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STABILITY OF BARLEY GENOTYPES FOR EARLINESS, YIELD AND RESISTANCE TO LEAF SCALD DISEASE IN ETHIOPIA

BY

LIJALEM GEBREWAHID EMBAYE

BSc (Horticulture) (Mekelle University)

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DECLARATION

I declare that this work has not been submitted for any degree and is not concurrently submitted for any degree other than that of Master of Science in Plant Breeding and Seed Systems of Makerere University. I also declare that this work is the result of my own investigations except where otherwise stated.

Signed_____ Date _____

Lijalem Gebrewahid Embaye

BSc. Horticulture, (MU, Ethiopia)

This dissertation has been submitted for examination with the approval of the following supervisors:

Signed……..……. Date ……..……

Dr. Richard Edema

B.Sc (Agric.) (MAK); M.Sc. (Agric.) (MAK); PhD, (The Ohio State University)

Signed……. Date……

Fetien Abay (PhD),

BSc (Agric.) (HARO, Ethiopia), M.Sc. (University of Wales, Bengor); PhD, (Norwegian University of Life Sciences)

DEDICATION

This thesis book is dedicated to all my colleagues, friends, and in particular to;

a) my parents, Mr. Gebrewahid Embaye and Mrs. Abeba Mesfin
b) my Wife, Mrs. Freweyni Tesfamichael, and
c) my sons Buruk Lijalem and Daniel Lijalem.

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ACRONYMS

BCC	Barley Core Collection
CSAE	Central Statistical Agency of Ethiopia
FAO	Food and Agriculture Organization
AUDPC	Area under disease progress curve
ANOVA	Analysis of variance
IPCA	Interaction Principal Component
ASV	AMMI Stability Value
SE	Standard Error
DH	Days to heading
DM	Days to maturity
TC	Tillering capacity
PH	Plant height
BY	Biological yield
SPS	Seeds per spike
TSW	Thousand seed weight
SL	Spike length
GY	Grain yield
ER	Early and resistant
LR	Late and resistant
LS	Late and susceptible
ES	Early and susceptible
BLUP	Best Linear Unbiased Prediction
SDI	Scald disease Incidence
NB	Net-Blotch
SP	sprayed
NSP	non-sprayed

ABSTRACT

Scald, caused by *Rhynchosporium secalis* (Oud) Davis, is the most widely distributed and destructive disease of barley (*Hordeum vulgare*) in Ethiopia. Developing genetic resistance in early maturing genotypes is a viable long term strategy to control the disease under unpredictable environments. In 2012 a field experiment was conducted to evaluate thirty-six barley genotypes for barley leaf scald disease resistance, earliness and yield performance in four locations (Mekelle, Korem, Debre-Birhan and Istayish) in the north and central part of Ethiopia. The experimental design was simple lattice with two replications and two spray treatment managements (sprayed and non-sprayed). In addition to the field experiment, data obtained from three environments from the previous experiment were incorporated to obtain deep insight in to the overall performance pattern. Data collected on yield and other agronomic traits including days to heading, days to maturity and disease score were subjected to different statistical analysis tools such as analysis of variance, regression analysis and additive main effect and multiplicative interaction (AMMI) model to evaluate consistency of scald resistance and maturity in barley in diverse environments in order to more effectively breed for stable resistance and earliness. The combined analysis of variance for 14 traits across three treated environments revealed that all but the most important traits central to this study, including days to heading, days to maturity, scald disease and grain yield had shown significant variation for location, genotype and genotype by location interaction. There was no significant variation among genotypes for spray treatments (management) in days to heading and days to maturity except for scald and grain yield. Location effect was the predominant source of variations accounted 70.32%. Besides, the broad-sense heritability estimates of heading days (0.30 on individual plot basis and 0.70 on entry mean across environment) pointed out that earliness is influenced by genotype. So, the possible reasons for most early maturing genotypes to behave susceptible could be early exposure of seedling to the heaviest release of spores from infected residues & adaptation pattern. Sprayed plot yielded up to 0.5 tonne per hectar more than unsprayed. This shows a major effect of scald on yield. The study had also discovered mean performance of genotypes for days to heading across seven environments to be varied between 71 and 96 of which G1, G26 and G34 were found to be early among others. Similarly, for grain yield the variation was between 2.56 and 4.42tha^{-1} of which G1 had the highest grain yield followed by G30. On contrary, G25 had the lowest grain yield. From trait association analysis, grain yield had significant ($P<0.001$) positive correlation with biological yield, harvest index as well as seed per

spike. Therefore, biological yield and numbers of seeds per spike had considerable contribution for yield to increase and can be used for indirect selection of high yielding genotypes.With regard to scald disease severity%, the lattice adjusted average performance was between 8.83% and 60.03% of which G6 had the lowest score. G4 on the contrary had the highest score. Though it requires further investigation, results suggested that three mega environments have been detected 1. Mekelle, Korem & Ayba, 2. Debre-birhan and 3. Istayish. The existence of high genotype by environment interaction at all environments was identified from the differential response of genotypes rank across locations. As a result, stability of the tested genotypes was determined by AMMI stability value (ASV) and yield stability index (YSI). Hence, based on AMMI2 biplot and genotype mean rank and AMMI stability value (ASV), G1, G6, G7, G15 and G34 were found stably combined scald resistance, earliness, and high yield across all environments. In summary, genotype by environment interaction can be minimized through selection of widely adapted genotypes.

