P<0.05)

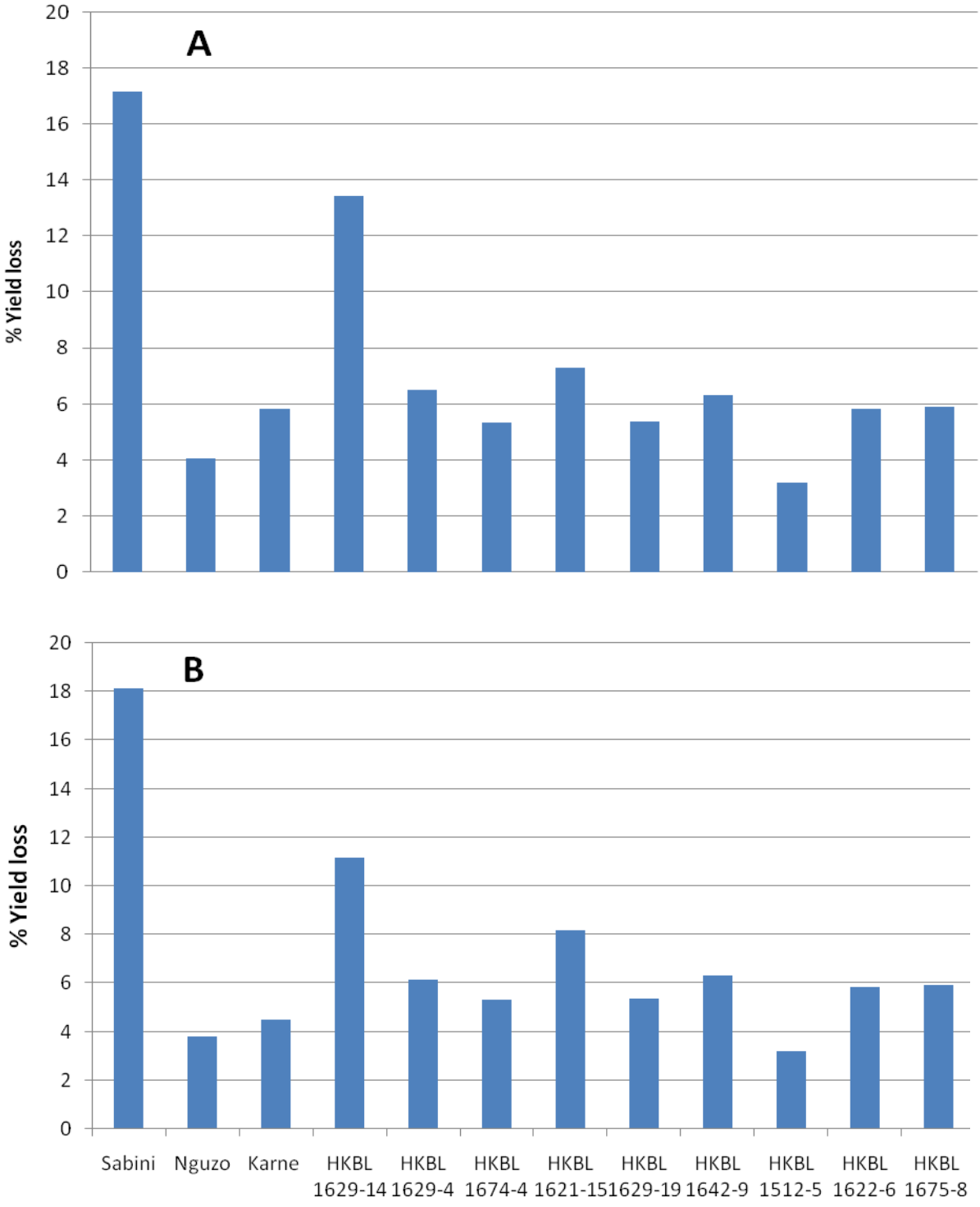

Figure 5; Percentage yield loss due to scald disease. A comparison of two sets of twelve cultivars/breeding lines of barley: (A) in Mau Narok Purko ranch and (B) Timau sites respectively.

4.3.4 Malting Quality (Total crude proteins)

The results showing the percentage protein content of the twelve varieties/breeding lines is summarized in Table 6 and 7. Generally there was no significant ($P<0.05$) difference in terms of percentage protein content among the twelve cultivars/breeding lines when left unsprayed (uncontrolled) and sprayed (controlled) using fungicide. Furthermore there was no significance difference in terms of the percentage protein content between the twelve varieties/breeding lines planted in Mau Narok and those ones planted in Timau.

