Genetic Diversity, Stability, and Combining Ability of Maize Genotypes for Grain Yield and Resistance to NCLB in the Mid-Altitude Sub-Humid Agro-Ecologies of Ethiopia

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Thesis Abstract

Maize (*Zea mays* L.) is the third most important cereal crops in the world after wheat and rice. In Ethiopia, maize remains the second largest food security crop after tef [*Eragrostis tef* (Zucc.) Trotter.]. The mid-altitude, sub-humid agro-ecology (1000 to 1800 m above sea level) is the most important maize producing environment in Ethiopia. However, productivity of maize is low, due to several biotic and abiotic constraints. Among the biotic constraints, *Turcicum* leaf blight disease of maize caused by *Exserohilum turcicum* Pass Leonard & Suggs shows high incidence of 95-100% and inflicts significant grain losses in the country. Therefore, high yielding, *Turcicum* leaf blight resistant and farmers-preferred maize varieties and their production technologies should be developed and made available to growers to enhance maize production and to achieve food security.

The objectives of this study were to: (1) assess farmer's preferences, and production constraints for maize in the mid-altitude, sub-humid agro-ecology of western Ethiopia, (2) determine the genetic variability among elite maize inbred lines and select promising parents for resistance to *E. turcicum,* (3) determine diversity among the elite germplasm lines using SSR markers, (4) determine combining ability and heterosis among elite maize inbred lines and their hybrids, and (5) investigate genotype x environment interaction and yield stability of experimental maize hybrids developed for the mid-altitude sub-humid agro-ecology of Ethiopia.

A participatory rural appraisal (PRA) research was conducted involving 240 maize farmers in three representative maize growing zones of western Ethiopia; West Shoa, East Wollega and West Wollega, each represented by two districts and two sub-districts. Maize was ranked number one both as food and cash crop by 82.9% of respondents. *Turcicum* leaf blight was ranked as number one devastating leaf disease by 46% of respondents. Breeding for improved disease resistance and grain yield, enhancing the availability of crop input and stabilizing market price during harvest time were recommended as the most important strategies to increase maize production by small-scale farmers in western Ethiopia.

Fifty inbred lines were evaluated for reaction to *Turcicum* leaf blight during the main cropping seasons of 2011 and 2012. Inbred lines were clustered into resistant (CML202, 144-7b, 136-a, 139-5j, 30H83-7-1, ILOO'E-1-9, SZYNA-99-F2, and 142-1-e), and susceptible (CML197, CML464, A7033 , Kuleni C1-101-1-1, CML443, SC22-430 (63), (DRB-F2-60-1-2) – B-1-B-B, Pool9A-4-4-1-1-1). Inbred lines (CML312, CML445, Gibe-1-158-1-1-1-1, CML395, and 124-b (113)) had intermediate response to the disease. Overall, inbred lines such as CML202, 30H83-7-1, ILOO'E-1-9-1, CML312, CML395 CML445 and 142-1-e were selected with better agronomic performance and resistance to leaf blight for breeding. Twenty selected elite parental inbred lines were genotyped with 20 polymorphic SSR markers. The genotypes used were clustered into five groups consistent with the known pedigrees. The greatest genetic distance was identified between the clusters of lines CML-202 and Gibe-1-91.

Eighteen selected inbred lines were crossed using the factorial mating scheme and 81 hybrids developed to determine combining ability effects and heterosis. Inbred lines with high GCA effect (CML 202, CML395, 124-b (113), ILOO'E-1-9 and CML 197) were selected as best combiners for hybrid development. Additionally five high yielding novel single cross hybrids with grain yield of > 8 t ha^{-1} and high SCA effects were identified such as CML395 X CML442, DE-78-Z-126-3-2-2-1-1 X CML442, ILOO'E-1-9-1-1-1-1-1 X CML312, X1264DW-1-2-2-2-2 X CML464 and SC22 X Gibe-1-91-1-1-1-1. These experimental hybrids are recommended for direct production or as hybrid testers for hybrid development.

Genotype x environment interaction (GEI) effects of 81 newly developed and three check maize hybrids were evaluated across 10 locations in the mid-altitude sub-humid agro-ecologies of Ethiopia. The AMMI-3 and GGE biplot models were used to determine stability. Hybrids such as G68, G39, G37, G77, G34 and G2 were identified as the most stable and high yielding at favorable environments such as Bako, Jima, Arsi Negelle and Pawe in Ethiopia. The genotype and genotype by environment interaction (GGE) biplot clustered the 10 environments into three unique mega-environments.

Environment I included Bako, Jima, Asossa, Ambo, Finote Selam, Haramaya and Pawe while environment II represented by Arsi-Negelle and environment III Areka and Hawassa.

In general, the study identified valuable maize inbred lines with high combining ability for breeding and novel single cross hybrids for large-scale production or as testers for hybrid development at the mid-altitude, sub-humid agro-ecologies of Ethiopia or similar environments in sub-Saharan Africa.

Declaration

I, Wende Abera, declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.

2. This thesis has not been submitted for any degree or examination at any other university.

3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

    a. Their words have been re-written but the general information attributed to them has been referenced.

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Signed

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Wende Abera Mengesha

As the candidate's supervisors, we agree to the submission of this thesis:

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Prof. Shimelis Hussein (Supervisor)

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Prof. John Derera (Co-Supervisor)

Acknowledgement

Dedication

This thesis is dedicated to my wife Gadise Tamiru, my children (Meti and Duretti), my father (Abera Mengesha), and my mother (Tirunesh Sintaro).

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Introduction to thesis

Maize (*Zea mays* L., 2n = 2x = 20) is one of the most important food crops world-wide, serving as staple food, livestock feed, and industrial raw material (Troyer, 2006). Among cereal crops, maize has the highest average yield per ha and remains third after wheat and rice in total area and production in the world (FAOSTAT, 2012). Maize grows in most parts of the world over a wide range of environmental conditions, with altitudinal ranges of 0 to 3000 meters above sea level (masl) (Dowswell et al., 1996).

Global importance and production of maize

According to the United Nations Food and Agriculture Organization's (FAO) (FAOSTAT, 2012), the world area of maize production was 176 million ha while that of wheat was 216 million ha and rice at 184 million ha during 2012. However, maize surpasses both wheat and rice in terms of productivity. For instance, the world maize production for 2012 was 875 million metric ton, while that of wheat was 606 million ton and rice 635 million ton. About 70% of the world maize production area is found in developing countries. However, these countries contribute to only 49% of the world's maize production (FAOSTAT, 2012). The share of Africa's maize production for 2012 was 69 million metric ton or about 8% of world production. In the developed world, maize is mostly used for animal feed (70%) and only a small percentage (5%) is consumed by humans. The developing countries consume about 62% of maize as food and 34% is used as feed. The remaining proportion is used for varied industrial uses and as seed. With a 43 kg per capita per year human consumption, maize contributes to 34% of the protein and 35% of the calories in Africa. In eastern and southern Africa alone, maize accounts for over 25% and 31% of the total calories consumed by humans with per capita annual consumption of 58 and 84 kg, respectively (FAOSTAT, 2012).

In sub-Saharan Africa maize is predominantly produced by small- to medium-scale farmers cultivating 10 ha or less (DeVries and Toenniessen, 2001). Small–scale farmers are resource limited and grow maize with diverse cropping system, where the crop faces many biotic and abiotic stresses (Hassan et al., 2001; Bänziger and Diallo, 2004).

In the region the use of agricultural input is extremely low resulting in poor maize yields of 1.3 t/ha (Bänziger and Diallo, 2004). Sub-Saharan African countries import approximately three million tons of maize annually to meet local demands (Pingali and Pandey, 2001; FAOSTAT, 2012). Consequently, maize productivity should be enhanced through the use of improved varieties along with their production technologies for food security and import substitution (Heisey and Edmeades, 1999; Reeves et al., 1999; Pingali and Pandey, 2001). Despite poor productivity, maize production area is fast increasing in sub-Saharan Africa including marginal areas (FAOSTAT, 2012).

In Ethiopia, maize is one of the most important cereal crops grown. The total annual production and productivity exceeds all other cereal crops except tef [*Eragrostis tef* (Zucc.) Trotter] in area coverage (Mosisa et al., 2012). Considering its importance, wide adaptation, total production and productivity, maize is regarded as one of the high priority food security crops in Ethiopia, the second-most populous country in sub-Saharan Africa after Nigeria (CSA, 2011).

In Ethiopia, maize production has increased during the years 1990 to 2010 (Table 1). In the 1980s, the total annual production remained below 2 million tons and production area was about 1 million ha (Worku et al., 2002). During the early 1990s the total area and production remained at 1.30 million ha and 2.34 million tons, respectively. During 1995 to 2000, yield ha^{-1}, total area and production of maize increased by 3.1, 7.1 and 11.3%, respectively. The Central Statistical Agency of Ethiopia (CSA, 2008) reported maize production at 1.69 million ha during the 2006/07 main cropping season. During this season the total maize production and average yield were at 3.77 million tons and 2.22 t ha^{-1}, respectively. During 2010 total area, production and yield of maize were at 1 963 000 ha, 4 986 000 tons and 2.5 t ha^{-1}, respectively (Table 1).

Table 1. Estimated total area, production and yield of maize in Ethiopia during 1990 – 2010 (Mosisa et al., 2012).

Year	Area ('000 ha)	Production ('000 tons)	Yield (t ha^{-1})
1990	1277	2056	1.61
1991	908	1159	1.28
1992	751	1234	1.64
1993	808	1391	1.72
1994	902	1113	1.23
1995	1104	1673	1.51
1996	1851	3105	1.68
1997	1688	2928	1.73
1998	1448	2344	1.62
1999	1308	2417	1.85
2000	1407	2525	1.80
2001	1323	2800	2.1
2002	1702	3086	2.2
2003	1336	2543	1.9
2004	1399	2407	1.7
2005	1526	3337	2.2
2006	1793	4030	2.2
2007	1767	3750	2.1
2008	1768	3933	2.2
2009	1772	3897	2.2
2010	1963	4986	2.5

Increased total area and production are attributed to the wide adaptation of maize to the varied agro-ecologies in the country. However, maize yields have remained low due to several biotic, abiotic and socio-economic constraints.

There are four major maize producing agro-ecologies in Ethiopia, of which the mid-altitude and lowland areas of the country are the major ones (Table 2). The predominant maize producing areas of the country are found mainly in the western, north western and southern parts of the country.

Table 2. The major maize producing agro-ecologies in Ethiopia (Worku et al., 2002).

Agro-ecologies	Altitude (m)	Rainfall (mm)
Mid-altitude sub humid	1000 – 1800	1000-1250
Moisture stress	500-1800	< 800
High altitude sub humid	1800-2400	1200-2000
Low altitude sub humid	< 1000	1200-1500

Production constraints of maize in Ethiopia

Table 3 summarizes the predominant biotic (diseases, weeds, insect and arthropod pests), abiotic (drought and nutrient deficiencies) and socio-economic (market price fluctuation, and unavailability of inputs) constraints that limit maize productivity in Ethiopia (Tesfa et al., 2004). Among the abiotic constraints, drought is the major problem, particularly in moisture stressed agro-ecologies. The second important abiotic stress is soil nutrient deficiency, which is prevalent including in the potential maize producing areas such as the mid-altitude sub-humid agro-ecology of the country (Worku et al., 2002). Nutrient deficiencies are associated with the low use of agricultural inputs due to unaffordability, poor cultural practice including limited crop rotation and fallowing (Tesfa et al., 2012).

Table 3. Major maize production constraints in Ethiopia

Agro-ecologies	Production constraints
Mid-altitude sub-humid	Diseases (blight, rust, gray leaf spot, ear rot), insects (stalk borer, storage pests), weeds (*Striga*), low soil fertility, lack of improved variety
Moisture stress	Drought, leaf diseases (rust, blight), insects (stalk borer, termites, storage insects), lack of improved variety, low soil fertility
High altitude-sub humid	Diseases (leaf blight, rust, grey leaf spot, ear rot), insects (stalk borer, storage insects), lodging due to poor stalk quality, low soil fertility, lack of improved variety
Low altitude-sub humid	Diseases (maize streak virus, grey leaf spot, rust), insects (storage pests, stalk borer), lodging, weeds

Among the biotic factors, diseases are the principal threats limiting maize production and productivity. Leaf diseases including *Turcicum* leaf blight caused by *Exserohilum turcicum* Pass Leonard & Suggs, grey leaf spot (*Cercospora zeae-maydis* Tehon & Daniels) and common leaf rust (*Puccinia sorghi* Schr.) (Tewabech et al., 2012) are the most important infectious diseases of maize in the country. Compared to other leaf diseases, *Turcicum* leaf blight also referred to as northern corn leaf blight (NCLB) is the most widely distributed and has high economic importance. The disease incidence ranges from 95 to 100% in areas with constant moisture and high humidity and the yield loss can reach up to 70%. *Turcicum* leaf blight is reported to cause devastating damage on most commercial varieties of maize released in the country (Tewabech et al., 2012).

Various options are available to control maize leaf blight such as the use of host plant resistance, cultural practices, and fungicides. Host plant resistance is the cheapest and most effective way to control leaf blight disease because chemical treatments are expensive, often ineffective, and sanitation practices are difficult to apply. The use of resistant varieties possessing qualitative and quantitative genes in combination or separately is cheapest and environmentally most friendly method. Development of resistant varieties against *Turcicum* leaf blight and other leaf diseases will boost maize productivity.

Among crops pests, arthropod pests are among the key constraints to maize production in Ethiopia (Girma et al., 2012). Further, the parasitic weed, *Striga*, and other annual and perennial weeds contribute to low yields (Temesgen et al., 2012). Three species of *Striga* have been reported in Ethiopia of which *Striga hermonthica* is the most prevalent across maize growing regions (Temesgen et al., 2012). The socio-economic constraints limiting maize production in Ethiopia include poor market access, lack of storage facilities, insufficient production and distribution of quality seeds, limited access to credit, limited production input supply, and limited research, development and extension capacity (Dawit et al., 2008).

Rationale of the research

The mid-altitude, sub-humid agro-ecology is considered to be the major maize growing zone in Ethiopia (Legesse et al., 2012). The region receives a fairly reliable average annual rainfall (1000–1500 mm year^{-1}) rendering it a region of high potential for maize production. However, maize production has remained low, with the estimated national average yield of 2.90 t ha^{-1} compared to the world average yield at 5 t ha^{-1} (FAOSTAT, 2012) due to several constraints, including foliar diseases. *Turcicum* leaf blight shows higher incidence of 95-100% and can result in a devastating yield loss in the country. Maize productivity could be enhanced through effective breeding using locally adapted germplasm as well as through a well-designed hybrid cultivar development program. Therefore, high yielding, *Turcicum* leaf blight resistant and farmer-preferred maize varieties and their production technologies should be developed and made available to growers to enhance maize production and to ensure food security in the country.

Research objectives

The specific objectives of the study were:
- To assess the present importance, production and productivity constraints, input availability and local market of maize in the mid-altitude, sub-humid agro-ecology of western Ethiopia.
- To determine the genetic variability among elite maize inbred lines and select promising parents for resistance to *NCLB*.
- To determine the level of genetic diversity among medium to late maturing maize inbred lines using genetic distances as measured by single sequence repeat (SSR) DNA markers.
- To determine combining ability and heterosis among 18 elite maize inbred lines and their hybrids in diverse tropical mid-altitude environments.
- To investigate genotype x environment interaction and yield stability of maize developed for the mid-altitude sub-humid agro-ecology.

Research hypotheses

The current study was therefore formulated based on the following hypotheses:

1. Smallholder maize farmers in mid-altitude sub-humid areas of Ethiopia are aware of disease problem and other complexes that hinder production and prefer varieties that combine tolerance to these constraints.

2. Considerable genetic variation for grain yield and NCLB tolerance exists.

3. Considerable genetic diversity for grain yield and resistance to NCLB are available among the elite and adapted maize genotypes in the mid altitude areas of Ethiopia.

4. The selected elite maize inbred lines adapted to mid-altitude sub-humid agro-ecology have good combining ability for grain yield and resistance to *Turcicum* leaf blight.

5. Grain yield in maize is affected by the change in environment.

Outline of this thesis

This thesis consists of six distinct chapters in accordance with a number of activities related to the above-mentioned objectives. Chapters 2-6 are written as discrete research papers intended for publication containing all the necessary information, some of which might have been presented in other chapters. Some overlap and unavoidable repetition may exist between the chapters and references.

Chapter	Title
-	Introduction to thesis
1	A review of the literature
2	Preferences and constraints of maize farmers in the development and adoption of improved varieties in the mid-altitude, sub-humid agro-ecology of western Ethiopia
3	Genetic variability of elite maize inbred lines for resistance to *Exserohilum turcicum* in the mid-altitude sub-humid agro-ecology of Ethiopia
4	Genetic interrelationships among medium to late maturing tropical maize inbred lines using selected SSR markers
5	Heterosis and combining ability of grain yield and Northern Corn Leaf Blight resistance among maize genotypes adapted to the mid-altitude sub-humid agro-ecologies
6	Genotype by environment interaction and yield stability of maize hybrids developed to the mid-altitude sub-humid agro-ecology of Ethiopia
7	An overview of the research findings

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Chapter 1

A Review of the Literature

1.1 Introduction

This chapter presents literature review in three different sections. The first section highlights maize (*Zea mays* L.) as a crop including its role among other cereals, origin and history, taxonomy and production trends worldwide, in Africa, and Ethiopia, and production constraints. The second section focuses on one of the most important production constraints that limits maize production in Ethiopia – northern corn leaf blight (NCLB) or *Turcicum* leaf blight disease. This is done by examining the importance, symptoms, distribution and control methods of the disease. This follows an in-depth description of the causative agent of maize leaf blight, *Exerohilium turcicum* (Pass.) Leonard and Suggs and its control methods. The third section covers breeding for NCLB resistance, the genetics of resistance to NCLB, maize breeding methods, and applications of molecular techniques in maize breeding.

1.2 Origin and history of maize

Maize (*Zea mays* L., 2n=2x=20), a member of the grass family *Gramineae (Poaceae)*, is one of the oldest cultivated crops. It was the principal food crop of the American Indians when Columbus arrived, and still remains the most important cereal food crop in Mexico, Central America, and many countries in South America and sub-Saharan Africa. Two locations have been suggested as possible centre of origin for maize, namely, the highlands of Peru, Ecuador, and Bolivia, and the region of southern Mexico and Central America. Many types of maize have been found in both areas. Several theories have been formulated to account for the origin of maize, but the exact relationship between Teosinte*, Tripsacum*, and early pod maize found in archaeological ruins has not yet been fully resolved (Poehlman, 1987).

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Today, maize is widely grown in most parts of the world, over a wide range of environmental conditions, between latitudes of 50^0 North and South of the equator. It grows from sea level to over 3000 m above sea-level (Dowswel et al., 1996). It is believed that maize was introduced to West Africa in the early 1500s by Portuguese traders and reached Ethiopia in the 1860s (Dowswell et al., 1996).

1.3 The biology of maize

Maize is a tall, monoecious, annual grass with overlapping sheaths and broad conspicuously distichous blades. The plants have staminate spikelets in long, spike-like racemes that form large, spreading, terminal panicles (tassels) and pistillate inflorescences in the leaf axils; spikelets occur in the axils in 8 to 16 rows, approximately 30 axils long, on a thickened, almost woody axis (cob) (Hitchcook and Chase, 1971).

Pollen is produced in the staminate inflorescence and eggs in the pistillate inflorescence. Maize is predominately cross pollinated by wind, but both self and cross pollination are possible. Pollen grain has a relatively thin outer membrane that gives little environmental protection; consequently, viability may be lost in a few minutes because of desiccation. Shed pollen usually remains viable for 10 to 30 minutes, but can be preserved under favorable conditions (Simmond and Smartt, 1999). The silk usually emerges at the top ear node 1 to 3 days after anthesis. Tassel development seems to control development of the ear shoot, and this dominance is greatest for genotypes that produce only one ear per plant in any environment. Prolific genotypes may have no dominance for the tassel, and their silks frequently emerge before the tassel begins to shed pollen (Hitchcook and Chase, 1971).

1.4 Importance of maize and production trends

Maize is the major food crop grown all over the world (FAO, 2011). It is utilized directly as a source of food and animal feed. In the developed countries maize is mainly used as animal feed; whereas, in sub-Saharan Africa and other developing countries it is the major staple food crop on which millions of people rely for their food and income generation.

Compared to all other cereals maize has the highest average yield per unit area. Globally maize stands third after wheat and rice in area and total production. The estimated area under maize production in the world is 144 376 477 ha, with an average yield of 4815.39 kg ha^{-1} (FAO, 2011).

Table 1.1 Maize area, production and productivity in different regions of the world in 2011.

Country/region	Area ('000 ha)	Total production ('000 t)	Yield (t ha^{-1})
China	29 883	166 035	5.6
Ethiopia	1 767	3 776	2.1
South Africa	2 799	11 597	4.1
USA	31 826	307 384	9.7
World	161 017	822 713	5.1
Africa	29 152	53 201	1.8
Eastern Africa	13 551	17 624	1.3
Central Africa	3 476	3 037	0.9
Northern Africa	1 072	6 731	6.3
Southern Africa	3 080	11 780	3.8
Western Africa	7 973	14 029	1.8

Source: FAO, 2011

The United States produces nearly one half of the total world production (Table 1.1). The next largest maize producing countries are China and Brazil.

Maize is grown on the global scale on 144 million ha, and has an annual production of about 700 million tons (FAO, 2011). In sub-Saharan Africa maize is produced in an estimated area of 26 117 628 ha with an average yield of 1771.22 kg ha^{-1}. In east Africa, maize occupies 11 734 616 ha production area with an average yield of 1478.09 kg ha^{-1}. The larger proportion of maize in Africa is produced by resource-limited, small-scale farmers. Yields in this farming system are very low because farmers are constrained by cash shortage to utilize the necessary inputs for maize production (Dawit et al., 2008). Due to increased demand maize production is spreading into marginal areas and this will likely engender risk to biotic and abiotic threats leading to minimal productivity. To achieve the growing need for maize in Africa it is necessary to boost productivity through reducing yield losses incurred by various stress factors including diseases and pests (Dagne et al., 2004).

The primary diseases of maize include seed and ear rots, seedling blights, root and stalk rots and foliar diseases (Pandurange et al., 1994). Simmonds and Smartt (1999) suggested that diseases caused by airborne fungi probably account for the greatest crop losses in maize. Foliar diseases are highly important constraints in tropical maize production (Tewabech et al., 2012).

De Vries and Toenniessen (2001) reported the serious impact of foliar diseases on the production and productivity of maize in east Africa. The authors summarized various reports estimating the severity and distribution of disease constraints in Africa and showed the extreme impact of maize streak virus (MSV) in lowland tropical agro-ecologies and, NCLB in highland agro-ecologies. Research conducted in the Republic of South Africa (RSA) has demonstrated yield reductions of 30 to 60% attributable to gray leaf spot (GLS), depending on cultivar and environmental conditions (Ward et al., 1997). Okporie (2008) and De Vries and Toenniessen (2001) documented the wide

distribution and persistence of foliar diseases such as GLS, MSV, and NCLB in both the mid-altitude and highland regions of eastern Africa.

In Ethiopia, the total annual production and productivity of maize exceeds all other cereal crops, though it is surpassed by tef [*Eragrostis tef* (Zucc.) Trotter], in area coverage (CSA, 2011). Due to its wide adaptation, total production, and productivity, maize is one of the high priority crops of the country to ensure food security. In the country, maize grows across various agro-ecologies due to its wide environmental adaptation (Legesse et al., 2012). Because of this wide adaptability, maize is cultivated on about 1.2 million hectare, accounting for 19.3% of the total area allocated to cereal crops production. Maize stands first in total national crop production and productivity in Ethiopia (CSA, 2011).

In Ethiopia, maize production has increased over the years (Figure 1.1). In the 1980s, the total area of production remained below 2 million tons. Maize production area slightly exceeded 1 million hectare only in 1987, 1988, and 1989 (Kebede et al., 1993; CSA, 2011). In the 1990s, maize production in Ethiopia increased with the total area exceeding 1.3 million hectare and production reached 2.34 million tons during 1996 to 2000. During the 1995 to 2000, annual increase on yield per hectare (Figure 1.1), total area, and production of maize were estimated at 3.1, 7.1and 11.3%, respectively. During 2006, the total maize production and average national yield of maize were at 3.77 million tons and 2.22 t ha^{-1}, respectively (CSA, 2007). Presently the national total maize production and average yield stand at 6.07 million tons and 2.95 t ha^{-1}, respectively (CSA, 2011).

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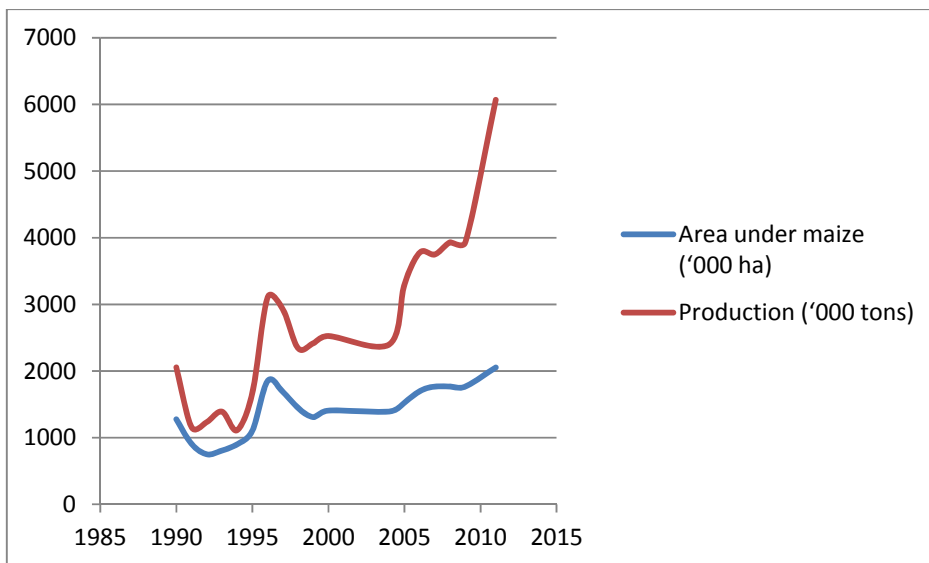
Figure 1.1 Trends of area, and production of maize in Ethiopia, 1990-2010 (CSA, 2011)
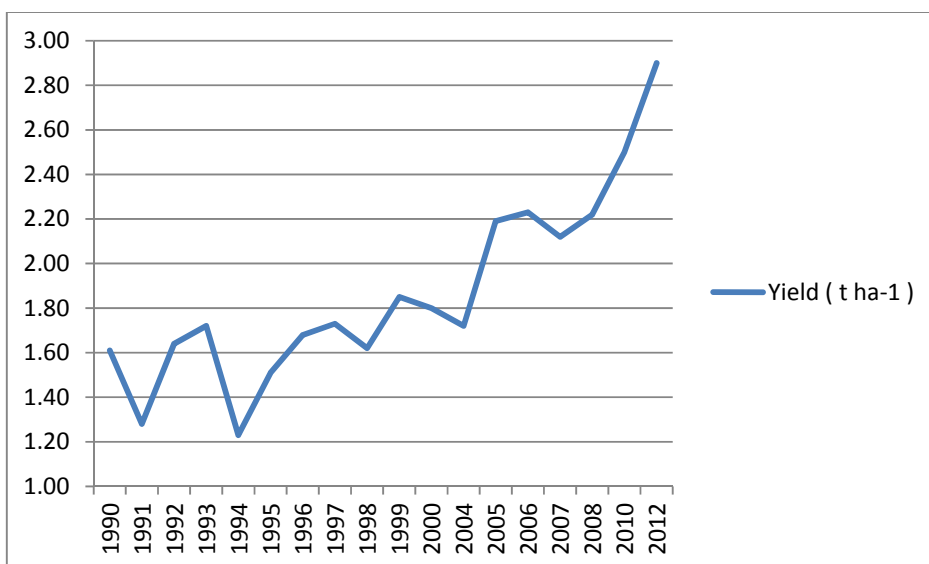
Figure 1.2 Trends of maize yields in Ethiopia, 1990-2010 (CSA, 2011).

1.5 Maize production constraints

Maize production is constrained by a number of abiotic and biotic stress factors. The major abiotic factors include nitrogen and drought-stress, both aggravated by land degradation. The biotic constraints include pests (stem borers, weevils, and termites)

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and diseases (northern leaf blight, gray leaf spot, maize streak virus, rust, and downy mildew) (Tefferi et al., 1996; Dagne et al., 2004).

1.5.1 Abiotic factors

Drought is the major crop production constraint in moisture stressed agro-ecolgies of Ethiopia. In the country 40% of the maize growing agro-ecology is challenged by recurrent drought. Consequently, this area contributes to less than 20% of the national maize production. In drought affected areas of the country maize yield is not exceeding 1.3 t/ha (Gezahegn et al., 2012). Drought stress occurs roughly once every ten years while this frequency is three out of five years in the drought stressed areas.

Soil nutrient deficiency is the major problem including in the potential maize producing areas such as the mid-altitude and sub-humid agro-ecologies of the country (Mosisa et al., 2002). Farmers in these agro-ecologies rarely apply chemical fertilizers or crop rotation or fallows to enrich soil fertility (Ransom et al., 1993). Nitrogen is the most important nutrient limiting crop production in the tropics (Mosisa et al., 2002). In sub-Saharan Africa, use of nitrogenous fertilizers by smallholder farmers to increase crop production has been limited. An increase in fertilizer prices due to the removal of government subsidies has further decreased fertilizer use. Also population increases and continuous mono-cropping in many areas have decreased or eliminated the use of traditional fallow systems to restore soil fertility (Wende et al., 2013).

1.5.2 Biotic factors

The most important biotic constraints to maize productivity include: insect pests, various weeds, and diseases.

1.5.2.1 Insect pests and weeds

Mammals such as monkeys, apes and wild pigs are among the key and serious pest inflicting substantial losses of maize production (Girma et al., 2012). There are about 100 weed species belonging to 24 families and 66 genera known to infest maize fields in the country (Temesgen et al., 2012). Maize is highly vulnerable to damage caused by the parasitic weed *Striga* (*Striga hermonthica*). The weed has been established for many decades in Ethiopia and regarded as the most yield limiting pest in most maize and sorghum growing regions (Temesgen et al., 2012).

1.5.2.2 Diseases

The major diseases of maize include NCLB caused by *Exserohilum turcicum* Pass Leonard & Suggs., GLS (*Cercospora zeae-maydis* Tehon & Daniels) and MSV (Meseret and Temam, 2008). Among the maize diseases, *Turcicum* leaf blight also known as NCLB is a wide spread disease causing a yield loss of up to 70% (Simmonds and Smartt, 1999; Tewabech et al., 2012).

The NCLB disease is caused by the ascomycete *Setospheria turcica* (Luttrell) Leonard and Suggs. (anamorph *Exserohilum turcicum* [Pass.] Leonard and Suggs.). *E. turcicum* was first reported on maize in Passerini in Italy in 1876. The disease was reported in New Jersey in the USA in 1878 and a serious outbreak occurred in Connecticut in 1889 (Ward et al., 1997). The northern corn leaf blight is favoured by mild temperatures and high humidity (Ullustrup, 1970). Heavy dews, cool temperature, and frequent rains are the environmental conditions conducive to disease development (Jordan et al., 1983). Levy (1991) reported that the disease is more aggressive in young susceptible plants with an optimum temperature for infection and lesion number at 20°C. Lesion length and inoculum concentration increase with extended dew period. Levy (1991) also reported that pathogenic fitness and environmental conditions are important factors in determining disease development and that epidemic depend on the ability of *E. turcicum* to infect, grow, and sporulate on maize plants.

Compared to other leaf diseases, Turcicum leaf blight (NCLB) has wide distribution and high economic importance in Ethiopia. Infection due to NCLB appears during the main and off seasons, but it is more serious during the main season particularly in wet and humid areas. Reportedly, NCLB is severe on most commercial varieties of maize so far released in the country (Tewabech et al., 2002). This necessitates the need for resistance breeding of maize to minimize damage caused by *Turcicum* leaf blight in particular and other foliar diseases in general.

Most causal agents of foliar diseases are widespread, but some tend to be more or less prevalent in particular regions, during certain seasons, due to environmental factors (Smith, 1999). In addition to periodic changes in the weather, changes in host-pathogen genetic inter-relationship may also be induced by the introduction of new cultivars or the occurrence of mutations and recombination in the pathogen (Ogliari et al., 2005). Emergence of new races of pathogen could attack both susceptible and previously resistant cultivars. Breeding for host plant resistance is cost effective, and environmentally friendly (Pratt et al., 2003) and should remain an integral part or the core component of future disease management strategies in maize. The strategy is crucial to meet the increasing demand of maize from time to time, and also to minimize disease epidemics that may arise with intensive production of maize in the future.

To meet the rising demand for maize there is a need to shift from a small scale, complex cropping system to a larger and more intensive one in diverse regions of the world (Dowswell et al., 1996). Expanded cultivation into less ideal environments, and change in management practices such as increased plant densities, double cropping, alteration in planting dates and fertility regimes, and the amount of crop residue left in the field, can influence disease incidence and severity. Increased production of genetically uniform cultivars will also create more favorable host-pathogen population dynamics (Simmonds and Smartt, 1999). These are compelling reasons for continued research and development of resistant maize varieties against foliar diseases including NCLB.

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1.6 General distribution of NCLB

Northern Corn Leaf Blight occurs throughout maize-producing regions of the world showing moderate temperatures and humidity (Smith, 1999). It is a potentially devastating disease that routinely limits maize productivity in sub-Saharan Africa, especially in the humid mid-altitude and highland regions (De Vries and Toenniessen, 2001).

1.7 Symptoms of NCLB

Early symptoms of NCLB are oval, water soaked spots on leaves with mature symptoms showing characteristic cigar shaped lesions that are 3 to 15 cm long. The lesions are elliptical and tan in color, developing distinct dark areas that are associated with fungal sporulation as they mature. Typically lesions first appear on lower leaves, spreading to upper ones and the ear sheaths as the crop matures. Under severe infection, lesions may coalesce, blighting the entire leaf. Lesion may vary slightly depending on the resistance status of the crop. For instance, lesion development on some hybrids with resistance genes may include long, chlorotic streaks that can be confused with other diseases such as Stewart's wilt (Levy, 1991; Muiru et al., 2007).

1.8 Physiologic races and growth cycles of NCLB

Two races of *E. turcicum* are commonly reported (Pandurange et al., 1994). Race 1 is avirulent to lines of maize carrying genes conferring resistance and denoted as *Ht1, Ht2, Ht3* and *HtN*. The second race is avirulent to lines with genes *Ht2, Ht3*, and *HtN* but virulent to lines carrying genes *Ht1A* or B (Pandurange et al., 1994). The *Ht1, Ht2,* and *Ht3* resistant genes occur as chlorotic lesions with minimum sporulation, while the *HtN* induced resistance is expressed as a delay in disease development after pollination (Leonard et al., 1989).