Key words: Barley, *Hordeum vulgare,* BCC, Scald disease, *Rhyinchosporium secalis*

CHAPTER ONE

INTRODUCTION

1.1 Background

Barley (*Hordeum vulgare*) is one of the major sources of food today for large numbers of people living in the cooler, semi-arid areas of the world where other cereals including wheat are less well adapted (Onwueme and Sinha, 1991). It is a cereal adapted to and grows in a wider range of environmental conditions from 70^0 N in Europe to arid regions near the Sahara and the plateau of Tibet than any other cereal (Onwueme and sinha, 1991). The global commercial production of barley as estimated by FAO in 2010 was 123 million metric tons, harvested from 47.9 million hectares. Germany and France were the top producers, followed by the Ukraine, Canada, and Australia (FAO, 2010). It was ranked fourth both in terms of quantity produced after wheat, rice, and corn. Moreover, it has considerable place in the world's food supply as human food, malt products, and livestock feed. Ethiopia is also among barley growing countries as mentioned by (Akar, *et al*., 2004). In Ethiopia, barley is a major traditional cereal crop representing about 8.43% of the total national cereal production, and it ranks 5th in the area, after maize, sorghum, tef and wheat, Central Statistics Agency of Ethiopian (CSAE, 2012). It is a very important crop particularly for the highlanders where options are limited to grow other crops because of frost damage and other abiotic factors. The major barley-growing areas in Ethiopia are Wello, Bale, Shewa, Gonder, Arsi and the Tigray highlands. Barley is mainly used for food and malt (CSAE, 2012). The total land under barley cultivation in the year of 2012 was 1,018,752.94 ha with annual production of 1.78 million tonnes, and average yield of 1.75 tons per hectare (CSAE, 2013). The barley landraces existing in the country are of varied morphology (two and six rows, irregular types) and color (black, white and pink). In addition to phenotypic diversity, the Ethiopian barley is important source of genes for barley yellow dwarf virus resistance, high lysine, drought resistance, resistance to diseases such as powdery mildew, leaf rust, spot blotch, septoria, loose smut and barley stripe mosaic virus (Demissie, 2006). In spite of its capacity to grow in diverse agro-ecology and multipurpose use, its productivity still remain very low due to biotic as well as abiotic factors. Low soil fertility, low soil pH, poor soil drainage and drought were among the most yield limiting abiotic factors (Mulatu and Grando, 2011) . Furthermore the most frequently occurring biotic stresses includes; scald (R. secalis), spot blotch

(*Helminthosporium sativum*), Powdery mildew (*Erysiphe graminis*), Rusts (*Puccinia* spp.) and the barley leaf stripe (*Pyrenophora graminea*) (Akar, *et al*., 2004). Scald, caused by *Rhynchosporium secalis* (Oud) Davis, is the most widely distributed and destructive disease of barley in Ethiopia, (Kiros, *et al*., 2004). The common characteristics of these symptoms are that they reduce the photosynthesis area. Often diseased plants have less ears and smaller and lighter grains. Use of resistant varieties is the best solution for farmers who cannot afford to purchase chemicals to control the disease.

1.2 Statement of the problem

In a country with dominating agrarian economy like Ethiopia, improving food security, which is strongly advocated by United Nations (UN) and included as one of the millennium development goal (MDGs), is mandatory. In Ethiopia, the demand of barley for food as well as brewing industry is dramatically increasing. Barley is considered one of the priority crops. However, the current production trend which is entirely dependent on the timely onset, duration, amount and distribution of rainfall that makes limited crop choice could not satisfy the ever increasing local demand. Many breweries imported 60% of their malt in 2012 (Heineken, 2013). Barley research commenced since 1955 in the country and most of the research activities were concentrated to high input areas (Mulatu and Grando, 2011). Therefore, deployment of the research results to the low input areas, which is dominated by subsistent farmer, was not effective and the rate of technology adoption was very slow. In general genetic, environment and socioeconomic factors have been reported by different researchers to be the pragmatic constraints that hinder the productivity of the crop (Mulatu and Grando, 2011). This is complicated by the fact that in recent times, according to climate trend analysis in Ethiopia significant rainfall reductions and increases in temperature have been recorded (Funk, *et al*., 2012). As a result, in many barley-growing parts of Ethiopia subsistence farmers are replacing their traditional long season barley landraces, with short season or early maturing varieties (Sinebo, *et al*., 2010). Yet, most of the early maturing barley genotypes are usually susceptible to barley scald (*Rhynchosporium secalis* (Oud.) J.J. Davis), (Leur and Gebre, 2003), which is one of the most widely-occurring and damaging fungal foliar diseases of barley (Akar, *et al*., 2004). As a result, the yield of such early maturing varieties has remained very low and contributed to widespread food insecurity. Where environmental conditions are favorable, yield losses from barley scald can reach up to 67%

(Yitbarek, *et al*., 1998). So there is an urgent need for improved barley varieties that combine adaptation to the wide environments. Full information related to scald disease, earliness and yield trait is not yet established. Such conditions necessitated execution of research on the barley core collection, obtained from the Ethiopian gene bank (EGB), to come up with genotypes endowed with resistance to barley leaf scald disease, early maturity and high grain yield.

