Genetic Analysis of Stem Rust Resistance among Ethiopian Grown Wheat Lines

By

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Thesis summary

Wheat (*Triticum aestivum* L.) is one of the major food crops in the world. Ethiopia is the second largest wheat producer in sub-Saharan Africa. However, wheat production in Ethiopia is constrained by many biotic and abiotic factors, and socio-economic constraints. Among the biotic stresses are the rust diseases: stem rust caused by *Puccinia graminis* f.sp. *tritici*, leaf rust (*P. triticina* Eriks) and stripe rust (*P. striiformis* Westend. f.sp. *tritici*)). Stem rust is considered to be the most destructive disease of wheat in the main wheat growing regions of Ethiopia. Losses may reach 100% on susceptible wheat cultivars when conditions are favorable for disease development. Use of resistant cultivars is the most effective, economical and environmentally safe control measure, especially for the resource poor farmers. Due to the frequent emergence of new stem rust races through mutation, migration and recombination of exsisting virulence genes, efforts to identify potentially new sources of effective resistance genes are of the highest importance followed by their incorporation into a desirable genetic background.

The objectives of the study were 1) to identify the primary threats to wheat production, farmers' selection criteria for wheat varieties, and disease management practices with emphasis on wheat rusts in the Arsi, Bale and West Shewa administrative zones of Ethiopia; 2) to identify possible sources of stem rust resistance among Ethiopian wheat lines; 3) to determine the levels of heterosis and combining ability, and to identify the best parents and crosses for breeding to stem rust resistance, high grain yield and desirable agronomic traits; 4) to introgress durable resistance genes from known resistance sources into farmers'-preferred and locally adapted but stem rust susceptible, improved wheat varieties.

A participatory rural appraisal (PRA) research was conducted involving 270 farmers in six districts of three administrative zones in Ethiopia. The participating farmers listed and prioritized their wheat production constraints. Wheat rust diseases, the high costs of fertilizers, lack of access to seeds of improved varieties and high seed prices were the major constraints reported by the respondents. The most important traits that farmers sought in wheat varieties were disease resistance and high grain

yield. Estimated yield losses due to stem rust disease were more than 60% in all the surveyed areas. Fungicide application was the main disease management practice used by the majority of respondent farmers.

Field and greenhouse experiments were conducted to identify possible sources of stem rust resistance among Ethiopian wheat lines. Two hundred fifty two wheat genotypes were evaluated for their resistance to stem rust at the seedling stage. Ninety one lines that exhibited intermediate and susceptible seedling reactions were field tested for their slow rusting characteristics. Among the 91, 38 lines that had high to moderate level of slow rusting were advanced to further field evaluation. Ten lines (H04-2, 204408-3, 214551-1, 231545-1, 7041-1, 7514-1, 226385-1, 226815-1, 7579-1, and 222495-1) were identified as good slow rusting lines while seven (237886-1, 227059-1, 203763-1, 226275-1, 227068-2, 226278-1 and 7994-1) were identified as moderately slow rusting lines.

Fifteen wheat hybrids were developed through a half diallel mating design involving six parents. The F1's and their parents were field evaluated for their stem rust reaction and agronomic performances at the Debere-Zeit Agricultural Research Center in Ethiopia, which is a well known hot spot area for stem rust. The analysis of variance revealed that tested genotypes had considerable genetic variability for all characters studied. The maximum positive mid-parent (31.45%) and better-parent heterosis (25.38%) were observed for grain yield. Plant height and days to maturity had maximum negative mid-parent heterosis levels of -11.01% and -8.02%, respectively. The majority of the crosses expressed negative heterosis over their mid-parent for AUDPC, indicating these crosses manifested resistance against stem rust. Significant general combining ability (GCA) effects were observed for all the characters studied. Furthermore, significant specific combining ability (SCA) effects were detected for most of the traits. Non-additive gene action was predominant for grain yield, thousand kernel weight and plant height. Additive gene action played a greater role in the inheritance of AUDPC, kernels per spike, number of tillers per plant and days to maturity. The study identified parental lines with good GCA effects for most of the characters, especially H04-2, Digelu and Danda'a. Crosses 231545-