Nguzo, Karne and HKBL1629-14 had the lowest protein content of 8.73%, 8.63% and 8.70% respectively. HKBL1675-8, HKBL1622-6, HKBL1512-5 and Sabini had a protein content of 9.40%, 9.57%, 9.80% and 9.90% slightly higher than Nguzo, Karne and HKBL1629-14 but not significantly different. HKBL1629-4, HKBL1674-4, HKBL1621-15, HKBL1629-19 and HKBL1642-9 recorded the highest protein content among the twelve cultivars/breeding lines of 10.13%, 10.13%, and 10.15%, 10.30% and 10.67% respectively. This was however not significantly different from the rest of the cultivars/breeding lines.

CHAPTER FIVE

DISCUSSION

Reaction of barley genotypes to scald

Scald disease pressure was higher in Timau than in Mau Narok probably because Timau had a more favorable environment for development of scald during the barley growing season in that it was cooler and more humid in Timau as compared to Mau Narok; evidenced by lower AUDPC figures for Mau-Narok in comparison with those of Timau in Appendix 2.0. The reason for the poor disease development in Mau Narok may be attributed to the fact that Mau Narok was dry during the barley cropping season with a low relative humidity of about 80 percent, and given that, studies have shown that scald requires a relative humidity of 90-100 percent at 15-21^{0}C for 24-48 hours to develop lesions (Zang *et. al*, 1987), these conditions were probably not met in Mau Narok. Of the 143 genotypes planted in Timau differences between MS, MR, S and R were easy to distinguish visually. The barley cultivars differed in their resistances to scald and these results are confirmed by other previous researchers (Albustan *et. al.*, 2008). Among the 143 barley genotypes tested, 33 percent were found to be resistant to moderately resistant to scald in the field. Sources of resistance to this disease have been identified in several countries (Robbertse et. al., 2000). However sources of complete resistance to scald have not been identified. This is probably because of the highly variable nature of *Rhynchosporium secalis* which may overcome host resistant gene(s) once they are deployed. Albustan *et. al.* (2008) found field resistance as 39 percent in their experiment. In the present study the group of entries that were evaluated as resistant included cultivar Nguzo and breeding line Steptoe among others. In the group of entries that were evaluated to be moderately resistant to scald were breeding lines HKBL 1512-5 and QSMO005 among others. A number of barley test lines also showed moderate reaction to scald; like breeding lines QSMO15 and Diamora among others. Ninety seven genotypes; translating to sixty nine percent, were evaluated as susceptible to moderately susceptible to scald under field conditions. Cultivar Sabini recorded a susceptible reaction to scald in both experimental sites of Timau and Mau-Narok. Scald susceptible genotypes gave lower

grain yield than those genotypes that recorded a scald resistant score. The scald susceptible genotypes produced less plump seeds than the scald resistant genotypes probably due to the fact that scald damage is mainly attributed to reduction of the 1000- kernel weight (Scott *et. al*., 1992; Meles *et. al*., 2004); a breeding line HKBL 1629-4 that scored as susceptible to scald in both Mau Narok and Timau recorded yields of 1.8 t/ha and 1.5t/ha respectively in Mau Narok and Timau. In comparison, cultivar Nguzo, that scored as resistant to scald recorded yields of 4.5t/ha and 3.8t/ha in Mau Narok and Timau respectively. Resistant genotypes showed lower scald severities and yield losses than susceptible genotypes Disease resistance can provide cost effective means for control of diseases such as scald and this study has shown that a large number of barley genotypes resistant to scald are available. The resistant genotypes determined in this study should be of value to plant breeders as scald resistant cultivars after their subsequent release or can be used in breeding programs to incorporate resistance in the locally popular but scald susceptible barley varieties. It should be however noted that the scald pathogen is highly variable and these identified resistances may become ineffective within a short time and therefore breeders must continue screening barley germplasm to identify new sources of resistance to the new races of the scald pathogen.

Effects of scald on yield and grain quality of barley

Scald infection significantly reduced the yield of the unprotected (not sprayed with fungicide) plots of the twelve test genotypes. The maximum yield reduction, expressed as a percentage of the protected plot yield was 17.16 (cv. Sabini), while the lowest yield reduction, also expressed as a percentage of protected plot yield was 3.22 (HKBL 1512-5). James *et. al*. (1968) reported that scald infections significantly reduced thousand kernel weights. This study has confirmed that severe infection with scald decreased barley yields mainly by reducing the plumpness of grain as a result of shriveling of the grain (Xi *et. al.* 2000).The percentage of yield loss depends on the susceptibility of the genotype. Highly susceptible genotypes had a relatively higher percent of shriveled grains as compared to the less susceptible genotypes. Scald resistant cultivars had less shriveled grain, and therefore less yield loss as a result of infection with scald. While the thousand kernel weights of both resistant and