1.3 Justification

Although Ethiopia is endowed with high diversity of barley landraces, most of the country's farmers still obtain very low yields due to biotic and abiotic factors. One of the important steps in this study is to evaluate barley genotypes retrieved from Ethiopian gene bank (EGB) in the view of higher yield, disease resistance and earliness stability in multi-locations in the highland and semi-highland areas of Ethiopia. The main reason why this study focused on barley crop is because farmers who operate barley-based farming systems in the highlands of Ethiopia have very few alternative crops. And it is an important crop that is adapted to marginal and stress affected environments and it is, therefore, of high importance to subsistence farmers of Ethiopia. Since barley is grown under low-input conditions in Ethiopia, it is compulsory to look for other options besides attempting to change the traditional farming practices, like requiring the use of fertilizer or earlier sowing, and so on. Earliness, which is considered as best option by the farmer to escape drought and major disease build up in the drought prone area are usually susceptible to scald disease. The presence of this disease interferes with selection of early maturing genotypes and cause substantial yield lost. Evaluation of genotypes has to be carried out in plots free from scald disease to understand well about the response. To do so, the genotypes should be planted in plots that are protected from scald by spraying of a fungicide, in addition to being planted in plots where the disease is encouraged by a susceptible spreader. Therefore, selection for stable disease resistance and early maturity can be done jointly. In addition, by comparing protected and diseased plots of the same genotype, it will be possible to quantify the impact of scald disease on the earliness and yield of the different genotypes in the different locations. A promising genotype should retain relatively high yield levels, early maturing and disease resistance not only in the environment in which it was initially selected but also in many other environments within its anticipated area of production. Therefore, testing the selected genotypes

in different locations will help to evaluate the mean performance of the genotypes and the relative consistency of genotypes in relation to earliness and resistance to leaf scald disease.

1.4 Objectives

1.4.1 General Objective

To evaluate barley genotypes for consistency of scald resistance, maturity and yield under diverse environments in order to more effectively breed for stable resistance and earliness.

1.4.2 Specific objectives:

1. To determine genotypic and genotype x environment effects on maturity, response to scald disease and yield in a set of Ethiopian barley core collection.
2. To determine the effect of scald disease on maturity and yield among genotypes from the Ethiopian barley core collection.

1.4.3 Research questions

1) a. Do any genotypes stably combine scald resistance, earliness, and high yield?

   b. How large is GxE in this set of genotypes and environments?

      i. Is there a difference in stability of the different genotypes for earliness, scald response, and yield?
      ii. Are any of the environments better than others at identifying genotypes with reliable expression of earliness, scald resistance, and yield?

2) Does scald affect earliness and yield in this set of genotypes and environments?

CHAPTER TWO

LITERATURE REVIEW

2.1 Taxonomy of Barley

Cultivated barley belongs to the genus Hordeum (Bothmer, 2003). Cultivated barley is descended from wild barley (*Hordeum spontaneum*) (Young, 2001) which is still found wild in the Middle East. Among the wild Hordeum, there are also tetraploid, and hexaploid species (Kling, 2004). Usually cultivated barley is an annual plant, but there are also many other perennial species(Kling, 2004). There are 32 species within the *Hordeum* genus, both the cultivated barley, *Hordeum vulgare* L. ssp. *Vulgare*, and its wild progenitor *H. vulgare* L. ssp. *Spontaneum* (C. Koch.) are diploid species, with 2n=14 (Kling, 2004). Barley can be classified in many ways: one way to classify barley is by type of inflorescent as two, four or six rows of grains on the head (Young, 2001; U.S. Grain Council, 2006). Spikelets are arranged in triplets alternate along the rachis. In wild barley, only the central spikelet is fertile, while the other two are not. 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. This produces six-row barleys (Daniel and Hopf, 2000). Recent genetic studies have revealed a mutation in one gene, *vrs1*, is responsible for the transition from two-row to six-row barley (Komatsuda, *et al*., 2006). Six row barley usually produce 25-60 grains, while two-row barley produces 25-30 grains (Gomez-Macpherson, 2001). Most cultivated barley is of the six-row type. Another way of classifying barley is based on beards (awns) covering the kernels (U.S. Grain Council, 2006). Awns are described along the following morphology (Database, 2003): Long awned, Short awned, (Normal) hooded, Elevated hooded, Subjacent hooded, Long awned in central row, and awnletted or awnless in lateral rows, Short awned in central row, and awnletted or awnless in lateral rows, Awnless or awnletted in central and lateral rows and Elevated hoods in central row, and awnless in lateral rows. Barley can also be described by: hulled or hulless (naked) (Kling, 2004; U.S. Grain Council, 2006), feed or malt type, height (dwarf), seed color (colorless, white, yellow, blue). Barley is grown for many purposes, but the majority of all barley is used for human consumption, animal feed, or malting (Kling, 2004). High protein barleys are generally valued for food and feeding, and starchy barley for malting. It can be rolled, ground, flaked or

pelleted, but by-products from malting and brewing are also used in feed production (Young, 2001). Barley is also used for pasture, green feed, hay, roughage and bedding (Young, 2001).