1 x H04-2, 7041-1 x H04-2, Digelu x Kubsa and Danda'a x Kubsa had significantly negative SCA effects for AUDPC. Progenies of these crosses will be selected in an ongoing stem rust resistance breeding program. In general, H04-2 and Danda'a were good general combiners for most of the important studied characters. Crosses that involved these lines performed well for most of the traits. Hence, Lines H04-2 and Danda'a could be exploited in wheat breeding programs to develop stem rust resistant and high yielding wheat cultivars.

Stem rust resistance genes were introgressed into locally adapted, high yielding susceptible wheat varieties, Kubsa (HAR1685) and Galama (HAR604), from two sources of adult plant resistance, Pavon 76 and Kenya Plume, using the single backcross-selected bulk breeding approach. The resistance sources were crossed with the adapted high yielding varieties and a single backcross was made with the recurrent parent. The resulting BC1 populations were selfed until the F3. Bulk selection was practiced from BC1- F3. The F3 populations, along with the recurrent parents, were evaluated in a replicated trial at Debre-Zeit Agricultural Research Center under high stem rust pressure to determine the genetic improvement attained in the populations for stem rust resistance and agronomic traits. All F3 populations, except the cross of Galama x Kenya Plume, were better performing for stem rust resistance and most agronomic traits studied when compared to the recurrent parents. The F3 progenies of Kubsa x Pavon 76 had superior mean values and high genetic gains for most agronomic attributes and stem rust resistance. These progenies will be advanced and selected in subsequent generations to develop locally adapted pure line wheat varieties with improved stem rust resistance and farmers'-preferred agronomic traits.

Overall, the present study attempted to understand farmers' wheat varietal preferences, farmers' wheat production constraints, identified slow rusting wheat lines among the Ethiopian bread wheat germplasm, identified promising lines and F1 hybrids with good combining ability for breeding towards stem rust resistance and high yields. Durable stem rust resistance genes were incorporated into locally adapted susceptible wheat varieties for further selection and future release to enhance wheat productivity in Ethiopia.

Declaration

I, Netsanet Bacha Hei, 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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5. This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the references sections.

Signed

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Netsanet Bacha Hei

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. Mark Laing (Co-Supervisor)

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Dedication

This thesis is humbly dedicated to my late mother, **Sr. Sisay Ketema**, who sacrificed everything to ensure my better future, wellbeing and welfare.

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Abbreviations

APR	Adult Plant Resistance
AUDPC	Area Under Disease Progress Curve
BPH	Better-Parent Heterosis
CI	Coefficient of Infection
CSA	Central Statistical Agency
CV	Coefficient of Variation
DM	Days to Maturity
FRS	Final Rust severity
GCA	General Combining Ability
GY	Grain Yield
IBCR	Institute of Biodiversity Conservation and Research/Ethiopia
Inf-rate	Infection Rate
KPS	Kernels Per Spike
LSD	Least Significant Difference
MPH	Mid-Parent Heterosis
PLH	Plant Height
PRA	Participatory Rural Appraisal
r	Correlation Coefficient
SCA	Specific Combining Ability
SE	Standard Error
TKW	Thousand Kernel Weight
TPP	Tillers Per Plant

Introduction to thesis

Importance of wheat

Wheat (*Triticum aestivum* L.) is one of the world's leading cereal grains used by more than one-third of its population as a staple food (Kumar et al., 2011). It is grown from below sea level to elevations exceeding 3000 m above sea level and at latitudes ranging from 30° and 60°N to 27° and 40°S (Nuttonson, 1955). Globally, it is cultivated on approximately 218 million hectares of land (EPI, 2013). Wheat is used for food and many industrial purposes.