susceptible genotypes in untreated plots were lower than in protected plots, the difference was significant only in susceptible genotypes. Fungicide protection led to significantly higher thousand kernel weights in susceptible genotypes. Resistant genotypes were higher yielding than susceptible genotypes in fungicide protected plots. There was no significant difference in the thousand kernel weight and the grain yield per plot of the protected and the unprotected plots of cultivars Nguzo and Karne and breeding lines HKBL 1512-5, HKBL 1622-6 and HKBL1674-4. This is probably because these cultivars /breeding lines recorded as resistant to scald. Nguzo and HKBL 1512-5 gave the highest kernel weights of 40.67g and 34.33g with grain yields of 3.88t/ha and 2.97t/ha respectively when unprotected with slight improvements in their kernel weights and grain yields from their fungicide protected plots. Cultivar Sabini and breeding lines HKBL 1675-8, HKBL 1629-4, HKBL 1642-9, HKBL 1629-19 and HKBL 1621-15 gave significant differences in the thousand kernel weight and grain yield between their protected and unprotected plots in terms of yield in kilograms per plot. Sabini and HKBL-1629-19 recorded kernel weights of 26.00g and 23.00g and yields of 2.97t/ha and 1.72t/ha respectively when unprotected but recorded higher kernel weight and grain yield when protected with fungicide. This result may be due the fact that cultivar Sabini and the respective breeding lines are susceptible to scald. This study confirms previous research (Aoki *et. al*., 2011), that barley cultivars differed in their yield loss as a result of scald attack depending on their level of resistance or susceptibility to the disease. This finding also shows that the yield of scald susceptible barley cultivars such as Sabini can be improved by applying appropriate fungicide sprays at recommended plant growth stage(s) when such cultivars are grown in scald prone environments. The quality of malt and feed grain can also be drastically affected by scald (Edney *et. al*., 1998; Khan & Crosbie, 1988) due to the loss of grain weight or plumpness. This study, however, did not find significant differences in percent protein (crude) among the twelve varieties when unprotected and protected with fungicide.

Seed to seedling transmission of scald

Low seed infection (nil and 20-25 percent) with the inoculum of *Rhynchosporium secalis* resulted in a low seed to seedling transmission of scald, however when the

seed borne infection was high (over 75 percent), the resultant seedlings scored a high severity rating for scald on the leaves. Fungicide treated seed of low infection (below 25 percent) resulted in scald clean seedlings but as the seed borne infection increased from 50 percent, seed dressing with fungicide did not reduce the seed to seedling transmission significantly because the resultant seedlings scored a high severity rating for scald. The main sources of primary inoculum of *Rhynchosporium secalis* are infected plant debris (Cladwell, 1937) and infected seed (Skoropad, 1959).The pathogen may also be soil borne (Kay & Owen, 1973). The relative importance of sources of primary inoculum is a matter of debate because the potential role of seed borne inoculum in the transmission of scald is not known but is thought to be of minor significance. Seed health testing for *Rhynchosporium secalis* has not been a routine in many seed health testing laboratories and besides, detection of seed infection with *Rhynchosporium secalis* is difficult due to the slow growth rate of the pathogen. The pathogen may also be present in 'symptomless' seed (Lee *et. al*., 1999).The experiment on seed transmission was conducted under greenhouse condition in an attempt to prevent contamination from any other sources. Infection of seedlings must have started from infected seeds only since the seedlings were protected from airborne inoculum and any soil borne inoculum if any. The study suggests that the extent of seed-to-seedling transmission of *Rhynchosporium secalis* the causal organism of barley scald increases with the amount or level of seed borne inoculum.The study has shown that the seed borne inoculum of *Rhynchosporium secalis* has an important role in the transmission of barley scald disease, especially in the dispersal of the pathogen (Salamati *et al*., 2000).The level of seed infection with *Rhynchosporium secalis* is positively related to the rate of seed-seedling transmission of the barley scald disease. Seed treatment with appropriate fungicides is effective at low levels of seed infection but as the level of seed borne infection increases, the benefits of fungicidal seed dress are drastically reduced. It is important to carry out seed health testing on seed barley that is suspected to be infected with the fungus to evaluate the level of seed borne infection and avoid using seed lots that show a high level of seed borne infection since seed borne infection may play an important role in scald epidemics in the field (Skoropad, 1959; Kay& Owen, 1973).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

The studies come up with the following conclusions:

1. Most of the cultivars and breeding lines of barley in Kenya are susceptible; there are also some scald resistant germplasm that are available. The following genotypes were found to be resistant; Nguzo, QSMO97, Karne, HKBL1512-5, SWISSHV95 and Chevron among others

2. Susceptible genotypes gave significantly higher yields when protected compared to resistant genotypes when protected. Scald infection had a significant effect on grain filling, measured by the percentage of plump kernels. Cultivar Sabini and HKBL1629-14 were recorded as susceptible to *Rhynchosporium secalis.*, consequently there were significant differences in the yield from their experimental plots which had fungicide treatment and those without fungicide treatment.

3. Yield losses ranged from 3 to 18% observed in the present study and the amount of yield loss depends on the degree of cultivar susceptibility, disease severity and cultivar response to foliar fungicide (Tebuconazole).