Turcicum leaf blight overwinters as mycelium and chlamydospore in infected crop debris. At the onset of the subsequent season, fungi in crop debris begin to sporulate in response to higher temperatures and humidity. Spores (conidia) are then disseminated by wind and rain splash to freshly planted maize. Conidia can be carried over vast distances by wind and germinate in temperature ranging from 17 to 27^{0}C and during periods of extended leaf wetness (6 to 18 hours) leading infection to maize tissue. Secondary cycles of disease occur where conidia produced in disease lesions are disseminated within the crop and to other fields by rain splash and wind (Madden et al., 2007).

1.9 Importance of NCLB in Ethiopia

According to Tewabech et al. (2012) the northern corn leaf blight has been reported to cause the highest grain yield loss of 50% and 1000 kernel weight loss of 16.4% on the susceptible cultivar OPV POOL 32C19 under artificial infestation in Ethiopia. In another experiment the same authors recorded grain yield losses of 34.08, 29.05 and 2.21% on varieties Abo-bako, Beletch, and BH660, respectively at Hawassa in Ethiopia.

1. 10 Resistance for foliar diseases

The defense mechanisms displayed by maize reflect the manner in which the host combats the different types of pathogens. Resistance to many generalist pathogens is usually of a quantitative nature. Quantitative resistance is also referred to as partial resistance or minor gene resistance. Resistance to pathogens that are specialists is usually of a more qualitative nature where "major" or "R" genes confer high levels of resistance. However this resistance may be more readily overcome following favorable mutation by the pathogen. Major gene resistance is ephemeral and typically fails within a period of time following deployment, although the longevity is difficult to predict (Leonard et al., 1989).

An example of the defeat of major-gene resistance in the U.S. maize breeding is the loss of effective control of northern corn leaf blight using *Ht* genes (Leonard et al., 1989). The *Ht* resistance was widely deployed, but virulent races of *E. turcicum* developed within a period of two decades (Smith, 1999). Awareness of the need to emphasize partial resistance (or quantitative resistance, as opposed to qualitative resistance) appears to be increasing. The redirection of breeding efforts towards increasing partial resistance is a positive development in long term disease management. Deployment of major-gene resistance is likely to continue, and new selection methods that enable the combination of major-genes with partial resistance are needed (Parlevliet, 1993).

Disease-resistance breeding, especially for partial resistance, is complicated by the variable nature inherent in both the incidence and severity of natural disease initiation and infection. The complexity arising from a host organism, a pathogenic microorganism, the interaction between them, and their interaction with the environment can make selection difficult (Parlevliet, 1993). Testing across locations or seasons and/or artificial inoculation systems is usually necessary to characterize accurately host resistance to foliar diseases in general and to NCLB in particular.

1.11 Types of resistances for NCLB

Resistance to NCLB is either quantitative or qualitative. Qualitative resistance is conferred by the genes *Ht1, Ht2, Htn1*, and *Htm* and is generally characterized by chlorotic lesions on leaves (Hooker, 1963; Hooker, 1981; Gevers, 1975; Robbins and Warren, 1993).

The *Ht* genes were widely used in U.S. maize research until the 1970s. After virulent races of NCLB matching *Ht* genes were encountered, the emphasis in U.S. maize breeding was switched to quantitative resistance, which has been durable for some time (Carson, 1995). Polygenic resistance has also been reported in Indian germplasm (Sharma and Payak, 1990). Earlier efforts were made to reflect additional sources of

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resistance in maize for *E. turcicum,* but breeders still lack information on the new sources of resistance, particularly in early maturity germplasm (Pandurange et al., 1994).

Pataky (1994) showed that high levels of partial resistance, with or without *Ht* genes, presented a spectacular approach in reducing damage from northern corn leaf blight on sweet corn, which also eliminates the severe yield-depressing chlorosis, associated with *Ht* gene resistance in very susceptible backgrounds. Studies by Carson (1995) indicated that the latent period is related to partial resistance, which suggested that selection for increased latent period length would be more beneficial than selecting for reduced disease severity. Selection for increased latent period length could be done in environments without severe disease epidemics, and also breeding material can be assessed at seedling stage for latent period length in the greenhouse during the off season.

Levy (1991) showed that isolates from different areas were different in parasitic fitness as indicated by infection efficiency, sporulation and lesion size. While isolates from the same location showed less variation. Inocula in previous crops have been found to be critical in epidemic build up for subsequent cropping, especially in non-tillage systems; in order to overcome the problem of the epidemic occurrence of the disease, different control methods are proposed and utilized.

1.12 Strategies to control NCLB

Numerous options have been recommended for the control and management of maize diseases. The availability, feasibility, and cost-effectiveness of each method will differ among production regions and settings, i.e., commercial or subsistence farming systems. Planting of resistant cultivars can effectively reduce the rate of disease development, and that practice is now widely recommended (Ward et al., 1997). Integrated Pest Management (IPM) strategies including host resistance, fungicides and

cultural practices, can serve a vital role in the control and management of NCLB (Pratt et al., 2003).

1.12.1 Cultural practices

Rotating maize with non-host crops can reduce disease pressure. Also timely removal of over wintering infected crop residue will reduce the amount of available inocula at the onset of the subsequent growing season. Recommended practices for the control of fungal diseases include conventional tillage that buries crop residues, crop rotation, and fungicide application (Pratt et al., 2003).

Effective disease management such as NCLB requires efficient tactics that focus on protection by limiting sources of primary inocula through crop rotation and residue management, and by reducing the rate of disease development. With limited options for crop protection in many sub-Saharan African farming systems, the challenge will be to increase the productivity and sustainability of maize and maize farming systems while at the same time protecting the natural resources on which future productivity depends (Rosegrant, 2001).

1.12.2 Use of fungicides

Fungicide application can effectively control NCLB when applied at the right time. Fungicides such as combinations of mancozeb and propoconazole should be applied when lesions first become visible on the lower leaves (Girma et al., 2008). In a cool and dry season, which is not favorable to *Turcicum* leaf blight, fungicide application may not be cost effective, particularly for grain production (Parlevliet, 1993). Therefore the uses of resistant cultivars supplemented with viable cultural practices are the most important and economical ways of disease control.

1.12.3 Use of resistant cultivars

Disease resistance to plant pathogens is evidenced by one of two major kinds of host responses. The host either resists the establishment of a successful parasitic relationship by restricting the infection site and the infection process, or it resists the colonization and growth of the parasite subsequent to a successful infection, even though the infection process, culminated by reproduction of the parasite, is completed. Resistance is considered in this context as an active, dynamic response of the host to a parasite and it excludes such passive phenomena as immunity or disease escape. Resistance to colonization and growth subsequent to infection is a host response characterized by the term tolerance and variously by the term field resistance, generalized resistance, nonspecific resistance, partial resistance, uniform resistance, horizontal resistance, multigenic or polygenic resistance, and minor gene resistance (Parlevleit, 1993).

Host resistance is the most efficient and cost effective means of disease resistance. Four genes offering major resistance gene to *Turcicum* leaf blight have been identified and are incorporated in many commercial hybrids (Carson, 1995). However, success of disease management using qualitative resistance will depend on the race of the pathogen present. Quantitative levels of host resistance are also available that restrict lesion development and sporulation. In order to achieve the optimum levels of host plant

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resistance to a pathogen, maize breeders have to follow viable breeding and selection methodologies which can efficiently accumulate the required resistant gene in the host (Pratt et al., 2003).

1.13 Farmer preferences in maize breeding

In developing new cultivars and extending them to farmers, the formal breeding sector has often encountered two setbacks (De Groote et al., 2002). First, most new cultivars have been unacceptable to farmers (Witcombe et al., 2003). Secondly, breeders have necessarily discarded many crosses because of traits considered undesirable yet these may be of interest to farmers (De Groote et al., 2002). This is because the breeders are not often well informed of the needs and preferences of farmers (De Groote et al., 2002).

Farmer preferences have emerged as a major factor in the improvement of maize for smallholder farming systems. As reported by Banziger and Cooper (2004), superior cultivars have not always been adopted, even where available, because they do not meet farmers' preferences. Apart from high yield and disease resistance, breeders may not know farmers' complex requirements. Small-scale farmers have some specially preferred traits, which may not be considered by breeding institutions.

According to Banziger and cooper (2004), regional programmes have a tendency to focus their breeding goals on the requirements of the commercial farming sector. Perhaps, the small-scale farmers might not have interest in growing some hybrids that have not been developed to meet their specific requirements (Kamara et al., 1996). Effective cultivar breeding for deployment in marginal areas should be based on the identified constraints and specific preferences for small-scale farmers.

1.14 Common breeding methods in maize

Maize improvement can be achieved by various breeding methods, depending on the end product, initial type and amount of materials available, in addition to the heritability of the required trait (Bänziger et al., 2006). Two major breeding strategies are normally used for maize improvement. Firstly, selection breeding, which is used to generate open pollinated varieties and mainly applies to traits with high heritability. This method also includes recurrent selection as one of the breeding procedures. The second breeding strategy is inbreeding and subsequent hybrid development.

1.14.1 Recurrent selection

Recurrent selection is a cyclic breeding procedure designed to improve mean performance of populations under selection. This can be accomplished by a gradual increase in the frequency of favorable alleles with the simultaneous maintenance of genetic variability (Bänziger et al., 2006).

Recurrent selection has been effective in gradually improving population performance as well as maintaining the performance of varieties (Tollenaar and Lee, 2002). Like any other method, recurrent selection requires critical selection of appropriate germplasm with accurate recording of pedigree information (Tollenaar and Lee, 2002). Under recurrent selection, populations are improved for specific quantitative traits before they can become popular sources of inbred lines (Sleper and Poehlman, 2006). In addition to being highly effective in improving traits with high heritability such as ear height, lodging resistance, resistance to diseases and days to maturity, recurrent selection is a simpler method (Troyer and Brown, 1972). Recurrent selection methods may be on an individual plant, family or progeny basis (Hallauer, 1992). The original population for improvement can be a landrace or one that is constructed by inter-mating inbred lines superior for the quantitative character that is to be improved. Recurrent selection operates by increasing the frequency of favorable alleles within a population (Hallauer and Miranda, 1988).

Different recurrent selection procedures have been developed for maize; however, their effectiveness depends on the stage of the breeding program, the population being improved, the breeding objective, and the trait of interest (Pratt et al., 2003). Pandey and Gardner (1992) reported that intra-population improvement methods have proven to be more effective than inter-population methods for improving population means *per se* for all traits. In related studies, Duvick and Cassman (1999) also reported that intra-population selection methods have been effective in improving drought tolerance in source populations. Furthermore, Tollenaar and Lee (2002) noted an increased probability of developing superior drought tolerant inbred lines from such populations. Similarly, Hallauer and Miranda (1988) noted that family based recurrent selection methods result in greater gains when the traits under selection are complex and of low heritability.

1.14.2 Mass selection

Mass selection is the oldest and simplest form of recurrent selection. Its simplicity and the completion of a cycle in the course of one year are its greatest advantages over other methods. Moreover, mass selection is most efficient for traits with high heritability (Hallauer and Miranda, 1988).

Mass selection has been shown to be highly effective in modifying highly heritable traits in maize (Smith, 1999). Selection effectiveness for yield improvement in a maize population is dependent upon the presence of additive genetic variation for yield (Levy, 1991). Mass selection has gained even greater importance, due to the introduction of the top cross system. The top cross system may minimize the yield disadvantage associated with conventional high oil corn hybrids. The top cross system uses the sterile version of a hybrid (90-95%) as a means to obtain a high yield, and high-oil population (5-10%) as a pollinator. Due to the effect of xenia, half of the oil content of the oil population is transferred to the sterile (female) version of the hybrid. In this way, it is possible to gain both high yield and high oil content (Smith, 1999).

1.14.3 Inbred line development

Inbred lines are pure lines developed through a series of selfing of selected heterozygous plants until homozygosity is reached. They ought to have desired traits and well defined heterotic groups. The first step in inbred line development is the selection of germplasm with the desired traits. According to Sleper and Poehlman (2006), superior lines for a particular quantitative trait can be extracted from recurrent selection populations designed to increase gene frequency of that trait, by repeated cycles of selection and inter-mating. Use of materials in which an increase in gene frequency for the character to be improved has been demonstrated in enhancing the development of superior inbred lines (Duvick and Cassman, 1999). Therefore, the success of inbred development is embedded in the characteristics and manipulation of the original germplasm selected for this purpose (Sleper and Poehlman, 2006).

1.14.4 Inbreeding and hybrid development

Inbreeding and hybrid development is another procedure through which the genetic constitution of plants can be manuiplated for the purpose of improving a particular population. The main purpose of inbreeding is the production of homozygous lines for subsequent crosses to develop hybrids to exploit hybrid vigor (heterosis). Falconer and Mackay (1996) noted that a crossing of inbred lines to produce hybrids plays a major role in crop improvement, most notably maize. Furthermore, they indicated that, in order to attain heterosis, the candidate lines for crosses need to be derived from different base populations; a cross between two unrelated base populations provides heterosis.

The degree of heterosis depends on the relative performance of the inbred lines and their crosses, as well as on the differential effect of the environment (Pandey and Gardener, 1992). Studies have shown that heterosis is greater in stress environments than under favorable conditions, due to the higher sensitivity of inbreds to stress than their hybrids (Ullustrup, 1970). This implies that it is more meaningful to characterize a

particular combination as showing heterosis for yield at a specific locality or under certain environmental conditions (Poehlman, 1987). More benefits other than hybrid production are achieved through inbreeding. For example, Wright (1980) and Falconer and Mackay (1996), reported the elimination of lethal and severely deleterious genes during inbreeding as a result additional improvements were registered in crossbreds over the base population.

1.15 Heterosis and heterotic groups in maize

Inbreeding and cross breeding constitute the major breeding strategies appropriate for improving populations with traits showing low heritability. These strategies exploit the phenomenon of hybrid vigour (heterosis). Heterosis, or hybrid vigour, is the measure of superiority of the F_1 compared to the average value of its parents. Heterosis exploits the high dominance present in the hybrids of characters with low heritability (Falconer and Macay, 1996). Maximum heterosis can only be attained when the genetic distance between the inbred materials used in the crosses is high. Falconer and Mackay (1996) further indicated that when inbred lines are crossed, the progeny show an increase in value for those characters that previously suffered a reduction from inbreeding; the fitness lost on inbreeding (inbreeding depression) tends to be restored on crossing. The relative importance of the types of genetic effects expressed in heterosis has led to the development of several methods of selection (Hallauer, 1992).

The classification of maize into heterotic groups guides breeders in determining the genetic distances between inbred lines and ultimately their potential vigour on crossing. Pratt et al. (2003) indicated that hybrid oriented breeding programs use different heterotic groups for specific regions. Widely adapted heterotic groups can, however, be used across regions. At least nine main heterotic groups of elite inbred lines are classified for the maize breeding programs in eastern and southern Africa (Halleur, 1992). However, the establishment of heterotic patterns is not fully achieved and hence knowledge on heterotic groups is yet to be utilized systematically by maize breeders in the tropics (Pratt et al., 2003). These authors emphasized the need to exploit the

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diverse maize germplasm in the tropics to enhance heterotic grouping patterns for maize hybrids and the development of synthetics that are resistant to biotic and abiotic constraints.

1.16 Combining ability studies

Combining ability is categorized into GCA, that measures the average performance of a line in all its crosses, expressed as a deviation from the overall mean of all the crosses, and SCA, the deviation of the line's performance from the expected value (Christie and Shattuck, 1992). SCA shows those situations in which the performance of a hybrid is relatively better or worse than would be expected on the average performance of the parents involved (Dowswell, 1996). Combining ability enhances crossbreeding by enabling the preliminary selection of inbred lines that perform well in crosses; ultimately an entire population of high performing hybrids can be generated (Falconer and Mackay, 1996). Generally, the GCA and SCA of inbred lines is an important aspect to consider when selecting materials for hybrid development and population improvement in maize breeding. Therefore, combining ability is used extensively in the breeding of several economic crop species (Gevers, 1975).

1.17 Mating designs in maize breeding

Successful trait improvement in maize requires knowledge of the number of genes involved in trait expression and their respective gene action. This is normally achieved by using a specific mating or genetic design. Various mating designs have been reviewed and genetic variance estimated in maize populations (Halleur and Miranda, 1988). The major mating designs in maize breeding include diallel crosses, North Carolina (NC) deign I (NC I), NC II, and NC III. However, adoption of any mating design requires validating the assumptions of random mating, absence of maternal effects, lack of epistasis, diploidy chromosome behaviour, and linkage equilibrium of genes when interpreting results from genetic studies (Jensen, 1970).

A diallel mating design (in which each line is crossed with every other line) is a commonly used experimental design for crossing inbred lines (Jenson, 1970). It is the most extensively used design to understand the nature of gene action involved in the expression of quantitative traits (Singulas et al., 1988). It allows estimations of general and specific combining abilities (Singulas et al., 1988). A complete diallel evaluates the variances due to the crosses, parents, and reciprocal effects (Falconer and Makay, 1996). However, Halleur and Miranda (1988) indicated that for ease of management of the crosses, diallel mating design is practically more applicable with few parents.

On the other hand, the North Carolina design I (NC I) which was introduced by Halleur (1992) enables the breeder to test a large number of plants from a population, and is usually important when using an unequal number of parents as male and female. The NC I provides a simple means of estimating additive genetic variance (V_A) and dominance variance (V_D) by allowing the between families statistics to be subdivided. The uniqueness of this design is that factors are nested in one another instead of being crossed in a factorial design. Hallauer and Miranda (1988) indicated that NC I is more frequently used in maize breeding than any other mating design other than the diallel.

With the North Carolina design II (NC II) all progeny families obtained from crossing males to females are raised. The NC II design estimates variance components in addition to GCA and SCA. Its major advantage is handling a larger number of parents in each experiment (Singulas et al., 1988). The male and female mean squares are estimated from the GCA, while the interaction between males and females is equivalent to the SCA variance of the diallel analysis (Halleur and Miranda, 1988). Similarly, dominance variance is estimated directly from the mean squares (Falconer and Mackay, 1996).

1.18 Genotype X environment interaction

In addition to genotype and environment main effects, performance of cultivars is also determined by genotype x environment interaction (G x E), which is the differential response of cultivars to environmental changes (Vargas et al., 2001). There are three

common types of G x E interaction, namely cultivar x location interaction; cultivar x year interaction; and cultivar x location x year interaction effects (Crossa, 1990). These G x E interactions are explained by variation in weather between and within seasons and soil properties, among other factors. For example, Troyer (1996) reported that cultivar x year interaction was larger than cultivar x location interaction due to differing soil moisture availability at flowering. Crossover interaction is the G x E interaction that changes the rank order for performance of cultivars. At times G x E does not change the rank order except for absolute differences of cultivar performance in the different environments. Crossover interaction causes problems in crop breeding because it impedes selection progress due to changing composition of cultivars selected in different environments (Cooper and Delacy, 1994; Crossa et al., 1995).

1.18.1 Stability of yield and yield components

Stable cultivars have little interaction with environments (Tollenaar and Lee, 2002). Becker and Leon (1988) defined two types of stability, namely static or dynamic. In static stability, cultivar yield does not change; but with dynamic stability cultivar yield changes in a predictable manner, and its stability is affected by the set of cultivars under evaluation (Becker and Leon, 1988; Tollenaar and Lee, 2002). Thus, static stability is an absolute measure, while dynamic stability is a relative measure. In cultivar selection, the best cultivar should effectively exploit the high inputs under favorable conditions and display acceptable grain yield under relatively low input systems. Finlay and Wilkinson (1963) suggested that dynamic stability could be preferred. This dynamic concept of stability is measured by the regression analysis as described by Finlay and Wilkinson (1963) and is sometimes referred as the parametric statistic.

Lin et al. (1986) reviewed the nonparametric statistics for evaluating G x E. These stability statistics are not influenced by the set of cultivars under evaluation. Lin et al. (1986) defined a stable cultivar as having a small variance and a similar deviation from the overall mean yield in all the environments. Lin and Binns (1988) also reported the

cultivar superiority index, which they defined as the mean square of the differences between the cultivar's response and the maximum response in different environments. Grain yield stability is influenced by the genetics of the cultivar. Eberhart and Russell (1966) reported that the use of genetic mixtures rather than homogeneous cultivars reduced G x E interaction due to population buffering in a heterogeneous population. Lee et al. (2003) reported that double cross hybrids had smaller G x E interaction, than single cross hybrids, which are more homogeneous. However, it is also possible that some single crosses could be more stable than the three-way and double cross hybrids (Eberhart and Russell, 1966). Grain yield stability can be improved through recurrent selection because it is heritable and largely controlled by additive gene action (Lee et al., 2003). In addition, stable cultivars can be identified through multi-location trials in targeted environments (Troyer, 1996). The high grain yield potential and adaptation of Pioneer hybrids to the USA were obtained through extensive multi-location trials (Duvick and Cassman, 1999; Evans and Fischer, 1999). It is, thus, prudent to evaluate regionally important germplasm under varied environments.

1.18.2 Additive Main Effects and Multiplicative Interaction (AMMI) model

Data from multi-location trials help researchers estimate yield more accurately, select better production alternatives, and understand the interaction of yield with environments. In breeding programs it is of interest to decide whether observed stability differences are due to chance or statistically significance difference. Significance testing is strongly advisable to determine the quality of stability estimates (Crossa et al., 1995).

A broad range of multivariate methods can be used to analyze multi-location yield trial data to asses yield stability. Although some of them overcome the limitations of linear regression, the results are often difficult to interpret in relation to GEI. Multivariate techniques are widely applied in stability analysis to investigate multivariate response of genotypes to environments. Among the multivariate analysis techniques, AMMI model is the powerful method in assessing GEI and stability or adaptation of genotypes from

multi-environment trials. AMMI is essentially effective where the assumption of linearity of responses of genotype to a change in environment is not fulfilled, which is an important aspect in stability analysis. The results can be graphed in a useful biplot that shows both main and interaction effects for both genotypes and environments (Gauch and Zobel, 1996).

The integration of certain ordination methods into "pattern" analysis and the bi-plot method are valuable tools for grouping environments or genotypes showing similar response patterns. The combination of analysis of variance and principal component analysis in the AMMI model, along with the prediction assessment, is a valuable approach for understanding genotype x environment interaction and obtaining better yield estimates. Agronomic predictive assessment with AMMI can be used to analyze the results of trials (Chukan, 2010).

AMMI analysis is helpful to choose the most stable hybrid and to group hybrids with a location where they have good specific adaptability. Adaptation to unsuitable conditions would also be shown. Thus the AMMI model was proved to be a useful tool in diagnosing the G x E interaction patterns and improving the accuracy of the response estimates in these trials. It provided more precise estimates of the true yield potential of both cultivars and specific environments where individual tests were evaluated. Increased accuracy in selection could help researchers identify specific cultivars with competitive yields across diverse environments (Zerihun, 2011).

1.18.3 GGE biplot analysis

The GGE (genotype and genotype by environment interaction) biplot analysis is increasingly being used in GEI studies in plant breeding research (Butran et al., 2004; Samonte et al., 2005). Visualization of "which won where" pattern of multi environment yield trial (MEYT) data is necessary for studying the possible existence of different mega environments (Gauch and Zobel, 1997; Yan et al., 2000; Yan, 2001). The polygon

view of a biplot is the best way to visualize the interaction patterns between genotypes and environments (Yan and Kang, 2003) to show the presence or absence of cross over GEI which is helpful in estimating the possible existence of different mega environments (Gauch and Zobel, 1997; Yan and Rajcan, 2002; Yan and Tinker, 2006). The polygon is formed by connecting the markers of genotypes that are further away from the biplot origin such that all genotypes are contained in the polygon (Kaya et al., 2006). The genotypes which are located on vertices of the polygon formed are either the best or poorest in one or more environments (Yan et al., 2000; Yan and Rajcan, 2002; Yan and Tinker, 2006). The vertex genotypes in each sector is also the best genotype for sites whose markers fell into the respective sector so that sites within the same sector share the same winning genotype (Yan, 1999; 2002; Yan et al., 2000). On the biplot, rays or lines that are drawn perpendicular to the sides of the polygon divide it into sectors.

1.18.4 Regression approach and its limitations in stability analysis

Crossa (1990) reviewed the limitations of regression analysis of stability. He reported that with few cultivars (less than 15) the mean of cultivars would not be independent of the marginal means of the environments. The regression analysis is not effective in the absence of a linear relationship between cultivar x environment interaction and the environmental means. Stability of a cultivar measured by regression analysis of a few and/or extreme environments would not provide reliable information, due to the high levels of bias. In the same vein, stability of a cultivar depends on the set of cultivars evaluated; hence application of the results from a regression analysis is limited to the specific set of environments and cultivars evaluated. Alternatives to the regression analysis are several nonparametric statistics. Huehn (1990) reviewed the rank analyses used in studying G x E interactions. These statistics have some advantages over the regression analysis such as reduction of bias caused by outlying cultivars and they are easy to interpret. In addition, the assumptions about the distribution of data, homogeneity of variances and linearity are not required for rank analyses (Huehn, 1990).

1.19 Conventional breeding approaches for disease resistance

When the gene action is additive, simple selection procedures e.g. recurrent selection should be effective in isolating inbred lines with multigenic resistance. Recurrent selection has been shown to be an effective means of concentrating genes for resistance. Jensen (1970) found that the greatest progress was made during the first two or three cycles of recurrent selection. With chlorotic-lesion type of resistance, homozygous resistant plants are readily selected in a homozygous condition following selfing and selection.

Susceptible lines can be improved by incorporating resistance genes through the backcrossing technique. In the case of resistance conditioned by few lesion-numbers, a large population needs to be grown, and linkage to other genes, such as affecting maturity, must be kept in mind. Since the chlorotic-lesion type of resistance is simply inherited, linkage to other genes is not much of a problem in the backcross program, but 6-10 backcrosses are needed to fully recover the character of the recurrent parent (Jensen, 1970).

1.20 Genetics of resistance to NCLB

The unique chlorotic lesion type of resistance on maize lines is characterized by chlorotic lesions and late developing lesions with small necrotic center surrounded by a light green margin (Ogliari et al., 2005). These lesions produced fewer spores compared to the rapidly developing necrotic susceptible lesions. This type of resistance was found to be controlled by a single dominant *Ht* gene. Homozygous dominant plants rarely have lesions. Ullstrup (1963) reported similar results on line P.I. 217407 where small lesions were surrounded by chlorotic halos with very limited sporulation in resistant genotypes. Further work by Hooker (1963) also concluded that the resistant chlorotic lesion type was conditioned by a single dominant gene in the dent corn inbred line GE440.

Similar symptoms were observed for susceptible and resistant lines, from 2-7 days, which appeared as minute white to light green flecks after inoculation of inbred lines and hybrid seedlings of GE440 with *E. turcicum* (Hilu and Hooker, 1963; Ullstrup, 1963). On susceptible lines, these flecks developed into lesions that wilted before developing necrosis. No wilting was seen on resistant cultivars. Disease development may take about 15 days. Sporulation was delayed 50-80 hrs and the population of spores per unit area may be reduced 60 times in the resistant lesions as compared to susceptible lesions (Ogliari et al., 2005). This is a situation normally seen in monogenic chlorotic lesion resistance but not in multigenic resistance. Carson (1995) reported that development of new races shorten the durability of the chlorotic resistant reactions which are controlled by single monogenic resistance genes. Polygenic resistance is normally expressed by reduced number of lesions and decrease in lesion size and amount of sporulation (Ullstrup, 1970). Singulas et al. (1988) reported that the average level of resistance, mean lesion area, the rate of increase in lesion size and the shape of the lesion are strongly influenced by host gene makeup as determined by contributions of each parent. In the race to develop resistant varieties to diseases the conventional methods of breeding needs to be supplemented by molecular marker technologies.

1.21 Application of molecular markers in maize breeding

Molecular markers play greater role in identifying different genes responsible for desirable traits like disease resistance. One method of using molecular markers for selecting desirable genes is in marker-assisted selection programs. Molecular markers therefore provide an important genetic tool where traditional studies have been difficult (Bagge and Lubberstedt, 2008).

Molecular markers provide a remarkable improvement in the efficiency of plant breeding (Bagge and Lubberstedt, 2008). Molecular markers allow the construction of high density linkage maps (Jones et al., 2003). Fine mapping of QTLs has sometimes

revealed the presence of tightly linked loci affecting the same trait (Bagge and Lubberstedt, 2008). Molecular markers can be used to test and select traits.

Several research groups mapped QTL responsible for quantitative resistance to NCLB (Keim et al., 1990). Jones et al. (2003) concluded lesion number in a segregating population is controlled by three QTL, while five QTL control disease severities in an environment of low disease severity. Molecular tools are also very important in determining the genetic distances of parental inbred lines, which will help in grouping the materials into different heterotic patterns in hybrid breeding. Knowledge on the genetic diversity and relationships among maize inbred lines is indispensable to identify promising combinations for exploitation of heterosis and establishment of heterotic groups for use as source materials in a breeding program. Molecular markers have proven valuable for genetic diversity analysis of many crop species. Their expression, unlike morphological markers, is not influenced by environmental factors; hence reflect the actual level of genetic difference existing between genotypes (Legesse et al., 2007)

1.22 Conclusion

Generally, this chapter reviewed important issues such as resistance breeding and management options for NCLB breeding strategies for the improvement of maize. It may serve as background information for maize breeders towards NCLB resistance and breeding for higher grain yield, using the conventional approach and molecular markers. Breeding information obtained out of sub-Saharan Africa might not have direct application, as the environment in which the application is going to be made, as well as the whole farming systems is quite different. There exists a huge gap between grain yield potential and the actual yield in the farmer's field, indicating the presence of opportunities for breeders in order to bridge the gap. In the temperate environments, improvement of grain yield is highly associated with improvement in the germplasm ability to tolerate biotic stresses such as disease. Therefore, it can be suggested that improving maize genotypes for their tolerance to biotic and abiotic stress would

contribute towards bridging the yield gap in sub-Saharan African environments. In terms of gene action, other types, in addition to the additive gene actions were also important in conditioning yield and other related traits. This scenario can indicate as simple selection only, which exploits additive effect, may not be enough to improve grain yield. Improving germplasm for stress tolerance and evaluating them for their stability across location and years is crucial for the identification of better performing varieties.

Genetic variation for resistance to NCLB was shown in both temperate and tropical adapted germplasm. It was also indicated that polygenic resistance is normally expressed by reduced number of lesions and decrease in lesion size and amount of sporulation and further stated that the average level of resistance, mean lesion area, the rate of increase in lesion size and the shape of the lesion are strongly influenced by host gene makeup as determined by contributions of each parent. Resistance was inherited mainly in an additive manner and was highly heritable; suggesting that resistance in regional maize can be enhanced by selection. High disease severity of up to 70% yield reduction was reported in Ethiopia, indicating that the disease has the potential of posing threat to regional food security. This review also suggested that cultivars with high resistance level with good stability would be obtainable by selecting germplasm directly in the hot spot areas followed by evaluation across the target environments.

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CHAPTER 2

Assessment of the preferences and constraints of maize farmers in the development and adoption of improved varieties in the mid-altitude, sub-humid agro-ecology of western Ethiopia

2.1 Abstract

Understanding farmers' production constraints and preferences is important in maize breeding, especially underlying successful adoption of improved varieties and their production packages. This study was conducted to assess the present importance, and productivity constraints of maize in the mid-altitude, sub-humid agro-ecology of western Ethiopia. Data was collected through a semi-structured questionnaire and focus group discussions, using 240 randomly selected respondent farmers, in twelve sub-districts, within three administrative zones. Maize was ranked number one as both food and cash crop by 82.9% of respondents. Most farmers (59%) use hybrids while 24% grow landrace varieties. Unavailability of improved seed and lack of production inputs were the two major constraints reducing maize productivity, as reported by 62% and 60% of respondents, respectively. A high proportion of respondents (80%) indicated that, unpredictable grain prices are the major market constraint as 97% of the respondents sell their maize crop in the local market. Northern corn leaf blight was reported to be important by 46% of respondents. Breeding for improved disease resistance and grain yield, enhancing the availability of crop input and stabilizing market price during harvest time are the most important strategies to increase maize production by small-scale farmers in western Ethiopia.

Keywords: farmers' constraints; maize; participatory research; production packages; *Zea mays*

2.2 Introduction

Maize (*Zea mays* L.) is the third important cereal crop globally after wheat and rice (FAO, 2011). The crop occupies a pivotal role in the world economy and is traded widely. Maize demand is projected to increase by 50% worldwide and by 93% in sub-Saharan Africa between 1995 and 2020 (FAO, 2007). In the past, much of the global use of maize has been for animal feed. However, maize is increasingly used for human consumption and accounts for 70% of the food consumed in sub-Saharan Africa (FAO, 2007). The recent volatile food market and rising prices for most food crops may increase the importance of maize production. In addition, because of its productivity and wide adaptation, maize remains an important source of food with great potential to improve the livelihoods of most poor famers in developing countries (FAO, 2011).

In developing countries most studies on agricultural technology development and diffusion are based on *ex-post* analysis of intervention programs (Bandiera and Rasul, 2006). Farmers are rarely consulted, *a priori*, about their specific circumstances, priority constraints, and their preferences with regards to production packages. Often the level of adoption is determined after costs are incurred and technologies are developed and diffused. Such top-down interventions usually result in a low level of acceptance by end users and minimal success for variety development programs (Feder et al., 1981). Prior identification of farmers' constraints and preferences is required in order to design more appropriate, acceptable and cost effective development intervention programs (Walter and Zewdie, 2008). In addition, the likely extent of future adoption of research results has a strong influence on the efficiency of research and on the results of research priority settings (Batz et al., 2003). Complementary contributions of farmers and scientists are essential for effective agricultural research and technology development, and their adoption (Chimdo et al., 2002).

Farmers' participation in setting research priorities and technology evaluation is crucial to scientists in order to design, test and recommend appropriate new production

technologies (Ashby, 1991). This can be achieved through participatory research that allows incorporation of farmers' indigenous technical knowledge, identification of farmers' criteria and priorities, and the definition of the research agenda, among others (Girma et al., 2005). Participatory rural appraisal (PRA) is a research tool to capture farmers' perceptions, constraints and preferences (De Groote and Bellon, 2000). These authors emphasized the effectiveness of PRA through seeking insights and promoting dialogue between scientists and farmers on production constraints, technology development and adoption. By integrating farmers' constraints and preferences into agricultural research, technologies can be developed that become widely adopted, resulting in more productive, stable, equitable and sustainable agricultural systems. This has led to the emergence of participatory plant breeding approaches in conventional plant breeding programs which integrates farmers' preferences and constraints (Ceccarelli et al., 2001). Participatory plant breeding approaches have been reported in cassava (Witcombe and Virk, 2001), barley technology adoption in Syria, Morocco and Tunisia (Ceccarelli et al., 2001), and maize in India (Joshi and Witcombe, 1996) and Ethiopia (Abebe et al., 2005).