2.2 Origin and Domestication of Barley (*Hordeum vulgare* L.)

Barley is considered to be the first domesticated cereals (Akar, *et al*., 2004). The exact origin of barley is uncertain. However, barley was under cultivation in the Middle East before 10,000 BC(Young, 2001; Kling, 2004). In China and India barley's cultivation probably occurred later (Kling, 2004). Barley was grown on the Korean Peninsula by 1500-850 BC (Young, 2001) along with millet, wheat, and legumes. Barley ranks fourth in world cereal crop production and is used for, in order of importance, animal feed, brewing malts and human food. Barley is a short season, early maturing grain found in widely varying environments globally. The immediate ancestor of cultivated barley, which is still abundant in nature, was first discovered in Turkey by the German botanist Carl Koch, and described by him as a separate species, *Hordeum spontaneum*. However, based on several criteria, the progenitor form is nowadays regarded as a subspecies (ssp.spotaneum (C.Koch) Thell.) within the same major species, H. vulgare L., as cultivated barley (ssp. Vulgare) (Von Bothmer, *et al*., 2003). With the development and advancement of molecular works, more precise information on origin and domestication of barley is underway. According to (Badr, *et al*., 2000), the monophyletic nature of barley has demonstrated based on allelic frequencies at 400 AFLP polymorphic loci studied in 317 wild and 57 cultivated lines and accordingly more similarities between wild populations and cultivated barley has been detected from Israel-Jordan region and indicated the area is most likely the first to brought barley in to domestication.

2.3 Barley cultivation and Utilization in Ethiopia

Ethiopia is considered as one of the twelve Vavilovian center of diversity of crops in the world due to its diverse agro-ecological as well as climatic features (Vavilov, 1951; Harlan, 1969). Barley was under cultivation in Ethiopia for at least the past 5000 years. So the country is considered as secondary centre of diversity for barley (Demissie, 2006). It is mainly used for food and malt (Fekadu, *et al*., 2005) in almost all parts of the country. Shewa, Arsi, Gojam, Gonder, Welo, Bale and Tigrai are the most important barley producing regions accounting for more than 85% of the country's total production (CSAE, 2012). Barley is utilized in different

forms according to various culture of the country such as *Kecha, Geat, tihn, enjera*, and *kolo,* accompanied by other cereals, legumes and animal products. Furthermore, it is also basic input in making local drink such as *siwa*. Nutrition wise, barley is a winner crop and called as "nutritional power house". This centuries-old grain is packed with fiber which is effective in lowering blood cholesterol and dipping of the risk of heart disease; it also contains important vitamins and minerals, and is slim on fat, and cholesterol-free (http//www.barleyfoods.org/nutrition.html). Because of wide use for various purposes, farmers in Ethiopia named it as "Gibse Yehl nigus" literally meaning "King of crops". Diverse barley landraces are adapted to different barley growing agro-ecologies of the country. These are result of diversity of physical characteristics of the land, farm size, agro-climatic futures and household characteristics (Abay and Bjørnstad, 2009). Ethiopian barley landraces have useful traits, especially for resistance to diseases, such as powdery mildew barley yellow dwarf virus, net blotch, scald and loose smut (Negassa, 1985). On the other hand, even though Ethiopia has a substantial number of barley land races that can confer resistance genes for scald disease resistance, the disease is still one of the most important. Kiros, *et al*., (2004) have described this situation in the following way: "Ethiopia, as the center of diversity, is conferring not only the resistance gene, but also many virulent races of the pathogen". It is the crop that matures early and an emergency crop bridging the critical food shortage occurs in September (Muhe and Assefa, 2011).

Five traditional barley production systems have been described in Ethiopia considering maturity level (Mulatu and Grando, 2011).

2.3.1 Late barley production system

This system is practiced in the high-altitude areas of Ethiopia which has relatively high rainfall distribution and is practiced during *Meher,* the main rainy season (June to October). Generally, barley genotypes used in this system are late type which required 5–6 months to reach maturity. Grain yields obtained from this system is high compared with the other system.

2.3.2 Barley production system with *guie* (soil burning)

This is a system that is practiced during the *Meher* season. It is important in the highlands of north and North West Shewa, where water logging is a major problem to barley production. To

alleviate this problem, farmers use *guie* (soil burning) and ploughing 3–5 times of fields that have been left fallow for at least five years to solve the stated problem. Early-maturing farmer cultivars, such as 'Demoye' and 'Magie', are used in this system, and the grain yield in the first year is generally high about 2.0 t/ha, but declines dramatically in subsequent years.