Ethiopia is the second largest wheat producer country in sub-Saharan Africa after South Africa (GAIN, 2012). In Ethiopia, wheat is cultivated on 1.6 million hectares and accounts for 13.25% of the crop land, with an annual production of 3.4 million metric tons. Wheat contributes about 14.85% of the cereal production in the country (CSA, 2013). In terms of area, wheat ranks fourth after teff (*Eragrostis tef* (Zucc.) Trotter), maize (*Zea mays* L) and sorghum (*Sorghum bicolor* (L.) Moench). In terms of total grain production, it ranks third after teff and maize (CSA, 2013). The crop is widely grown by subsistent farmers and over one-third of cereal farm households are dependent on wheat farming (Shiferaw et al., 2013).

Both bread (*T. aestivum* L.) and durum (*T. turgidum* var. *durum*) wheat varieties are grown in the wheat growing areas of the country. Bread wheat is an introduced crop whereas durum wheat is an indigenous crop. However, bread wheat cultivation is expanding due to its high yield and wide adaptability (Ashamo et al., 2012; Shiferaw et al., 2013). According to ECEA (2008) the major wheat producing regions in Ethiopia include Oromia, Amhara, Southern Nations Nationalities and Peoples' Region (SNNPR) and Tigray. Almost all wheat (99%) comes from these regions. The Bale Zone of Oromia region is included among the wheat belts in eastern Africa. Bale Zone constitute about 142,415 hectares of land devoted to wheat production, predominantly by subsistence farmers and a few profit oriented state farms (CSA, 2013). The mean wheat yield in the country is estimated to be 2.1 t ha^{-1} (CSA, 2013), which is well below the world mean of 3.0 t ha^{-1} (Hawkesford et al., 2013). This is due to losses caused by biotic, abiotic and socioeconomic constraints (Abebe et al., 2012; Haile e al., 2012).

Constraints to wheat production

The major biotic factors that limit wheat production in the country include diseases, pests and weeds (Abebe et al., 2012). Among the abiotic factors, soil fertility and moisture stress are the principal wheat production limiting factors in Ethiopia (Bogale et al., 2011a; Bogale et al., 2011b; Haile et al., 2012). Among the diseases, rusts (stem rust (*Puccinia graminis* f.sp. *tritici*), leaf rust (*P. triticina* Eriks) and stripe rust (*P*. *striiformis* Westend. f.sp. *tritici*) are the most important diseases reducing wheat production in Ethiopia. Of the three rusts, stem rust has been the most important disease of wheat in main wheat growing regions of Ethiopia (Admassu et al., 2012; Denbel et al., 2013).

Stem rust is caused by the fungus *Puccinia graminis* f.sp. *tritici Ericks* and Henn. The fungus is an obligate parasite, requiring living host tissue for growth and reproduction. It is heteroecious in its life cycle and heterothallic in mating type (Kolmer, 2013). In the absence of living host tissue, the fungus survives for only a short period as spores under field conditions. Symptoms of stem rust are brick red pustules formed on stems and to a lesser extent on leaves of susceptible plants. In epidemic situations, it causes yield losses reaching up to 100% on susceptible cultivars (Park, 2007; Hodson, 2014). Stem rust causes yield losses in several ways. The fungus absorbs nutrients from the plant tissues that would be used for grain development in a healthy plant. By the time rust pustules break through the epidermal tissue, the rust will have affected the plant transpiration, making the metabolism less efficient. Interference with the vascular tissues results in shrivelled grains. Stem rust also can weaken wheat stems, so plants lodge, or fall over, in heavy winds and rain (Craigie, 1957; Bushnell and Rowell, 1968; Roelfs, 1985).