In Ethiopia, maize is the second largest food security crop after tef [*Eragrostis tef* (Zucc.) Trotter.]. It is predominantly grown by small-scale farmers in the mid- and low-altitude, sub-humid agro-ecologies. It is primarily produced and consumed by the small-scale farmers that comprise about 80% of Ethiopia's population (Dawit et al. 2008). Maize has increasingly become a popular crop in the country with steady growth in production area and yield (Doss et al., 2003). The use of improved maize varieties has increased from 5% in 1997 to 20% in 2006 (CSA, 2001, 2004, 2006; Byerlee et al., 2007). The mid-altitude, sub-humid agro-ecology is the most important maize producing environment in Ethiopia (Birhane and Bantayehu, 1989; Kebede et al., 1993). This region is considered to be the major maize growing zone in the country. The region lies at an altitudes of between 1000-1800 meters above sea level and receives a fairly reliable average annual rainfall (1000–1500 mm year^{-1}), rendering it a region of high potential for maize production. However, maize production has remained low, with the

estimated national average yield of 2.5 t/ha due to several constraints: biotic (inadequate improved varieties, pests and diseases), abiotic (low soil fertility, land and water degradation, and drought) and socio-economic (input unavailability, lack of storage facility, poor access to markets) (CSA, 2010). Therefore, maize production components such as farmer-preferred, improved varieties, farming technologies, farm inputs, and access to markets should be developed and made available to enhance maize production, in order to achieve food security.

Bako National Maize Research Project was established under the Ethiopian Institute of Agricultural Research (EIAR) to coordinate maize research and development nationally. The project is situated in the region of mid-altitude, sub-humid maize growing agro-ecology. It is aimed at development and popularization of improved maize varieties, together with their management technologies. In the past there was a limited participatory research approach in maize technology development and/or improvement by the project. Consequently, there is little formal documentation of farmers' production constraints and preferences which are needed to guide maize varietal development in the mid-altitude, sub-humid agro-ecology of western Ethiopia. Therefore, the objective of the study was to assess the present importance, and production and productivity constraints, of maize in the mid-altitude, sub-humid agro-ecology of western Ethiopia. The study will serve as the basis for formulating research and development strategies to increase maize production and productivity by resource poor famers in these and similar environments.

2.3 Materials and methods

2.3.1 Study sites and sampling

A survey study was conducted in three representative maize growing zones of western Ethiopia; West Shoa, East Wollega and West Wollega. In each zone two districts were selected. Two sub-districts were selected per district providing a total of 12 study sites.

The study zones are situated in the mid-altitude, sub-humid agro-ecology where maize is grown as the predominant cereal crop. The zones receive the main rain from May to September in most years. Most farming activities follow the main rainy season which has a uni-modal distribution. Soils are varied and generally of low fertility. This is regarded as one of the major problems for cereal production in the zones. As a result of the high intensity of rainfall in the study areas, the soils are acidic (Wakene et al., 2012). The study sites were selected based on the importance of maize in the livelihoods of small-scale farmers, and the prevalence of major maize production constraints, including diseases, during production seasons. The survey was conducted during January, February and March 2011. According to the recent population census (CSA, 2010), the population demography and area of the study districts are shown in Table 2.1. Overall, Ilu Galaan is the most densely populated (244 per km^2) and Manasibu (86 per km^2), the least (Table 2.1).

Table 2.1 Area, population demography and population density of the study zones and districts (CSA, 2010).

Zone and district	Area (Km^2)	Males (000)	Females (000)	Total (000)	Density per km^2
West Shoa					
Ilu-Galaan	332.04	40.83	40.25	81.08	244.20
Bako-Tibe	1 044.52	60.22	66.67	126.89	121.48
East Wollega					
Gobu Sayyo	337.53	22.01	23.87	45.88	135.95
Sibu-Sire	1 132.50	61.32	54.00	115.31	101.82
West Wollega					
Najo	958	68.71	71.14	139.86	145.99
Manasibu	1 668.10	69.77	74.61	144.38	86.56

2.3.2 Sampling procedures

Multi-stage random sampling techniques were applied to select the study sites that represent the diverse ecological and socio-economic environments, and varying maize production systems, in the mid-altitude, sub-humid agro-ecological zones of western Ethiopia. The major criteria used during selection of the study sites were the relative importance of maize, and the severity of maize diseases.

2.3.3 Data collection

Data sources

Both primary and secondary data were utilized in the study. Primary data was collected through interviews of male and female farmers, key informants and focus group discussions. The key informants included maize researchers, experienced farmers in the villages, local leaders and agricultural agents. The facilitators used pictures showing disease symptoms and cards that had drawings representing various traits and constraints to assist the responding farmers during the discussions. The secondary data was obtained from the zonal and district agricultural offices of the respective districts included in the study areas. The offices of agriculture in the respective districts were the main sources for the secondary data. Eighty respondents were available per zone, per two districts (Table 2.2). Data was collected using a total of 86 variables. Consequently, principal component analyses (PCA) was used for data reduction and to identify the most important variables based on the magnitude of contributions to the total variation in the data set.

Table 2.2 Study zones, districts and sub-districts and number of sampled maize farmers in the mid-altitudes sub-humid areas of western Ethiopia

Zone	District	Sub-district	Female	Male	Total
West Shoa	Ilu-Galan	Siba-Biche	2	18	20
		Jato dirqi	2	18	20
	Bako-Tibe	Tulu Sangota	1	19	20
		Bacharra	2	18	20
East Wollega	Anno	Qeejo	1	19	20
		Anno	2	18	20
		Bakanisa			
	Sibu-Sire	Biqila	1	19	20
		Chari	1	19	20
West Wollega	Manasibu	Teyiba	2	18	20
		Bengua	-	20	20
	Nejo	Goori	1	19	20
		Dilla	-	20	20
Total			15	225	240

2.3.4 Administering questionnaire

A semi-structured questionnaire was administered to 240 respondent farmers from 12 sub-districts of three administrative zones. The sub-districts and respondent farmers were randomly selected in the major maize producing areas of each zone. Both male and female maize farmers were mobilized for focus group discussion through the local administrators, development agents and extension staff of the respective sub-districts. Checklists were developed and used to guide discussions with farmer groups and individual key informants. The objectives of the project and the significance of contributions from various actors were explained to both groups and communication procedures established.

The farmers were encouraged to use a language they were most familiar with. A member of the research team most versed with Afan Oromo, the local language, facilitated the group discussions. For ease of focusing the discussions and reaching a consensus, farmers were allowed to form discussion groups. Sex and age were the important criteria farmers used in categorizing themselves into discussion groups.

Amongst other variables, farmers were asked to list maize varieties that they grow and provide the relative proportions of the varieties, to list and rank their criteria for variety selection in terms of their relative importance, and the main constraints to maize production. The interviews in all the study areas were held by five researchers, selected from different disciplines of the Bako Agricultural Research Centre.

2.3.5 Data analysis

Data from questionnaires of individual interviews was coded, captured and analyzed using the SPSS computer package (SPSS, 2005). Descriptive statistics, chi-square tests, and PCA analyses were performed using the same computer package.

2.4 Results and discussion
2.4.1 Demography, socio-economic characters and main food crops grown

Of the total interviewed farmers, 93.8% were males, while 6.2% were females. Of all the respondents 98.8% were household heads and almost all the respondents (99.6%) were farmers in occupation. Among total respondents 53.8% were educated up to primary level, 25% secondary level and 19.6% without any education. The mean household age in the study areas was 41, with the maximum and minimum ages of 73 and 20, respectively. The major five crops grown in the study zones are summarized in Table 2.3. Most of the respondents (82.9%) ranked maize as the number one food crop. Other important crops in the zones included tef, sorghum, pepper, wheat and pearl millet (Table 2.3).

In the present study, farmers in all the study areas grow an assemblage of different varieties in order to avoid the risk of crop failure caused by growing the same variety. Girma and Tanto (2008) reported that most farmers in Ethiopia grow a diverse assemblage of genotypes (landraces) to minimize risks of crop failure and to increase food security because the landraces are well-adapted to production environments, and

carry farmers preferred traits. Further, in the Hararghe highlands of eastern Ethiopia, Mulatu and Zelleke (2002) found that farmers preferred landrace varieties over improved cultivars despite the higher yield potential of the latter. Both studies contended that landraces possess farmer-preferred attributes, resulting from long term human selection.

Table 2.3 Major crops grown, area of production and rank in the study zones of the mid-altitude sub-humid areas of western Ethiopia

| Zone | Crop | Production | |
		Area (ha)	Rank
West Shoa	Maize	118 344	2
	Tef	181 018	1
	Pepper	36 818	5
	Wheat	57 425	4
	Sorghum	77 758	3
East Wellega	Maize	121 854	1
	Tef	74 497	2
	Wheat	9 840	5
	Sorghum	51173	3
	Pearl millet	15 471	4
West Wellega	Tef	34 040	3
	Wheat	2 927	5
	Maize	73 960	1
	Sorghum	55 926	2
	Pearl millet	33 294	4

2.4.2 Maize varieties grown

Table 2.4 summarizes maize varieties grown across the three zones. Farmers grow an assortment of maize types [hybrids, landraces and improved open pollinated varieties (OPVs)], either on the same or different fields. Farmers grow both landraces (often referred to as local varieties) or improved varieties, to meet multiple objectives. Many of the respondents (60%) grow hybrid varieties, whilst 24% grow landraces (Table 2.4). Among the respondents, 39% grow the maize hybrid BH660 and 15% grow BH540. BH660, BH540, Pioneer hybrids and BH543 are the most widely grown hybrid varieties, in their order of importance in the study areas (data not shown). In some instances, the same landrace varieties are known by different names. The names of the landrace maize varieties are often descriptive, referring to certain key identifiable characteristics, especially grain color, appearance, growth habit and the perceived place of origin.

Table 2.4 Widely grown maize types and names with corresponding frequency and percentage of farmers in the mid-altitude sub-humid zones of western Ethiopia

Type	Name	Frequency	Percent
Hybrid	Different Hybrids	143	59.6
Landrace	Landraces	58	24.2
Hybrid and OPV	BH-660 and OPV	2	0.8
Hybrid and Landrace	BH-660 and Landrace	37	15.4
Total		240	100

2.4.3 Farmers' preferences of maize varieties

Farmers said that they use many criteria in selecting maize varieties for production. Overall, the major and common selection criteria of maize varieties and corresponding number of farmers are presented in Table 2.5. Farmers' perceptions and rating of the different criteria varied across the study sites. The most important criteria across the

studied districts were high yield, resistance to disease and insect pests, lodging resistance, ability to perform well under low soil fertility, and a combination of these. There were highly significant (P< 0.01) differences among the respondents in their preference to maize variety traits in all the 12 sub-districts (Table 2.5).

Table 2.5 The important farmers preferred traits of maize varieties in the mid-altitude sub-humid zones of western Ethiopia

Farmers preferred traits	Farmers	
	Number	Percent
Improved yield	54	22.5
Disease resistance	2	0.80
Insect resistance	6	2.50
Yield and disease resistance	41	17.10
Yield, insect and disease resistance	21	8.80
Yield, disease, insect and lodging resistance	57	23.80
Yield, disease and lodging resistance	21	8.80
Yield, insect and lodging resistance	2	0.80
Yield and lodging resistance	12	5.00
Disease and insect resistance	24	10.00
Total	240	100
Chi-square	32.65	
Significance level	0.000	

2.4.4 Major constraints to maize production

Table 2.6 summarizes the main constraints to maize production in the studied zones. Prioritization of the constraints was based on number of households affected, severity of the constraint, importance of the constraint in attainment of household objectives, frequency of occurrence of the constraint and the likelihood of a solution to be provided by the research team.

Table 2.6 Major maize production constraints in the mid-altitude, sub-humid zones of western Ethiopia as revealed by five randomly selected farmers averaged over 12 sites

| Constraints | Farmers group across 12 sites | | | | | Total | Importance |
	A	B	C	D	E		
Early cessation of rain	3.25	2.50	2.50	2.75	2.37	13.37	4
Diseases	3.50	3.00	3.37	3.50	3.62	16.99	3
Insect pests	2.25	2.37	3.12	2.75	2.50	12.99	5
Weeds	2.37	2.50	1.62	1.75	2.00	10.24	5
Input costs	4.87	4.62	5.00	4.87	4.87	24.25	2
Labour shortage	2.00	1.37	1.37	2.00	1.12	7.87	5
Low soil fertility	4.90	4.77	4.87	5.00	5.00	24.54	1

Key: 1 = most important, 5 = least important; A, B, C, D, E = five different farmers pooled across 12 sites.

The most important farmers' constraints in maize farming, as revealed by semi-structured questionnaire, were low soil fertility, high input prices (especially fertilizers and seed), leaf diseases, and early cessation of rain. Other important constraints were insect pests (termite and stalk borer), volatile grain prices, and unavailability of fertilizer (Tables 2.7 and 2.8).

Northern corn leaf blight (NCLB) and gray leaf spot (GLS) are the most important reported maize diseases. Farmers in the study area (46.7%) indicated NCLB as the major leaf disease on maize (Table 2.7). GLS is ranked as the second most important leaf disease in the area, as reported by 17.9% of the respondents. Price fluctuation of maize grain is also one of the major constraints in the maize market, impacting on the production of maize in the study areas.

Farmers in the study areas (80.8%) indicated that the unpredictable grain price was the major financial problem (Table 2.8). Furthermore, a widespread lack of storage facilities and high transportation costs affected the marketing of maize. During harvest times (November/December) the farm price of a 100 kg of maize is at Birr200 (≈12 USD), that increases to birr 500-600 during June/July. In the present study, the major market outlets for the sale of maize were local markets as indicated by 97.1% of the respondent farmers. Only a small proportion of farmers (<3%) sold their products to other market outlets, such as unions, grain trade enterprises and farmer to farmer.

Table 2.7 Major diseases and insect pests in the study area

	Farmers	
Name	Frequency	Percent
Northern corn leaf blight (NCLB)	112	46.7
Gray leaf spot (GLS)	43	17.9
Maize streak virus (MSV)	5	2.1
Termite	1	0.4
Stalk borer	4	1.7
NCLB,GLS, termite and stalk borer	1	0.4
NCLB,GLS and stalk borer	2	0.8
NCLB and GLS	49	20.4
NCLB, termite and stalk borer	3	1.2
NCLB , GLS and MSV	2	0.8
NCLB , GLS and termite	1	0.4
GLS and rust	1	0.4
NCLB and MSV	7	2.9
GLS and MSV	1	0.4
NCLB, GLS and *Phaosphaeria* leaf spot	5	2.1
NCLB and termite	2	0.8
NCLB, MSV and stalk borer	1	0.4
Total	240	100.0

In this study NCLB was reported as the number one disease followed by GLS. This contradicts Dagne et al. (2004) who reported GLS to be the number one leaf disease, followed by NCLB in the mid-altitude, sub-humid maize growing areas. The present result suggests that a shift in disease levels has occurred making NCLB a research priority. Declining soil fertility was also identified as a major limitation in the production of maize in these zones. Farmers indicated that a decline in soil fertility was exacerbated by mono-cropping and limited soil conservation practices.

Table 2.8 Input and marketing constraints affecting maize production

Constraints	Farmers	
	Frequency	Percent
Market		
Grain price fluctuation and low price	193	80.8
Storage facility	3	1.2
Price fluctuation and transport cost	14	6.0
Price fluctuation, transport cost and storage facility	12	5.0
Price fluctuation and storage facility	8	3.3
No sales of produce	7	2.9
Transportation cost	2	0.8
Total	240	100.0
Fertilizer		
Readily available	25	10.4
Moderately available	93	38.8
Poorly available	100	42.0
Not available	21	8.8
Do not use	1	0.4
Total	240	100.0

The present study indicated that the unpredictable grain price was the major bottleneck in the maize grain market, which was further aggravated by a lack of, or poor infrastructure, including postharvest storage facilities and transport systems. In the study by Dawit et al. (2008), the volatility of the maize price was seen as one of the major challenges in maize production. Furthermore, this affected the allocation of land for maize seed production and the total quantity of maize seed produced over the last decade.

Farmers considered high input costs, as compared to grain prices, as a key constraint because they believed that lower inputs costs would lead to alleviation of many other constraints. Dawit et al. (2008) found similar constraints in their studies of maize seed systems in the drought prone areas of Ethiopia. In some instances, farmers did not explicitly indicate the constraints they face. However, they said that they lack cash to buy the optimum crop input. Hailu (1992) reported the unavailability of agricultural inputs as the main bottleneck in maize production and productivity in Ethiopia. The prevalence of low quality agricultural inputs in the market is also one of the major constraints that farmers face. Maize seeds, for instance, are often adulterated or not true to type. Walter and Zewdie (2008) found that unavailability and untimely supply of seed are the major production constraints in maize growing areas of sub-Saharan Africa.

2.4.5 Focus group discussion

Focus group discussion was conducted to identify locally preferred maize varieties, and the traits associated with the respective varieties. Farmers listed the varieties they grew, ranked them and identified traits they preferred in maize, giving reasons for their choice of the selected varieties. Further, the focus group discussion identified important constraints in maize production. Farmers identified, listed and ranked the problems based on their priority of the constraint in the study areas.

A maximum of 20 and a minimum of 15 farmers group participated in group discussion across all the study areas. The gender composition of the participating farmers was considered but most respondents were male farmers. Household leaders in almost all of the study areas were male farmers. Maize was the major cereal crop in terms of area under production in all of the study areas, except in west Shoa, where it was surpassed by tef. Maize was also number one in yield per hectare and total production in the mid-altitude, sub-humid areas of western Ethiopia (CSA, 2010; CSA, 2011). The responding farmers preferred maize because of its adaptability and also for food security. Most of the farmers in the study areas utilized improved maize seed, except in their homestead where they use own seeds of local variety called 'Burre'.

The farmers in the study areas recognized the value of improved maize varieties because of higher yield, lodging resistance, flour quality, and market preferences. Farmers in the majority of the study areas indicated that some improved maize varieties are more susceptible to diseases and insect pests than the landrace varieties, which they considered to be more adapted to the production environments. Often the main maize growing season coincides with the severe outbreaks of leaf diseases, including NCLB, GLS and MSV. Farmers indicated that there were little traditional or modern control methods applied for the control of leaf diseases on maize, especially NCLB and GLS.

2.4.6 Principal component analysis

PCA is most useful in data reduction and projection, and allows maintenance of smaller number of principal components than original variables. PCA is a powerful tool for pattern recognition, classification, modeling, and other aspects of data evaluation (Heberger et al., 2003). The current study used 86 variables to establish maize production constraints, using 240 respondent farmers. According to the principal component analysis (PCA), 21 principal components (PC) were identified with Eigen values >1 (Sneath and Sokal, 1973) and contributed to 69% of the total variation (Table

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2.10). As a result, these components and variables that were highly correlated were identified as important contributors to the variation presented in the data sets of this study (data not shown).

The most important variables well-correlated with PC1 (r≥0.5) were: Which constraints limit your maize production, rank of maize among cereals produced for the last three years; maize productivity; availability of fertilizer; and widely grown maize varieties. Considering the PC2 and PC3, the important variables were: accessibility to inputs; improved quality required from maize varieties; availability of improved seed for the production of maize; and criteria for selecting sites for maize (Table 2.9). Though PC1, PC2 and PC3 were the most important components and made a significant contribution to the variance (≈70%) in the data set, other components among the 21 were also relevant. Some of the important variables well-correlated with the rests of the PCs were: market for maize produce; trends of maize production; land utilization; area for maize; types of cereals grown, and preference for maize (data not shown). These were also important variables identified and retained through the PCA in the study. These variables could be used in the future studies on the preferences of farmers in similar maize growing environments.

Table 2.9 Important Principal components with variables having r> 0.6

Variables	Principal Component	r>0.6
Which constraints limit your maize production	PC1	0.825
How is the accessibility to inputs for maize production?	PC2	0.877
what improved qualities would you like the improved maize cultivars to have	PC3	0.676
Total area cultivated last production year	PC4	0.868
Which cereal crops do you grow?	PC5	0.814
What is the origin of maize in your community	PC8	0.747
For how long have you been cultivating and consuming maize	PC10	0.611
Where do you market your maize producer?	PC14	0.806
What was the trend of maize production,	PC18	0.664
Do you like maize production	PC20	0.846

Table 2.10 The Eigen values, percent of variance and cumulative variance explained by the 21 Principal Components (PC)

PC	Initial Eigen values		
	Total	% of Variance	Cumulative %
1	6.380	10.999	10.999
2	3.776	6.510	17.509
3	2.652	4.572	22.081
4	2.406	4.148	26.229
5	2.311	3.985	30.214
6	2.007	3.460	33.674
7	1.937	3.340	37.014
8	1.839	3.171	40.185
9	1.573	2.713	42.898
10	1.532	2.642	45.540
11	1.511	2.605	48.145
12	1.406	2.424	50.570
13	1.357	2.340	52.910
14	1.345	2.318	55.228
15	1.295	2.233	57.461
16	1.262	2.176	59.637
17	1.190	2.052	61.689
18	1.114	1.922	63.610
19	1.110	1.913	65.523
20	1.058	1.824	67.348
21	1.045	1.802	**69.149**

2.5 Conclusion

The present findings showed that maize production in the mid-altitude, sub-humid areas of western Ethiopia is constrained by a myriad of related factors. The most important diseases, in decreasing order of importance, are NCLB, GLS and MSV. This study indicated a shift in the order of importance of major maize diseases in the study areas when compared with previous studies (Dagne et al., 2004). Recurrent future studies are required every 4-5 years in order to collate information on the relative importance of maize diseases and other relevant constraints in the area. Maize breeding cannot incorporate all the desired attributes instantaneously. But the key attributes should be included in a particular variety, and other candidate varieties should be bred focusing on the preferences of different groups of farmers. Equally farmers should have access to improved seed and other inputs with reasonable price. To overcome the setback of low grain prices following maize harvests, farmers should be provided with technical and financial support to store their maize product till market prices improve.

Farmers have diverse perceptions and complex combinations of criteria to select maize varieties. From this study, farmers- preferred attributes included high yields, resistance to disease and insect pests, fair seed costs and the ability of a variety to give a reasonable yield with little application of external inputs, especially fertilizer and pesticides. To increase maize production, research should be inclusive and take into consideration the farmers' circumstances and preferences. Maize varieties and crop management packages developed should meet farmer's needs. Incorporation of farmers' preferences and production constraints in selection of maize varieties in any breeding project would increase the likelihood of adoption of the varieties. The findings from this study will form the basis for farmer-oriented maize breeding in the mid-altitude, sub-humid zone of western Ethiopia and similar areas in Sub-Saharan Africa.

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CHAPTER 3

Genetic variability of elite maize inbred lines for resistance to *Exserohilum turcicum* in the mid-altitude sub-humid agro-ecology of Ethiopia

3.1 Abstract

Maize (*Zea mays* L.) is the most important staple food crop in sub-Saharan Africa. Maize productivity is curtailed by a number of stress factors including diseases and insect pests, among others. The leaf blight disease caused by *Exerohilium turcicum* (Pass.) Leonard and Suggs is one of the most economically important diseases of maize. The objective of this study was to determine genetic variability among elite maize inbred lines and select promising parents for resistance breeding to *E. turcicum*. Field established plants were artificially inoculated at 4 - 6 leaf stages during 2011 and 2012 at Bako research centre in Ethiopia. Data on disease severity and incidence, AUDPC and yield were recorded. Inbred lines showed significant differences for *E. turcicum* reactions and separated into classes of resistant, intermediate or susceptible. Mean disease severity ranged from 2.04 for inbred line 136-a to 3.25 for Kuleni-C1-101-1-1-1. The upper leaf area infection varied from 3.3% for inbred line143-5-I and 136-a to 100% for Kuleni-C1-101-1-1-1. The minimum AUDPC score was 238 and the maximum at 700, for lines 136-a and Kuleni-C1-101-1-1-1, respectively. Overall, inbred lines CML-202, 144-7-b, 139-5-j, 136-a, 30H83-7-1-1-1-2-1, ILoo'E-1-9-1-1-1-1-1 and 142-1-e, were identified as promising sources of resistance against *E. turcicum* for breeding at the mid-altitude sub-humid agro-ecology of Ethiopia or similar agro-ecologies. The selected inbred lines may be used in general varietal development, disease management and to enhance maize productivity.

Keywords: *Exerohilium turcicum*, inbred line, maize, resistance breeding

3.2 Introduction

Maize (*Zea mays* L.) is the third most important cereal crop after wheat and rice (FAO, 2011). During 2006 the world maize production was 144 million hectares while that of wheat was 216 million and rice 154 million hectares (FAO, 2008).The crop occupies a pivotal role in the world economy and is traded widely. Maize demand is projected to increase by 50% worldwide and by 93% in sub-Saharan Africa between 1995 and 2020 (Rosegrant et al., 2001; FAO, 2007). In the past, much of the global use of maize has been for animal feed. However, maize is increasingly used for human consumption and accounts for 70% of the food consumed in sub-Saharan Africa (FAO, 2007). Because of its productivity and wide adaptation, maize remains an important source of food with great potential to improve the livelihoods of most poor farmers in developing countries (FAO, 2011).

Maize productivity is limited due to a number of biotic and abiotic stresses. The major abiotic stresses affecting maize production included drought and soil nutrient deficiency and the biotic stresses are: infectious diseases and pests such as maize stem borer, weevils and termites (Mosisa et al., 2012; Girma et al., 2012). The major diseases of maize include *Turcicum* leaf blight caused by *Exserohilum turcicum* Pass Leonard & Suggs, grey leaf spot (*Cercospora zeae-maydis* Tehon & Daniels) and common leaf rust (*Puccinia sorghi* Schr.) (Tewabech et al., 2012). Among the maize diseases, *Turcicum* leaf blight also known as northern corn leaf blight (NCLB) is a wide spread disease with incidence ranging from 95 – 100% in areas with constant moisture and high humidity occurring in the main rainy season (Juliana et al., 2005; Tewabech et al., 2012). Also, the disease causes qualitative changes in the seed resulting to decreased sugar content, germination capacity and heavily infected plants are predisposed to stalk rot (Bowen and Pedersen, 1988; Cardwell et al., 1997; Muiru et al., 2007). The disease is ranked as the number one problem and is considered a high research priority of maize in Ethiopia (Wende et al., 2013).

The NCLB of maize is one of the widely distributed and economically important diseases of maize in sub-Saharan Africa (Tewabech et al., 2012; Wende et al., 2013). Infection of the disease appears during both off and main production seasons. However, disease epidemics are more pronounced during the main-season especially in constantly wet and humid areas. Therefore, strategic breeding to develop resistant varieties is crucial in areas where the disease reaches epidemic proportions (Girma et al., 2008).

Different options are available to control maize leaf blight such as the use of host plant resistance, cultural practices, and fungicides (Girma et al., 2008; Meseret and Temam, 2008). Host plant resistance is the cheapest and most effective way to control leaf blight disease because chemical treatments are expensive, often ineffective, and sanitation practices are difficult to apply. The use of resistant varieties possessing qualitative and quantitative genes in combination or separately is the cheapest and environmentally friendly method (Juliana et al., 2005; Dagne et al., 2008). Two forms of host plant resistance are distinguishable: qualitative and quantitative. Qualitative resistance is race-specific and governed by a single or few gene(s) whereas quantitative resistance is race-non-specific and polygenic (Singh et al., 2004; Ogiliari et al., 2005). Qualitative resistance genes such as *Ht*1, *Ht*2, *Ht*3, *Htn* and *Htm* are reportedly dominant or partially dominant and confer nondurable resistance. This form of resistance may break down due to emergence of virulent races of the pathogen through genetic mutation and recombination events (Freymark et al., 1994; Weilz and Geiger, 2000; Juliana et al., 2005; Ogiliari et al., 2005). *E. turcicum* exhibits a wide range of variability (Yeshitela, 2003) and new races overcoming previously resistant varieties are documented (Juliana et al., 2005). Most breeding programs rely on qualitative resistance conferred by *Ht* genes.

Resistance conferred by the *Ht* gene(s) are characterized by chlorotic and necrotic lesions or lesions surrounded by a yellow to light brown margin, without spore formation, which limits the growth and spread of the disease (Jungenheimer, 1976;

Hooker et al., 1977; Weilz and Geiger, 2000; Singh et al., 2004). Typically, resistance conferred by the *Htn* gene is expressed as a delay in lesion formation and presence of fewer lesions (Gevers, 1975; Ogiliari et al., 2005). Varieties with the *Htn* gene generally remain free from lesion development until pollination (Leonard, 1989). Polygenic resistance conferred by minor genes is not absolute when compared to qualitative or monogenic or oligogenic resistance. However, minor gene resistance is more durable and chances of new pathological races breaking the resistance are relatively minimal (Ojulong et al., 1995; 1996). Polygenic resistance effectively reduces the rate of disease increase (Vanderplank, 1963; Parlevliet, 1979). Genotypes with this resistance can vary from highly resistant showing a few lesions to more susceptible reaction types where large sporulating lesions are present (Elliott and Jenkins, 1946; Meyer et al., 1991).

Breeding for resistance or tolerance to *E. turcicum* is the most economically viable option to release varieties for resource-constrained farmers. This is achieved through incorporation of resistance genes into the existing elite genotypes. The option serves as one of the major components in the integrated management of the maize leaf blight. Disease severity, disease incidence, lesion size, and area under disease progress curve are the most common parameters used in the evaluation of maize genotypes for resistance to *Turcicum* leaf blight (Adipala, 1994; Pratt et al., 2003).

Due to the economic importance of *Turcicum* leaf blight disease various national and international programs are actively involved in breeding for resistance. However, some of the commercial varieties as well as elite parental inbred lines are reportedly vulnerable to *Turcicum* leaf blight (Njuguna et al., 1990; Muthinda 1997; Welz and Geiger, 2000). There is a continued need to identify new sources of resistance through artificial inoculation or natural epidemics of the disease among available breeder's genetic stocks and introduced germplasm for breeding, disease management and to enhance maize productivity (Girma et al., 2008).

In Ethiopia maize productivity is low, with the estimated national average yield of 2.5 t ha^{-1} due to several abiotic and biotic constraints (CSA, 2010). Bako national maize research project in Ethiopia is involved in maize research and development. The project, situated in the region of mid-altitude, sub-humid maize growing agro-ecology, aims to enhance maize productivity through effective breeding using locally adapted germplasm as well as through well-designed hybrid cultivar development. Recently the project embarked on a dedicated resistance breeding program to develop leaf blight resistant varieties through incorporation of resistant genes into well-adapted but susceptible germplasm for sustainable production across the mid-altitude sub-humid agro-ecologies. Therefore, the objective of this study was to determine the genetic variability among 50 elite maize inbred lines and select promising lines with leaf blight resistance and adaptation to the mid-altitude sub-humid agro-ecologies. The selected lines may be used in resistance breeding programs to minimize losses incurred by *Turcicum* leaf blight in maize.

3.3 Materials and Methods
3.3.1 Plant material and study site

The study used 50 elite inbred lines adapted to the mid-altitude agro-ecologies. The lines were acquired from the Ethiopian maize research program (Bako) and the international maize and wheat improvement center (CIMMYT) (Table 3.1). Inbred line CML-197 sourced from CIMMYT served as susceptible check. All the lines were stable and homozygous and descended through controlled selfing.

The study was conducted at Bako in Ethiopia. Bako is the national maize research coordination centre situated at an altitude of 1650 metre above sea level, longitude 37^0 09 E, latitude 09^0 06N and has nitosol soil type. It receives an annual rainfall of 1200 mm. Bako is one of the major maize producing environments in the country, representing the mid-altitude sub humid agro-ecological zone.

3.3.2 Field experiments

Inbred lines were evaluated using a 5 x 10 alpha lattice design with three replications. Trials were conducted for two years (2011 and 2012) during the main rainy season of Ethiopia, i.e., May through September. Each plot was 5.1 m in length. The inter-row spacing was 75 cm and intra-row spacing at 30 cm. Phosphorus (100 kg ha^{-1}) was applied once at planting. A 100 kg ha^{-1} Nitrogen fertilizer was applied in two splits; half at planting and the rest at 37 days after emergence. Urea and diammonium phosphate (DAP) were used as source of nitrogen and phosphorus fertilizer, respectively. All the trial management practices were based on the recommendation of the location.

Table 3.1 Descriptions of the inbred lines used in this study

No.	Pedigree	Source
1	CML395	CIMMYT
2	Pool9A-4-4-1-1-1	Bako
3	Gibe-1-158-1-1-1-1	Bako
4	124-b(109)	Bako
5	SC22	Bako
6	Iloo'E-1-12-4-1-1	Bako
7	30H83-7-1-1-1-2-1	Bako
8	DE-78-Z-126-3-2-2-1-1	Bako
9	139-5-j	Bako
10	(LZ-955459/LZ955357)-B-1-B-B	Bako
11	SZSYNA-99-F2-3-6-2-1	Bako
12	CML442	CIMMYT
13	30H83-7-1-5-1-1-1-1	Bako
14	Gibe-1-20-2-2-1-1	Bako
15	30H83-56-1-1-1-1-1	Bako
16	Gibe-1-91-1-1-1-1	Bako
17	SZSYNA-99-F2-2-7-3-1-1	Bako
18	CML 202	CIMMYT
19	CML 312	CIMMYT
20	SC-715-121-1-3	Bako
21	SC-715-13-2-1	Bako
22	142-1-e	Bako
23	(CML 205/CML208//CML 202)-X2-1-2-B-B-B	CIMMYT & Bako
24	(DRB-F2-60-1-2)-B-1-B-B-B	Bako
25	DE-105-Z-126-30-1-2-2-1	Bako
26	Kuleni-0080-4-2-1-1-1-1	Bako
27	A7033	Bako
28	ILoo'E-1-9-1-1-1-1-1	Bako
29	Gibe-1-186-2-2-1	Bako
30	F7215	Bako
31	CML 464	CIMMYT
32	CML 445	CIMMYT
33	SC-715-154-1-1	Bako
34	136-a	Bako
35	CML 444	CIMMYT
36	SZSYNA-99-F2-81-4-3-1	Bako
37	BH6609(F2)-10-2-1-2-1	Bako
38	CML 197	CIMMYT
39	SZSYNA-99-F2-80-3-4-1	Bako
40	DE-78-Z-126-3-2-1-2-1	Bako
41	X1264DW-1-2-1-1-1-1	Bako
42	Pool9A-128-5-1-1-1	Bako
43	Iloo'E-5-5-3-1	Bako
44	SC-22-430(63)	Bako
45	124-b(113)	Bako
46	144-7-b	Bako
47	CML 443	CIMMYT
48	30H83-5-1-4-2-1-1	Bako
49	143-5-I	Bako
50	Kuleni-C1-101-1-1-1	Bako

Bako = Bako national maize research coordination centre
CIMMYT= International maize and wheat research centre

3.3.3 Leaf blight inoculums collection and preparation

Isolates of *E. turcicum* were obtained from diseased maize samples collected from maize fields where the disease is prevalent. The infected leaves were cut into small sections (1 square centimeter) and surface sterilized using 2.5% Sodium hypochlorite for 2 - 3 minutes. The plant tissues were then rinsed with sterile distilled water, blot dried and plated on PDA in petri-dishes and incubated at room temperature for 3 - 4 days. Pure cultures were prepared by sub-culturing from the isolation plates and the cultures incubated for 7 - 10 days to obtain sufficient growth. Inoculum was prepared by flooding the cultures with sterile distilled water, scrapping the surface with microscopic slides to dislodge the conidia and then filtered using cheese cloth. The concentration of the conidia suspension was then adjusted to 10^5 conidia per ml using a haemocytometer.