2.3.4 Early-barley production system

This system is practiced during the *Meher* (main) season, and is important in both the mid- and high-altitude areas. Currently, this system is becoming dominant due to erratic and unbalanced rainfall distribution in the country. Therefore, early varieties are grown that require 3.5–4 months to mature, such as 'Saesa' The yield of early barley in a normal year varies from 0.7 to 1.5 t/ha.

2.3.5 *Belg* barley production system

This system is practiced in north and North West Shewa, North Wollo, Bale and a few areas in Arsi and in the south and eastern part of Tigrai. Planting is usually carried out in February to early March and harvested in early July. Early-maturing cultivar that matures 3–4 months is usually used for cultivation. The yield of *Belg* barley in a normal year varies from 0.8 to 1.2 t/ha.

2.3.6 Residual barley production system

This system is important in some parts of Gojam, North and South Gonder, and West Shewa. Early-maturing varieties are common used in this system. Planting is carried out between September and October, immediately after harvest of the main-season barley crop. The seed of the main-season barley is re-sown in the same field, in the main-season fallow field, or in any other field where the main-season crop has failed. Fertilizer is not generally applied in this system. Harvesting is carried out from December to February. Grain yield from this system is generally low, less than 1.0 t/ha, and mainly used as seed for the next season.

2.4 Barley Core Collection (BCC)

The definition of BCC (Roland von Bothmera, 2004), is a selected and limited set of accessions. It optimally represents the genetic diversity of cultivated barley and the wild species of *Hordeum*, covering the three gene pools. The core should include as much as possible of its genetic diversity.

The BCC is mainly developed to achieve four main objectives.

(1) increase the knowledge about the barley gene pool; (2) increase the efficiency of evaluation and thus of utilisation of existing collections; (3) provide a manageable and representative, highly diverse selection of the available barley germplasm for use in research and plant breeding; (4) provide adequate standards, e.g., for studies of genetic diversity in barley.

A collection of more than 12,500 barley accessions are found in the institute of Bio-diversity Conservation (IBC, Addis Ababa, Ethiopia) (Roland von Bothmera, 2004). The use of barley core collections representing at world, regional and local level is always successful in locating sufficient sources of resistance (Bockelman, *et al*., 1981).

2.5 Biology of the causal agent of barley scald

Scald or leaf blotch is common disease of barley worldwide. It is caused by the haploid imperfect fungi (ascomycete) *Rhynchosporium secalis* (Oudem.) J. J. Davis, *i.e*. without known sexual stages since no teleomorph has been described for the fungus. It is most prevalent in temperate area where the relative temperature is low combined with humid weather condition as well as in tropical areas where there is high rainfall and temperatures are low because of the altitude difference Gilchrist-Saavedra and McNab (2006). Nevertheless, the fungus has a very high genetic diversity Mcdermott, *et al*., (1989), which clearly contradict with the result of asexual propagation. Therefore, there could be some form of sexual recombination as it was described by (Salamanti, *et al*., 2000). Barley, rye, and other grass species are the main hosts of the pathogen and so the pathogen can cause significant yield losses during cool and wet condition (Martens, *et al*., 1984 and Mathre, 1997). The mycelium is hyaline to light gray and develops sparsely as a compact stroma under the cuticle of the host plant (Mathre, 1997). Condia (2-4 x 12-20 µm) are borne fixed on cells of the fertile strom with characteristics of host infection and hyphal growth in the absence of a sexual reproduction. According to (Linde, *et al*., 2003) the fungus is heterothallic implying that sexual reproduction does take place but no sexual fruiting bodies have been described. The fungus persists on dead leaves and other plant residues to initiate primary infection. Seed borne spores may contribute to initial infections Bockelman, *et al*., (1981). However, left over residues from previous year crops are considered the most important source of primary inoculums. Spore production is abundant during moist period and secondary spread of the inoculums takes place via wind or splashing rain. The disease may develop rapidly during cool weather and in severe cases may virtually cause defoliation by

coalescing of the lesions. Yield loss can be considerable (Yitbarek, *et al*., 1998). Sporulating potential of fungal material on crop residues left in the field could survive for up to a year. Overwintering mycelia will produce spores when environmental conditions are favorable, serving as primary inoculums to initiate an epidemic. Two-celled colorless conidia characteristically beak-shaped are produced on a stroma at the surface of a lesion and germinate at temperature between15-21°C and at about 95 % humidity and germination can reach up to 80% within 24 hours when environmental conditions are conducive. The conidia can germinate with several germ tubes from one or both cells and appresoria develop at the tips of the germ tubes (Shipton, *et al*., 1974) and penetrate the cuticle through penetration pegs (Ayesu-Offei, 1970). Infection is followed by formation of a subcuticular mycelium, which develops into a stroma. The epidermal cell layer then penetrated through hyphae particularly at the junction of guard and epidermal cells. Then after infection causes stomata to open more to light, due to an alteration of the turgor relations between guard cells and the surrounding epidermal, cuticle starts to separate and crackdown. The scald like lesions of this disease is conspicuous on the leaf blades and sheaths. Infection causes mesophyll cells to collapse and the ovate to irregular lesions are first water-soaked in appearance, but soon fade to a bleached, scalded appearance and are surrounded by a brown-pigmented ring (Bockelman, *et al*., 1981). Means of control are crop rotation, elimination of crop residue, and resistant varieties. But the latter is most effective and durable. The disease was first reported in Ethiopia by Stewart & Dagnachew, 1967 cited in (Mulatu and Grando, 2011). It primarily affects the leaves, but it can also affect the glumes, awns and grain. Symptoms in the field are easily recognized because they start as oval, elongated or elliptical lesions, with dark brown reddish edges, and grey or yellowish-brown centers.