P. graminis f.sp. *tritici* can exist in the form of many races that vary in pathogenicity, aggressiveness and virulence. Such physiologic specialization in wheat stem rust was first reported by Stakman and Piemeisal (1917). New races may develop due to mutation or recombination between different races within the same rust group (Singh and Rajaram, 2006). Existing races may spread faster, or become more virulent, if environmental conditions change. Consequently, specific genes that confer resistance can lose their effectiveness quickly. Stem rust outbreaks may occur when new pathogen races arise against which the existing genes for resistance are ineffective. East African highlands are among the global hot spots for the development of the new wheat stem rust races (Singh et al., 2008a). For example, the pathogen race Ug99 that has been called the most virulent strain of wheat stem rust in 50 years, was identified in Uganda in 1999 (Pretorius et al., 2000). Since then the race has spread to East Africa, the Middle East and South Asia (Nazari et al., 2009; Singh et al., 2009). Ug99 was first reported in Ethiopia in 2003 (Singh et al., 2008b) and is potentially a major threat to wheat production in the country (Periyannan et al., 2013).

Control measures of stem rust

Attempts have been made to minimize or control stem rust through fungicides, cultural practices and genetic control through the host. Fungicides have been widely investigated for stem rust control. With early disease detection and immediate application of fungicides economic levels of control can be achieved (Peterson, 2001). Fungicides reduce subsequent rust severity on plant parts that were slightly infected at the time of fungicide application, but they can not protect plant parts that are already heavily infected (Beard et al., 2004) because the plant tissues are already damaged. Although effective, fungicides are unaffordable for small-scale farmers.

Several cultural methods can be used to reduce the intensity of an epidemic or provide long term partial control (Goyal and Prasad, 2010). The use of early maturing cultivars, early planting and destruction of volunteer wheat and other susceptible grasses, can be effective in reducing the amount of initial inoculums and early infections. However, early planting can lead to early heading and in some environments there is a risk of head frosting that can be as damaging as the rust. Besides, early planting may actually increase the chance of overwintering of the rust under milder climates (Roelfs and Bushnell, 1985). Roelfs et al. (1992) stated that the success of implementing cultural control method depends on the level of knowledge of the epidemiology of stem rust in a particular area.

Breeding for stem rust resistance is the most economical and effective management method of stem rust (Rouse and Jin, 2011). Resistance to stem rust in wheat has been based mainly on race-specific resistance (Ayliffe et al., 2008). Varieties having such type of resistance generally show complete resistance to a specific physiological race (pathotype) under most environmental conditions. However, hybridization among pathotypes or mutations in the pathogen population may produce a new race that has the virulence to infect the previously resistant variety (Singh and Rajaram, 2006). Hence, the useful life of cultivars with resistance conferred by race-specific genes may be short. Consequently some recent breeding programs have focused on developing cultivars with more durable resistance.

Rationale of the research

The use of resistant cultivars, which is effective and affordable to farmers without extra expenses for disease management, has been the major strategy for control of stem rust in many countries (Broers and Denial, 1994; Singh et al., 2002; Rouse and Jin, 2011). Accordingly, the major efforts of the national and regional wheat improvement research programs of Ethiopia focus on the development of resistant varieties. However, the varieties so far released do not possess sufficient resistance to withstand the persistent challenge of stem rust pathogen because the resistance has not been durable (Admassu et al., 2012). The major cause of the ineffectiveness of wheat varieties against stem rust is the rapid evolution of new virulent pathotypes and the deployment of the same R gene (s) in many cultivars (Admassu et al., 2012).

Wheat genetic materials with related parentage and largely race-specific major gene resistance have been backbone of the wheat improvement programs of Ethiopia (Badebo, 2002; Aida, 2005). Consequently, the available diversity of local wheat accessions has not been well exploited as the source of parental populations for wheat breeding. According to Jaradat (2011), local wheat genetic materials have been largely displaced by high yielding cultivars in many developing countries. However, landraces and old cultivars may also provide valuable traits needed to meet the challenges of the future, such as adapting our crops to changing climatic conditions or outbreaks of diseases (Xepapadeas et al., 2012). Wheat genotypes are genetically variable in their ability to tolerate biotic and abiotic stresses. Selection within local germplasm tolerant to stresses can be a useful component of breeding strategies for wheat improvement. Jaradat (2011) also noted that participatory plant breeding and variety selection are more successful in satisfying the needs of the farmer and the final consumer.