4. *Rhynchosporium secalis* has no effect on protein content because all the affected lines/cultivars recorded below 10.5% which is within the acceptable range of below 11.5% 5. 5. Scald may be controlled by use of proper fungicides but the greatest opportunity for reduction of crop losses due to scald infections can be offered by resistant cultivars.

6. *Rhynchosporium secalis* is a seed borne disease and the seed borne transmission has shown to play a big role in the dispersal and development of scald in barley. The level of seed infection with the pathogen is highly correlated with the rate of transmission of the disease to the resultant seedlings.

7. Seed treatment of infected barley seeds with suitable fungicides has shown to significantly reduce the rate of seed to seedling transmission of this disease; however it is important to note that, the effectiveness of seed treatment increases if used on clean seed i.e. seeds previously harvested from a crop with low scald infection (<20%

scald infection). As the seed borne infection increases beyond 50%, the benefit of seed treatment with fungicide to control scald greatly diminishes.

7. Scald can be effectively controlled by use of appropriate foliar fungicides.

The following recommendations can be made from the results of this study:

1. It is possible to get breeding lines/cultivars that are resistant to *Rhynchosporium secalis* through screening. These breeding lines/cultivars can be advanced to breeding and selections nurseries made within preliminary and advanced yield trials for future variety release or they can be used as males in the crossing block to impove the popular but scald susceptible local cultivars (females).

2. Resistant cultivars offer the greatest opportunity for reduction of crop losses due to scald hence screening of more cultivars/breeding lines from different parts of the world should be done to identify more sources of resistance.

3. It is necessary to regularly screen commercial barley varieties and promising lines to determine if previously resistant genotypes have maintained their resistance and identify new sources of resistance to scald.

4. Seed treatment with suitable fungicides is beneficial in the control of seed borne to seedling transmission of scald, particularly when the seed borne infection is at low levels.

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APPENDICES

Appendix 1.1; Rainfall and temperature data recorded in Mau Narok during the experiment period (2011-2012)

Year	Month	Rainfall (mm)	Maximum (^{o}C)	Minimum (^{o}C)
2012	July	42.2	23.0	7.0
	August	56.3	25.0	9.0
	September	45.1	25.0	8.0
	October	74.8	22.0	10.0
	November	62.2	23.0	9.0
	December	76.7	23.0	10.0
2012	January	42.9	23.3	9.0
	February	157	25.0	10.5
	March	184.1	23.0	10.0
	April	140.4	23.0	10.0
	May	180.8	22.0	11.0
	June	51.8	22.0	9.0

Appendix 1.2: Rainfall and temperature data recorded in Timau during the experiment period (2011-2012)

Year	Month	Rainfall (mm)	Maximum (^{o}C)	Minimum (^{o}C)
2012	January	42.9	23.3	9.0
	February	157	25.0	10.5
	March	184.1	23.0	10.0
	April	140.4	23.0	10.0
	May	180.8	22.0	11.0
	June	57.8	22.0	10.0

	July	136.1	21.0	8.0
	August	140.7	21.0	9.0
	September	112.2	23.0	8.0
	October	89.9	22.0	10.0
	November	74.1	23.3	11.0
	December	12.3	26.2	11.0

Appendix 2.0: The reaction of 143 barley genotypes to Rhynchosporium secalis in Mau Narok and Timau

No.	Cultivar/ Breeding line	**Mau Narok**				**Timau**			
		1	2	3	4	1	2	3	4
		Average disease severity (%)	AUDPC	Infection rate/day	Average Grain Yield (t/ha)	Average disease Severity (%)	AUDPC	Infection rate/day	Average Grain Yield (t/ha)
1.	QSMO93	44.0	102.5	0.151	3.3	50.0	566.9	0.178	3.1
2.	Chevron01	43.0	55.3	0.194	3.1	52.5	626.3	0.161	2.7
3.	USDA344	20.0	249.0	0.157	2.9	57.5	758.1	0.162	3.4
4.	QSMO89	45.0	440.3	0.044	2.8	75.0	1020.0	0.173	2.5
5.	Steptoe01	58.0	123.0	0.142	2.1	42.5	553.8	0.120	1.9
6.	Steptoe02	63.0	308.6	0.099	3.3	62.5	830.0	0.152	3.2
7.	09N6-08	35.0	349.5	0.126	2.8	70.0	867.5	0.183	2.0
8.	QSMO08	58.3	232.6	0.130	3.2	58.6	746.09	0.161	3.0
9.	Steptoe03	34.5	234.7	0.140	3.0	30.5	207.4	0.120	3.5
10.	QSMO94	30.0	26.5	0.192	2.5	40.0	543.1	0.164	2.9
11.	Steptoe04	8.0	1.5	0.217	3.0	15.0	136.5	0.159	3.6
12.	21481	19.0	3.3	0.288	2.1	10.0	136.3	0.186	3.0
13.	QSMO97	6.0	0	0.208	4.3	17.5	11.4	0.105	3.8
14.	Steptoe05	10.0	0	0.236	3.4	22.5	16.3	0.094	3.8
15.	Steptoe06	60.0	187.3	0.149	2.4	42.5	553.8	0.122	2.8
16.	USDA620	28.0	15.5	0.210	2.0	6.25	53.0	0.200	2.5
17.	USDA386	11.0	6.3	0.207	2.7	10.2	104.8	0.194	2.3
18.	USDA384	40.0	24.5	0.150	2.8	21.25	275.0	0.109	2.2
19.	QSMO86	25.0	27.0	0.119	3.6	22.5	158.1	0.224	3.1