Figure 1: An overview of scald infected barley leaf at Debre-birhan site
(Photo: Lijalem G.sep.2012)

The disease affects barley, rye, triticale and a number of grasses, particularly ryegrasses. There are specialized forms of the pathogen which are generally restricted in their host range. Rhynchosporium is a polycyclic barley disease, normally involving several pathogen generations during a growing season. The conidia produced on crop debris or seed can act as a source of primary inoculum to initiate epidemics and secondary disease spread from infected leaves by rain splash-dispersed conidia.

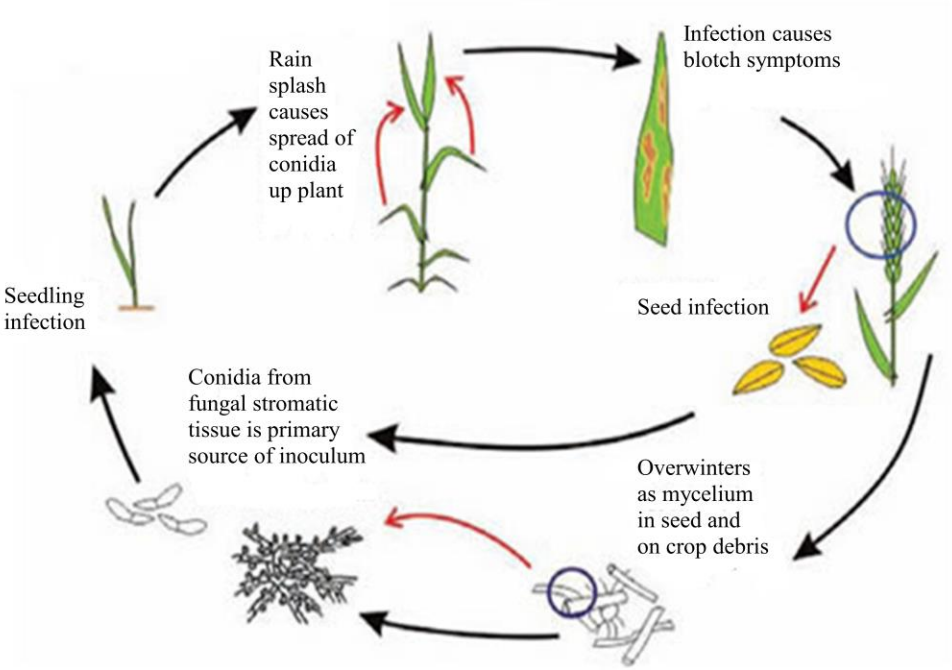

Figure 2: Life cycle of scald disease
Source: Cereal disease Encyclopedia

2.6 Relationship between heading time and scald disease in barley

Character including yield, plant height, days to heading, days to maturity, etc. are quantitative characters and are governed by several genes; each gene has small effect, which is usually cumulative. These characters are considerably affected by the environment. Quantitative characters show continuous variation and it is not possible to classify them in to distinct classes like qualitative character. In breeding, selection is based on phenotypic expression. And the effectiveness of selection is largely depending on the extent of phenotype due to genotype. Consequently it is crucial important for the breeder to quantify the extent of influence by environment on quantitative character (Singh, 1993). Winter barley varieties require a period of cold stimulus (vernalisation) to initiate floral development. While spring barleys varieties on the other hand do not require vernalisation. Vernalization is the induction of the flowering process by extended exposure of the shoot apex to low temperatures. Most barley varieties grown in Ethiopia are spring type. Flowering in many barley varieties does affect by the day length and temperature. Heading (flowering) period, which is one quantitative trait controlled by many genes, is a basic adaptive trait and a critical developmental lever in the transition from vegetative