Therefore, keeping in view the rapid evolution and spread of new virulent races of stem rust, the frequent failure of new varieties with major gene stem rust resistance, and the take off durable stem rust resistance in the Ethiopian bread wheat improvement programs, there is a need to identify durable sources of resistance from locally adapted wheat germplasm, and to understand the genetics of stem rust resistance in wheat.

To enhance the potential for adoption of new wheat varieties by farmers, farmers' constraints and their preferences need to be identified and be included in breeding of new cultivars.

Research objectives

The specific objectives of the study were:

i) To determine wheat production constraints, farmers' varietal preferences, and disease management practices, with special emphasis on wheat rusts.

ii) To identify slow rusting resistance to stem rust in Ethiopian wheat lines.

iii) To determine the levels of heterosis and combining ability, and to identify the best parents and crosses for breeding to stem rust resistance, high grain yield and desirable agronomic traits

iv) To introgress durable resistance genes from known resistance sources into farmers'-preferred and locally adapted but stem rust susceptible, improved wheat varieties.

Research hypotheses

This study was carried out to test the following hypotheses:

i) In wheat growing areas of Ethiopia, farmers are aware of wheat disease problems and other constraints to wheat production, and prefer varieties that combine resistance to these constraints.

ii) Many wheat lines have slow rusting resistance to stem rust in Ethiopian germplasm.

iii) The selected sources of resistance to stem rust combine well for wheat stem rust resistance, grain yield performance and other agronomic traits.

iv) Additive genetic effects control durable resistance to stem rust.

v) Stem rust resistance genes from donor parents can be transferred into locally adapted wheat varieties, and will increase rust resistance in the progeny.

Outline of the thesis

This thesis consists of four distinct chapters in accordance with a number of activities related to the above-mentioned objectives. Chapters 2-5 are written as discrete research papers, each following the format of a stand-alone research paper (whether or not the chapter has already been published). This is the dominant thesis format adopted by the University of KwaZulu-Natal. Some overlap and unavoidable repetition of references and some introductory information between chapters may exist.

The referencing system used in the chapters of this thesis is based on the Harvard system of referencing (De Montfort University), and follows the specific style used in "Southern Forests: a Journal of Forest Science". The exception to this is Chapter 3, which was published in the journal of "Phytopathology". In this case, Chapter 3 has followed the referencing and formatting style used by "Phytopathology".

Chapter	Title
-	Introduction to thesis
1	A review of the literature
2	Appraisal of farmers' wheat production constraints and breeding priorities in stem rust prone agro-ecologies of Ethiopia
3	Assessment of Ethiopian wheat lines for slow rusting resistance to stem rust of wheat caused by *Puccinia graminis* f.sp *tritici*
4	Heterosis and combining ability analysis of stem rust resistance and grain yield and related traits in bread wheat
5	Introgression of durable resistance genes into farmers'-preferred and locally adapted stem rust susceptible wheat varieties
6	An overview of the research findings

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CHAPTER 1

A review of the literature

1.1 Introduction

Wheat (*Triticum aestivum* L.) is a staple food crop worldwide (Kumar et al., 2011). However, its production and productivity is limited by a number of abiotic, biotic and socio-economic constraints. Wheat stem rust caused by *Puccinia graminis* Pers. f.sp. *tritici* Eriks and Hann is one of the major production constraints of both bread wheat (*Triticum aestivum* L., 2n=6x=42, AABBDD) and durum wheat (*T. turgidum* var. *turgidum* L., 2n=4x=28, AABB). The use of resistant cultivars remains the most economical and environmentally friendly management strategy of stem rust, especially for resource poor farmers. However, most of the wheat varieties released thus far have not possessed durable resistance and became susceptible shortly after their deployment. In most cases, failures of resistance have been attributed to the emergence of new virulent pathotypes and the narrow genetic base in the cultivated wheat cultivars. Hence, there is a need for development of novel wheat cultivars with durable stem rust resistance and farmers'-preferred agronomic attributes. Novel sources of resistance and understanding the genetics of durable stem rust resistance are prerequisites for durable resistance breeding in wheat. This review highlights the origin, history and biology of wheat, the current state of wheat production, its importance, and its production constraints. It emphasises the importance and threat of stem rust and its control strategies, and provides background information on the genetics and breeding of stem rust resistance in wheat.