20.	HKBL1629-14	8.0	1.5	0.217	3.8	15.0	136.5	0.159	4.3
21.	USDA261	48.0	125.8	0.163	2.7	22.5	273.8	0.115	3.0
22.	USDA709	25.9	37.8	0.194	2.9	16.5	200.58	0.155	3.0
23.	USDA1771	30.2	110.7	0.119	2.0	23.0	201.4	0.134	2.5
24.	HKBL1595-5	63.0	263.3	0.130	4.2	55.5	845.0	0.109	4.0
25.	Steptoe07	55.0	226.5	0.120	2.3	65.3	902.5	0.157	2.0
26.	USDA341	58.0	218.3	0.135	2.0	40.0	479.4	0.145	2.3
27.	USDA708	45.0	153.5	0.118	3.0	62.5	837.5	0.159	2.9
28.	HKBL1675-3	30.0	61.8	0.195	3.4	20.0	265.0	0.187	3.0
29.	USDA143	50.0	144.0	0.147	2.5	42.5	570.6	0.168	2.8
30.	USDA622	45.0	343.5	0.078	3.0	67.5	900.0	0.165	2.6
31.	USDA1811	45.0	245.0	0.130	1.9	57.5	947.5	0.115	2.5
32.	USDA396	63.0	226.5	0.175	2.2	65.0	951.3	0.155	3.0
33.	USDA382	45.0	332.5	0.078	1.8	57.5	711.3	0.190	2.2
34.	USDA345	53.0	246.3	0.115	2.3	47.5	616.3	0.127	2.4
35.	HKBL1591-8	50.2	223.7	0.130	2.0	52.7	729.7	0.152	2.3
36.	07MB-405	43.5	334.5	0.143	2.4	40.4	556.5	0.154	3.0
37.	HKBL1629-5	20.5	223.4	0.155	2.2	18.4	334.5	0.144	2.9
38.	USDA1472	38.0	123.0	0.142	2.3	42.5	553.8	0.120	2.0
39.	HKBL1629-12	17.0	308.6	0.099	4.0	19.5	830.0	0.152	3.7
40.	Q218619	65.5	224.7	0.118	3.0	63.3	345.8	0.119	3.2
41.	UT03B1953-64	6.0	0	0.208	4.3	17.5	11.4	0.105	3.8
42.	USDA623	10.0	0	0.236	3.4	22.5	16.3	0.094	3.8
43.	USDA380	60.0	187.3	0.149	2.4	42.5	553.8	0.122	2.8
44.	USDA1648	28.0	15.5	0.210	2.0	6.25	53.0	0.200	2.5
45.	HKBL1629-4	59.0	556.3	0.133	1.8	60.2	454.3	0.136	1.5
46.	08-MN-49	33.3	324.5	0.154	2.6	30.4	466.5	0.145	2.0
47.	Q21861DHP	45.0	343.5	0.078	2.0	67.5	900.0	0.165	2.3
48.	HKBL1629-19	45.0	245.0	0.130	2.4	57.5	947.5	0.115	2.3
49.	HKBL1621-15	53.0	226.5	0.175	3.0	65	951.3	0.155	2.4
50.	USDA383	45.0	332.5	0.078	3.2	57.5	711.3	0.190	2.8
51.	HKBL1622-6	70.0	623.7	0.119	2.3	73.2	614.5	0.115	2.8
52.	08-WA-01	56.1	453.4	0.132	2.1	50.1	554.7	0133	1.7
53.	Steptoe08	0	0	0	2.5	5	1.0	0.01	2.7
54.	USDA340	8.0	1.5	0.217	3.0	15	136.5	0.159	2.4
55.	HKBL1673-9	19.0	3.3	0.288	3.4	10	136.3	0.186	3.2
56.	USDA399	6.0	0	0.208	3.8	1.75	11.4	0.105	2.4
57.	Steptoe09	10.0	0	0.236	2.4	2.5	16.3	0.094	2.0
58.	HKBL1642-9	60.0	187.3	0.149	3.0	42.5	553.8	0.122	2.7
59.	HKBL1675-8	43.1	443.5	0.143	2.4	40.0	334.6	0.133	2.3
60.	HKBL1674-4	58.7	456.9	0.154	1.8	54.3	334.8	0.143	1.6
61.	HKBL1629-10	55.3	556.3	0.145	1.5	56.0	554.8	0.133	1.6