to reproductive growth that ensures plants set their flowers at an optimum time for pollination, seed development, and dispersal. Selection of this century old crop by farmer and breeder based on flowering time, and yield had contributed to wide adaptability (Mulatu & Grando, 2011). As a result, early flowering genotype has evolved as an adaptation to short growing seasons. Environmental and endogenous cues interaction has been reported as means of controlling flowering time (Dean, 1998). The photoperiod response gene including *Ppd-H1* locus located on chromosome 2HS that is capable of heading about 20 day earlier than *ppd-H1* plants under LD conditions (16 h of light) has been reported as genetic source for earliness (Turner, 2005). A second major photoperiod response gene, *Ppd-H2*, has been mapped on the long arm of the chromosome 1H. Three vernalization genes (*Vrn-H1, Vrn-H2 and Vrn-H3*) have been demonstrated and interact to control flowering time in response to temperature. Earliness per se (*eps*) is also considered as intrinsic difference in flowering time of fully vernalized plants grown under long day conditions. The diversity of Ethiopian landraces increases as one goes from lowland to highland and maximum diversity attained at moderate altitude 2,460 m.a.s.l. (Tanto, *et al*., 2010). The presence of high morphological variation within regions and at relatively higher altitude, (Tanto, *et al*., 2010) could possibly contribute variation in heading as well as disease resistance. According to (Leur and Gebre, 2003) an experiment carried out for 155 samples of varieties collected from various regions of Ethiopia displayed a highly negative correlation between days to heading and disease severity rates ($r=0.94$ at $p<0.001$). *(Yitbarek, et al*., 1998), on the other hand indicated that scald severity was negatively correlated with altitude. (Engels, 1994), in his work on "genetic diversity in Ethiopian barley in relation to altitude", he found out that the mean diversity index for all characters under the study increased with altitude and significant difference have been obtained for days to maturity and plant height. Similarly, (Yitbarek, *et al*., 1998) reported that populations collected from higher altitudes have shown more resistance to scald than those from lower altitudes. According to (Leur and Gebre, 2003), early-maturing varieties have been found to be highly susceptible to scald disease in general, even though some of these have also shown resistance. Most of the studies have indicated that early maturing varieties are more susceptible to scald disease than late maturing varieties.

Area under disease progress curve (AUDPC)

For disease data, the most useful measurement of disease progress depends upon both the host and the pathogen of interest. Disease incidence, or the number of diseased plants express as a proportion of the total number of plants assessed, is one common measurement. Disease incidence is often a useful measure for quantifying diseases for perennial crops, such as citrus, or for estimating crop losses due to a disease such as stalk rot in corn, where entire plant death from a single infection is possible. Disease severity, usually defined as the percentage (area) of diseased tissue present on an affected plant, is another common measure, particularly for evaluation of foliar diseases where the amount of disease present on the plant may be correlated to a yield loss estimate. AUDPC is a measure of the total amount of disease over a period of time, determined from graphs of disease vs. time, which can be used to compare epidemics quantitatively. And it helps understand patterns of disease progress for different diseases, cultivars, management strategies, and also helps in determining how plant diseases may best be managed. As it has been reported by (Viljanen_Rollinson, 2001), plant epidemiologists apart from using simple descriptive type statistics to see disease increase pattern, AUDPC was also further used to assess yield lost and partial or quantitative resistance.

$$\text{AUDPC} = \sum_{i=1}^{N_i-1} \frac{(y_i + y_{i+1})}{2}(t_{i+1} - t_i)$$

Where, y_i = disease severity on the i^{th} date, t_i= i^{th} day, and n=number of scoring dates. Then the average disease severity at the midpoint between each two time points was calculated and multiplied that average by the length of time between the two points and summed those products across all time intervals.

2.7 Breeding for disease resistance and yield in barley

Stress refers to adverse conditions for crop growth and production imposed by either environmental factors (abiotic) or biological factors (biotic)(Singh, 1996). Biological factors includes disease, insect and parasitic weeds. However, the environmental factor includes deficiency or excess of nutrient, moisture, temperature etc. Adaptation to various environmental conditions has been improved through the use of wild species. For example, tolerance to cold in

rye, wheat, onion, potato, tomato, grapes, strawberry etc. in Russia and improvement in yield have been noticed 25-30% (Singh, 1996). To most effectively utilize resistance and yield potential a certain amount of basic information is required from the pathogen as well as the host. Genetic resistance refers to those heritable features of host plant that suppress development of pathogen and give high yield than other varieties under similar environmental conditions (Singh, 1996). Vertical or specific resistance (qualitative resistance) refers to the resistance of a host to the particular race of a pathogen and governed by one or few genes. The host with vertical resistance controls only one race, therefore, it is known as non-uniform resistance and less durable. Horizontal resistance (polygenic resistance) on the other hand provides protection from several races of disease. It has low heritability and therefore identification of resistance types is difficult(Singh, 1996). A collection of barley genotypes can be tested against the pathogen across wide environments to determine the differential response of genotypes. This, in turn, gives important information on the effectiveness of particular resistance genes which is useful in breeding program. That in turn is helpful in determining the inheritance level of the genotypes which is useful for further crossing a resistance line to susceptible variety and then examining the segregation in the subsequent generations to choose the best way of introgression of the resistant gene to the susceptible verities (Bockelman, *et al*., 1981). In Ethiopia in the early 1970s, exotic cultivars were under study and appeared to show narrow adaptation and were susceptible to pests and diseases. Then after the research directions have been changed towards evaluation and selection of local landraces consequently many accessions were evaluated and were selected based on different agronomic traits. These selections were used by national and international research organizations to develop improved varieties(IBC, 2007). Resistance gene incorporation is the most effective, economical and eco-friendly way in protecting crops (Wenzel, *et al*., 1996). However, evaluation of genotypes performance in wider environments is usually complicates selection for the best and consistent performer in wide environment (Kang, 1998). Environmental effects, with variable agro-ecological potential are not direct concern of any breeding program to recommend plant varieties (Annicchiarico, 2002). However, deferential responses of genotypes to variable environmental conditions which are primarily associated with performance change of genotypes, normally limit accurate resistance as well as yield estimate and detection of superior stable genotypes (J.Crossa, 1991). Furthermore, according to FAO's paper written by Annicchiarico,P (Annicchiarico, 2002) genotypes shown inconsistency across