1.2 Origin and genetic inter-relationships of species of wheat

Common or bread wheat is believed to have originated in south-western Asia where it has been grown for more than 9000 years (Zohary and Hopf, 2000). The genetic relationships between wild and domesticated einkorn wheat (*T. monococcum,* 2n=2x=14; AA) and emmer wheat (*T. turgidum,* 2n=4x=28; AABB*)* suggest that south-eastern Turkey is the most likely site of its origin and first domestication (Dubcovsky and Dvorak, 2007). During the Neolithic period, the cultivation of wheat began to spread beyond its center of origin. Wheat spread into central Asia and Africa by about 3000 years ago. It reached Africa initially through Egypt (Feldman, 2001). Today, wheat is widely grown in many parts of the world, across a wide range of environmental conditions, from sea level to over 3000 m above sea level (masl). Wheat production is most successful between the latitudes of 30° and 60°N and 27° and 40°S, although it can be grown beyond these lim its (Nuttonson, 1955).

1.3 Biology of wheat

Bread wheat is a member of the tribe *Triticeae* of the family *Poaceae* to which all the major cereals belong. It is an autogamous allo-hexaploid species (2n=6x=42). Three genomes, designated as A, B and D, were involved in its evolution (El-Twab, 2006). Bread wheat is evolved through wide-hybridization of diploid grass species *Aegilops tauschii* (2n=14, DD) with the cultivated tetraploid durum wheat *T.turgidum* (2n=4x=28; AABB) (Salamini et al., 2002; Dubcovsky and Dvorak, 2007).

Wheat has intermediate plant height with annual growth habit. The terminal floral spike consists of perfect and clestogamous flowers, leading to strict self fertilisation, although cross fertilisation may occur up to 5% (Skymo, 1999). The vegetative state of the plant is characterized by formation of tillers. Tillers are lateral branches produced with primary stem (Setter and Carlton, 2000). Spikes are made up of two rows of spikelets and each spiklet consist three to four florets. Florets are enclosed by lemma and palea and contain three anthers supported by filaments and a feathery stigma (Setter and Carlton, 2000).

1.4 Wheat production and its importance

Wheat ranks first among cereals in total production (Al-Musa et al., 2012; Sarwar et al., 2013). It is cultivated on approximately 218 million hectares of land worldwide (EPI, 2013).The total wheat production in 2012 was more than 654 million tonnes.

The largest wheat producing countries are China, India, the United States and Russia, in that order (Table 1.1). These countries account for nearly 50% of the world wheat production (EPI, 2013). The remainder of the production is spread throughout the rest of wheat producing counties worldwide.

Table 1.1. The leading wheat producing countries of the world during 2012

Country	Production (t)	Percent contribution to world production
China	120 600 000	18.44
India	93 900 000	14.34
USA	61 755 000	9.44
Russia	37 700 000	5.76

Source: (EPI, 2013).

Bread wheat is becoming an important food security crop in Africa. South Africa is the leading wheat producer in sub-Saharan Africa followed by Ethiopia (GAIN, 2012). In Ethiopia an estimated area of 1.6 million hectares is under wheat production (CSA, 2013). Wheat is fourth in the area of production after teff (*Eragrostis tef* (Zucc.) Trotter*),* maize (*Zea mays L*) and sorghum (*Sorghum bicolor* (L.) Moench), accounting for 13.25% of the crop area. It ranks third in total grain production after teff and maize, with an annual production of 3.4 million tons (Table 1.2) (CSA, 2013). Wheat is grown from 1500 to 3000 masl. The most suitable areas, however, fall between 1800 and 2500 masl (Winch, 2007). Arsi and Bale Administrative Zones of the Oromia Regional State in Ethiopia are among the major wheat production environments. The Bale Zone is recognized as one of the wheat belts of eastern Africa. About 142, 415 ha are devoted to wheat production in Bale Zone (CSA, 2013). In Ethiopia wheat is produced largely under rainfed conditions.