62.	Nguzo	5.0	10.3	0.104	4.5	6.0	20.0	0.173	3.8
63.	HKBL1595-1	38.0	123.0	0.142	3.4	42.5	553.8	0.120	3.0
64.	HKBL1512-5	17.0	308.6	0.099	4.0	19.5	830.0	0.152	3.7
65.	QSMO55	65.0	349.5	0.126	2.5	70.0	867.5	0.183	2.0
66.	QSMO42	58.3	232.6	0.130	2.8	58.6	746.09	0.161	3.0
67.	QSMO33	45.5	443.7	0.133	2.5	48.9	554.3	0143	2.2
68.	Steptoe09	18.0	218.3	0.135	2.6	10	479.4	0.145	3.0
69.	QSMO70	45.0	153.5	0.118	2.9	62.5	837.5	0.159	3.5
70.	09N2-52	30.0	61.8	0.195	2.4	20	265.0	0.187	2.8
71.	09N2-64	50.0	144.0	0.147	2.0	42.5	570.6	0.168	2.4
72.	09MT-38	45.0	343.5	0.078	3.1	67.5	900.0	0.165	3.6
73.	Q21861	45.0	245.0	0.130	2.9	57.5	947.5	0.115	3.4
74.	QSMO15	60.4	307.2	0.120	2.5	61.5	421.1	0.119	2.8
75.	Steptoe10	45.0	332.5	0.078	1.8	57.5	711.3	0.190	2.2
76.	QSMO19	53.0	246.3	0.115	2.3	47.5	616.3	0.127	2.4
77.	QSMO18	50.2	223.7	0.130	2.0	52.7	729.7	0.152	2.3
78.	QSMO29	43.5	334.5	0.143	2.4	40.4	556.5	0.154	3.0
79.	Chevron02	19.0	366.7	0.288	3.4	10.0	136.3	0.186	3.2
80.	15517	6.0	0	0.208	3.8	1.75	11.4	0.105	2.4
81.	14934	10.0	0	0.236	2.4	2.5	16.3	0.094	2.0
82.	08-UT-47	60.0	187.3	0.149	3.0	42.5	553.8	0.122	2.7
83.	SWISSHV65	6.5	228.4	0.123	4.1	8.0	324.5	0.112	3.9
84.	21486	23.7	456.0	0.135	3.2	18.7	348.4	0.145	3.0
85.	CLHO14977	17.6	445.4	0.145	3.8	15.8	554.9	0.154	3.2
86.	08-AB-16	45.0	245.0	0.130	2.9	57.5	947.5	0.115	3.4
87.	SHECH/HAR	40.7	554.7	0.144	2.3	44.5	334.6	0.143	2.1
88.	Chevron03	48.0	125.8	0.163	2.7	22.5	273.8	0.115	3.0
89.	P1347245	25.9	37.8	0.194	2.9	16.5	200.58	0.155	3.0
90.	24767	30.2	110.7	0.119	2.0	23.0	201.4	0.134	2.5
91.	Chevron04	7.6	334.8	0.133	4.4	8.8	223.7	0.124	4.0
92.	Steptoe11	20.4	234.4	0.145	2.8	33.3	342.3	0.134	3.1
93.	SWISSHV67	45.6	445.6	0.145	2.5	48.8	554.7	0.143	2.3
94.	05WA-328.8	18.0	218.3	0.135	2.3	10	479.4	0.145	2.8
95.	Chevron05	45.0	153.5	0.118	3.0	62.5	837.5	0.159	2.8
96.	08-AB-17	30.0	61.8	0.195	2.5	20	265.0	0.187	2.7
97.	06WA-466.6	50.0	144.0	0.147	2.8	42.5	570.6	0.168	3.0
98.	SWISSHV63	45.0	343.5	0.078	2.7	67.5	900.0	0.165	3.1
99.	ND25161	45.0	245.0	0.130	3.0	57.5	947.5	0.115	2.6
100.	08-UT-91	63.0	226.5	0.175	2.8	65	951.3	0.155	2.7
101.	Chevron06	45.0	332.5	0.078	3.1	57.5	711.3	0.190	3.2
102.	14938	53.0	246.3	0.115	3.5	47.5	616.3	0.127	3.0
103.	08-BA-69	50.2	223.7	0.130	3.2	52.73	729.7	0.152	2.8
104.	QSMO37	40.7	554.7	0.144	2.3	44.5	334.6	0.143	2.1
105.	25030	44.2	218.7	0.139	3.2	47.2	265.4	0.130	3.0
106.	08-WA-64	85.0	440.3	0.044	2.8	75.0	1020.0	0.173	2.4
107.	QSMO16	18.0	123.0	0.142	3.3	12.5	553.8	0.120	3.5
108.	14942	63.0	308.6	0.099	2.6	62.5	830.0	0.152	3.0