3.3.4 Leaf blight inoculation

Field grown maize plants were inoculated at the 4 - 6 leaf stage of growth during mid-July, the middle of the main rainy season in Ethiopia. Inoculations were accomplished by spraying the maize plant with the conidia suspension until runoff using a hand atomizer. The inoculation was done during the evening when there was sufficient moisture in the air. To promote condition favorable for disease development, fine mist water was sprayed over the inoculated plants.

3.3.5 Data collection and analysis
3.3.5.1 Disease assessment

Northern corn leaf blight was visually assessed in the field 2 - 3 weeks after inoculation during each year. Ten randomly selected plants were tagged and used for successive disease assessments. Plants were rated at 10-day intervals for percent incidence, lesion length, and lesion width. Lesion sizes (expressed in centimeters) of two lesions of

the 10 plants were measured at 10 day intervals to determine the rate of lesion expansion. Monitored lesions were marked with a marker so that the lesions could be traced and measured.

Disease severity was rated using CIMMYT's method (www.CIMMYT.org) with 1 - 5 scoring scale: where

1.0 = very slightly infected, one or two restricted lesions on lower leaves or trace.

2.0 = slight to moderate infection on lower leaves, a few scatter lesions on lower leaves.

3.0 = abundant lesions on lower leaves, a few on middle leaves.

4.0 = abundant lesions on lower and middle leaves extending to upper leaves.

5.0 = abundant lesions on all leaves, plant may be prematurely killed by blight.

3.3.5.2 The area under disease progress curve (AUDPC)

Data on northern leaf blight was recorded at 10-day intervals starting from disease on-set (7 times) each year during the entire growing period. To ensure consistent disease evaluation in the field, a disease progress curve was drawn. This curve was developed from 10 days severity reading in both years. By constructing a curve, symptom development and disease severity could be compared over years. The area under disease progress curve was used to quantify the beginning of the epidemic and the time until the blight reached its peak. The derived disease parameter, AUDPC was calculated according to the equation of Campbell and Madden (1991) using the following formula:

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

Where n is the number of observations, ti days after planting for the ith disease assessment and yi disease severity.

Analysis of disease development could be performed when greater quantification was needed for resistance evaluation. The disease progress curve represents an integration of all host, pathogen and environmental effects occurring during disease development and provides an opportunity for greater in depth analysis, when comparing small differences among test entries.

3.3.5.3 Yield and thousand seed weight

Grain yield (t ha^{-1}) was calculated using the average shelling percentage of 80% adjusted to 12.5% moisture. Data on grain yield was analyzed with GenStat release 14.2 computer software, VSN international Ltd (Payne et al., 2008). Analysis of variance was done for the individual trials as well as combined analysis for the two years. Thousand seed weight (in gram) was measured from a random sample of 1000 seeds of each inbred line.

3.3.5.4 Data analysis

Analysis of variance of all the disease and agronomic data were conducted with the GenStat release 14.2 computer software program (Payne et al., 2008) to determine significant differences.

3.4 Results and discussion
3.4.1 Disease development

Disease ratings were significantly different among the tested inbred lines (p < 0.001) both on severity and lesion length. Out of the 50 inbred lines tested during the two years, 11 were considered resistant, 26 intermediate, and 13 susceptible (Tables 3.2 and 3.3) with an average disease severity rating of 2.48. Mean values of the disease assessment varied due to year and genotypic differences. The combined analysis of variance indicated highly significant (p<0.001) difference of year x line interaction for

grain yield, disease severity and incidence. The effects of year, line and line x year interaction on disease scores, grain yield and thousand seed weight were highly significant (p < 0.001). Disease progress was faster on susceptible inbred lines in both test years (Figure 3.2). The disease incidence on susceptible inbred lines were high and reached a maximum at 88.48% on the control line CML-197 (Table 3.2), while on resistant inbred lines (SZSYNA-99-F2-81-4-3-1, 144-7-b, 30H83-7-1-1-1-2-1 and CML 202) the incidence was lower at the end of the growing period during the two years of screening (Tables 3.2 and 3.3). No accession was immune to blight, but inbred lines such as CML 197, SC-22-430(63), Kuleni-C1-101-1-1-1, SC22 and 124b (109) were more susceptible compared to others (Table 3.2).

Table 3.2 Maize leaf blight reactions, grain yield and thousand seed weight of 50 inbred lines tested during 2011 at Bako research centre in Ethiopia

No.	Inbred lines	Severity score (0-5)	Reaction type	Incidence %	Lesion length (cm)	AUDPC	TSW	Yield(t ha^{-1})
1	CML 202	2.0	R	46.81	9.88	408.3	223.3	2.22
2	CML442	2.7	I	78.43	13.40	612.5	223.3	2.40
3	CML 312	2.4	I	61.52	10.35	385.0	276.7	3.03
4	CML 464	2.2	I	55.64	13.82	595.0	223.3	3.79
5	Gibe-1-91-1-1-1-1	2.5	I	71.32	14.57	408.3	321.7	2.90
6	CML 445	2.5	I	65.20	14.02	571.7	213.3	3.34
7	CML 443	2.9	S	69.61	13.48	595.0	211.7	2.07
8	Gibe-1-158-1-1-1-1	2.5	I	66.42	11.37	507.5	281.7	3.43
9	A7033	2.9	S	68.63	15.37	641.7	273.3	2.58
10	(CML 205/CML208//CML 202)-X2-1-2-B-B-B	2.7	S	83.58	15.88	571.7	300.0	5.60
11	CML395	2.4	I	71.08	14.07	420.0	338.3	4.96
12	CML 444	2.5	I	69.12	18.28	443.3	260.0	2.95
13	DE-78-Z-126-3-2-2-1-1	2.7	S	67.89	14.48	536.7	280.0	4.14
14	30H83-7-1-1-1-2-1	2.0	R	53.19	10.90	495.8	210.0	3.14
15	ILoo'E-1-9-1-1-1-1-1	2.0	R	56.62	15.62	420.0	346.7	4.83
16	SZSYNA-99-F2-81-4-3-1	2.0	R	42.40	10.77	466.7	315.0	2.46
17	X1264DW-1-2-1-1-1-1	2.9	S	70.59	15.00	571.7	213.3	1.94
18	124-b(113)	2.6	I	59.80	15.27	606.7	365.0	3.53
19	SC22	2.8	S	85.78	14.72	501.7	271.7	3.56
20	SC-715-121-1-3	2.5	I	67.40	13.47	396.7	336.7	3.45
21	DE-105-Z-126-30-1-2-2-1	2.0	R	61.27	14.55	420.0	235.0	2.89
22	Gibe-1-20-2-2-1-1	2.7	S	69.12	18.78	501.7	301.7	2.62
23	Kuleni-0080-4-2-1-1-1-1	2.0	I	61.52	16.38	449.2	326.7	3.72
24	Pool9A-4-4-1-1-1	2.7	S	68.63	21.35	670.8	288.3	4.85
25	30H83-5-1-4-2-1-1	2.5	I	63.97	16.27	484.2	308.3	4.27
26	Iloo'E-5-5-3-1	2.6	I	74.26	13.48	560.0	328.3	4.41
27	SZSYNA-99-F2-2-7-3-1-1	2.0	R	57.35	11.77	478.3	206.7	2.77
28	SC-715-154-1-1	2.2	I	65.20	11.97	402.5	280.0	5.89
29	BH6609(F2)-10-2-1-2-1	2.3	I	61.76	11.83	402.5	300.0	3.98
30	143-5-I	2.3	I	60.29	15.48	420.0	325.0	6.84

31	144-7-b	1.9	R	58.09	12.87	385.0	330.0	4.45
32	(LZ-955459/LZ955357)-B-1-B-B	2.4	I	67.16	12.20	431.7	256.7	2.98
33	139-5-j	2.0	R	53.43	13.78	385.0	258.3	2.56
34	30H83-56-1-1-1-1-1	2.3	I	57.35	10.22	495.8	205.0	3.57
35	SZSYNA-99-F2-80-3-4-1	2.6	I	73.53	20.05	525.0	293.3	3.15
36	124-b(109)	2.9	S	81.86	15.48	536.7	310.0	5.54
37	F7215	2.4	I	63.73	14.72	455.0	393.3	3.86
38	136-a	1.8	R	51.47	13.82	238.0	396.7	4.41
39	DE-78-Z-126-3-2-1-2-1	2.6	I	70.83	14.85	595.0	286.7	3.83
40	Gibe-1-186-2-2-1	2.5	I	51.96	14.88	350.0	373.3	2.70
41	Pool9A-128-5-1-1-1	2.7	I	71.43	13.12	595.0	278.3	2.45
42	30H83-7-1-5-1-1-1-1	2.0	R	52.45	12.05	379.2	220.0	2.60
43	SZSYNA-99-F2-3-6-2-1	2.6	I	70.83	12.33	618.3	256.7	2.36
44	SC-715-13-2-1	2.4	I	61.76	12.87	420.0	248.3	2.34
45	SC-22-430(63)	3.0	S	80.15	11.57	478.3	311.7	2.48
46	Kuleni-C1-101-1-1-1	3.0	S	75.49	17.07	700.0	258.3	2.84
47	Iloo'E-1-12-4-1-1	2.4	I	51.96	10.30	443.3	276.7	2.43
48	(DRB-F2-60-1-2)-B-1-B-B-B	2.8	S	75.98	16.23	600.8	270.0	2.66
49	142-1-e	2.0	R	62.01	15.02	595.0	323.3	3.94
50	CML 197	3.0	S	88.48	18.07	525.0	271.7	50
	LSD	0.4260	-	18.513	7.504	129.93	72.64	1.465
	Pr>f	**	-	**	**	**	**	**
	CV (%)	3.3	-	17.6	10.6	16.2	15.9	25
	Overall mean	2.486	-	65.49	14.16	493.9	284.1	3.52

R= Resistant (1.0 – 2.0); I = Intermediate (2.0 – 2.5); Susceptible (2.5 - 5); TSW= Thousand seed weight

Table 3.3 Maize leaf blight reactions, grain yield and thousand seed weight of 50 inbred lines tested during 2012 at Bako research centre in Ethiopia

No.	Inbred lines	Severity score (0-5)	Reaction type	Incidence %	Lesion length (cm)	Lesion width (cm)	TSW	Yield(t/ha)
1	CML 202	2.4	R	40.69	12	1.33	173	2.15
2	CML442	2.7	S	72.55	13.67	1.67	210	2.67
3	CML 312	2.5	I	64.22	12.33	0.83	220	3.25
4	CML 464	1.9	R	52.45	13	1.03	207	3.01
5	Gibe-1-91-1-1-1-1	2.5	S	74.02	20.33	1.5	260	3.76
6	CML 445	2.4	I	65.69	14.33	1.17	207	3.36
7	CML 443	2.9	S	64.71	13	1	183	1.93
8	Gibe-1-158-1-1-1-1	2.4	I	58.82	12	1.57	270	2.93
9	A7033	2.8	S	58.82	13.33	1.33	240	2.41
10	(CML 205/CML208//CML 202)-X2-1-2-B-B-B	2.6	S	86.76	22.33	1.83	237	5.83
11	CML395	2.3	I	70.59	21.67	2	313	5.04
12	CML 444	2.6	S	65.69	23.33	2	230	2.67
13	DE-78-Z-126-3-2-2-1-1	2.7	S	65.2	18.33	1.5	250	2.6
14	30H83-7-1-1-1-2-1	1.9	R	39.71	13.33	1.67	187	2.91
15	ILoo'E-1-9-1-1-1-1-1	2.1	I	54.41	23.67	1.33	293	4.62
16	SZSYNA-99-F2-81-4-3-1	1.7	R	27.94	14	1	257	2.01
17	X1264DW-1-2-1-1-1-1	2.4	I	72.55	19.33	1.33	183	1.92
18	124-b(113)	2.3	I	45.1	16.33	1.67	303	3.13
19	SC22	2.0	I	91.18	16.67	2	230	3.14
20	SC-715-121-1-3	3.0	S	70.1	16	2.17	270	2.64
21	DE-105-Z-126-30-1-2-2-1	1.5	R	69.61	20.67	1.83	230	3.42
22	Gibe-1-20-2-2-1-1	2.5	I	77.45	25	1.33	287	3.08
23	Kuleni-0080-4-2-1-1-1-1	4.3	I	58.33	20.33	1.33	283	3.8
24	Pool9A-4-4-1-1-1	2.4	I	62.25	25.67	1.67	270	5.35
25	30H83-5-1-4-2-1-1	2.5	I	67.16	22	2	260	4.39
26	Iloo'E-5-5-3-1	2.6	S	77.94	14.33	1	260	3.5
27	SZSYNA-99-F2-2-7-3-1-1	2.2	I	55.88	15	1.5	170	2.88
28	SC-715-154-1-1	2.0	I	73.53	15.67	1.83	217	5.01
29	BH6609(F2)-10-2-1-2-1	2.3	I	54.9	15.33	1.53	243	1.74

30	143-5-I	2.1	I	51.96	18	2.17	273	5.95
31	144-7-b	1.8	R	59.31	18	1	333	3.47
32	(LZ-955459/LZ955357)-B-1-B-B	2.3	I	67.65	16.67	1.33	200	2.72
33	139-5-j	2.0	I	44.12	19.33	1.07	237	1.8
34	30H83-56-1-1-1-1-1	2.2	I	50.98	13	0.83	203	2.93
35	SZSYNA-99-F2-80-3-4-1	2.8	S	76.47	27.67	1.83	237	3.38
36	124-b(109)	3.0	S	82.84	19.33	1.33	270	5.59
37	F7215	2.5	I	62.75	21.33	1.07	273	2.92
38	136-a	1.7	R	42.16	17.33	1.33	363	3.62
39	DE-78-Z-126-3-2-1-2-1	2.5	S	65.2	19	1	237	3.54
40	Gibe-1-186-2-2-1	2.8	S	49.02	19.33	1.33	360	2.3
41	Pool9A-128-5-1-1-1	2.7	S	68.36	15.33	1.67	223	2.87
42	30H83-7-1-5-1-1-1-1	1.9	R	50	16.67	2	193	2.84
43	SZSYNA-99-F2-3-6-2-1	2.3	I	63.24	15.33	2	233	2.36
44	SC-715-13-2-1	2.5	I	66.67	16.33	1.17	210	2.34
45	SC-22-430(63)	3.0	S	89.71	14.33	1.67	227	2.05
46	Kuleni-C1-101-1-1-1	2.8	S	68.63	7.67	1.17	237	3.07
47	Iloo'E-1-12-4-1-1	2.2	I	33.33	10.67	1.33	243	1.57
48	(DRB-F2-60-1-2)-B-1-B-B-B	2.7	S	67.65	21	2	230	2.48
49	142-1-e	1.8	R	49.51	16.33	1.5	287	4.29
50	CML 197	3.5	S	98.53	19.67	2.67	213	4.41
	LSD	0.396	-	19.159	9.013	0.902	47	1.3
	Pr>f	**	-	**	**	*	**	**
	CV (%)	10.1	-	18.8	32.1	36.9	11.9	24.7
	Overall mean	2.43	-	62.93	17.31	1.51	245	3.23

R= Resistant (1.0 – 2.0); I = Intermediate (2.0 – 2.5); Susceptible (2.5 - 5); TSW= Thousand seed weight

3.4.2 Lesion length

There were highly significant differences (p < 0.001) for lesion length among inbred lines tested in 2011 and 2012. Pool9A-4-4-1-1-1, SZSYNA-99-F2-80-3-4-1 and CML 197 had larger lesion length compared other inbred lines, whereas the lesion length of CML 202 and CML 312 was small and consistent in both years (Tables 3.2 and 3.3). Muriithi (1992) reported the presence of resistance to *E. turcicum* in maize germplasm. The author described reduced percent leaf area and small lesion size as manifestations of resistance to maize blight disease. The significant difference detected among genotypes in this study across years is attributable to factors affecting the development of leaf blight disease in maize. Among the factors are favorable climatic conditions, genotypic difference, inoculation method employed and proper disease rating. Levy (1991) indicated that for northern leaf blight development, pathogenic fitness and environmental conditions were the important factors.

3.4.3 Disease severity and AUDPC

Disease severity scores in both years were significantly different (p < 0.01) (Tables 3.2 and 3.3). During the two testing years, inbred lines with low severity score were CML 202, 144-7-b, and 142-1-e. Inbred lines with high severity score values suggesting their susceptibility were CML 197, Kuleni-C1-101-1-1-1, and SC-22-430(63) (Tables 3.2 and 3.3). Final severity score and AUDPC value provided adequate evaluation of the reaction of the testing inbred lines to *E. turcicum* (Table 3.2). AUDPC was significantly different (p≤0.001). Higher area under disease progress curves were recorded on susceptible than resistant lines (Table 3.2). In the case of the resistant inbred lines the severity of the disease was slightly increasing with time, as opposed to the susceptible ones, where the disease severity increase was high as time elapsed. This suggests that susceptible inbred lines showed higher severity scores towards maturity unlike resistant inbred lines (Figures 3.1 and 3.2).
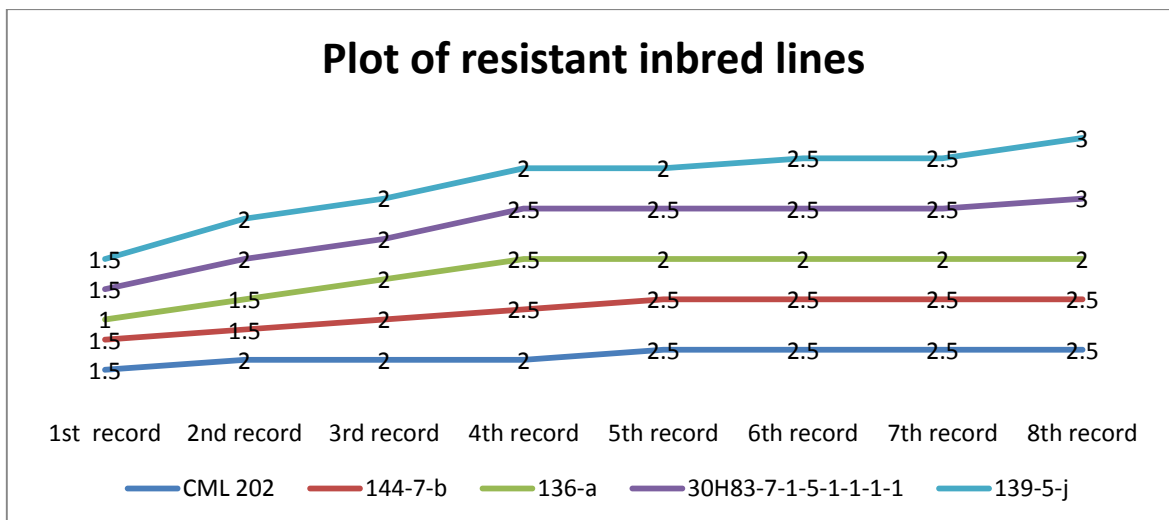
Figure 3.1 Disease severities of five selected resistant inbred lines against record time
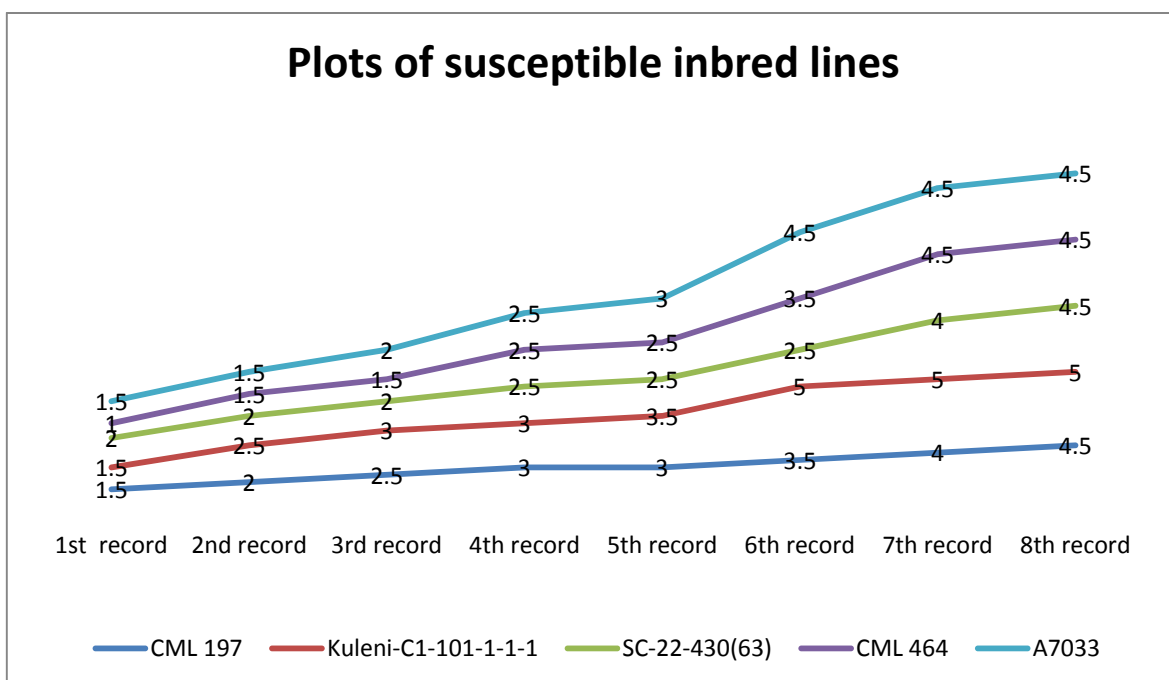
Figure 3.2 Disease severities of five selected susceptible inbred lines against record time

Considering the AUDPC values, inbred lines identified as susceptible including Kuleni-C1-101-1-1-1, Pool9A-4-4-1-1-1, and A7033 had extremely high records at 700, 671, and 642, respectively. Lines identified as resistant such as 136a and Gibe-1-186-2-2-1

had consistently lower AUDPC values of 238 and 350, respectively (Table 3.2). The severity of the disease was slightly higher in 2011 than 2012 (Tables 3.2 and 3.3). This might be associated with low rainfall at flowering in 2012, which created unfavorable conditions for blight development. However, the environmental conditions were generally favorable for northern leaf blight development during the two years (Table 3.4).

Previous studies (Muiru et al., 2007) showed that the dropper inoculation was efficient and eliminated chances of disease escape for blight evaluation. Likewise the present study found that this inoculation technique was easy to employ and reliable. Clear differences were notable between genotypes, as the lesions on resistant accessions remained at the point of inoculation and on the intermediate entries, size of lesion was relatively small. During flowering period, the rating remained relatively low and constant, but the susceptible genotypes still exhibited a moderate increase in diseased leaf tissue. Selection of less susceptible individual progenies can result in the accumulation of minor genes thereby increasing the level of resistance (Bowen and Pedersen, 1988; Ceballos et al., 1991; Ojulong et al., 1996; Pratt et al., 1997).

Table 3.4 Average monthly rainfall, temperature and relative humidity at Bako during 2011 and 2012

Month	2011			2012		
	Rainfall	Temperature (c^0)	RH (%)	Rainfall	Temperature	RH (%)
January	15.90	20.20	58.00	0.00	20.40	52.70
February	2.00	20.90	50.90	4.40	21.80	47.50
March	58.80	21.90	53.90	16.20	23.00	48.90
April	68.10	20.40	52.40	30.70	24.00	62.50
May	222.20	21.30	58.50	92.8	23.00	55.60
June	295.00	19.90	67.50	153.30	20.20	66.90
July	224.10	19.30	69.30	138.20	19.50	76.00
August	294.60	19.10	75.60	263.60	19.70	64.00
September	131.30	20.00	65.90	157.50	20.10	74.40
October	53.20	20.20	59.80	6.00	21.00	50.50
November	60.10	20.00	59.80	17.10	20.30	49.70
December	0.00	19.80	54.50	6.70	21.5	45.70
Total	1425.30			886.50		

RH = Relative humidity

3.4.4 Correlation analysis

The result of Pearson correlation analysis indicated significant and positive relationship ($p < 0.05$) between the disease parameters assessed during test years (Tables 3.5 and 3.6). However, non-significant and negative correlations were observed between AUDPC and thousand seed weight ($r=-0.26$, $p<0.09$), indicating that disease pressure reduced seed weight (Table 3.5).

Correlation of disease parameters with yield and seed weight was determined for the year 2011 and 2012 (Tables 3.5 and 3.6). There were significantly negative and non significant correlations between the disease assessment parameters and thousand seed weight in both years. Yield, had weak and non-significant positive correlations with the disease parameters (Tables 3.5 and 3.6) during both years. This indicated that all the parameters effectively measured the disease progress and had a negative effect on yield and seed weight.

Table 3.5 Pair-wise Pearson correlation matrixes among selected agronomic and disease parameters in 50 maize inbred tested for NCLB reactions, in 2011

	TSW	AUDPC	Incidence	Lesion length	Severity	Yield
TSW	1					
AUDPC	-0.26*	1				
Incidence	-0.05ns	0.54**	1			
Lesion length	0.31ns	0.36**	0.43**	1		
Severity	-0.08ns	0.67**	0.85**	0.41**	1	
Yield	0.51*	-0.09ns	0.09ns	0.34**	-0.13ns	1
	TSW	AUDPC	Incidence	Lesion length	Severity	Yield

TWS = Thousand seed weight, AUDPC = Area under disease progress curve

Table 3.6 Pair-wise Pearson correlation matrixes among selected agronomic and disease parameters in 50 maize inbred tested for NCLB reactions, in 2012

	TSW	Incidence	Lesion width	Lesion length	Severity	Yield
TSW	1					
Incidence	-0.14_{ns}	1				
Lesion width	-0.02_{ns}	0.38^{**}	1			
Lesion length	0.35_{ns}	0.32^{*}	0.34^{*}	1		
Severity	-0.16_{ns}	0.75^{**}	0.24_{ns}	0.14_{ns}	1	
Yield	0.30^{**}	0.32_{ns}	0.31_{ns}	0.40^{*}	-0.03_{ns}	1
	TSW	Incidence	Lesion width	Lesion length	Severity	Yield

TWS = Thousand seed weight

The minimum spanning tree (Figure 3.3) clustered the tested inbred lines using the four disease parameters in this study (AUDPC, disease severity, incidence and lesion length). Accordingly, inbred lines with similar reaction types were clustered on the same branch (Figure 3.3). Among the inbred lines, the most susceptible ones were CML-197 (entry 50), Kuleni-C1-101-1-1-1(46), and 124-b (109) (36) and they were allocated on the same branch. Consequently, these lines were considered susceptible by all the four disease parameters. Inbred line CML-197, a susceptible check in the trial, was allocated to this group. Resistant inbred lines such as CML202, and pool9A-128-5-1-1-1 were allocated on the same position in the tree (Figure 3.3). The resistant inbred lines will be used for the formation of hybrids to incorporate NCLB resistance.
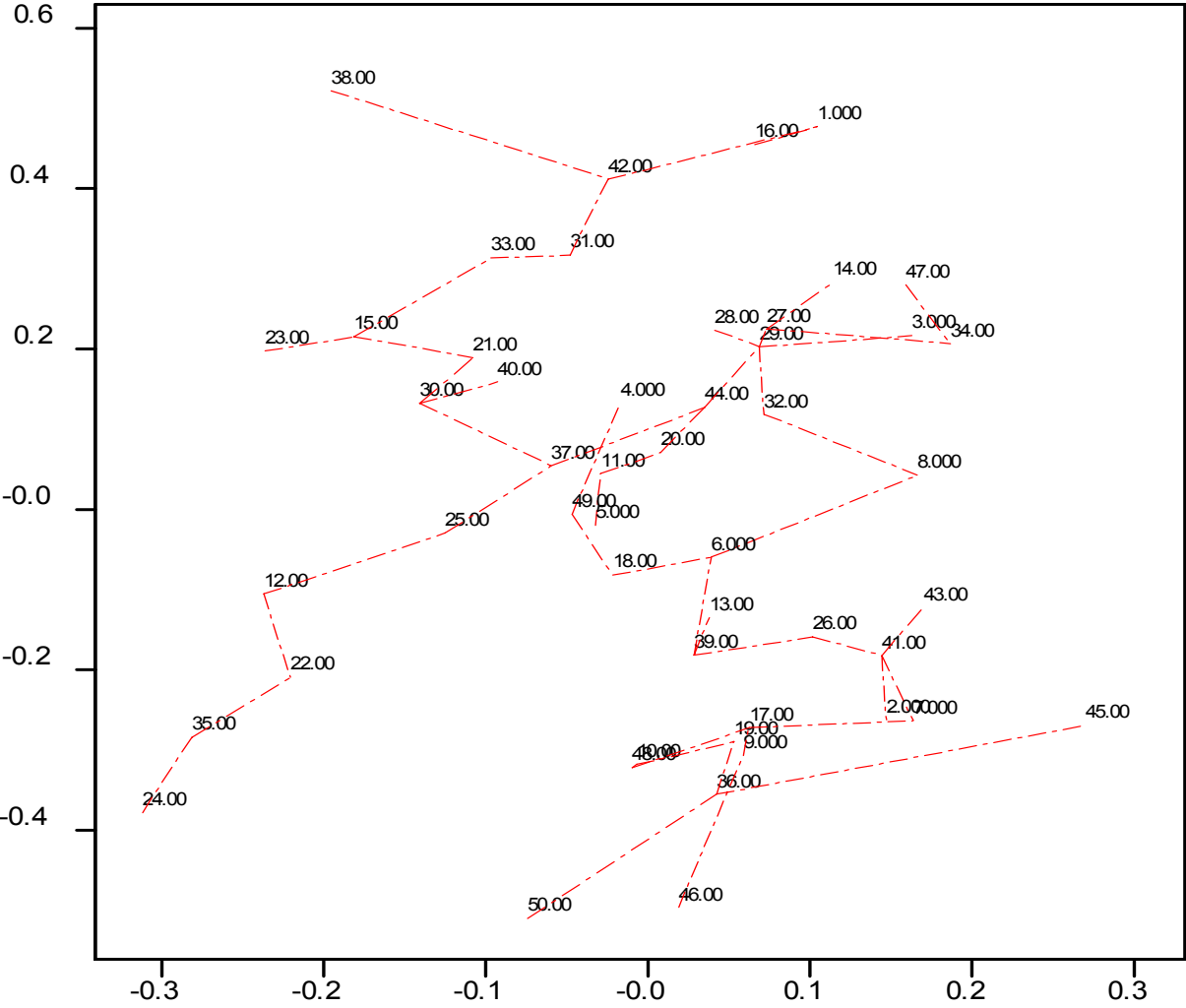
Figure 3.3 Clustering trees of 50 elite maize inbred lines for their reaction to NCLB using four disease parameters (AUDPC, disease severity, incidence and lesion length)

3.5 Conclusions

The resistant inbred lines reported here appear adapted and have potential to be used as source material in the breeding of disease resistant, high yielding and stable hybrids in the mid-altitude areas of Ethiopia and similar environment. Maize genotypes identified as resistant should be screened across locations and years or under controlled environments. The type of resistance, mechanism of resistance, and the location of the gene(s) for the resistance of these screened inbred lines remain unknown. The biotechnology methods currently available can be used to locate the gene (s) and incorporating it into cultivars with desired agronomic characteristics.

Progeny testing and selfing of individual plants derived from single ears of the resistant genotypes are being conducted to examine the inheritance of resistance to NCLB. From the current evaluation it appeared that there are potential losses incurred by northern leaf blight on maize yield. Therefore, it is justifiable for establishment of a resistance breeding program to develop varieties with increased adult plant resistance, which is the most effective and affordable way to overcome the problem of leaf diseases of maize in the mid-altitude agro-ecology of Ethiopia and similar environments in Africa.

As most of these lines identified as resistant have proven to have good combining ability, they can be considered as superior germplasm that provide disease resistance to NCLB in the mid-altitude sub-humid agro-ecologies of Ethiopia and similar areas in sub-Saharan Africa, without having a yield penalty associated with the resistance.

Due to large genotype-by-environment interactions associated with resistance, screening of introduced materials under various environments is worthwhile. Thus, the selected maize genotypes identified as resistant should also be screened across other locations and years under controlled conditions. Deployment of resistant lines in combination with appropriate production inputs and other integrated disease management practices including modern cultural practices are suggested strategies that could reduce inoculum pressure, especially for resource-constrained small scale farmers of east Africa.

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CHAPTER 4

Genetic interrelationships among medium to late maturing tropical maize inbred lines using selected SSR markers

4.1 Abstract

Understanding the genetic relationships among breeding lines is fundamental in crop improvement programs. The objectives of this study were to apply selected polymorphic single sequence repeat (SSR) DNA markers and cluster medium to late maturing tropical elite maize inbred lines for effective hybrid breeding. Twenty elite inbred lines were genotyped with 20 SSR markers. The analysis detected a total of 108 alleles. The unweighted pair group method with arithmetic mean allocated the inbred lines into five clusters consistent with the known pedigrees. The tested inbred lines that were adapted to mid-altitude, sub-humid agro-ecologies were classified in different clusters, except for a few discrepancies. The greatest genetic distance was identified between the clusters of lines CML-202 and Gibe-1-91-1-1-1-1. The analysis determined the genetic grouping present in the source population, which will assist in effective utilization of the lines in tropical hybrid maize breeding programs to exploit heterosis.

Keywords · genetic diversity; inbred lines; SSR markers; Tropics; *Zea mays*

4.2 Introduction

In Ethiopia, maize is the second largest food security crop after tef [*Eragrostis tef* (Zucc.) Trotter] in area coverage but it remains first in quantity of production. Maize has increasingly become popular in the country, with a steady growth in production area and total yield (Doss et al., 2003; CSA, 2011). The use of improved maize varieties has increased from 5% in 1997 to 20% in 2006 (Byerlee et al., 2007; Dawit et al., 2008). However, maize yields have remained low with an estimated national mean yield of 2.95 t ha^{-1} (CSA, 2011) due to several biotic, abiotic and socio-economic constraints. Maize productivity could be enhanced through effective breeding using locally adapted germplasm as well as through well-designed hybrid cultivar development program geared to exploit heterosis.