time has a negative effect on farmers' income and, in the case of staple crops, contributes to food insecurity at national and household level . Therefore, there is a need to further evaluate in a wide range of environments to use opportunities, especially in the selection and adoption of genotypes showing positive interaction with the location (Freeman, 1972). And as well to identify the possible strategy based on magnitude of the interaction; if the interaction is large and repeatable then breeding for specific adaptation become the first choice. In the absence of genotype by environment interaction genotypic main effects is the only entity that can provide information. Nevertheless, differences between genotypes may vary widely among environments in the presence of GE interaction effects. Taking in to consideration GE interactions as an essential source of variation, appropriate techniques should put in place in order to analyze GE and explore the potential opportunities and disadvantages and to better understand the type and size of GE. GE interactions occurred normally when there are wide variation between genotypes for physiological and morphological characters and wide variation between environments. Heritability estimate is usually used by breeder in order to verify the extent of genetic and environment influence on traits of interest.

2.8 Statistical analysis

Analyzing information involves examining it in ways that reveal the relationships, patterns, trends, etc. that can be found within it. Which means subjecting data to statistical operations can tell us not only what kinds of relationships seem to exist among variables, but also to what extent confident is someone by the answers he/she is getting. Analysis of variance (ANOVA), one of the statistical tools used in several ways to develop and confirm an explanation for the observed data (Gomez & Gomez, 1984), used normally to evaluate the importance of one or more factors by comparing the response variable means at the different factor levels to identifying the presence of significant variation. Further, joint regression models, AMMI (Additive Main effect and Multiplicative Interaction) models, factorial regression models including environmental covariates and pattern analysis are some of the models adopted by researchers to carry out adaptation analysis based on yield.

The use of AMMI model however was found useful for yield trials as described by Gauch (Gauch, 1988; Gauch, 1992). (Zobel, *et al*., 1988) compared the performance of AMMI analysis with the ANOVA approach and regression approach and found that ANOVA was found to fail detecting a significant interaction component and while regression approach accounts only a

small portion of the interaction sum of squares only when the pattern fits a specific regression model. In contrast, the principal-component approach of AMMI, graphically displayed as a biplot, can give additional understanding of the pattern of response of genotypes and the suitability of different testing sites for differentiating among genotypes.

2.8.1. AMMI model

During evaluation of genotypes, plant breeders usually face challenge from the so called genotype x environment interactions. This interaction normally leads best performing genotype to be inconsistent across environments and so complicates selection of elite materials in the breeding program. Nevertheless, it is possible to minimize GxE through selection of widely adapted genotypes or positively exploited through selection of specifically adapted genotypes (Ceccarelli, 1989). In order to select best performing elite breeding material, genotypes have to be assessed in multi-environment (METs). Therefore, collected data have to be analyzed using appropriate techniques so as to explore the potential opportunities and shortcomings. Additive main effects and multiplicative interaction (AMMI) model is the most frequently utilized statistical analysis for better understanding of the type and size of the GE interactions in many breeding programs (Gauch, 1988; Gauch, 1992). The method is used when analyzing data from a series of trials. A principal components model is fitted to the residuals from the ANOVA and the resulting scores, called interaction principal component analysis (IPCA) scores, are calculated for both the genotypes and the environments. The AMMI biplot analysis display of PCA scores plotted against each other provides visual inspection and interpretation of the GEI patterns graphically. Integrating biplot display and genotypic stability statistics enables genotypes to be grouped based on the similarity of performance across diverse environments.

AMMI analysis requires estimation of genotype and location main effects by ANOVA analysis. Residuals from additively of these effects (i.e. GL effects) are then partitioned into:

- the multiplicative term of the model, of which the estimated parameters relate to the statistically significant axes of a double-centered principal components analysis performed on the GE interaction matrix; and
- a deviation from the model term:

$$GE_{ij} = \sum u_{in}\, v_{jn}\, l_n + d_{ij} = \sum (u_{in} \sqrt{l_n})\, (v_{jn} \sqrt{l_n}) + d_{ij}$$

where u_{in} and v_{jn} are eigenvectors (scaled as unit vectors, i.e. $\sum u_{in}^2 = \sum v_{jn}^2 = 1$) of the genotype $_i$ and the location j, respectively, and l_n is the singular value (i.e. the square root of the latent root or eigenvalue) for the principal component (PC) axis n; and d_{ij} is the deviation from the model.