109.	Steptoe	65.0	349.5	0.126	3.4	70.0	867.5	0.183	3.1
110.	Chevron07	58.3	232.6	0.130	2.9	58.6	746.09	0.161	2.8
111.	QSMO54	45.0	440.3	0.044	2.8	75.0	1020.0	0.173	2.5
112.	SWISSHV67	14.2	218.7	0.139	3.2	17.2	265.4	0.130	3.0
113.	SWISSHV50	28.0	15.5	0.210	2.0	6.25	53.0	0.200	2.5
114.	Chevron08	25.5	349.7	0.128	2.6	20.2	453.3	0.125	2.3
115.	SWISSHV68	18.0	218.3	0.135	2.3	10	479.4	0.145	2.8
116.	08-UT-73	13.0	217.8	0.130	2.7	16.1	208.7	0.137	2.5
117.	QSMO-49	38.0	123.0	0.142	3.4	42.5	553.8	0.120	3.0
118.	QSMO002	17.0	308.6	0.099	4.0	19.5	830.0	0.152	3.7
119.	QSMO005	20.0	365.6	0.133	3.0	19.5	544.5	0.138	2.6
120.	08-N2-22	45.0	322.5	0.078	1.8	57.5	711.3	0.190	2.3
121.	Q21861	53.0	246.3	0.115	2.3	47.5	616.3	0.127	2.4
122.	QSMO059	17.4	223.6	0.123	3.8	15.6	445.8	0.133	3.3
123.	QSMO057	44.6	335.5	0.144	2.5	45.0	455.8	0.154	2.6
124.	QSMO061	48.0	125.8	0.163	2.7	22.5	273.8	0.115	3.0
125.	14942	25.9	37.8	0.194	2.9	16.5	200.58	0.155	3.0
126.	14905	30.2	110.7	0.119	2.0	23.0	201.4	0.134	2.5
127.	ND25882	23.3	445.8	0.145	2.9	26.0	333.5	0.143	2.3
128.	08-WA-42	50.0	144.0	0.147	2.8	42.5	570.6	0.168	3.0
129.	23027	45.0	343.5	0.078	2.7	67.5	900.0	0.165	3.1
130.	Chevron09	12.5	155.8	0.134	3.6	13.9	334.3	0.127	3.2
131.	QSMO79	50.0	144.0	0.147	2.7	42.5	570.6	0.168	2.5
132.	Chevron10	45.0	343.5	0.078	3.3	67.5	900.0	0.165	3.0
133.	Rawson	45.0	245.0	0.130	2.5	57.5	947.5	0.115	2.4
134.	04WA-122.9	50.2	223.7	0.130	2.0	52.7	729.7	0.152	2.3
135.	08-N2-47	43.5	334.5	0.143	2.4	40.4	556.5	0.154	3.0
136.	08-N2-87	11.5	155.8	0.123	3.5	12.8	223.1	0.127	3.1
137.	08-N2-48	22.4	345.7	0.145	3.0	17.8	234.2	0.133	2.6
138.	Diamora	63.3	502.1	0.230	3.2	62.1	443.1	0.210	2.9
139.	P1386458	8.0	1.5	0.217	2.8	15	136.5	0.159	3.0
140.	FEG192-1	19.0	3.3	0.288	3.8	18.0	136.3	0.186	3.4
141.	QSMO90	6.0	5.8	0.208	4.1	1.75	11.4	0.105	4.3
142.	SWISSHV66	21.5	144	0.236	3.5	2.5	23.3	0.094	4.0
143.	Q21861	60.0	187.3	0.149	2.3	42.5	553.8	0.122	2.8

Appendix 3: ANOVA tables for percent severity of scald on barley lines at various sites

Appendix 3.1; ANOVA table for scald severity (% infection) in Mau Narok (sprayed)

Source of variation	d.f.	S.S.	M.S.	V.R.	F pr.
Rep stratum	2	0.30	0.152	0.36	
Treatment	11	15.60	1.418	3.34	0.008
Residual	22	9.33	0.424		
Total	35	25.33			

Appendix 3.2; ANOVA table for scald severity (% infection) in Mau Narok (unsprayed)

Source of variation	d.f.	S.S.	M.S.	V.R.	F pr.
Rep stratum	2	0.56	0.143	0.25	
Treatment	11	17.50	1.516	2.45	0.016
Residual	22	8.90	0.325		
Total	35	26.96			

Appendix 3.3; ANOVA table for scald severity (% infection) in Timau (sprayed)

Source of variation	d.f.	S.S.	M.S.	V.R.	F pr.
Rep stratum	2	3.85	1.92	3.05	
Treatment	11	19.50	1.77	2.81	0.019
Residual	22	13.90	0.63		
Total	35	37.25			

Appendix 3.4; ANOVA table for scald severity (% infection) in Timau (unsprayed)