Genetic gain during selection is dependent on the presence of genotypes possessing favorable alleles for important traits, which in turn depends on the available genetic diversity (Fehr, 1987). Maize (*Zea mays* L.) breeding programs are heavily dependent on phenotypic evaluations of traits of economic importance (Smith et al., 1997). However, the presence of favorable alleles is difficult to detect among germplasm using phenotypic evaluations. Further, for effective breeding and management of genetic diversity, germplasm collections need to be well-characterized and genetic pools should be classified into distinguishable clusters based on genetic diversity. The genetic grouping of inbred lines will enable the breeder to predict the performance of maize hybrids to be developed from different intergroup crosses. The presence of discrete genetic groups among inbred lines is attributed to increased allelic diversity. This may provide a high degree of hetrozygosity in the hybrid associated with increased hybrid vigor. However, complementary combining ability tests on yield and yield components are required among the inbred lines to confirm the genetic group that may be generated through molecular data (Hallauer and Miranda, 1988; Xiao et al., 1996).

The efficiency of selection procedures determines the ability to identify promising inbred lines for the development of outstanding maize hybrids (Hallauer and Lopez-Perez, 1979; Hallauer, 1990). The best hybrid combinations can be identified using information derived from a variety of methods such as genetic analysis with mating designs (Han et al., 1991;

Gonzalez et al., 1997; Terron et al., 1997), pedigree analysis and molecular markers (Smith et al., 1997). Each method of analysis has its own advantages and disadvantages. Marker-assisted selection (MAS) and DNA fingerprinting techniques have been effectively used to increase the efficiency of conventional breeding by reducing the time taken in varietal development (Welz and Geigerb, 2002).

Assigning elite inbred lines into well-differentiated genetic clusters can reduce the creation and evaluation of many undesirable crosses (Terron et al., 1997). Molecular markers assist in characterizing inbred lines and in establishing distinct cluster of genotypes based on genetic diversity, which is useful in maize breeding programs (Melchinger and Gumber, 1998; Reif et al., 2003). Previous studies successfully applied molecular markers to allocate maize germplasm into heterotic groups (Lee et al., 1989; Livini et al., 1992; Dubreuil et al., 1996). Pejic et al. (1998) compared different markers for their effectiveness in estimating genetic grouping among maize inbred lines. The authors showed that SSR markers revealed the highest level of polymorphism due to their co-dominant nature and high numbers of alleles per locus. The SSR are powerful markers with which to discriminate between inbred lines, including those related by pedigree. These markers are relatively inexpensive, easy to use and can be automated to allow for gene mapping to specific chromosome location (Senior and Heun, 1993; Konstantinov and Drinic, 2000).

Comparative studies of maize inbred lines using different molecular markers were mostly carried out on germplasm from temperate regions (Smith et al., 1997; Pejic et al., 1998; Melchinger, 1999). Genetic analysis of tropical maize germplasm using molecular markers is scanty (Yuan et al., 2000; Barbosa et al., 2003). In Ethiopia maize inbred lines are primarily selected based on maturity groups which range from early to late but the majority of production areas are planted with medium to late maturing varieties. The maize breeding program in the country establish the genetic relationships of inbred lines through phenotypic characterization. As such there have been no prior studies on genetic clustering of mid-altitude elite maize inbred lines using molecular data. Previous studies focused on genetic diversity analysis of the highland maize germplasm that are not adapted to the major maize growing areas of the country which are predominantly found in the mid-altitude sub-humid

agro-ecologies (Legesse et al., 2007). Consequently, it is important to develop systematic genetic groupings using novel and elite medium to late maturing maize inbred lines of diverse geographical origin with well-adaptation to the mid-altitude sub-humid agro-ecologies. This will assist in developing varieties in a reduced timeline in Ethiopia and other tropical maize breeding program. The efficiency of genetic classification of inbred lines based on the genetic distances estimates depends on the availability of polymorphic SSR markers, among others.   Therefore, the objective of this study was to apply selected polymorphic single sequence repeat (SSR) DNA markers and cluster medium to late maturing tropical elite maize inbred lines for effective hybrid breeding.

4.3 Material and Methods
4.3.1 Plant materials

The study used twenty maize inbred-lines of which eight were obtained from the international maize and wheat improvement center (CIMMYT) developed for the mid-altitude and sub-humid agro-ecologies. The remaining lines, also well adapted to mid-altitude were developed by the Ethiopian maize research program (Bako) (Table 4.1). The local inbred lines were developed from three commonly used heterotic groups in the country that include: 1) Kitale synthetic II, 2) Ecuador 573 and 3) Pool 9A. Lines were selected for their vigor, disease resistance and general adaptability to the mid-altitude areas of the country. These inbred lines are widely used by maize researchers for the development of hybrids and composite maize varieties in the country

Table 4.1 Description of maize inbred lines used in the study

Entry	Pedigree	Origin
1	CML 202	CIMMYT
2	CML 442	CIMMYT
3	CML 312	CIMMYT
4	CML 464	CIMMYT
5	Gibe-1-91-1-1-1-1	BAKO
6	CML 445	CIMMYT
7	CML 443	CIMMYT
8	CML 197	CIMMYT
9	A-7033	BAKO
10	CML 205/208//202-X-2-1-2-B-B-B	BAKO
11	CML 395	CIMMYT
12	F-7215	BAKO
13	DE-78-Z-126-3-5-5-1-1	BAKO
14	30H83-7-1-1-1-2-1	BAKO
15	I100E-1-9-1-1-1-1-1	BAKO
16	SZYNA99F2-81-4-3-1	BAKO
17	X1264DW-1-2-1-1-1	BAKO
18	124-b (113)	BAKO
19	SC22	BAKO
20	SC715-121-1-3	BAKO

4.3.2 DNA sampling

All the plants were tagged individually before sampling. DNA collection was done on three to four week old plants, using Whatman FTA cards. The sampling protocol was done according to the modified protocols of FTA paper technology (Mbogori et al., 2006). FTA® classic card (Whatman Inc., Clifton, NJ) is a Whatman paper that has been impregnated with a patented chemical formulation that lyses cells, then captures and immobilizes nucleic acids in the paper matrix. In addition, they contain compounds for denaturing, chelating and trapping free radicals which prevent damage of the nucleic acids (http://www.whatman.com). One FTA classic card measures 750 x 130 mm and each was labelled prior to the day of sampling. For sampling, the second or third leaf was excised from the plant, wrapped round the FTA paper strip, and placed in a small polythene bag. A pair of pliers was used to press the leaf sample extract on to the FTA paper until both sides were soaked. Adequate quantities of sap were collected onto FTA cards by placing the plant samples directly onto the FTA paper and applying moderate pounding/pressure with a pestle (Lange et al., 1998; Lin et al., 2000). Ethanol (70%) was used to clean the pliers between samples to prevent cross contamination. The FTA card was then hung on a drying line using a paper clip for air drying at room temperature for 2 to 5 h. Later samples were stored in an air tight plastic container. Ten samples from each of the 20 entries were taken and their bulks were utilized for the analysis.

4.3.3 SSR analysis

Samples on FTA cards from the twenty inbred lines (10 samples per entry) were analysed at the INCOTEC-PROTEIOS laboratory in South Africa (Incotec, SA Pty. Ltd. South Africa). All samples were used in bulked amplification, using DNA from 20 individual leaf samples. Samples were bulked to rule out variation within entry. A single punch of each card per submission was taken and homogenized in the Finnzymes dilution buffer (Kit). 2uL of each of bulked sample was used in the polymerase chain reaction (PCR).

Twenty SSR markers were used in this study (Table 4.2). The primer sequences used for PCR amplification were selected from the Maize GDB database (http://www.agron.missouri.edu). The microsatellite loci used were chosen based on the size of the repetitions and their location, to obtain a representative sampling of the whole genome (Table 4.2). PCR products were fluorescently labelled and separated by capillary electrophoresis on an ABI 3130 automatic sequencer (Applied Biosystems, Johannesburg, South Africa). Analysis was performed using GeneMapper 4.1. The positions of the primers were obtained from maize GDB website, and synthesized by Sigma Genosis. PCR was done for all of the 20 primers (Table 4.2). Euclidian distances were calculated between bulked samples, using the program GGT 2.0 (van Berloo, 2007). The data matrices of the genetic distances were used to create the dendrogram using the unweighted pair group method with arithmetic mean allocated (UPGMA). The assay efficiency index referred to as polymorphism information content (PIC) was calculated using the following: PIC = 1-$\sum$fi, where fi is the frequency of the ith allele (Smith et al., 1997).

Table 4.2 Details of the 20 SSR loci used in this study: repeat type, bin location (Bin no.), number of alleles, PIC values and heterozygosity (He) when testing 20 maize inbred lines

SSR locus	Repeat types	Bin no.	No. alleles	PIC value	He
Umc1568	TCG	1.02	6	0.6833	0.7250
Bnlg176	___	1.03	4	0.3092	0.3378
Bnlg182	___	1.03	6	0.5510	0.5888
Phi 037	AG	1.08	1	0.0000	0.0000
Bnlg 108	___	2.04	4	0.4253	0.4637
Nc003	AG	2.06	6	0.7429	0.7778
Umc2214	CTT	2.1	8	0.7075	0.7350
Bnlg602	___	3.04	6	0.4701	0.4900
Umc2038	GAC	4.06	4	0.6311	0.6925
Phi085	AACGC	5.06	4	0.6695	0.7222
Umc1153	TCA	5.09	8	0.6683	0.7036
Bnlg238	___	6	8	0.7689	0.7922
Umc1296	GGT	6.07	1	0.0000	0.0000
Phil015	AAAC	8.08	7	0.5112	0.5938
Umc1367	CTG	9.05	2	0.4949	0.5850
Phi054	AG	10.03	6	0.8028	0.8255
Umc1677	GGC	10.05	7	0.3047	0.3750
Bnlg2190	AG	10.06	11	0.8224	0.8395
Bnlg240		8.06	7	0.7777	0.8025
umc2361	CCT	8.06	2	0.3743	0.4986

4.4 Results and Discussion

4.4.1 Genetic polymorphisms

The twenty SSR primers identified 108 alleles among the 20 maize inbred lines. The number of alleles scored across SSR loci ranged from 1 to 11, with a mean of 5.4 alleles (Table 4.2). A number of loci (Phi 037, Umc1296) revealed one allele, and the maximum numbers of alleles were detected at the Bnlg 2190, Umc2214 and Umc1153 loci. The PIC estimated for all loci ranged from 0.0000 to 0.8028 with a mean of 0.54 (Table 4.2). Expected heterozygosity (He) values, as a measure of allelic diversity at a locus, varied from 0.0000 to 0.8395 with an average of 0.5774. These values were well-correlated with the number of alleles (Table 4.2). Ten SSR loci (Umc1568, Nc003, Umc2214, Umc2038, Phi085, Umc1153, Bnlg238, Phi054, Bnlg2190, and Bnlg240) manifested a PIC value of more than 0.6, reflecting their potential to detect differences between the inbred lines.

The genetic diversity of the study material is the most important factor limiting the number of alleles identified per micro satellite locus during screening. However, other factors such as the number of SSR loci and repeat types, and the methodologies employed for the detection of polymorphic markers, have been reported to influence allelic differences. In this study, the mean number of alleles (5.4) was similar to those reported in maize. Warburton et al. (2002), using 85 SSR loci found 4.9 alleles, whereas Vaz Patto et al. (2004) detected 5.3 alleles with 80 SSR loci. With regard to the SSR loci used in this study the values closely agree with the findings reported by Legesse et al. (2007) and Pinto et al. (2003), who reported 3.85 and 4.16 alleles using 27 and 30 polymorphic SSR loci, respectively. The mean PIC value determined in the present investigation agrees well with earlier research on the use of SSR markers on maize inbred lines (Senior et al., 1998; Heckenberger et al., 2002). PIC demonstrates the usefulness of the SSR loci and their potential to detect differences among the inbred lines based on their genetic relationships. The dinucleotide SSR loci (phi054, nc003, bnlg2190) identified the largest mean number of alleles (7.67) and mean PIC (0.79), as compared to tri-, tetra- and penta-nucleotide repeats in this study, which is also in close agreement with previous observations in maize (Smith et al., 1997; Senior et al., 1998; Enoki et al., 2002; Legesse et al., 2007).

In this study, automated analysis was used for screening the microsatellites, resolving allelic variation better than the analysis of gel electrophoresis. This may be particularly important for SSR loci containing dinucleotide repeats whose amplification products are in the ranges of 130 to 200 bp, because PCR products differing by two base pairs cannot be resolved with agarose gel electrophoresis (Senior et al., 1998; Sibov et al., 2003). The mean PIC value determined in our investigation agrees well with the earlier findings based on SSR markers in maize inbred lines (Senior et al., 1998; Heckenberger et al., 2002; Vaz Patto et al., 2004).

The ability to measure genetic distances between the inbred lines that reflect pedigree relationship ensures a more stringent evaluation of the adequacy of marker profile data. The fact that the minimum genetic distance was revealed between CML-202 and I100E-1-9-1-1-1-1-1 (0.28) was a good indication confirming the power of SSR markers to distinguish closely related inbred lines (Table 4.3). Similar findings have been reported for maize inbred lines using SSR markers (Boppenmeier et al., 1992; Lanza et al., 1997; Smith et al., 1997; Li et al., 2002; Legesse et al., 2007).

Table 4.3 The matrix of Euclidian genetic distances among 20 maize inbred lines analyzed using 20 SSR markers

Line	CML-202	CML 442	CML 312	CML 464	Gribe-1-91-1-1-1	CML 445	CML 443	CML 197	A-7033	CML 205/208//202-X-2-1-2-B-B-B	CML 395	F-7215	DE-78-Z-126-3-5-5-1-1	30H83-7-1-1-1-2-1	I100E-1-9-1-1-1-1-1	SZYNA99F2-81-4-3-1	X1264DW-1-2-1-1-1	124-b (113)	SC22
CML 442	5.9																		
CML 312	5.0	4.8																	
CML 464	5.8	4.8	4.5																
Gribe-1-91-1-1-1	6.6	3.9	5.9	5.8															
CML 445	5.6	5.9	5.6	4.4	5.3														
CML 443	5.9	5.4	4.3	5.5	5.1	5.7													
CML 197	5.2	5.7	4.3	4.7	6.3	5.9	3.2												
A-7033	6.4	4.8	5.3	5.5	4.7	5.2	4.6	4.8											
CML 205/208//202-X-2-1-2	4.8	5.1	5.5	5.4	5.6	5.7	5.7	5.4	6.3										
CML 395	4.3	6.4	4.7	5.1	5.8	4.6	4.7	4.6	5.2	5.5									
F-7215	4.5	5.6	5.0	4.8	6.5	5.2	4.9	4.4	5	5.1	4.2								
DE-78-Z-126-3-5-5-1-1	5.8	4.8	6.3	5.3	4.8	5.5	4.8	4.7	4.3	4.5	5.7	5.2							
30H83-7-1-1-1-2-1	4.7	5.8	3.7	4.2	5.9	5.3	5.2	4.6	5.5	4.9	2.7	4.6	6.2						
I100E-1-9-1-1-1-1-1	2.8	5.7	4.9	5.3	5.9	5.2	4.8	3.8	5.4	4.7	3.8	4.8	4.8	4.3					
SZYNA99F2-81-4-3-1	6.0	3.9	4.4	4.7	4.3	5.1	4.2	5.2	3.7	5.1	5.7	4.9	4.8	5.2	5.9				
X1264DW-1-2-1-1-1	3.9	5.7	4.8	6.1	5.1	5.2	4.0	5.0	5.5	5.2	4.6	5.2	5.3	5.2	3.6	4.6			
124-b (113)	4.2	5.2	5.3	5.7	4.1	4.9	4.6	5.2	4.6	5.2	4.8	5.5	4.5	5.5	4.0	4.2	3.3		
SC22	6.0	5.7	5.3	4.5	5.4	4.3	4.8	3.8	3.7	6.1	4.2	4.8	4.7	4.8	5.1	5.1	6.0	5.2	
SC715-121-1-3	4.1	4.6	5.3	5.7	5	5.5	5.5	5.4	5.2	4.9	5.2	5.1	4.0	5.6	4.3	5.1	4.2	3.9	5.2

4.4.2 Cluster analysis

The dendrogram constructed using the UPGMA clustering algorithm based on SSR data matrices grouped the inbred lines into five categories (Figure 4.1). This information will be used together with their pedigree records and combining ability analysis, in order to identify optimal crosses and to separate inbred lines into different heterotic groups. The closest distance was found between the clusters of CML-202 and IL00E-1-9-1-1-1-1-1 lines, and the greatest distance was found between the cluster of CML-202 lines and the cluster of Gibe-1-91-1-1-1-1 lines. Cluster I consisted of mid- altitude adapted inbred lines, together with lines from CIMMYT. Most of the mid-altitude inbred lines in this group originated from the heterotic group Kitale Synthetic II and constitute the largest group in the cluster. In Cluster II, CIMMYT inbred lines CML312 and CML395 were grouped along with two local inbred lines, with two sub-divisions in the main group. Cluster III contained two major sub-groups, one containing CIMMYT inbred lines and the other, local inbred lines. Cluster IV included two CIMMYT inbred lines (CML-464 and CML-445). These inbred lines are very close in their pedigree information and they are grouped under heterotic group AB, confirming the presence of relationship between the pedigree and the SSR marker groupings in this study. In another cluster, two CIMMYT inbred lines (CML-443 and CML-197) were grouped closely, as revealed on the UPGMA dendrogram (Figure 4.1). These two inbred lines were also grouped in the same heterotic groups A and AB, based on their heterosis. Cluster V consisted of one CIMMYT inbred line and two locally adapted mid-altitude inbred lines.

Majority of the tested inbred lines (60%) were previously developed by the Bako maize breeding program in Ethiopia. Genetic admixtures or incomplete pedigree records may be encountered in breeding programs. As a result, discrepancies in classification of germplasm may occur when comparing molecular results with classification based on pedigree relatedness. The effects of selection, genetic drift and mutation may contribute to these discrepancies. The technique of clustering

inbred lines can create apparent discrepancies, when one inbred line that is related to two inbred lines from separate clusters is then grouped with the inbred to which it is most closely related (Ajmone-Marsan et al., 1998; Senior et al., 1998).
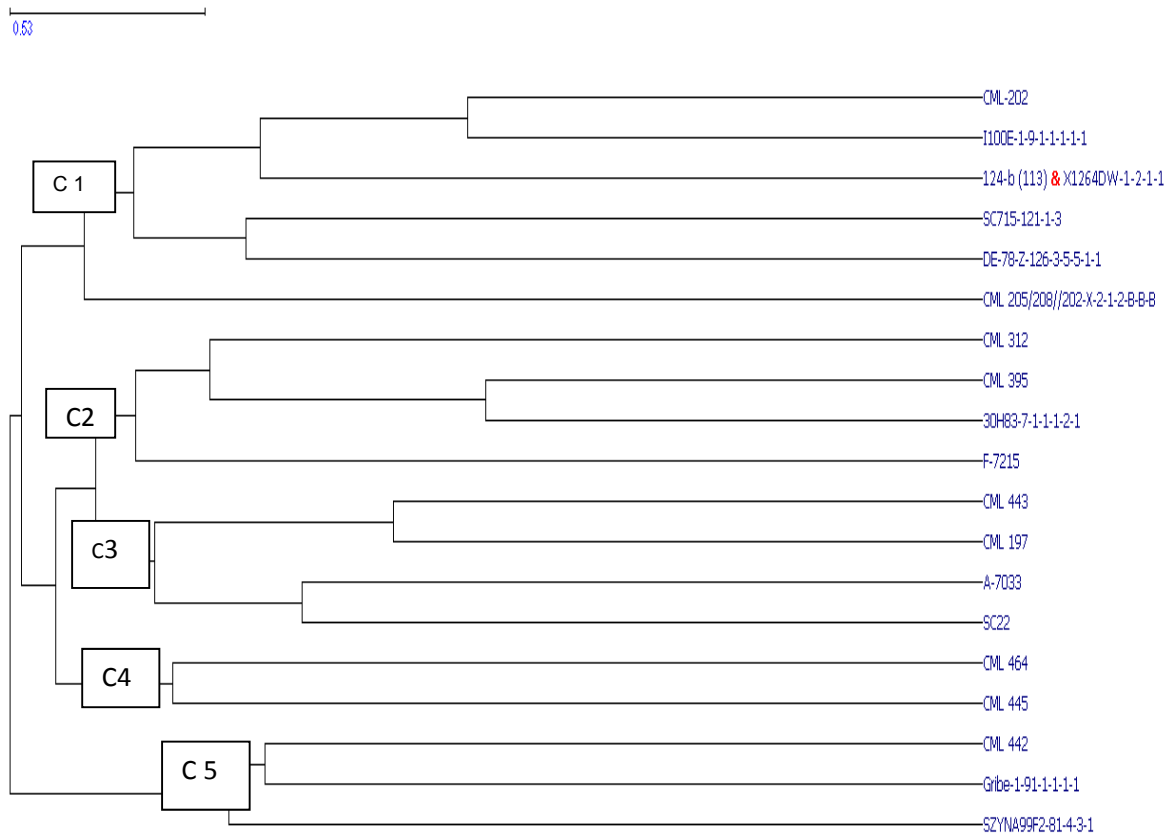
Figure 4.1 Dendrogram showing genetic relationship among 20 maize inbred lines tested using 20 SSR markers. The five clusters among the inbred lines are denoted from C1 to C5

4.5 Conclusions

SSR markers separated most of the inbred lines into five distinguishable clusters, which generally agreed with the existing pedigree records. This result agrees with the previous findings on maize (Pejic et al., 1998; Enoki et al., 2002; Legesse et al., 2007) who used SSR markers to group maize inbred lines. The high PIC value across all loci is the most relevant evidence confirming the potential for SSR markers to discriminate between inbred lines of diverse sources and even between closely related genotypes. A number of loci have been identified with high PIC values, indicating their usefulness for diversity analysis of maize inbred lines under consideration. This study effectively differentiated and allocated the inbred lines into distinct groups based on genetic distance estimates generated through selected polymorphic SSR primers. The separation of elite mid-altitude maize inbred lines into genetically distinct groups may be associated with high heterotic response and increased combining ability useful for hybrid development. The genetic grouping is valuable information for tropical maize breeding programs in Africa.

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CHAPTER 5

Heterosis and combining ability of elite maize inbred lines for grain yield potential and reaction to Northern Corn Leaf Blight in the mid-altitude sub-humid agro-ecologies

5.1 Abstract

Yields of maize (*Zea mays* L.) are remarkably low in sub-Saharan Africa and farmers have limited access to improved varieties. Hybrid maize varieties are known for their enhanced productivity due to heterosis. The objective of this study was to determine combining ability and heterosis among 18 elite maize inbred lines and their hybrids in diverse tropical mid-altitude environments (1000 – 1800 masl). Nine elite inbred lines were crossed as females with nine male lines using the North Carolina design II mating scheme and 81 hybrids were generated. The hybrids, parents and three standard check varieties were evaluated using the alpha lattice design with two replications across seven environments in the mid-altitude sub-humid agro-ecologies in Ethiopia. Results indicated significant ($P \leq 0.05$) differences among genotypes for grain yield, Northern Corn Leaf Blight (NCLB) resistance and yield related traits. The new hybrids were predominantly holding the top 20 ranks for grain yield, and displayed up to 250% high parent heterosis (HPH) and mid-parent heterosis (MPH). Similarly, -14% and -25% high parent and mid-parent heterosis were recorded for NCLB, respectively. General combining ability (GCA) and specific combining ability (SCA) effects were significant ($P \leq 0.05$) for most traits, indicating the significance of both additive and non-additive gene effects, respectively. The SCA x site interaction was not significant as a result the top yielding hybrids were generally stable across environments. Inbred lines such as CML 395, 30H83-7-1, ILO'OE-1-9, 124-b (113), CML202, CML312, and Gibe-1-91 were selected as promising parents and resulted significant SCA effects for grain yield. Lines CML395 and ILO'OE-1-9 had negative and significant GCA effects for NCLB resistance. Best performing hybrids such as CML-312 x CML395, CML197 x CML395, CML443 x DE-78-Z-126-3 were identified with average grain yield of > 8 t ha^{-1} and high specific combining ability. The new hybrids can be used for direct production or as testers in hybrid development.

Keywords: general combining ability; heterosis; hybrid; maize; specific combining ability

5.2 Introduction

Maize (*Zea mays* L.) is one of the world's leading cereal crops. It is the major food security crop in sub-Saharan Africa, where the rapidly growing population is needing more supplies. In Ethiopia, maize was grown on over 2 million hectares, resulting in total annual grain production of 6.5 million tons with an average yield of 3 t ha^{-1} during 2012 (CSA, 2012). It is a primary crop in the majority of farming systems and a staple food for the rural population predominately grown in the mid-altitude sub-humid agro-ecology of the country. Of all cereal crops cultivated in Ethiopia, maize stands first in total production and productivity and second in area coverage after tef [*Eragrostis tef* (Zucc.) Trotter] (CSA, 2012).

Hybrid breeding has been highly effective in increasing maize yields globally (Banziger and Cooper, 2001). The world average maize yield is 5.1 t ha^{-1} and that of USA 10.3 t ha^{-1}, Germany (9.7 t ha^{-1}), Canada (8.4 t/ha), and South Africa (4.96 t ha^{-1}) (FAOSTAT, 2010). In Ethiopia, the national average grain yield increased from about 1.6 t ha^{-1} in 1990 (Worku et al, 2001) to 3 t ha^{-1} in 2012 (CSA, 2012). Increased yields are in part due to improved agronomic practices and increased inputs. However, increased yields can be significantly realized through genetic improvements. Thus strategic maize breeding is required to develop hybrid varieties with increased yield and disease resistance which are well adapting to the different maize agro-ecologies of the country.

Development and identification of inbred parents to form superior hybrids is the most costly and time-consuming phase in maize hybrid development. *Per se* performance of maize inbred lines does not predict the performance of maize hybrids for grain yield (Hallauer and Miranda, 1988). Heterosis or hybrid vigor is an important predictor of the hybrid value in hybrid breeding programs. Adequate genetic distance or genetic divergence between parents plays a crucial role in the expression of heterosis. When heterosis or some of its components are significant for economic traits, it may be concluded that there is genetic divergence among the chosen parents. Information on the genetic diversity and distance among the breeding lines, and the correlation

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between the genetic distance and hybrid performance are important for determining breeding strategies, classifying the parental lines, defining heterosis, and predicting hybrid performance (Xingming et al., 2001; Legesse et al., 2009).

The combining ability of inbred lines is critical and determines their potential value in hybrid or synthetic variety development to enhance yield and stress tolerance. Crosses between inbred lines from different heterotic groups result in vigorous F_1 hybrids than lines sampled from similar heterotic groups. Equally important is the nature of gene action involved in expression of both quantitative and qualitative traits of economic importance. Falconer (1989) observed that general combining ability (GCA) is directly related to the breeding value of the parent and is associated with additive genetic effects, while specific combining ability (SCA) is associated with non-additive genetic effects predominantly contributed by dominance, or epistatic or genotype x environment interaction effects (Rojas and Sprague, 1952).

In maize hybrid programs the GCA and SCA effects are important indicators of the potential value of inbred lines and hybrids, respectively. The use of selected inbred lines sampled from unrelated heterotic groups aided by combing ability tests would result in the development of high yielding hybrids. Testers of hybrid value or heterosis between parental inbred lines can increase the efficiency of hybrid breeding programs (Hallauer and Miranda, 1988). The North Carolina mating design II (factorial mating design) is one of the most widely used genetic designs in maize breeding programs to identify best parents for hybrid formation, identify superior hybrids, and to assign lines to new heterotic groups (Hallauer and Miranda, 1988).

Biotic and abiotic stresses are the major constraints to maize production. Among the biotic factors, the northern corn leaf blight (NCLB) disease of maize caused by *Exserohilum turcicum* Pass Leonard & Suggs is the number one leaf disease causing considerable yield loss in maize in Ethiopia (Wende et al., 2013). As a result there is a continued need to identify new sources of resistance to develop resistant hybrids using

the available breeder's genetic stocks and introduced germplasm to enhance maize productivity (Girma et al., 2008).

Various studies were conducted and estimated combining ability in maize using commercial inbred lines (Dagne, 2002; Hadji, 2004; Berhanu, 2009; Dagne et al., 2010; Zeleke and Tuna, 2010; Tolera, 2013) in Ethiopia. In an attempt to develop promising maize hybrids with increased yield and NCLB resistance the Bako research program in Ethiopia developed elite maize inbred lines adapted to tropical mid-altitude environments. Consequently, detailed information on the combining ability and heterosis of the newly developed inbred lines needs to be determined for hybrid breeding. Therefore, the objective of this study was to determine the combining ability and heterosis among 18 elite maize inbred lines and their hybrids in diverse tropical mid-altitude environments in Ethiopia. The study may assist to identify promising hybrids for direct production or further breeding.

5.3 Materials and methods
5.3.1 Plant materials and mating design

The study used 81 single cross hybrids generated from crosses of two sets of parents consisting of nine female and nine male inbred lines using the North Carolina design II or factorial design. The 18 parents are adapted to the mid-altitude agro-ecology which are elite maize inbred lines developed by the Bako national maize research project in Ethiopia and CIMMYT. The details of the lines are summarized in Table 5.1. The inbred lines used in the crosses were selected from previous evaluation studies. The lines are adapted to mid-altitude and sub-humid agro-ecologies, possess a considerable level of resistance to NCLB, and have great *per se* performance. Further, the lines were selected from varied heterotic groups and they have resistant, susceptible and moderately resistant background. Three locally adapted hybrids (BH540, BH543 and BHQPY545) were included as comparative controls.

Table 5.1 List of parental inbred lines used in the study

Serial Number	Pedigree	Origin	Role in cross	Reaction type
1	CML395	CIMMYT	F1	Resistant
2	F7215	Bako	F2	Susceptible
3	DE-78-Z-126-3-2-2-1-1	Bako	F3	MR
4	30H83-7-1-5-1-1-1-2-1	Bako	F4	Resistant
5	ILOO'E-1-9-1-1-1-1-1	Bako	F5	MR
6	SZSYNA99F2-81-4-3-1	Bako	F6	Susceptible
7	X1264DW-1-2-2-2-2	Bako	F7	Susceptible
8	124-b(113)	Bako	F8	MR
9	SC22	Bako	F9	Susceptible
10	CML202	CIMMYT	M1	Resistant
11	CML442	CIMMYT	M2	MR
12	CML312	CIMMYT	M3	Resistant
13	CML464	CIMMYT	M4	MR
14	Gibe-1-91-1-1-1-1	Bako	M5	MR
15	CML445	CIMMYT	M6	Resistant
16	CML443	CIMMYT	M7	Resistant
17	CML197	CIMMYT	M8	Susceptible
18	A-7033	Bako	M9	Susceptible

*F and M denote female and male parents, respectively; MR = moderately resistant

5.3.2 Description of experimental sites

Eighty one experimental and three check hybrids were evaluated at seven locations (Table 5.2) in the mid-altitude sub-humid agro ecologies: namely; Bako, Jimma, Finote Selam, Areka, Arsi Negelle, Ambo, and Asosa Agricultural Research Centers in Ethiopia during the main cropping season of 2012. All the locations are among the principal maize evaluation sites in Ethiopia and believed to represent the maize growing regions of the country.

Table 5.2 Descriptions of the 7 locations used for hybrid testing

Site	Site code	Region	Latitude (^{0} north)	Longitude (^{0} east)	Altitude	Annual rainfall (mm)	Temperature	
							Max	Min
Bako	E1	Oromia	9.12	37.08	1,650	1,211	27.9	12.9
Jima	E2	Oromia	7.67	36.83	1,725	1,448	27.2	11.4
Arsi Negele	E4	Oromia	7.19	38.39	1,940	900	25.3	11.2
Areka	E5	SNNPS	7.07	37.68	1,750	1,401	25.8	12.5
Asosa	E6	Beni Shangul	10.07	34.52	1,560	1,247	27.8	14.4
Ambo	E8	Oromia	8.57	38.07	2,225	1,115	25.4	11.7
Finote Selam	E10	Amhara	10.40	37.16	1,853	1,125	25.5	11.2

SNNPS = Southern nations and nationalities people state

5.3.3 Experimental design and data collection

The hybrids were established using a 7 x 12 alpha lattice design with two replications at each location. Each plot consisted of one row with 5.1 m in length. The spacing between rows was 75 cm, while spacing between plants was 30 cm. Two seeds per hill were sown and later thinned to one to achieve the desired plant density of 44,444 ha^{-1}. Trials received 100 kg ha^{-1} P_2O_5 at planting and 100 kg ha^{-1} N in two splits (at planting and knee height). The trials were conducted under rain-fed condition and other management practices were followed according to the recommendations of the specific areas.

Data collected included grain yield (t ha^{-1}), yield related traits and NCLB resistance. Grain weight from all the ears of each experimental plot was measured and used to calculate grain yield at 12.5% moisture level. The NCLB reaction was recorded using a 1-5 visual scale, where 1 indicated highly resistant and 5 a highly susceptible reaction to the disease; the plant aspect (PASP) were rated using a scale of 1 – 5, where 1 is very good and 5 is bad. Ear height (EHT) was measured from the ground level to the upper most ear-bearing node and expressed in centimeters. Likewise plant height (PHT) was determined from the soil surface to the first tassel branch and expressed in centimetres. NCLB, PASP, EHT and PHT were determined from ten randomly selected and tagged plants from each experimental unit. The measurements on plant and ear height were made two weeks after pollen shedding ceased. A pre-emergence herbicide,

Primagram–Gold 660 SC was applied at the rate of three litres per hectare (3 l ha^{-1}) to control weeds.

5.3.4 Data analysis

The data collected were analyzed using the lattice procedure of SAS statistical package (SAS, 2002). Accordingly, significance tests were conducted in each and across locations using the ANOVA. For the analysis of variance tests entries were used as fixed factor while location, replication and incomplete blocks within replication were considered as random factors.

5.3.5 Estimation of combining ability effects

Both GCA and SCA effects were estimated from inbred parents and crosses, respectively. The standard checks were excluded while analyzing combining abilities. The GCA effects of females and males, the SCA effect of crosses, and their interactions with the environment were determined following the factorial mating design and using the following model:

Yijk = μ + *gi* + *gj* + *Sij* + *ek* + (*ge*)ik + (*ge*)jk + (*se*)ijk

Where *Yijk*= the performance of the hybrid, made with *ith male* and *jth female*, in the *kth* location, μ= the overall mean, gi= the effect of the i*th* male, gj= the effect of the jth female, sij= the interaction of the ith male with the jth female, ek= the effect of the kth environment, (ge)ik= the interaction of the gi and ek, (ge)jk= the interaction of the gj and ek, (se)ijk= the interaction of sij and ek.

According to Hallauer and Miranda (1988), male and female main effects represent two independent estimates of GCA, which are designated GCA$_M$ + GCA$_F$. The F x M is equivalent to SCA effect.

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The significance of GCA mean squares of males [GCA (M)] and that of the females [GCA (F)] in each location was determined using the M x F interaction as the error term while the significance of the M x F interaction (SCA) was determined using the error mean square as error term. Since the combining ability mean squares were calculated based on cross means of each genotype from each location, error mean squares calculated for crosses above were used to test the significance of GCA and SCA interactions with location (Singh and Chaudhary, 1985; Sharma, 1998; Dabholkar, 1999). The proportional contributions of Males (GCAM), Females (GCAF), and their interaction (SCA MxF) to the sum square of crosses were calculated as the ratio between the sum of squares of each component and the cross sum of squares (Singh and Chaudhary, 1985; Sharma, 1998).

5.3.5.1 Estimation of General Combining Ability Effects

The GCA effects of Females and Males were calculated as a deviation of the Male or Female mean from all hybrids mean following Singh and Chaudhary (1985).

5.3.5.2 Estimation of specific combining ability effects

The SCA effects were calculated as a deviation of each cross mean from all hybrid means adjusted for corresponding GCA effects of parents. The SCA effects were computed as suggested by Singh and Chaudhary (1985).

5.4 Results and discussion

5.4.1 Combined analysis of variance

The analysis of variance for each location showed highly significant differences (P<0.01) among genotypes and crosses (data not shown) for grain yield, NCLB, PASP, EHT and PHT. The results from combined analyses of variance for grain yield, NCLB resistance and yield related traits are presented in Table 5.3. Highly significant differences (P<0.01) were detected among genotypes and crosses. Further significant differences (P<0.05) were detected among females, males, and females x males. These sources of variations had significant interactions with the environment for grain yield and non-significant for NCLB resistance and plant aspect. There were also highly significant differences (P<0.01) among females, males and females x males x environment on plant height and ear height (Table 5.3). Non significant differences were observed in checks x environment for all the traits studied. Similar significant differences following combined analyses were previously reported by various researchers (Gudeta, 2007; Bayisa et al., 2008; Kanyamasoro et al., 2012) in their studies using different sets of maize inbred lines.

The significance of the mean squares of the GCA effects of females and males and the SCA effects of F x M indicated the importance of both additive and non additive gene effects, respectively. This is consistent with the findings of other researchers (Meseka et al., 2006; Alam et al., 2008; Dagne et al., 2012). The GCA sum of squares of females were greater than the GCA sum of squares of males for all traits except plant height, indicating that females contributed more favorable genes than males towards higher values of these traits.

Table 5.3 Mean squares from the combined ANOVA on grain yield, northern corn leaf blight resistance and yield related traits of 84 maize genotypes (81 hybrids derived from 9 x 9 factorial cross combinations with three checks) evaluated across seven environments in Ethiopia

Source	DF	Mean square				
		YLD	NCLB	EHT	PASP	PHT
Genotypes (G)	83	6.75**	0.45**	1112**	1.62**	1708.7**
Environments (Env)	6	437.60**	16.95**	3193**	37.49**	140831.9**
G X Env	498	2.356	0.1959	171.4*	1.16	362.5*
Crosses	80	3.83	0.23	564.31**	0.24	801.18
GCA_F	8	17.28*	0.89	3007.25**	0.38	1901.2**
GCA_M	8	7.48*	0.57	1723.93**	1.22	4105.86**
SCA (females x males)	64	1.69*	0.11	113.99**	0.10	250.59**
Checks	2	3.07	0.14	311.9	1.54**	383.6
Checks X Env	12	0.748	0.1133	243.6	0.4306	201.2
GCA (females) x env	48	2.37*	0.22	167.87**	0.67	269.35**
GCA (males) x env	48	2.28	0.11	144.59**	0.74	296.16**
SCA x env	384	0.79	0.085	69.29	0.56	162.68
Pooled error crosses	566	2.402	0.2287	149.9	1.249	315.8
Pooled error genotypes	587	2.361	0.2285	149.2	1.211	310.6
Pooled error checks	20	1.29	0.23	135.8	0.1952	172.9
$SSGCA_F$ %		45.14	38.03	73.5	15.84	23.73
$SSGCA_M$ %		19.54	24.36	4.21	50.84	51.25
SSSCA %		35.32	37.61	22.3	33.34	25.02

*P $\leq$ 0.05, ** P $\leq$ 0.01, DF=degrees of freedom, YLD=Grain yield, NCLB=Northern corn leaf blight, EHT=Ear height, PASP=Plant aspect, PHT=Plant height

The GCA/SCA ratio was greater than unity in this study indicating that additive gene effects were more important for all the traits (Table 5.3). Significant mean squares (P<0.01) of environments for traits revealed that the responses of the genotypes across

all the environments were different. The significance of mean squares due to genotypes (P<0.01) indicated the existence of genetic variability among test genotypes.
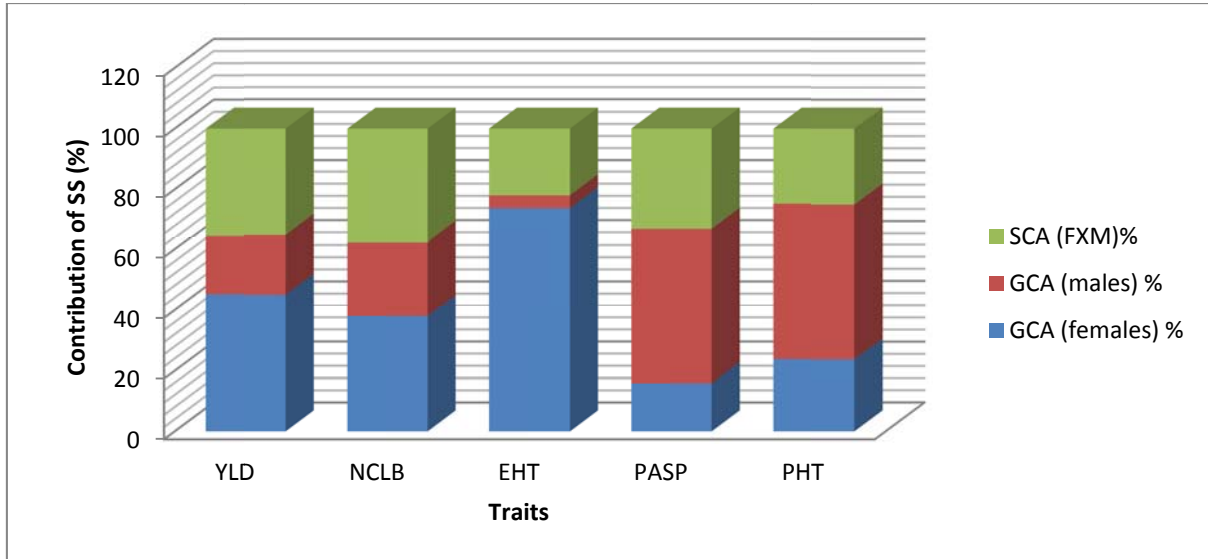
Figure 5.1 Relative contribution of sum squares for GCA (females or males) and SCA (females x males) to total sum of squares for yield (YLD), northern corn leaf blight reaction (NCLB), ear height (EHT), plant aspect (PASP), and plant height (PHT)

The mean values of the 81 crosses and three standard checks of grain yield, NCLB reaction, plant height, and ear height and plant aspect are presented in Table 5.4. These values were averaged across seven environments. The relative contribution of sum squares of GCA to the total sums of squares was higher than the contribution of SCA (Figure 5.1)

Table 5.4 Mean values of grain yield (t ha^{-1}), NCLB reaction, plant height (cm), ear height (cm) and plant aspect of 81 single cross experimental hybrids and three standard checks of maize evaluated across seven locations in Ethiopia

Entry	Crosses	YLD	NCLB	PHT	EHT	PASP
1	CML395 x CML202	6.12	2.3	212.6	108.97	2.93
2	F7215 x CML202	7.34	2	237.1	125.03	2.68
3	DE-78-Z x CML202	7.28	2.2	212.28	104.03	2.40
4	30H83-7x CML202	6.91	2.1	223.45	107.81	2.93
5	ILO'OE x CML202	7.54	2	224.45	110.38	2.35
6	SZYNA x CML202	6.3	2.2	237.45	120.55	2.53
7	X1264D x CML202	6.89	2.3	232.75	108.61	2.63
8	124b(113) x CML202	6.94	2	225.74	111.17	2.63
9	SC22 x CML202	7.22	2.1	217.4	109.22	2.48
10	CML395 x CML442	6.95	2.4	204.88	98.73	2.70
11	F7215 x CML442	7.51	2.2	229.68	107.23	2.58
12	DE-78-Z x CML442	6.71	2.3	203.8	93.56	2.43
13	30H83-7x CML442	6.78	2.2	226.48	104.31	2.78
14	ILO'OE x CML442	7.67	2.2	214.45	101.21	2.23
15	SZYNA x CML442	7.08	2.3	216.22	107.49	2.75
16	X1264D x CML442	6.68	2.5	227.78	103.32	2.30
17	124b(113)x CML442	7.21	2.2	223.8	111.09	2.58
18	SC22 x CML442	7.43	2.2	218.9	106.83	2.35
19	CML395 x CML312	5.79	2.6	211.7	111.98	2.83
20	F7215 x CML312	6.31	2.2	247.05	126.58	2.63
21	DE-78-Z x CML312	6.93	2.3	217.67	110.69	2.48
22	30H83-7x CML312	6.59	2.2	229.57	110.9	2.85
23	ILO'OE x CML312	5.64	2.4	212.32	103.98	2.88
24	SZYNA x CML312	6.81	2.1	245.2	120.81	2.58
25	X1264D x CML312	6.14	2.4	243	115.78	2.80
26	124-b(113)x CML312	6.47	2.4	230.8	113.16	2.80
27	SC22x CML312	6	2.5	227.47	116.17	2.68
28	CML395x CML464	6.9	2	225.78	121.03	2.63
29	F-7215x CML464	6.2	2	237.2	125.75	2.70
30	DE-78-Z x CML464	7.3	2.1	212.41	112.07	2.38
31	30H83-7x CML464	6.68	2	235.1	114.44	2.65
32	ILO'OE x CML464	7.83	1.9	222.49	111.77	2.58
33	SZYNA x CML464	6.55	2.1	235.6	124.62	2.45
34	X1264D x CML464	7.4	2.2	236.6	124.78	2.53
35	124-b(113)x CML464	7	2.1	236.55	120.14	2.60
36	SC22x CML464	6.92	2.3	222.75	117.54	2.63
37	CML395 x Gibe-1-91	8.33	2.2	227.75	122.54	2.35
38	F-7215x Gibe-1-91	6.99	2.1	244.2	135.13	2.45
39	DE-78-Z x Gibe-1-91	8.38	2.1	214.16	116.35	2.40
40	30H83-7x Gibe-1-91	6.37	2.3	229.8	114.64	2.55
41	ILO'OE x Gibe-1-91	6.09	2	221.4	113.68	2.50
42	SZYNA x Gibe-1-91	7.09	2.1	238.03	128.29	2.55
43	X1264D x Gibe-1-91	7.18	2.2	239.43	126.4	2.55
44	124b(113) x Gibe-1-91	6.34	2.3	230.4	120.47	2.60
45	SC22x Gibe-1-91	6.65	2.2	216.98	110.98	2.58

		YLD	NCLB	PHT	EHT	PASP
46	CML395x CML445	6.13	2.3	209.38	103.56	2.75
47	F-7215x CML445	6.05	2.3	224.28	109.19	2.65
48	DE-78-Z x CML445	6.41	2.3	225.45	101.47	2.55
49	30H83-7x CML445	6.52	2.2	220.65	98.01	2.95
50	ILO'OE x CML445	6.77	2.1	208.02	103.6	2.73
51	SZYNA x CML445	7.38	2.1	226.7	106.17	4.35
52	X1264D x CML445	7.09	2.2	230.7	108.81	2.75
53	124-b(113)x CML445	6.09	2.3	218.28	99.81	2.90
54	SC22x CML445	6.17	2.3	210.28	102.35	2.83
55	CML395x CML443	6.13	2.5	213.35	110.93	3.05
56	F-7215x CML443	5.9	2.2	224	111.55	2.95
57	DE-78-Z x CML443	6.85	2.2	203.63	99.46	2.60
58	30H83-7x CML443	6.07	2.4	218.91	103.43	3.03
59	ILO'OE x CML443	7.1	2.2	217.02	106.25	2.70
60	SZYNA x CML443	5.69	2.3	218.6	113.44	2.73
61	X1264D x CML443	6.44	2.6	220.28	108.15	2.78
62	124-b(113)x CML443	5.86	2.3	215.3	105.64	3.15
63	SC22x CML443	5.43	2.4	207.85	101.9	2.88
64	CML395x CML197	7.3	2.6	227.45	118.69	2.58
65	F-7215x CML197	6.63	2.3	238.65	130.53	2.65
66	DE-78-Z x CML197	5.78	2.6	197.7	104.97	2.75
67	30H83-7x CML197	7.57	2.1	241.62	124.32	2.73
68	ILO'OE x CML197	8.77	2	226.25	109.73	2.45
69	SZYNA x CML197	6.42	2.2	234.53	122.35	2.55
70	X1264D x CML197	7.53	2.4	237.05	121.27	2.65
71	124-b(113)x CML197	6.68	2.3	227.09	116.29	2.73
72	SC22x CML197	6.6	2.5	217.58	112.86	2.45
73	CML395x A7033	6.82	2.2	223.7	122.95	2.90
74	F7215x A7033	5.32	2.4	235.99	129.36	2.88
75	DE-78-Z x A7033	6.67	2.3	216	112.96	2.60
76	30H83-7x A7033	5.06	2.3	233.48	119.24	3.15
77	ILO'OE x A7033	7.67	2.2	225.75	116.08	2.75
78	SZYNA x A7033	5.86	2	239.1	126	2.75
79	X1264D x A7033	6.14	2.2	240.5	128.16	2.90
80	124-b(113)x A7033	5.03	2.3	228.88	121.09	2.93
81	SC22x A7033	6.16	2.4	227.75	118.99	2.90
82	BH540	6.11	2.2	230.9	117.55	2.73
83	BH543	6.97	2.2	227.2	120.67	2.38
84	BHQPY545	6.09	2.2	221.48	112.17	2.83
Cross mean		6.67	2.24	224.9	113.45	2.69
CV		24	20.1	7.5	10.20	40.2
LSD (0.05)		ns	ns	*	*	ns
Maximum		8.77	2.8	257.43	144.04	4.250
Minimum		5.03	1.9	205.21	98.73	2.250

*=significant at P < 0.05, ns=non-significant
YLD=Grain yield, NCLB=Northern corn leaf blight, PHT=Plant height EHT=Ear height, PASP=Plant aspect.

Plant height (PHT) ranged from 197 to 247 cm with a mean of 225 cm while ear height (EHT) varied from 93.56 to 135.13 cm with a mean of 113.45 cm. The lowest mean for both PHT and EHT were observed in hybrids 124-b (113) x CML442 and F7215 x CML442, while the highest means were observed in the hybrid ILOO'E-1-9-1-1-1-1-1 X CML202. Overall, hybrids 124-b (113) X CML442 and F7215 X CML442 were selected for shorter ear and plant height, which are the most preferred traits for lodging tolerance. Hybrids had plant aspect varying from 2.23 to 4.35, with a mean of 2.69. The highest and lowest mean values for plant aspect were observed in hybrids SZSYNA99F2-81-4-3-1 X Gibe-1-91-1-1-1-1 and F7215 X CML464, respectively. Hybrid F7215 X CML464 was selected for better plant aspect displaying smaller values. Hybrids displayed grain yield ranging from 5.03 to 8.77 t ha^{-1} with a mean of 6.67 t ha^{-1}. The three check hybrids used in this experiment BH540, BH543 and BHQPY545, had mean grain yield of 6.11, 6.97 and 6.09 t ha^{-1}, respectively.

The mean grain yield of six hybrids exceeded that of the best check (BH543) in this experiment by above 10%. These hybrids were: 124-b(113) X CML464, ILOO'E-1-9-1-1-1-1-1 X CML442, ILOO'E-1-9-1-1-1-1-1 x CML202, 30H83-7-1-5-1-1-1-2-1 X CML464, SC22 X CML464 and F7215 X CML464 that provided 25.83, 20.23, 19.51, 12.34, 10.19, and 10.04% yield advantage over BH543, respectively (Table 5.4). Therefore, these hybrids are the best candidates for wide area production if they show stable yields across years.

5.4.2 General combining ability of females

The GCA effects of the nine female parents for grain yield, NCLB reaction, plant height, ear height and plant aspect are summarized in Table 5.5. CML395, 30H83-7-1-5-1-1-1-2-1, ILOO'E-1-9-1-1-1-1-1 and 124-b (113) had significantly positive mean GCA effects for grain yield. Therefore, these lines are good combiners for grain yield. In contrast, SC22 had a significantly negative GCA effect for grain yield and was not a good combiner. In line with the current study, several authors reported either positive or

negative significant GCA effects of experimental maize hybrids for grain yield (Dagne, 2002; Ahmad and Saleem 2003; Gudeta, 2007; Mosisa et al., 2008; Legesse et al., 2009; Mosa, 2010; Zeleke and Tuna, 2010).

Inbred parents CML395 and ILO'OE-1-9-1-1-1-1-1 showed significantly negative GCA effects for NCLB reaction. Therefore, these lines are good combiners for breeding to NCLB resistance. Inbred lines DE-78-Z-126-3-2-2-1-1, 30H83-7-1-5-1-1-1-2-1, ILOO'E-1-9-1-1-1-1-1, 124-b (113) and SC22 had significantly positive GCA effects for plant height and ear height. Whereas lines F7215, SZSYNA99F2-81-4-3-1 and X1264DW-1-2-2-2-2 had significantly negative GCA effects for these traits. Thus the female parents with significantly negative GCA effects are good combiners for both traits. CML395 showed no significant GCA effects for ear height, but did show it for plant height.

Regarding plant aspect all female lines except SZSYNA99F2-81-4-3-1 and SC22 showed non-significant (both negative and positive) GCA effect. Therefore, the remaining seven lines were not good combiners for plant aspect. Recently torrential rain with high wind speed is a common phenomena in the mid altitude agro-ecology of Ethiopia attributed to global climate change. Consequently, short plant height maize varieties are needed with reduced ear height to circumvent lodging under this agro-ecology. Therefore, inbred lines which had significantly negative GCA effects were considered as good combiners for hybrid breeding. Similar findings reported lower plant height and ear height reduced lodging in maize (Zeleke and Tuna, 2010; Mosa, 2010; Rahman et al., 2012).

Table 5.5 Estimates of the GCA effects of nine maize inbred lines used as female parents on grain yield, NCLB reaction, plant height, ear height and plant aspect tested at seven environments

Females	YLD	NCLB	EHT	PHT	PASP
CML395	0.97141**	-0.72101*	0.10988ns	2.02725**	-0.13384ns
F7215	0.43728ns	0.02963ns	-9.30123**	-7.21878**	-0.12038ns
DE-78-Z-126-3-2-2-1	-0.66668ns	0.11201ns	1.58924**	5.31534**	0.0308ns
30H83-7-1-1-1-2-1	0.68443*	-0.22862ns	6.06226**	3.76693**	-0.19415ns
ILO'OE-1-9-1-1-1-1-1	0.71697*	-0.73942*	7.41464**	4.66376**	-0.20768ns
SZYNA99F2-81-4-3-1	-0.36224ns	0.00423ns	-9.43774**	-4.43307**	0.75121*
X1264D-1-2-1-1-1	-0.54875ns	0.1082ns	-7.12504**	-9.34894**	0.22383ns
124-b(113)	0.88713**	0.13796ns	3.90511**	2.23042**	-0.05753ns
SC22	-0.98954**	-0.00299ns	6.78289**	2.99709**	0.80774*

*=significant at P < 0.05, **=significant at P< 0.01
YLD=Grain yield, NCLB=Northern corn leaf blight, PHT=Plant height EHT=Ear height, PASP=Plant aspect

5.4.3 General Combining Ability of males

The GCA effects of the male parents for grain yield, NCLB reaction, plant height, ear height and plant aspect are presented in Table 5.6. Three lines: CML202, CML312 and Gibe-1-91-1-1-1-1 had significantly positive GCA effects for grain yield, whereas CML197 had significantly negative GCA effect. Consequently, the three lines were selected with good combining abilities for grain yield improvement.

Likewise, the GCA effects of the male lines for Northern corn leaf blight reaction, plant height, ear height and plant aspect is presented in Table 5.6. The GCA effects of all male parents were significantly positive or negative for plant height and ear height. Lines with significantly negative effects for the two traits are: CML312, Gibe-1-91-1-1-1-1, CML464 and CML202. These lines are good combiners and useful for breeding. The GCA effect of none of the males except CML464 and CML445 were significant for plant aspect (Table 5.6). CML202 and Gibe-1-91 showed significant negative effect for NCLB which makes them the ideal inbred lines in breeding for NCLB resistance; however A7033 showed significant positive effect and thus, not ideal inbred line for breeding against this pathogen.

Table 5.6 Estimates of the GCA effects of nine maize inbred lines used as male parents on grain yield, NCLB reaction, plant height, ear height and plant aspect tested at seven environments

Males	YLD	NCLB	EHT	PHT	PASP
CML202	0.52649*	-0. 6749*	0.59559*	-6.9188**	0.05529ns
CML442	-0.30272ns	-0.0545ns	9.54956**	10.4622**	-0.01031ns
CML312	0.79554**	0.01217ns	-8.13616**	-13.2743**	-0.23603ns
CML464	-0.1697ns	-0.05235ns	-2.68695**	5.1003**	0.57764*
Gibe-1-91-1-1-1-1	0.73887**	-0.55997*	-4.23457**	-4.6783**	-0.11223ns
CML445	-0.1143ns	-0.07664ns	5.07496**	5.5304**	0.58926*
CML443	-0.21065ns	0.12892ns	1.71305**	8.5812**	-0.11607ns
CML197	-0.54541*	0.01828ns	0.73051**	1.0765**	0.0485ns
A7033	-0.21811ns	0.61661*	-2.606**	-5.8791**	0.00396ns

*=significant at P < 0.05, **=significant at P< 0.01
YLD=Grain yield, NCLB=Northern corn leaf blight, PHT=Plant height EHT=Ear height, PASP=Plant aspect

5.4.4 Specific Combining Ability effects

Estimates of the specific combining ability effects of the 81 hybrids averaged across seven test locations for grain yield, NCLB reaction, plant height, ear height and plant aspect are presented in Table 5.7.

Hybrids including CML395 X CML442, DE-78-Z-126-3-2-2-1-1 X CML442, ILOO'E-1-9-1-1-1-1-1 X CML312, X1264DW-1-2-2-2-2 X CML464 and SC22 X Gibe-1-91-1-1-1-1 had significantly positive SCA effects for grain yield. These hybrids were selected with increased grain yield level for direct production or further breeding. Three of the nine female parents [ILOO'E-1-9-1-1-1-1-1, X1264DW-1-2-2-2-2, and 124-b (113)] had significantly positive SCA effects when crossed to male parent CML202 for grain yield implying that there was positive interaction of genes between the two sets of parents. Hybrids with high SCA effects exhibited dominance genetic effects, the basis for the expression of heterosis though epistasis genetic effect may not be ruled out. High heterosis is contributed by the complementarities of the inbred lines used in the crosses.

Table 5.7 Estimates of specific combining ability effects of 81 maize hybrids on grain yield, NCLB reaction, plant height, ear height and plant aspect when tested at seven environments

Crosses	YLD	NCLB	EHT	PHT	PASP
CML395 x CML202	-0.39538ns	-1.24696*	-0.1765ns	-2.3542**	0.28415ns
F7215 x CML202	1.45811**	-0.99534ns	3.4695**	3.2648**	0.04261ns
DE-78-Z x CML202	1.24557*	-0.9163ns	-0.9734ns	1.1442*	-0.1241ns
30H83-7x CML202	1.24653*	-0.0732ns	-2.3369**	-7.0733**	0.16609ns
ILO'OE x CML202	-0.45347ns	0.08442ns	3.1393**	5.0481**	-0.10161ns
SZYNA x CML202	-0.0503ns	0.18394ns	4.1584**	5.1251**	-0.2371ns
X1264D x CML202	-0.49395ns	0.15267ns	-3.9226**	0.9315ns	-0.05777ns
124b(113) x CML202	0.13224ns	-1.48812*	-1.8257**	-4.4209**	0.02952ns
SC22 x CML202	1.21065*	-1.13503*	-1.5321**	-1.6653**	-0.0018ns
CML395 x CML442	-0.33982ns	0.07775ns	-3.6083**	-4.2082**	0.10984ns
F7215 x CML442	1.15795*	-0.02884ns	-6.1194**	0.768ns	0.01458ns
DE-78-Z x CML442	-0.6603ns	0.07593ns	-2.148**	-0.5669ns	0.2103ns
30H83-7x CML442	-0.27649ns	0.11902ns	-0.0972ns	2.4013**	0.07406ns
ILO'OE x CML442	-0.01792ns	-0.05907ns	0.7504ns	1.6942**	-0.18835ns
SZYNA x CML442	0.07383ns	0.04331ns	-1.0305ns	-12.7574**	0.06387ns
X1264D x CML442	-0.04125ns	0.01632ns	-3.2686**	1.7489**	-0.18366ns
124b(113)x CML442	-0.05078ns	-0.10161ns	8.4568**	4.3966**	0.02877ns
SC22 x CML442	1.25478*	-0.1428ns	7.0647**	6.5235**	-0.1294ns
CML395 x CML312	-0.613ns	0.1168ns	1.2441*	-7.2138**	-0.03348ns
F7215 x CML312	1.44621**	-0.0255ns	5.1187**	4.2339**	0.02412ns
DE-78-Z x CML312	0.27367ns	-0.0136ns	2.6473**	1.2418*	0.04798ns
30H83-7x CML312	0.35462ns	-0.12336ns	-0.5019ns	-2.8185**	-0.10054ns
ILO'OE x CML312	-1.61109**	0.29426ns	-6.6115**	-10.7471**	0.27833ns
SZYNA x CML312	0.31494ns	-0.47479ns	-0.9638ns	8.9656**	-0.27559ns
X1264D x CML312	-0.33871ns	-0.03749ns	-3.3877**	3.8005**	0.12603ns
124-b(113)x CML312	1.12605*	0.01315ns	-0.5194ns	0.4339ns	-0.06869ns
SC22x CML312	0.34732ns	0.05053ns	2.9743**	2.1037**	0.00185ns
CML395x CML464	-0.09554ns	-0.10257ns	0.5139ns	0.9061ns	-0.00081ns
F-7215x CML464	-0.22633ns	0.02228ns	-1.8543**	-3.7463**	0.1825ns
DE-78-Z x CML464	1.22113*	-0.00511ns	0.16ns	-1.8812**	-0.01064ns
30H83-7x CML464	-0.46935ns	0.03085ns	-2.8035**	1.1585*	-0.00145ns
ILO'OE x CML464	0.15208ns	-0.11511ns	-4.4988**	-3.1772**	0.08857ns
SZYNA x CML464	-0.03474ns	0.0837ns	0.1203ns	0.5426ns	-0.20207ns
X1264D x CML464	0.04303ns	-0.11114ns	3.325**	-1.5225**	0.03369ns
124-b(113)x CML464	-0.02935ns	-0.0005ns	1.7076**	9.568**	-0.10945ns
SC22x CML464	1.13907*	0.19759ns	3.3298**	-1.8479**	0.01966ns
CML395 x Gibe-1-91	1.28335*	-0.12463ns	0.2901ns	5.5807**	-0.15958ns
F-7215x Gibe-1-91	-0.20601ns	-0.03836ns	7.3076**	6.6997**	-0.09083ns
DE-78-Z x Gibe-1-91	1.56145**	-0.01931ns	0.5219ns	-4.3066**	0.04274ns
30H83-7x Gibe-1-91	-0.44903ns	0.02021ns	-2.1702**	-3.2955**	-0.17878ns
ILO'OE x Gibe-1-91	1.11668*	-0.00788ns	-0.4511ns	1.3974*	0.14009ns
SZYNA x Gibe-1-91	-0.19871ns	0.06593ns	2.025**	0.4886ns	-0.12154ns
X1264D x Gibe-1-91	0.06764ns	-0.04677ns	2.1012**	-0.5765ns	0.10136ns
124b(113) x Gibe-1-91	-0.28903ns	0.14958ns	-1.2162*	0.214ns	0.03622ns
SC22x Gibe-1-91	-0.58633ns	0.00124ns	-8.4083**	-6.2019**	0.23033ns
CML395x CML445	-0.24744ns	-0.01471ns	-2.8718**	-3.7368**	-0.23604ns
F-7215x CML445	1.17034*	0.11799ns	-2.8543**	-6.5463**	-0.34929ns

DE-78-Z x CML445	0.07208ns	-0.00939ns	4.0743**	21.1759**	-0.34343ns
30H83-7x CML445	-0.08268ns	0.01942ns	-3.6035**	-1.4701**	-0.23224ns
ILO'OE x CML445	-0.17697ns	-0.01225ns	7.8584**	-6.463**	-0.27123ns
SZYNA x CML445	1.24049*	0.00442ns	-1.594**	1.5426**	1.81842**
X1264D x CML445	-0.19744ns	-0.07257ns	1.4822**	0.1632ns	-0.15753ns
124-b(113)x CML445	-0.30411ns	-0.04765ns	-6.4781**	-5.4749**	-0.1201ns
SC22x CML445	1.22573*	0.01474ns	3.9869**	0.8093ns	-0.10856ns
CML395x CML443	1.19907*	0.00275ns	2.5012**	1.2791*	0.00192ns
F-7215x CML443	-0.42173ns	0.02116ns	-4.767**	1.0839ns	0.03295ns
DE-78-Z x CML443	0.06287ns	-0.12765ns	0.8616ns	2.5347**	-0.10048ns
30H83-7x CML443	1.55526**	0.04045ns	0.5123ns	1.7315**	0.01699ns
ILO'OE x CML443	0.26668ns	-0.02693ns	2.4171**	6.5529**	0.0863ns
SZYNA x CML443	-0.38157*	-0.01741ns	-0.3067ns	-6.5844**	-0.33548ns
X1264D x CML443	0.24478*	0.22704ns	-0.1162ns	-5.4352**	0.01814ns
124-b(113)x CML443	0.26097ns	0.01267ns	0.6806ns	-2.6304**	0.34057ns
SC22x CML443	-0.41633ns	-0.13209ns	-1.7829**	1.468**	-0.06089ns
CML395x CML197	1.54605*	0.17656ns	0.5139ns	8.9569**	-0.04972ns
F-7215x CML197	-0.27903ns	-0.08717ns	4.7028**	0.4188ns	0.11017ns
DE-78-Z x CML197	-1.13014*	0.54259ns	-3.24**	-17.2161**	1.36803*
30H83-7x CML197	1.32367*	-0.18217ns	11.525**	11.5093**	0.05907ns
ILO'OE x CML197	0.25224ns	-0.28526ns	-5.3559**	3.0593**	-0.14905ns
SZYNA x CML197	-0.24459ns	-0.01503ns	-1.4083**	0.3077ns	-0.37612ns
X1264D x CML197	1.099176*	0.04013ns	-0.475ns	-1.8431**	0.10993ns
124-b(113)x CML197	-0.53633ns	0.02577ns	-1.8496**	-2.4526**	-0.06978ns
SC22x CML197	-0.36363ns	0.08458ns	-4.4131**	-2.7399**	-0.00253ns
CML395x A7033	0.36272ns	-0.17892ns	1.5933**	0.7902ns	0.08372ns
F7215x A7033	-0.89951ns	0.15378ns	-5.0035**	-6.1765**	0.03319ns
DE-78-Z x A7033	1.15367*	-0.12717ns	-1.9035**	-2.1257**	-0.09039ns
30H83-7x A7033	-0.40252ns	0.14878ns	-0.5242ns	-2.1431**	0.1968ns
ILO'OE x A7033	1.63176**	0.12783ns	2.752**	2.6354**	0.11696ns
SZYNA x A7033	-0.21935ns	-0.07407ns	-1.0004ns	2.3696**	-0.33439ns
X1264D x A7033	0.22414ns	-0.1682ns	4.2616**	2.7331**	0.0098ns
124-b(113)x A7033	1.36034*	0.05672ns	1.0441ns	0.3664ns	-0.06705ns
SC22x A7033	-0.27125ns	1.36124*	-1.2194*	1.5505**	0.05134ns
SE	0.55	0.55	0.55	0.55	0.55

*=significant at P < 0.05, **=significant at P< 0.01
YLD=Grain yield, NCLB=Northern corn leaf blight, PHT=Plant height EHT=Ear height, PASP=Plant aspect

Hybrids such as CML395 X CML202, CML395 X CML197 and CML395 X A7033 showed significantly negative SCA effects on NCLB reaction indicating their resistance reaction against this pathogen. All other hybrids were either non-significant or had significantly positive (SC22 X A7033) SCA effect for NCLB. Hence they are not good hybrids for NCLB management. Conversely, hybrids including CML395 X CML464, CML395 X CML197, CML395 X A7033, F7215 X CML202, F7215 X CML445, SC22 X CML442, SC22 X CML312, DE-78-Z-126-3-2-2-1-1 X Gibe-1-91-1-1-1-1, 30H83-7-1-5-1-1-1-2-1 X CML442, 30H83-7-1-5-1-1-1-2-1 X Gibe-1-91-1-1-1-1, ILOO'E-1-9-1-1-1-1-

1 X CML464, ILOO'E-1-9-1-1-1-1-1 X A7033, SZSYNA99F2-81-4-3-1 X CML202, SZSYNA99F2-81-4-3-1 X CML442, SZSYNA99F2-81-4-3-1 X CML197, X1264DW-1-2-2-2-2 x CML445 and 124-b (113) x CML197 had significantly negative SCA effects for plant height and ear height. These hybrids were selected for lodging tolerance. Hybrids such as CML395 x CML442, CML395 X Gibe-1-91-1-1-1-1, CML395 X CML445, F7215 X CML197, F7215 X A7033, DE-78-Z-126-3-2-2-1-1 X CML442, DE-78-Z-126-3-2-2-1-1 X CML312, DE-78-Z-126-3-2-2-1-1 X A7033, ILOO'E-1-9-1-1-1-1-1 X CML442, SZSYNA99F2-81-4-3-1 X CML312, X1264DW-1-2-2-2-2 X Gibe-1-91-1-1-1-1, 124-b (113) x CML464 and SC22 x CML443 had significant positive SCA effects for plant and ear height. As such these crosses are not desirable owing to their likely vulnerability to lodging. Hybrids such as SZSYNA99F2-81-4-3-1 X CML445 and 124-b (113) X CML464 had significantly positive SCA effects for plant aspect and are thus not recommended as good candidates for production. Sixty five of the 81 hybrids had significant SCA effects for plant height and 33 of them displayed significantly positive while 32 had significantly negative SCA effects (Table 5.7). Likewise, 30 of the 81 hybrids had significantly negative SCA effects for ear height suggesting increased lodging tolerance (Table 5.7). The hybrids which had negative SCA effects for plant aspect were desirable and maintained for further breeding.