Source of variation	d.f.	S.S.	M.S.	V.R.	F pr.
Rep stratum	2	4.75	1.87	4.05	
Treatment	11	18.56	1.47	2.93	0.007
Residual	22	12.76	0.56		
Total	35	36.07			

Appendix 4: ANOVA tables for 1000 kernel weight of barley lines grown at various sites

Appendix 4.1; ANOVA table for 1000 kernel weight in Mau Narok (sprayed)

Source of variation	d.f.	S.S.	M.S.	V.R.	F pr.
Rep stratum	2	5.89	1.98	3.07	
Treatment	11	17.75	1.54	2.05	0.015
Residual	22	11.45	0.65		
Total	35	35.09			

Appendix 4.2;ANOVA table for 1000 kernel weight in Mau Narok (unsprayed)

Source of variation	d.f.	S.S.	M.S.	V.R.	F pr.
Rep stratum	2	4.75	1.17	3.06	
Treatment	11	17.56	1.69	2.73	0.017
Residual	22	13.76	0.57		
Total	35	36.07			

Appendix 4.3; ANOVA table for 1000 kernel weight in Timau (sprayed)

Source of variation	d.f.	S.S.	M.S.	V.R.	F pr.
Rep stratum	2	3.85	1.92	3.05	
Treatment	11	19.50	1.77	2.81	0.019
Residual	22	13.90	0.63		
Total	35	37.25			

Appendix 4.4; ANOVA table for 1000 kernel weight in Timau (unsprayed)

Source of variation	d.f.	S.S.	M.S.	V.R.	F pr.
Rep stratum	2	0.58	0.143	0.15	
Treatment	11	18.50	1.416	3.45	0.009
Residual	22	9.90	0.335		
Total	35	27.98			

Appendix 5: ANOVA tables for grain yield of barley lines grown in various sites

Appendix 5.1; ANOVA table for grain yield in Mau Narok (sprayed)

Source of variation	d.f.	S.S.	M.S.	V.R.	F pr.
Rep stratum	2	6.89	1.98	3.07	
Treatment	11	18.75	1.54	2.05	0.004
Residual	22	12.45	0.65		
Total	35	38.09			

Appendix 5.2; ANOVA table for grain yield in Mau Narok (unsprayed)

Source of variation	d.f.	S.S.	M.S.	V.R.	F pr.
Rep stratum	2	0.58	0.153	0.15	
Treatment	11	19.51	1.116	3.46	0.012
Residual	22	8.91	0.337		
Total	35	28.00			

Appendix 5.3; ANOVA table for grain yield in Timau (sprayed)

Source of variation	d.f.	S.S.	M.S.	V.R.	F pr.
Rep stratum	2	5.89	1.98	3.07	
Treatment	11	17.75	1.57	2.04	0.107
Residual	22	11.45	0.65		
Total	35	35.09			

Appendix 5.4; ANOVA table for grain yield in Timau (unsprayed)

Source of variation	d.f.	S.S.	M.S.	V.R.	F pr.
Rep stratum	2	3.85	1.95	3.07	
Treatment	11	16.51	1.78	2.91	0.018
Residual	22	13.91	0.73		
Total	35	35.27			

Appendix 6: ANOVA tables for protein content of barley lines grown at various sites

Appendix 6.1; ANOVA table for protein content in Mau Narok (sprayed)

Source of variation	d.f.	S.S.	M.S.	V.R.	F pr.
Rep stratum	2	0.30	0.152	0.36	
Treatment	11	15.600	1.418	3.34	0.008
Residual	22	9.33	0.424		
Total	35	25.33			

Appendix 6.2; ANOVA table for protein content in Mau Narok (unsprayed)

Source of variation	d.f.	S.S.	M.S.	V.R.	F pr.
Rep stratum	2	3.85	1.92	3.05	
Treatment	11	19.50	1.77	2.81	0.019
Residual	22	13.90	0.63		
Total	35	37.25			

Appendix 6.3; ANOVA table for protein content in Timau (sprayed)

Source of variation	d.f.	S.S.	M.S.	V.R.	F pr.
Rep stratum	2	4.79	1.45	4.08	
Treatment	11	19.60	1.88	2.35	0.003
Residual	22	14.90	0.48		
Total	35	39.29			

Appendix 6.4; ANOVA table for protein content in Timau (unsprayed)

Source of variation	d.f.	S.S.	M.S.	V.R.	F pr.
Rep stratum	2	3.89	1.92	3.05	
Treatment	11	19.50	1.77	2.81	0.013
Residual	22	14.90	0.63		
Total	35	38.29			

Appendix 7: ANOVA table for seed borne infection-seedling transmission

Source of variation	d.f.	S.S.	M.S.	V.R.	F pr.
Category	3	1.96	0.65	77.61	.0001
Treatment	1	0.76	0.76	90.30	.0001
Category*Treatment	3	0.32	0.11	12.86	.0002
Total	23	3.18			