5.4.5 Heterosis

Heterosis is the best indicator of the superiority of a hybrid when compared with its inbred parents. Two types of heterosis are distinguishable, mid parent and high parent heterosis. High parent heterosis is presented in Figure 5.2 for grain yield and reaction to NCLB. Thirty three hybrids displayed positive mid-parent heterosis of up to 250% (Figure 5.2), among them DE-78-Z-126-3-2-2-1-1 X CML445, SZSYNA99F2-81-4-3-1 X CML445, X1264DW-1-2-2-2-2 X CML445, X1264DW-1-2-2-2-2 X CML443, and DE-78-Z-126-3-2-2-1-1 X CML464 displayed MPH of greater than 200% (Figure 5.2). Thirty three crosses displayed positive high parent heterosis of up to 235% for grain yield (Figure 5.3). Hybrids such as DE-78-Z-126-3-2-2-1-1 X CML445, SZSYNA99F2-81-4-3-

1 X CML445, X1264DW-1-2-2-2-2 X CML445 and SC22 X CML445 displayed positive high parent heterosis of more than 200% (Figure 5.3). Locally originated and adapted inbred lines such as Gibe-1-91-1-1-1-1, DE-78-Z-126-3-2-2-1-1, ILO'OE-1-9-1-1-1-1-1, SZYNA-99-F2-81-4-3-1 and x1264DW-1-2-2-2-2, in combinations with CIMMYT derived lines (CML202, CML442, CML312, CML445, CML464) featured in 33 of the hybrids with positive mid parent heterosis for grain yield. This highlights that the newly bred hybrids can perform better than the standard checks in grain yield and resistance to NCLB, which can be recommended for large scale production. Therefore, hybrids selected based on their high parent and mid-parent heterosis can be selected for release or for further breeding.

Evaluation of a large number of hybrid combinations in multi-location trials facilitates verification of the hybrid yield potential and heterosis (Gudeta, 2007). For NCLB, negative heterosis is desirable but for yield positive heterosis is a prerequisite for selection. Thirty two out of 81 hybrids had negative (< -10) mid-parent heterosis for NCLB reaction (Figure 5.5). Six hybrids [124-b(113) X CML197, 124-b(113) X CML464, 124-b(113) X CML442, X1264DW-1-2-2-2-2 X CML464, F7215 X CML197 and CML395 X CML197] displayed mid parent heterosis of <-20 (Figure 5.5). The observed negative mid parent heterosis ranged from -0.06 to -26% for NCLB. The standard heterosis (SH) for NCLB showed high negative heterosis of up to -14%, indicating that significantly large numbers of hybrids are better than the standard check (Figure 5.4). Less than -8 standard heterosis was observed on six hybrids (CML395 X Gibe-1-91-1-1-1-1, F7215 X Gibe-1-91-1-1-1-1, 30H83-7-1-5-1-1-1-2-1 X CML202, ILOO'E-1-9-1-1-1-1-1 X CML445 and SZSYNA99F2-81-4-3-1 X Gibe-1-91-1-1-1-1), indicating their better performance than the three adapted checks in their reaction to NCLB. These hybrids are recommended for large-scale production or as testers in hybrid breeding.
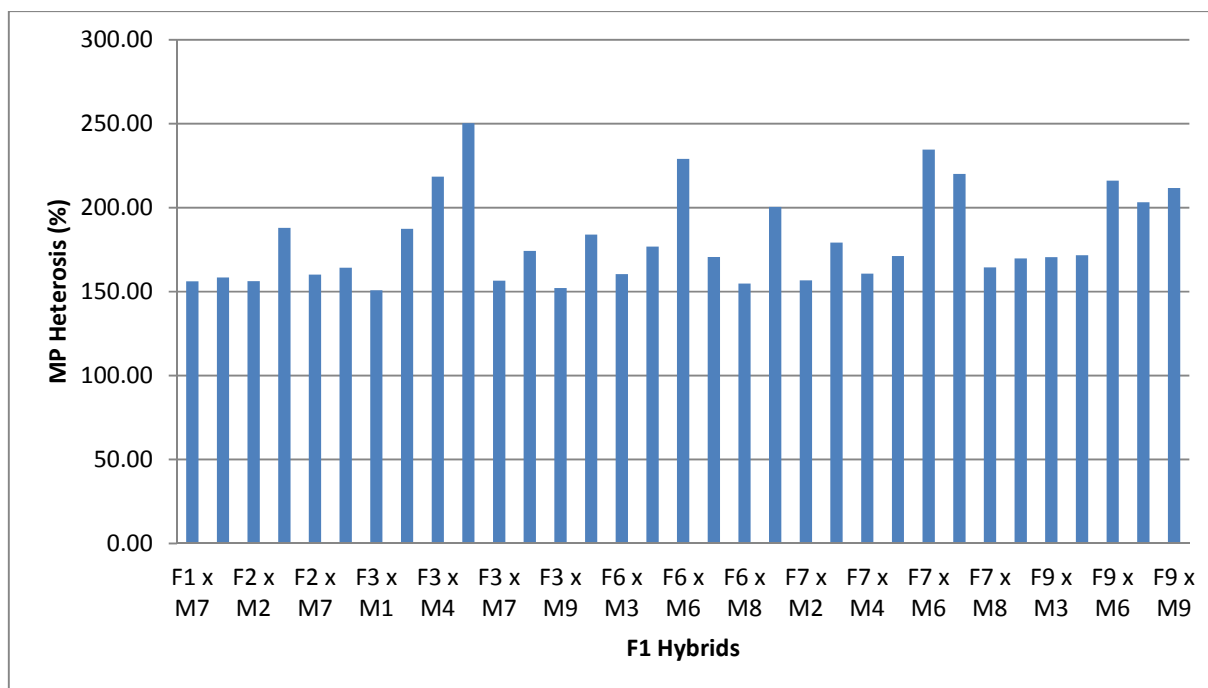
Figure 5.2 Positive mid parent (MP) heterosis (%) of grain yield among 33 selected single cross maize hybrids tested at seven locations. Note: the codes for Male (M) and Female (F) parents are given in Table 1
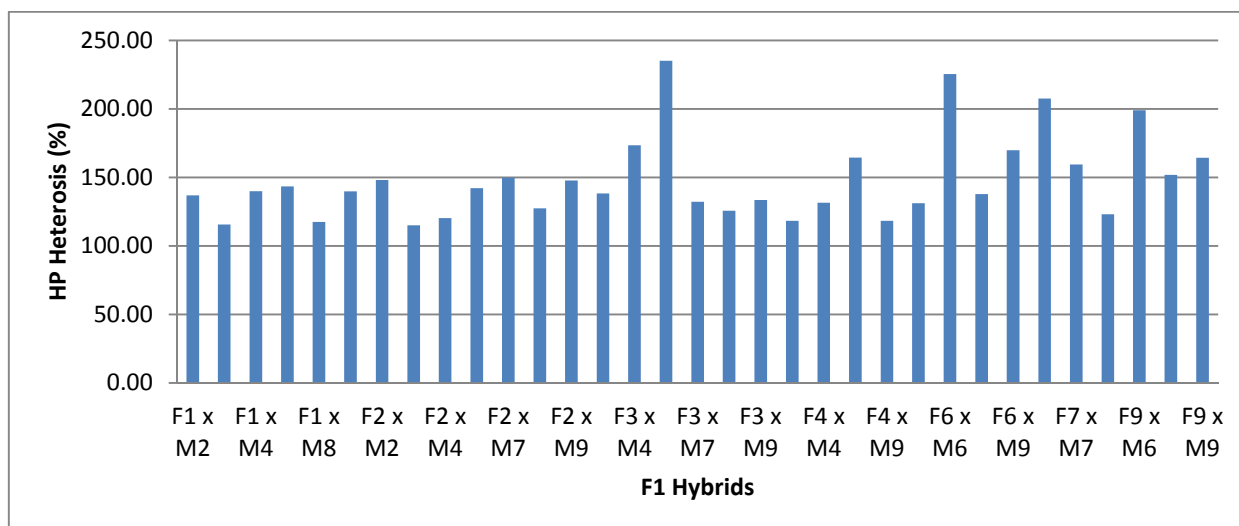
Figure 5.3 Positive high parent (HP) heterosis (%) of grain yield among 33 selected single cross maize hybrids tested at seven locations. Note: the codes for Male (M) and Female (F) parents are given in Table 1

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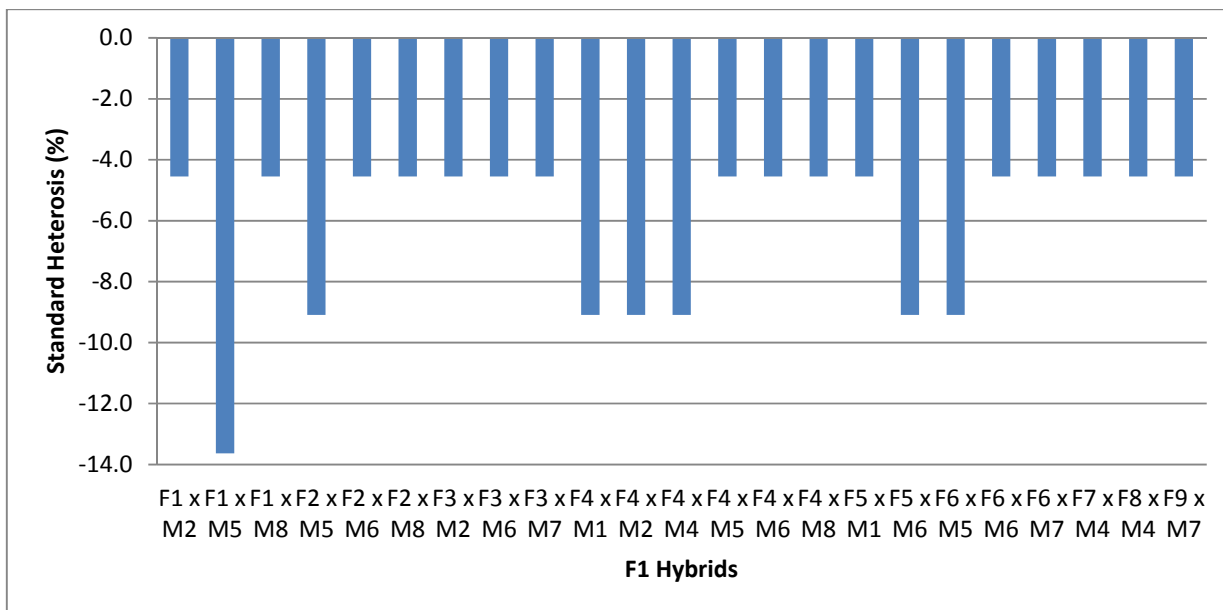
Figure 5.4 Negative Standard heterosis (%) of northern corn leaf blight reactions among 23 selected single cross maize hybrids tested at seven locations. Note: the codes for Male (M) and Female (F) parents are given in Table 1
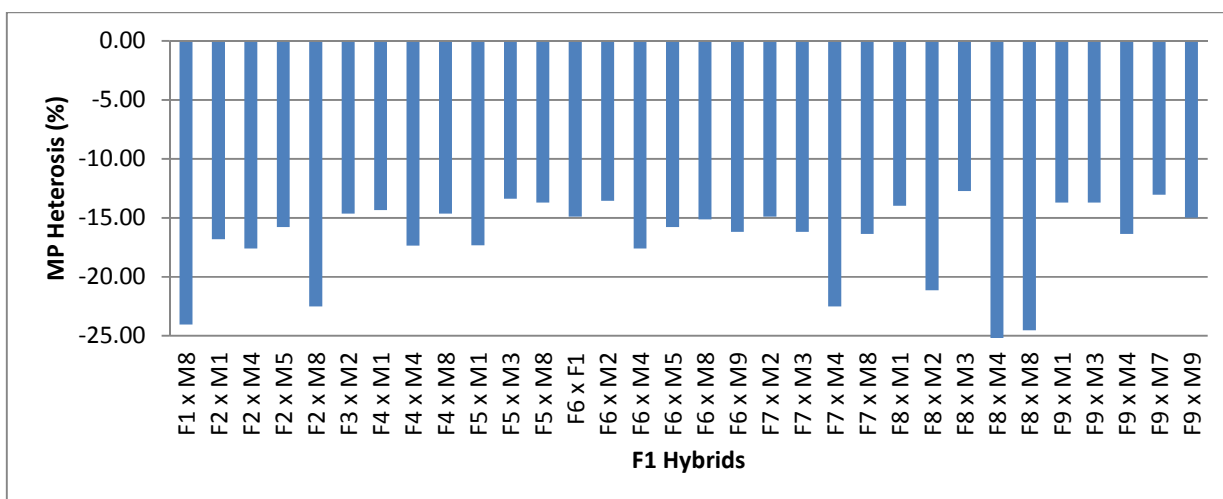
Figure 5.5 Negative mid parent (MP) heterosis (%) of northern corn leaf blight reactions among 32 selected single cross maize hybrids tested at seven locations. Note: the codes for Male (M) and Female (F) parents are given in Table 1

5.5 Conclusions

In the present study inbred lines such as CML395, 30H83-7-1-1-1-2-1 and ILO'OE-1-9-1-1-1-1-1 when used as female, and lines CML202, CML312 and Gibe-1-91-1-1-1-1 when used as male had high positive GCA effects for grain yield. These lines made significant contributions to increased and positive heterosis in most crosses involved. Inbred parents with considerable resistance to NCLB were CML202, CML 312, Gibe-1-91-1-1-1-1, ILO'OE-1-9-1-1-1-1-1, CML395, and CML445. However, only lines CML395 and ILO'OE-9-1-1-1-1 had significant negative GCA effects, and hence were good combiners for NCLB. Almost all inbred lines used in this study had significantly negative GCA effects for plant height and ear height which are good combiners for both traits.

GCA effects accounted for 65% and the SCA for 35% of the hybrid sum of squares for grain yield. This indicates the predominance of additive genetic effect over the non-additive in this study. Similarly, GCA effects accounted for 62.44, 77.71, and 74.98% for NCLB, EHT and PHT, respectively, indicating the relative prevalence of additive gene action over non-additive gene effect. The results indicate the predominance of genes with additive over non-additive gene effects for NCLB resistance, grain yield and other agronomic traits investigated. The preponderance of the additive gene effect for NCLB resistance indicates the possibility of breeding through recurrent selection and backcrosses to incorporate candidate genes to susceptible parents.

The hybrids with significant negative SCA effects for NCLB were CML395 x CML202, 124 b(113) x CML202, SC22 x CML202 and SC22 x A7033. Hybrids CML395 x Gibe-1-91-1-1-1-1, F7215 x Gibe-1-91-1-1-1-1, 30H83-7-1-5-1-1-1-2-1 x CML202 displayed a high negative standard heterosis for NCLB and performed better than the check hybrids. Similarly, hybrids like CML395 x CML197, F7215 x CML197, 124-b (113) x CML442 and 124-b(113) x CML464 had a high negative mid-parent heterosis.

Outstanding hybrids such as CML-312 x CML395, CML197 x CML395, CML443 x DE-78-Z-126-3-2-2-1-1 were identified with average yield of > 8 t ha^{-1} and high specific combining ability for direct production or as testers in hybrid development. Furthermore, single cross hybrids such as ILOO'E-1-9-1-1-1-1-1 x CML445, SZYNA99F2-81-4-3-1 x CML445 and X1264DW-1-2-2-2-2 x CML445 had high parent heterosis of >200%. Hybrids DE-78-2-126-3-2-2-1-1 x CML445, SZYNA99F2-81-4-3-1 x CML445 and X1264DW-1-2-2-2-2 x CML445 showed positive mid-parent heterosis up to 250%. Inbred parent CML445 contributed towards high positive heterosis in most of the crosses involved. Therefore, the significant SCA effects observed in reducing disease, increasing grain yield, reducing plant height and ear height indicates that dominance gene effects can be utilized in the development of superior hybrids. The selected hybrids based on their SCA effects and increased heterosis can be recommended for direct release and as testers for breeding. Likewise, selected inbred lines with high GCA effects are valuable germplasm for hybrid development in the mid-altitude and sub-humid agro-ecologies of Ethiopia or similar environments in sub-Saharan Africa.

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CHAPTER 6

Genotype by environment interaction of maize hybrids in the mid-altitude sub-humid agro-ecology of Ethiopia

6.1 Abstract

Genotype x environment interactions (GEI) indicates the inconsistency in relative performance of genotypes over environments. Assessment of the stability of genotypes across different environments is useful for recommending cultivars for known conditions of cultivation. The objectives of the study were to investigate GEI of 84 maize (*Zea mays* L.) hybrids developed for the mid-altitude sub-humid agro-ecology. The hybrids were tested across 10 different locations representing the mid-altitude sub-humid agro-ecology in Ethiopia. Additive main effects and multiplicative interaction (AMMI) and GGE (genotype and genotype by environment interaction) biplot were used to assess the stability of hybrids. There were highly significant differences between genotypes, environments and GEI. The first three interaction principal component axes (IPCA) of the AMMI-3 model accounted for 63.49% of the total G x E interaction sum of squares for grain yield. The AMMI biplot clearly depicted the genotypes on the bases of their adaptation patterns. Hybrids G68, G39, G37, G77, G34 and G2 were found to be more stable and responsive to favorable environments. Among them G2 is more stable across locations with mean yield ranging from 4.54 – 9.02 t/ha, and G68 was picked as best hybrid across 4 locations. Hybrids G25 and G80 showed higher yield stability in the low yielding environments. The GGE biplot allocated Hawassa as the most discriminating environment for the new hybrids as indicated by the longest distance between its marker and the origin and gave information on the performance of the hybrids. Overall, the study identified candidate hybrids G68, G2, G77 and G67 with high yield and average stability for release in the mid-altitude sub-humid agro-ecology.

Key Words: Agro-ecology, AMMI, G x E Interaction, GGE biplot, Maize, Grain-yield, Stability

6.2 Introduction

Maize (*Zea mays* L.) is the principal grain crop and globally ranks third after wheat and rice. It remains the food security crop in eastern and southern Africa (ESA) predominantly grown by the resource-constrained and small-scale farmers. Newly developed superior maize cultivars should exhibit great yield potential and average stability over a wide range of environmental conditions for release. High genotype by environmental interaction (GEI) leads to differential response and stability among genotypes. In maize breeding, choice of a suitable candidate cultivar is subject to two considerations: (1) high grain yield across a wide range of environments and (2) consistent performance over environments. Consistency of performance is dependent upon the GEI. Cultivars, which show less GEI are described as more stable or well buffered. Stability of yield is defined as the ability of a genotype to avoid substantial fluctuations in yield over a range of environments (Kang, 1998).

Cultivar performance is a function of the genotype and the nature of the production environment (Cooper and Byth, 1996). Environmental factors have greater effect on quantitative than qualitative traits. Consequently, performance tests of potential cultivars are conducted in multiple years and locations (Bernardo, 2002). In addition to genotype and environment main effects, performance of cultivars is also determined by the GEI, which is a differential response of cultivars to environmental changes (Hallauer and Miranda, 1988; Crossa et al., 1990; Vargas et al., 1999). Various causes have been described as sources of GEI in sub-Saharan Africa maize growing environments; for example, temperature, rainfall, length of growing season, drought, sub-soil pH and socio-economic factors that result in sub-optimal input application (Banziger et al., 2006). Biotic factors are also among the contributing factors for the presence of GEI (Butran, 2004). The relative magnitude of GEI provides information concerning the likely area of adaptation of a given genotype. It is also useful in determining efficient methods of using time and resources in a breeding program (Ceccarelli, 1989; Kang, 1998).

Large GEI is expected when genotypes are grown under a wide range of environments and outside their normal zone of adaptation (Beck et al., 1991).

Yield trials frequently have both significant main effects of genotype and environment and GEI (Zobel et al., 1988). The existence of GEI necessitates that breeders evaluate genotypes in more than one environment to obtain repeatable rankings of genotypes (Hallauer and Miranda, 1988). However, GEI becomes of practical significance only when crossover interactions occur (Baker, 1988; Crossa and Cornelius, 1997). Crossover interactions occur in evaluation trials when ranks of cultivars change across environments (Russell et al., 2003). In varying environments, genotypes that provide high average yields with minimum GEI have been gaining importance over increased yields (Ceccarelli, 1989; Gauch and Zobel, 1997; Kang, 1998). The definition of a stable cultivar varies with the type of stability analysis used, but generally breeders want cultivars with high mean yield that respond to improved environments (Hallauer and Miranda, 1988).

The conventional method of partitioning total variation into components due to genotype, environment, and GEI conveys little information on the individual patterns of response (Zobel et al., 1988). To optimize growers' yields, the growing region must be subdivided into relatively homogenous mega-environments and appropriate genotypes must be targeted for each of these mega-environments (Gauch and Zobel, 1997). The usual analysis of variance (ANOVA) fails to detect a significant interaction component, principal component analysis (PCA) fails to identify and separate the significant genotype and environment main effects, and linear regression (LR) accounts for only a small portion of the interaction sum of squares (Zobel et al., 1988). Since ANOVA, PCA, and LR are sub-cases of the more complete AMMI model (Zobel et al., 1988), AMMI offers a more appropriate first model of choice when main effects and interaction are both important (Zobel et al., 1988; Crossa and Cornelius, 1997; Gauch and Zobel, 1997). AMMI increases the precision of yield estimation and selection of higher yielding genotypes than treatment means (Crossa et al., 1990). AMMI has no specific

experimental design requirements, except for a two way data structure (Zobel et al., 1988).

The International Maize and Wheat Centre (CIMMYT) and several other national maize breeding programs constantly develop, evaluate and select the best performing maize varieties for the mid-altitude sub-humid agro-ecology of east and central African (ECA) countries. This is especially important in Ethiopia where the national average maize yields are very low at 2.9 t/ha due to several production constraints, which are the major cause for the presence of GEI and low yield stability. The Bako National Maize Research Project of Ethiopia under the Institute of Agricultural Research (EIAR) coordinates maize research and development nationally. Candidate maize hybrids developed nationally need to be tested for their adaptability and comparative yield stability at the target environments to make recommendations based on the interests of the farmers in the target areas.

In the past maize breeders at EIAR in collaboration with CIMMYT actively engaged in the development of suitable maize varieties, both hybrids and Open pollinated varieties (OPVs), targeting all maize growing agro-ecologies in the country. The estimation of GEI and yield stability analysis of maize genotypes in Ethiopia have been previously reported (Wende, 2003; Gezahegn et al., 2008; Mosisa and Habtamu, 2008; Solomon et al., 2008). However, the type and number of test genotypes and the number of environments included were not sufficiently addressed; as a result there is no information on the GEI and stability in grain yield performance of newly bred single cross hybrids developed for the mid-altitude sub-humid agro-ecology. Therefore, the objectives of the study were to investigate GEI and stability among 81 new and three existing maize (*Zea mays* L.) hybrids across 10 representative locations of the mid-altitude sub-humid agro-ecology and to determine the pattern of grouping of the genotypes and the environments based on grain yield response.

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6.3 Materials and methods

6.3.1 Materials

The study used eighty one experimental maize hybrids including three locally released standard checks (Table 6.1).

Table 6.1 Description of the newly developed maize hybrids and checks tested across ten locations in the mid-altitude sub-humid agro-ecology in Ethiopia

Entry	Entry code	Source	Entry	Entry code	Source	Entry	Entry code	Source
G1	CML-SC-1	CML/NP	G29	CML/BK-29	CML/NP	G57	CML/BK-57	CML/NP
G2	CML/BK-2	CML/NP	G30	CML/BK-30	CML/NP	G58	CML/BK-58	CML/NP
G3	CML/BK-3	CML/NP	G31	CML/BK-31	CML/NP	G59	CML/BK-59	CML/NP
G4	CML/BK-4	CML/NP	G32	CML/BK-32	CML/NP	G60	CML/BK-60	CML/NP
G5	CML/BK-5	CML/NP	G33	CML/BK-33	CML/NP	G61	CML/BK-61	CML/NP
G6	CML/BK-6	CML/NP	G34	CML/BK-34	CML/NP	G62	CML/BK-62	CML/NP
G7	CML/BK-7	CML/NP	G35	CML/BK-35	CML/NP	G63	CML/BK-63	CML/NP
G8	CML/BK-8	CML/NP	G36	CML/BK-52	CML/NP	G64	CML-SC-64	CML/NP
G9	CML/BK-9	CML/NP	G37	CML/BK-37	CML/NP	G65	CML/BK-65	CML/NP
G10	CML-SC-10	CML	G38	BK-SC-38	NP	G66	CML/BK-66	CML/NP
G11	CML/BK-11	CML/NP	G39	BK-SC-39	NP	G67	CML/BK-67	CML/NP
G12	CML/BK-12	CML/NP	G40	BK-SC-40	NP	G68	CML/BK-68	CML/NP
G13	CML/BK-13	CML/NP	G41	BK-SC-41	NP	G69	CML/BK-69	CML/NP
G14	CML/BK-14	CML/NP	G42	BK-SC-42	NP	G70	CML/BK-70	CML/NP
G15	CML/BK-15	CML/NP	G43	BK-SC-43	NP	G71	CML/BK-71	CML/NP
G16	CML/BK-16	CML/NP	G44	BK-SC-44	NP	G72	CML/BK-72	CML/NP
G17	CML/BK-17	CML/NP	G45	BK-SC-45	NP	G73	CML/BK-73	CML/NP
G18	CML/BK-30	CML/NP	G46	CML-SC-46	CML	G74	BK-SC-74	NP
G19	CML-SC-19	CML	G47	CML/BK-47	CML/NP	G75	BK-SC-75	NP
G20	CML/BK-20	CML/NP	G48	CML/BK-48	CML/NP	G76	BK-SC-76	NP
G21	CML/BK-21	CML/NP	G49	CML/BK-49	CML/NP	G77	BK-SC-77	NP
G22	CML/BK-22	CML/NP	G50	CML/BK-50	CML/NP	G78	BK-SC-43	NP
G23	CML/BK-23	CML/NP	G51	CML/BK-51	CML/NP	G79	CML/BK-79	CML/NP
G24	CML/BK-24	CML/NP	G52	CML/BK-52	CML/NP	G80	BK-SC-80	NP
G25	CML/BK-25	CML/NP	G53	CML/BK-53	CML/NP	G81	BK-SC-81	NP
G26	CML/BK-26	CML/NP	G54	CML/BK-54	CML/NP	G82	BH540	NP
G27	CML/BK-27	CML/NP	G55	CML-SC-55	CML	G83	BH543	NP
G28	CML-SC-28	CML	G56	CML/BK-56	CML/NP	G84	BHQPY545	NP

SC = Single cross; G1 – G81 = Candidate single cross hybrids; G82 – G84 = Standard checks; CML/NP = CIMMYT and National program; NP = National program; CML = CIMMYT

The parental inbred lines of the 81 newly developed hybrids were selected based on their heterotic groups, per se performance, and their reaction to major foliar diseases. The check hybrids (BH540, BH543 and BHQPY545) were released by the national maize project and adapted to the mid altitude sub-humid maize growing areas of

Ethiopia. All the hybrids are categorized under the medium maturity group (140-145 days) and adapted to the mid-altitude sub-humid agro-ecology which includes areas with altitudinal ranges of 1000-2000 meter above sea level and receiving an annual rainfall between 1000-1200 mm.

6.3.2 Description of the experimental sites

The study was conducted across 10 locations representing the mid-altitude sub-humid maize producing mega-environments of Ethiopia (Table 6.2). All the locations are among the principal maize testing sites in Ethiopia and believed to represent the maize belt regions of the country. Table 6.2 describes the study sites.

Table 6.2 Description of the 10 locations for testing 84 maize hybrids

Site	Site code	Region	Latitude (^{0} north)	Longitude (^{0} east)	Altitude	Annual rainfall (mm)	Temperature	
							Max	Min
Bako	E1	Oromia	9.12	37.08	1,650	1,211	27.9	12.9
Jima	E2	Oromia	7.67	36.83	1,725	1,448	27.2	11.4
Hawassa	E3	SNNPS	7.03	38.28	1,708	945	26.7	12.3
Arsi Negele	E4	Oromia	7.19	38.39	1,940	900	25.3	11.2
Areka	E5	SNNPS	7.07	37.68	1,750	1,401	25.8	12.5
Asosa	E6	Beni Shangul	10.07	34.52	1,560	1,247	27.8	14.4
Pawe	E7	Beni Shangul	11.15	36.05	1,050	1,585	32.1	16.4
Ambo	E8	Oromia	8.57	38.07	2,225	1,115	25.4	11.7
Haramaya	E9	Oromia	8.37	42.02	2,050	820	23.4	8.9
Finote Selam	E10	Amhara	10.40	37.16	1,853	1,125	25.5	11.2

SNNPS = Southern nations and nationalities peoples state

6.3.3 Experimental design and data measurement

The hybrids were established using a 7 X 12 alpha lattice design with two replications at each location in 2012. Each Plot consisted of one row with 5.1 m in length; the whole row was used for data recording. The spacing between rows was 75 cm, while spacing between plants was 30 cm. Two seeds per hill were sown and later thinned to achieve

the desired plant densities of 44,444 ha^{-1}. Trials received 100 kg ha^{-1} P$_2$O$_5$ at planting and 100 kg ha^{-1} N in two splits (at planting and knee height). The trials were conducted under rain-fed conditions and other management practices were followed according to the recommendations of the specific areas. Grain yield (t ha^{-1}) was the major character measured for the analysis. Grain weight from all the ears of each experimental plot was measured and used to calculate grain yield at 12.5% moisture level.

6.3.4 Data analysis

Yield data were subjected to AMMI and GGE biplot analyses using GenStat software version 14 (Payne, 2008). The AMMI model, which combines the standard analysis of variance with principal component analysis (Zobel et al., 1988), was used to investigate the nature of GEI. The AMMI model first fits additive effects for the main effects of genotypes and environments, using the additive analysis of variance procedure. Subsequently the program fits multiplicative effects for GEI by principal component analysis (Zobel et al., 1988; Gauch and Zobel, 1996, 1997). Biplots (GGE - biplot, 2009) were used to illustrate the relationships among genotypes, environments and genotypes and environments. Both AMMI and GGE biplot methods were used to investigate the G, E and GEI effects on grain yield of maize hybrids. These methods were described in detail by Yan (2002), Yan and Hunt (2001) and Gauch (2006).

6.4 Results and discussion

6.4.1 Combined analysis of variance
The combined analyses of variance (ANOVA) of the 84 maize hybrids evaluated across 10 locations according to the AMMI model are presented in Table 6.3. The ANOVA indicated highly significant differences (P $\leq$ 0.01) for environments, genotypes and genotypes x environment interaction. The IPCA are ordered according to decreasing importance. The F-test was highly significant (P $\leq$ 0.01) for the first three IPCA axes.

Table 6.3 Analysis of variance (ANOVA) based on the AMMI model for grain yield (t ha^{-1}) of 84 maize hybrids tested across ten locations using two replications

Source of variation	DF	SS	MS	Total variation explained (%)	G X E explained (%)	Cumulative (%)
Total	1679	7583	4.52			
Genotypes (G)	83	600	7.23**	8.21		
Environments (Env)	9	2819	313.24**	39.00		
Reps within Env.	10	299	29.87**			
G x Env	747	1970	2.72**	26.00		
IPCA 1	91	528	5.80**		26.80	26.80
IPCA 2	89	447	5.03**		22.69	49.49
IPCA 3	87	275	3.16**		14.00	63.49
IPCA 4	85	209	2.75ns		11.00	
Residuals	395	1012	1.45			
Error	830	1833	2.21			

DF= degrees of freedom; SS=Sum of square; MS= Mean square

* and ** denote significant differences at $P \leq 0.05$ and $P \leq 0.01$, respectively.

IPCA= Interaction principal component axis

The total explained variation, ranged from 8.21% for genotypes, 39.00% for environments and 26.00% for G x E. The highly significant variance of the environment indicates its major contribution in influencing yield performance of maize. The variation due to G x E was three fold than variation ascribed to genotype main effect. Out of the total eight IPCA (data not presented), the first three IPCA axes explained 63.49% of the G x E interaction. The first IPCA captured 26.80% of the total interaction sum of squares in 12% of the interaction degrees of freedom. The second IPCA explained 22.69% of the interaction sum of squares in 11.90 % of the interaction degrees of freedom, and the third IPCA explained 14 % of the GE interaction sum of squares in 11.6% of the interaction degrees of freedom (Table 6.3).

Table 6.4 and Table 6.5 present the AMMI analyses data with the IPCA1, IPCA2 and IPCA3 scores for the hybrids and the test environments, respectively. The IPCA scores indicate how far the individual genotype or environment deviates from the zero (origin). The more deviation from zero (either negative or positive direction) the more unstable

they are. The tables also show graph ID of the hybrids and the environments. In Figure 6.1 the IPCA1 scores for both the hybrids (lower case) and the environments (upper case) are plotted against the mean yield for the hybrids and the environments, respectively.

Table 6.4 IPCA1, IPCA2, and IPCA 3 scores and graph IDs for the 84 maize hybrids sorted based on mean yield (t/ha) when evaluated in ten environments

Graph ID	Hybrid	Mean GY (t/ha)	IPCA1	IPCA2	IPCA3
G1	1	5.978	-0.09192	0.23162	0.83826
G2	2	7.311	-0.10405	0.11565	0.02907
G3	3	7.312	-0.48175	1.14128	-0.65878
G4	4	6.838	0.22974	0.11690	-0.12639
G5	5	7.396	0.05051	0.81772	-0.34682
G6	6	6.093	-0.26508	-0.47066	0.38159
G7	7	6.393	0.01892	0.39346	-0.21634
G8	8	6.871	0.16397	0.14015	0.55093
G9	9	6.676	-0.21870	0.04960	-0.15144
G10	10	6.543	-0.12048	-0.07344	0.26384
G11	11	7.239	-0.05835	-0.13454	0.41570
G12	12	6.642	0.27016	-0.05924	0.58849
G13	13	6.743	-0.20992	-0.34565	-0.10752
G14	14	7.355	-0.36492	0.07035	0.61979
G15	15	6.679	-0.05050	0.21566	0.29945
G16	16	7.064	0.31220	0.21587	0.05776
G17	17	7.036	0.09204	-0.50228	0.14986
G18	18	6.915	-0.30350	-0.20241	0.33603
G19	19	5.650	0.69396	-0.06183	0.26260
G20	20	5.933	-0.42511	-0.48957	-0.25700
G21	21	7.050	0.54363	0.60062	-0.72879
G22	22	6.836	0.89799	0.07673	-0.40139
G23	23	5.641	0.28894	0.01503	-0.18521
G24	24	6.986	0.38091	0.02704	0.30469
G25	25	6.163	-0.09977	-0.17226	-0.36231
G26	26	6.701	0.42429	-0.43784	-0.31403
G27	27	5.761	-0.54390	-0.39576	-0.62139
G28	28	6.742	0.00495	0.15196	0.50116
G29	29	6.299	0.39897	0.09612	-0.09116
G30	30	7.410	-0.40538	-0.53749	0.03118
G31	31	6.724	0.36579	-0.08398	0.08520
G32	32	7.285	-0.46206	0.76381	0.01077
G33	33	6.066	-0.72282	0.03752	0.59269
G34	34	7.335	0.67173	0.47983	0.10183
G35	35	6.496	-0.40272	-0.19975	0.42169
G36	36	6.669	-0.29377	-0.19346	-0.30607
G37	37	7.547	-0.62421	0.07719	-0.08813
G38	38	6.687	0.14305	0.15560	0.44230
G39	39	7.746	-0.50208	0.10331	-0.23598
G40	40	6.849	1.37852	0.36239	-0.02252
G41	41	6.232	-0.32509	1.60943	-0.12624

G42	42	6.876	-0.26338	0.59283	0.07854
G43	43	7.007	0.02033	-0.01876	-0.10141
G44	44	6.265	-0.37935	0.22106	0.48450
G45	45	6.962	-0.07952	0.48328	0.08559
G46	46	6.137	0.20116	-0.29166	-0.06274
G47	47	6.172	-0.22056	-0.14058	-0.57452
G48	48	6.363	0.15094	-0.19594	0.02970
G49	49	6.064	-0.19408	0.20112	-0.22058
G50	50	6.556	-0.70220	0.18151	0.15163
G51	51	6.678	-0.33439	0.06694	0.30484
G52	52	6.650	-1.04305	-0.28925	0.51290
G53	53	6.429	0.56075	-0.20203	-0.04181
G54	54	6.267	0.45643	-0.09584	-0.29973
G55	55	6.416	1.18041	0.49605	0.48690
G56	56	5.904	0.19333	-0.20283	-0.04768
G57	57	6.738	0.08974	-0.21979	-0.07299
G58	58	5.894	0.23786	-0.57389	0.04232
G59	59	7.151	0.01426	0.31476	-0.28188
G60	60	5.668	0.38717	-0.28821	0.50942
G61	61	6.364	0.26565	-0.38604	0.39349
G62	62	5.998	0.26283	0.17439	0.33187
G63	63	5.468	0.15499	-0.53755	-0.02632
G64	64	7.221	-0.60707	-0.50412	-0.97508
G65	65	6.523	-0.54202	-0.91028	-0.57635
G66	66	5.639	-0.28601	-0.64535	-0.09463
G67	67	7.398	0.01118	-0.14229	0.35035
G68	68	8.127	-1.04694	0.53166	0.64505
G69	69	5.999	-0.06652	0.11871	0.34321
G70	70	6.883	-0.78788	0.47792	-0.31246
G71	71	6.861	0.65538	-0.18160	0.05205
G72	72	6.713	0.15225	0.31682	-0.56381
G73	73	6.624	0.07084	-0.15670	-0.47010
G74	74	5.298	0.20887	-0.89596	-0.30904
G75	75	6.731	-0.17822	0.41616	-0.54807
G76	76	5.008	0.04919	-0.06324	-0.47677
G77	77	7.343	-0.19069	0.58595	-0.43286
G78	78	5.653	0.01953	-0.03571	0.18316
G79	79	6.066	0.02847	-0.48696	-0.09220
G80	80	5.492	-0.13226	-0.69570	0.44174
G81	81	5.892	0.20944	-0.10092	-0.44225
G82	82	6.305	0.73650	-0.01507	-0.06865
G83	83	7.005	0.49684	0.04897	0.12538
G84	84	6.436	-0.01439	-0.65657	-0.39806

Table 6.5 The IPCA1, IPCA2 scores and the graph ID for the ten environments, sorted on environmental mean yield

Graph ID	Environments	ENV. mean	IPCA1	IPCA2	IPCA3
E1	1	8.027	-1.09773	0.78827	0.30266
E2	2	7.415	-0.50139	1.14466	-1.74512
E3	3	7.386	3.47741	1.18906	0.36031
E4	4	8.189	0.36602	-2.70698	0.95825
E5	5	4.662	0.58905	-0.52975	-0.21569
E6	6	6.720	-0.85951	1.47135	0.54051
E7	7	6.819	0.07394	-1.32691	-1.98329
E8	8	5.034	-0.89845	0.10737	0.95842
E9	9	6.901	-0.53800	-0.23654	-0.59071
E10	10	4.458	-0.61135	0.09947	1.41469

Table 6.6 First four AMMI selections per environment

| Environments	Mean	1st	2nd	3rd	4th
E1	8.02	G68	G3	G41	G32
E2	7.42	G77	G5	G39	G21
E3	7.39	G40	G55	G34	G22
E4	8.19	G17	G30	G11	G18
E5	4.66	G34	G39	G40	G30
E6	6.72	G68	G32	G3	G5
E7	6.82	G64	G84	G65	G47
E8	5.03	G68	G14	G52	G67
E9	6.90	G39	G37	G68	G64
E10	4.46	G68	G14	G67	G52

Table 6.6 summarizes the first four hybrids considered as best in all the 10 environments. Accordingly, G68 was selected as number one in four environments and selected number three in one environments followed by G67, G40 and G30 which were identified as best in two environments.

6.4.2 AMMI biplot

The plot (Figure 6.1) of the hybrids and the environments clearly shows the pattern of associations. The IPCA scores of a genotype in the AMMI analysis (Table 6.4 and Figure 6.1) are an indication of the stability of a genotype over environments. The greater the IPCA scores, either positive or negative, the more specifically adapted a genotype is to certain environments. The more IPCA scores approximate to zero, the more stable the genotype is over all environments sampled. Accordingly, hybrids G45, G2, G28 and G17 can be considered as the most stable hybrids, as their IPCA scores are closer to zero (Table 6.4 and Figure 6.1)

From the biplot (Figure 6.1), environments are distributed from lower yielding in quadrants I (top left) and IV (bottom left) to the higher yielding in quadrants II (top right) and III (bottom right) (Figure 6.1). The higher yielding environments classified according to the AMMI 1 model were E1 (Bako), E2 (Jima), E4 (Arsi Negelle) and E3 (Hawassa), whereas, the lower yielding environments are E5 (Areka), E8 (Ambo) and E 10 (Finote Selam). As a result, Areka is generally categorized under low yielding maize environment as compared to the other two (Bako and Jima), which were relatively categorized under high yielding environments. It is further noted that Arsi Negele (E4) was the most favourable environment and E10 (Finote Selam) the less favourable among the ten environments included in this study. This situation is clearly indicated in Figure 6.1, where the two environmental variations are plotted far apart from the mean. The observed yield differences across the locations were due to many factors, like low temperature and prevalence of NCLB at Ambo, and also NCLB pressure at Areka.

The hybrids categorized under favorable environments with above-average mean yields (7 – 8 t ha^{-1}) were G68, G39, G37, G77, G34 and G2 among them G2 is found to be more stable. Genotypes grouped under low yielding environments were shown at the lower left quadrant of the biplot (Figure 6.1). Generally G33 was the most unstable genotype identified by the AMMI model (Figure 6.1). Genotypes that were close to each other tend to have similar performance and those that were close to environment indicates their better adaptation to that particular environment. Hence hybrid G68 was better adapted to Bako and hybrids G14 and G30 showed similar performance as they were close to each other.
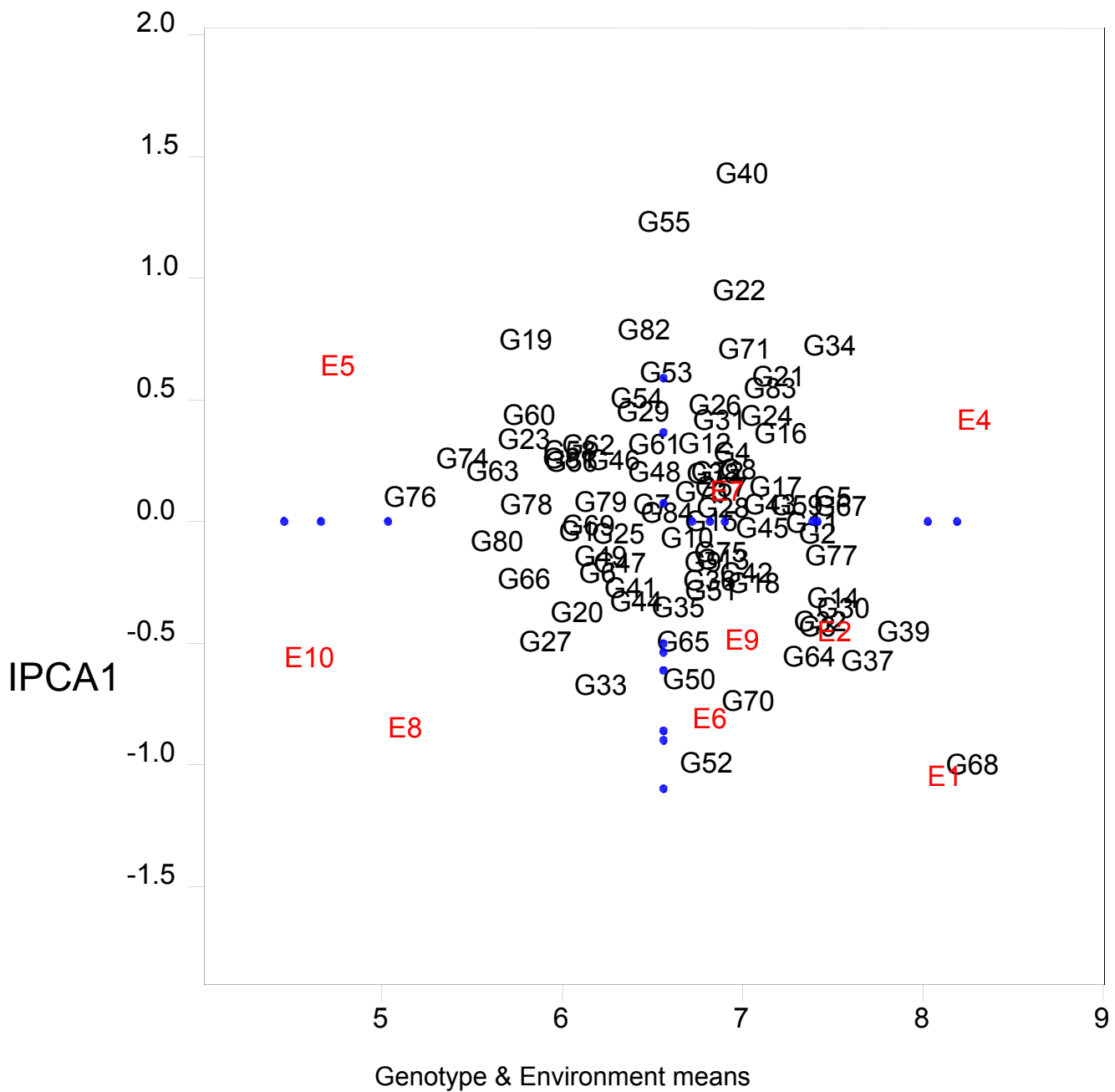
Figure 6.1 AMMI 1 biplot for grain yield of maize hybrids showing genotypes (G1-G84) and environments (E1-E10) plotted against their IPCA1 scores

The three IPCA axes can be taken as adequate dimensions for the data (Table 6.3 and 6.4). However, only the first two IPCA axes were plotted against one another to help investigate the G x E interactions pattern of each genotype (Figure 6.2). Among the test environments, Bako had the best yield potential and a good stability. Hybrids G68, G39 and G30 had the best association with the Bako and Jima, with the average yields of more than 7.5t/ha. The hybrids with low stability or associated with one or two sites would have a disadvantage of not adapting to other sites. It is therefore important to release for farmers these promising hybrids with good general stability that would also not only adapt, but also be productive in unstable environments. According to the AMMI analysis the hybrids G45, G2, G67, G5 and G11 were the most stable and they were more adapters to E2 (Jima) and E7 (Pawe) environments. While G30, G39, G14, G64 and G37 were most stable in E2 (Jima), G34, G21, G24, G83 and G16 were adapters to E4 (Arsi Negele).

6.4.3 GGE biplot

In addition to AMMI, the GGE biplot was also utilized to investigate the GEI in this study. The GGE biplot revealed the GEI of both the hybrids and the environments, This was done by plotting the most discriminating environment, by revealing which hybrid won where and finally by identifying the ideal genotypes in each mega environments (Figure 6.2, 6.3 and 6.4). An ideal genotype should have both high mean yield performance and high stability across environments (Kaya et al., 2006; Yan and Tinker, 2006). Furthermore, the ideal genotype is a genotype to be on average environment coordinate (AEC) on positive direction and has vector length equal to the longest vectors of the genotypes on the positive side of AEC with the longest vector length of high yielding genotypes and they are indicated by an arrow pointed to it (Kaya et al., 2006; Yan and Tinker, 2006). In this regard the hybrid G68 was revealed as an ideal genotype and allocated Hawassa as the most discriminating environment (Figure 6.2 and 6.4). It also divided the environment used in this study into three mega environments namely mega environment 1 (Bako, Jima, Asosa, Ambo, Finote Selam, Haramaya and Pawe), mega

environment 2 (Hawassa and Areka) and mega environment 3 (Arsi Negelle). Different hybrids were identified as winning genotypes in different mega environments. Accordingly hybrid G68 was the winning genotype in mega environment 1, G40 was winner in mega environment 2 and G52 was the winning hybrid in the third mega environments. Thus these three winning hybrids can be recommended for production in their respective mega environments.

The GGE biplot generated using the first two principal component scores showed a clear association between genotypes and environments (Figure 6.2). The biplot showed that Hawassa was the most discriminating environment for the genotypes as indicated by the longest distance between its marker and the origin. This environment provided adequate information on the performance of the hybrids. However, due to its high IPCA score, genotype variability at this environment may not exactly reflect the average genotypic performance across environments. Considering the environments closer relationships was observed between Haramaya and Ambo, which were both transitional highlands with similar production factors. Pawe was identified as stable environment as its IPCA2 score and its vector was near to the source (zero). Arsi Negele and Hawassa were projected in the opposite direction (Figure 6.2), indicating that hybrids better performed in Hawassa may not have the same trend at Arsi Negele, this was because the two locations are situated in different production zones, Hawassa in mid-altitude where as Arsi-Negele in the highland transitional areas.
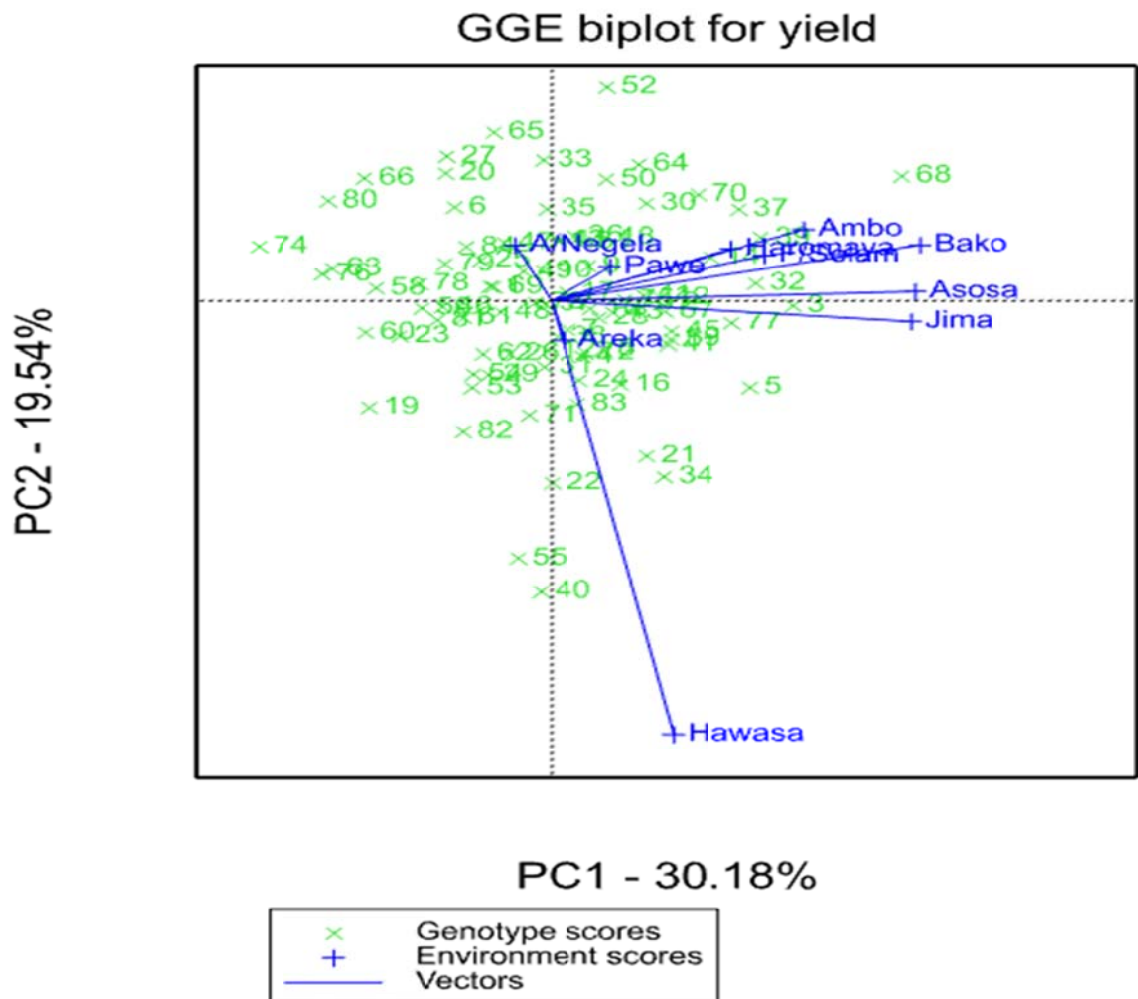
Figure 6.2 GGE biplot on grain yield of 84 maize hybrids tested at 10 locations plotted with PC1 and PC2 showing genotypes, environments and their vectors.

The GGE biplot (Figure 6.2) also indicated the relationship among the maize hybrids. Hybrid G74 was different from others as it is located far apart from the other hybrids in the biplot. This hybrid is also the most unstable. Hybrids which were positioned closer to the origin of the biplot (G67, G77, G3) indicate their stability in performance across environments, while those positioned far apart (G74, G40, G55) are unstable. Hybrid G19 was more adapted to low yielding environment (Areka) and hybrids G68 was more close to Bako, the high yielding environment. Generally hybrids which lies nearer to each other and those projecting in similar direction, designate their proximity in the grain yield performance (Figure 6.2).
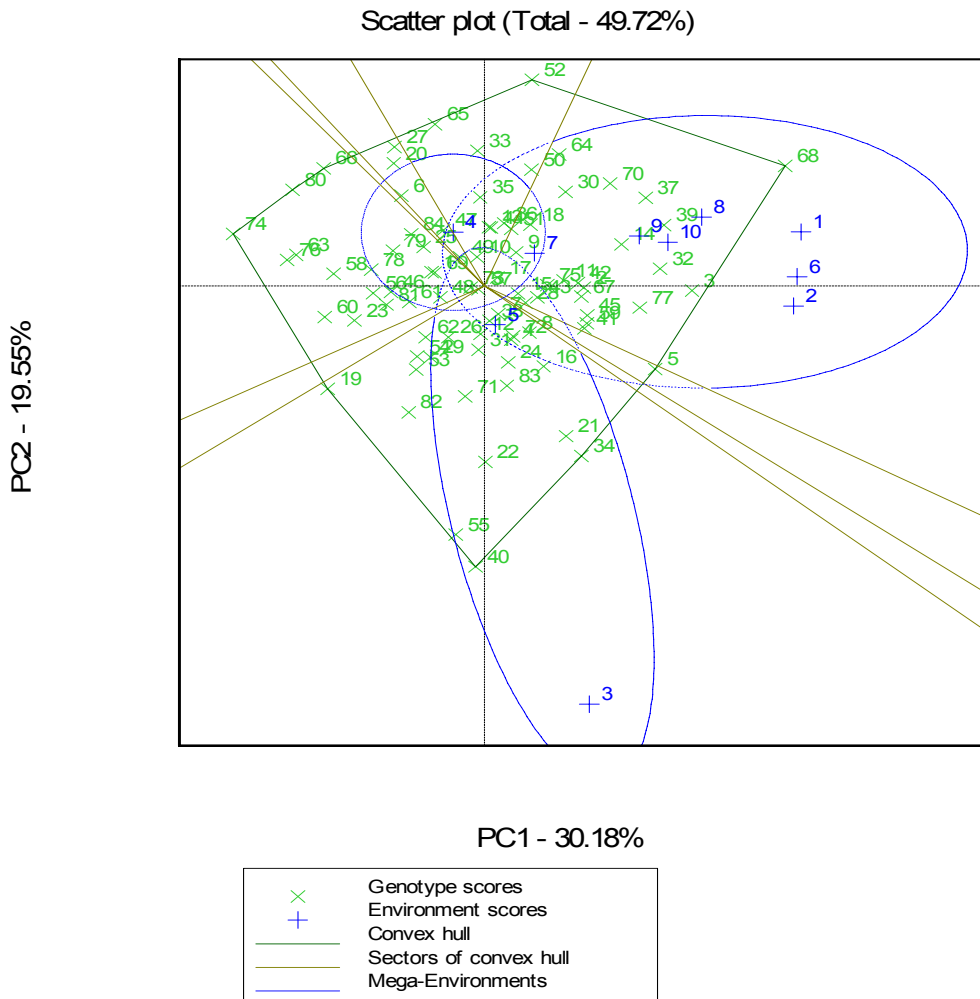

Figure 6.3 Polygon views of the GGE biplot based on symmetrical scaling for which won where pattern of genotypes and environments.

Mega environment classification and winning genotypes

Figure 6.3 presents schematic view of which hybrid won where. Accordingly, nine lines divide the biplot into nine sectors, out of these; environments fall into 3 of them. Seven environments (E1 (Bako), E2 (Jima), E6 (Asosa), E7 (Pawe), E8 (Ambo), E9 (Haromaya) and E10 (Finote Selam)) fell in one sector comprising one large mega-environment, and the vertex genotype for this sector was G68 implying that this genotype was the winning genotype for these environments. Sector 2 contained two environments (E3 and E5). The remaining environment (E4) was contained in the last smaller mega environment and G52 hybrid being the winner.

It appears that there exist three possible mega environments. The first mega environment was consisting of seven environments, with G68 as a winner genotype. The second mega environment was smaller compared to the first and it comprises environments E3 (Hawasa), and E5 (Areka) with a winning genotype G40. The third and the smallest mega environment contained E4 (Arsi Negelle), and the winner genotype was G52.

Comparison biplot (Total - 50.45%)
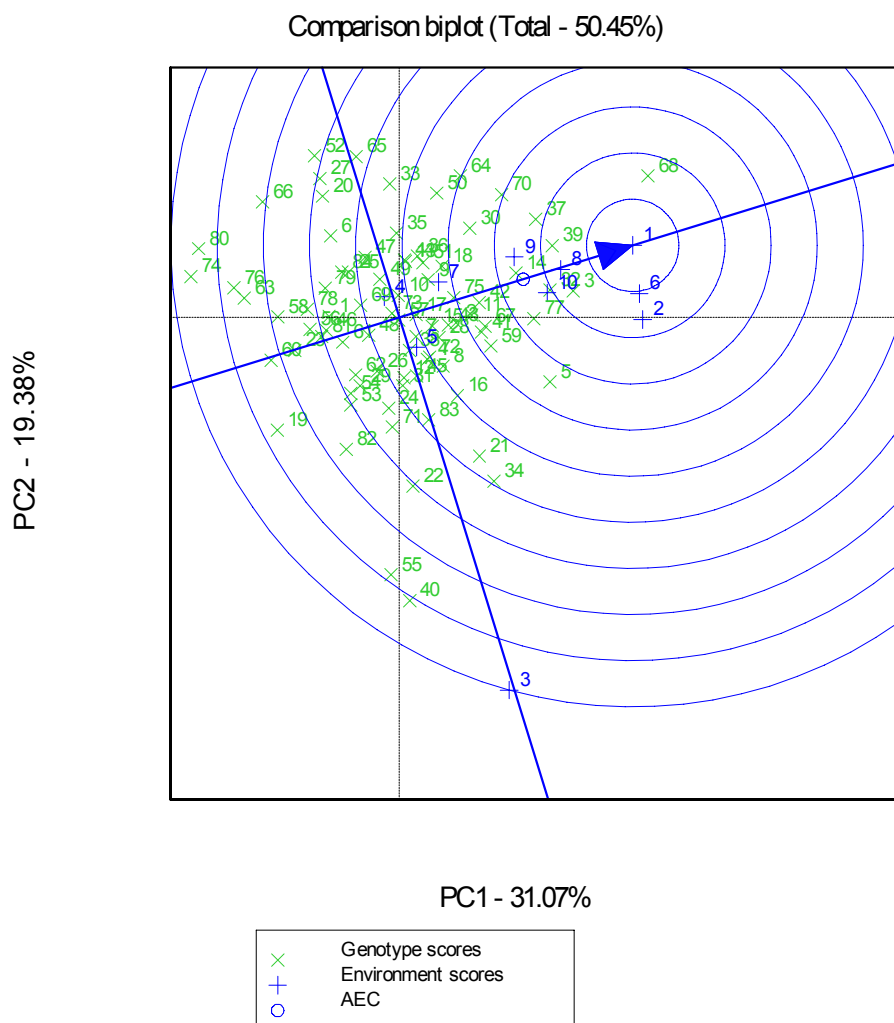
Figure 6.4 Ranking of genotypes relative to an ideal genotype. The ideal genotype can be used as a reference for genotype evaluation.

The ideal genotype can be used as a reference for genotype evaluation. In this study, G68, G3 and G39 were ideal genotypes (the center of concentric circles) and genotypes located closer to the ideal genotypes are more desirable than the others (Figure 6.4). Genotypes grouped in the concentric circle next to ideal genotype were more desirable. However, genotype G74 and G80 were undesirable (Figure 6.4). The present study used the AMMI and the GGE models and summarized patterns and relationships of genotypes and environments successfully. These models are reportedly useful to provide a valuable prediction assessment (Ezatollah et al., 2012). However, Becker and

Léon (1988) stated that multivariate methods are too sophisticated to provide a simple measure of yield stability which allows a ranking of genotypes. In the present study the models have clearly demarcated the pattern of adaptation of hybrids to environments and can be used to identify the superior genotypes in relation with the environments.

Three hybrids namely G68, G40 and G52 were identifies as stable hybrids by both AMMI-3 and GGE biplot methods of stability analysis, and thus they can be recommended for further testing across years or be recommended for production in the mid-altitude sub-humid areas of Ethiopia and similar environments in Sub-Saharan Africa.

6.5 Conclusions

The results of this study indicated that grain yield performances of newly developed hybrids were highly influenced by environmental effect followed by GEI. Mosisa and Habtamu (2008) also reported the influence of environment and GEI using different sets of maize genotypes across locations in Ethiopia. The magnitude and genotypic differences had least contribution to the total variation. The magnitude of GEI effect was about more than three times than that of genotype. Maize hybrids showed crossover GEI. Among hybrids, G68, G39, G37, G77, G34, G40 and G2 were selected with high yield (> 7tha^{-1}) and average stability. These candidate hybrids can be recommended in the mid-altitude areas of Ethiopia. Regarding testing environments, there exist three possible mega maize growing environments in the mid-altitude sub-humid maize growing areas of Ethiopia. These include: mega environment 1, 2 and 3, each of them consisting of different environments where the evaluations of the hybrids made. Therefore, the maize breeding program of Ethiopia should consider these three mega environments separately in order to maximize the yield potential of maize and provide specific recommendation in the mid-altitude areas of the country. Moreover, additional yield trials may be required to better investigate the magnitude of GEI and yield stability of maize hybrids across all the maize growing agro-ecologies in addition to the mid-altitude areas.

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Chapter 7
An Overview of the Research Findings

7.1 Introduction and objectives of the study

Maize is one of the leading food security crops in Ethiopia widely grown in the mid-altitude sub-humid agro-ecologies. Potential maize yields are considerably low due to biotic, abiotic and socio-economic constraints in the country. Among the biotic constraints, *Turcicum* leaf blight caused by *Exserohilum turcicum* Pass Leonard & Suggs inflicts a significant yield loss. Crop productivity is the function of the genetic potential, the growing environments and genotype by environment interactions. Identification of useful and farmers-preferred maize germplasm is an important step to develop improved varieties with high yield potential, *Turcicum* leaf blight resistance and yield stability across target growing environments. This chapter summarised the research objectives and highlighted the core findings and their implications for maize breeding towards high-yield potential and resistance to *Turcicum* leaf blight.

In summary, the objectives of this study were:

- To assess the present importance, production and productivity constraints, input availability and local market of maize in the mid-altitude, sub-humid agro-ecology of western Ethiopia.
- To determine the genetic variability among elite maize inbred lines and select promising parents for resistance to *E. turcicum*.
- To determine the level of genetic diversity among medium to late maturing maize inbred lines using genetic distances as measured by single sequence repeat (SSR) DNA markers.
- To determine combining ability and heterosis among 18 elite maize inbred lines and their hybrids in diverse tropical mid-altitude environments.

- To investigate genotype x environment interaction and yield stability of maize developed for the mid-altitude sub-humid agro-ecology.

7.2 Research findings in brief

7.2.1 Assessments of the preferences and constraints of maize farmers in the development and adoption of improved varieties in the mid-altitude, sub-humid agro-ecology of western Ethiopia

A participatory rural appraisal (PRA) research was conducted using semi-structured questionnaire and focus group discussions and involving 240 randomly selected farmers, in twelve sub-districts, within three administrative zones in Ethiopia. The study indicated that;

- Maize was identified as the number one crop both for food and as a source of cash and the majority of the farmers grew hybrid maize varieties.
- Unavailability of improved seed and lack of production inputs as well as unpredictable grain prices were among the major constraints reducing maize production and productivity in the mid-altitude sub-humid areas of Ethiopia.
- *Turcicum* leaf blight is identified as the major leaf disease in the mid-altitude maize growing areas in Ethiopia.
- Farmers-preferred maize varieties were high yielding, disease resistant and stable performing across production areas and year.
- Breeding for improved disease resistance and grain yield, enhancing the availability of crop input and stabilizing market price during harvest time are the most important strategies to increase maize production by small-scale farmers in western Ethiopia.

7.2.2 The genetic variability of elite maize inbred lines for resistance to Exserohilum turcicum in the mid-altitude sub-humid agro-ecology of Ethiopia

Fifty Inbred lines were evaluated in the field using the RCBD design with three replications. The study revealed that;

- Inbred lines showed significant differences for *Turcicum* leaf blight reactions which were allocated into resistant, intermediate or susceptible groups.

- Inbred lines such as CML-202, 144-7-b, 139-5-j, 136-a, 30H83-7-1-1-1-2-1, ILoo'E-1-9-1-1-1-1-1 and 142-1-e, were identified as promising sources of resistance against *E. turcicum* for mid-altitude sub-humid agro-ecology of Ethiopia or similar agro-ecologies.

7.2.3 Genetic interrelationships among medium to late maturing tropical maize inbred lines using selected SSR markers

Twenty selected maize inbred-lines were genotyped using twenty polymorphic SSR markers selected from the Maize GDB database (http://www.agron.missouri.edu). It was found that;

- The inbred lines were allocated into five genetic clusters consistent with the known pedigrees except for a few discrepancies.

- The SSR primers identified 108 alleles and the number of alleles scored across loci ranged from 1 to 11, with a mean of 5.4.

- The greatest genetic distance was observed between the clusters of lines CML-202 and Gibe-1-91-1-1-1-1.

- The genetic grouping present in the source population will assist in effective utilization of the lines for tropical hybrid maize breeding programs to exploit heterosis.

7.2.4 Heterosis and combining ability of grain yield and Northern Corn Leaf Blight resistance among maize genotypes adapted to the mid-altitude sub-humid agro-ecologies

The study used 81 single cross hybrids generated from crosses of two sets of parents consisting of nine females and nine males using the North Carolina Design II mating scheme. The study showed that;

- Top 20 promising experimental hybrids were identified that displayed high grain yield and up to 250% high parent (HP) and mid-parent heterosis (MPH).

- General combining ability (GCA) and specific combining ability (SCA) effects were significant ($P \leq 0.05$) for most traits, implying significance of both additive and non-additive gene effects, respectively.

- Inbred lines such as CML 395, 30H83-7-1, ILO'OE-1-9, 124-b (113), CML202, CML312, and Gibe-1-91 were promising parents with significant and positive GCA effects and their hybrids displayed significant SCA effects for grain yield potential.

- Lines such as CML395 and ILO'OE-1-9 displayed negative and significant GCA effects for *Turcicum* leaf blight resistance. Moreover, considerable amount of hybrids had -14% and -25% standard and mid-parent heterosis for *Turcicum* leaf blight resistance, respectively.

- Outstanding experimental hybrids such as CML-312 x CML395, CML197 x CML395, CML443 x DE-78-Z-126 were identified with average yield of > 8 t ha^{-1} and high specific combining ability. These hybrids can be used for direct production or as testers in three-way hybrid development.

7.2.5 Genotype by environment interaction and yield stability of maize hybrids developed to the mid-altitude sub-humid agro-ecology of Ethiopia

The study evaluated 81 newly developed maize hybrids and three local checks across 10 locations representing the mid-altitude sub-humid maize producing environments of Ethiopia. It is indicated that;

- Highly significant differences were detected between genotypes, environments and genotype x environment interaction (GEI).

- The AMMI biplot well-depicted the genotypes on the basis of their adaptation patterns. The GGE biplot also clustered the environments into mega-environments and identified the winning genotype in each mega-environment.

- Hybrids G68, G39, G37, G77, G34 and G2 were found to be more stable and responsive to favourable environments such as Bako, Jima, Arsi Negelle and Pawe. Hybrids G25 and G80 showed higher yield stability in the low yielding environments (Ambo, Areka and Finote Selam).

- Hawassa was identified as the most discriminating environment for the newly developed hybrids.

- Overall, the study identified candidate single cross hybrids such as G68, G2, G77 and G67 with high yield (> 7.5 t ha^{-1}) and average stability for release in the mid-altitude sub-humid agro-ecology.

7.3 Implications of the research findings to breeding maize for higher yield and resistance to *Turcicum* leaf blight

The following implications for breeding were noted:

- Farmers' participation in maize varietal selection and identification of breeding priorities is important for better dissemination, acceptance and impact of improved varieties and production technologies. Their views and priorities will be considered by the maize program in Ethiopia.

- There is considerable genetic diversity for grain yield potential and *Turcicum* leaf blight resistance between selected elite inbred lines which is useful for maize breeding in the mid altitude sub-humid maize growing areas in Ethiopia.

- The SSR genetic markers were useful and provided five distinct genetic groups enabling breeders to design targeted crosses for hybrid development to exploit heterosis, and maintain diversity between clusters.

- The importance of both additive and non-additive effects in controlling grain yield, *Turcicum* leaf blight resistance and other agronomic traits suggested that breeding gain can be realized through hybridization and selection strategies in the program.

- Overall, the study developed promising new single cross hybrids with yield advantages and leaf blight resistance for breeding and large-scale production in the mid-altitude sub-humid areas of Ethiopia. These can be evaluated in similar agro-ecologies in sub-Saharan Africa.