Both bread and durum wheat are important food crops in Ethiopia. Ethiopia is a center of diversity for durum wheat (Zohary, 1970). However, bread wheat production is expanding in the country, because of its higher productivity and broader adaptation (Ashamo, 2012; Shiferaw, et al., 2013). The mean wheat yield in the country is estimated around 2.1 t ha^{1} (CSA, 2013), which is considerably lower than the potential yield of 8-10 t ha^{-1} (Mollah et al., 2009).

Wheat is predominantly grown by subsistence farmers in Ethiopia (Alemu et al., 2014). There are about 4.6 million farm households who are directly dependent on wheat farming as a major source of food and cash in Ethiopia (Shiferaw et al., 2013). The straw is used as animal feed during the dry season and for thatching roofs.

Table 1.2. Area, yield and production of wheat of private peasant holdings in Ethiopia during the main cropping seasons of 2010 to 2013.

Year	Area (ha)	Yield (t ha^{-1})	Production (t)
2010	1 683 565.26	1.83	3 075 643.60
2011	1 553 239.89	1.84	2 855 681.74
2012	1 437 484.73	2.03	2 916 333.69
2013	1 627 647.16	2.11	3 434 706.12

Source: CSA (Central Statistical Agency of Ethiopia), 2010, 2011, 2012 and 2013

1.5 Major production constraints of wheat in Ethiopia

Wheat production is constrained by a number of abiotic and biotic factors (Nelson, 2013). Among the abiotic factors, drought, nutrient deficiencies, and waterlogging in vertisol areas are identified as major threats. Among the biotic stresses, grass weeds and diseases are the major constraints reducing wheat production (Yami, 2012).

1.5.1 Abiotic factors

Drought is one of the major abiotic constraints on wheat production in drought prone areas of Ethiopia (Bogale et al., 2011a). More than 50% of the total arable land in Ethiopia is classified as semi-arid or arid (Berhanu, 2004). In such areas, moisture stress is an important yield limiting factor for crop production. Moreover, because of degradation and poor vegetation cover, soils in semi-arid and arid areas have low fertility, with poor water holding capacity (Berhanu, 2004).

Nutrient deficiency is also one of the important abiotic stresses in wheat production in the country. It is probably the most widely spread problem in Africa due to the limited use of chemical fertilizers, lack of crop rotation or fallowing. According to Schneider and Anderson (2010), soil fertility related constraints such as expensive or limited access to nitrogen fertilizer, nitrogen deficiency, and soil fertility depletion were present in over 40% of wheat growing areas in sub-Saharan Africa, and accounted for 20% of the total yield gap. In Ethiopia, soil depletion is a widespread problem, which led to stagnant crop yields despite the use of modern inputs such as enhanced seed and some fertilizers (Bayu, 2012).

Water logging is another important abiotic factor that threatens wheat production in the vertisol areas, mostly in the highlands above 1500 masl in Ethiopia (Kebede and Bekele, 2008). It impedes the performances of cereal crops during the main rainy season in the highlands of Ethiopia. In such areas, early planting is not possible, which in turn reduces the length of the growing cycle and consequently the yield (Kebede and Bekele, 2008).

1.5.2 Biotic factors

Diseases are among the most important yield limiting factors in wheat production. Wheat in Ethiopia is attacked by a number of diseases that reduce the quality and quantity of grain. Among the major wheat diseases are the wheat rusts: stem rust (*Puccinia graminis* f.sp. *tritici*), leaf rust (*P. triticina* Eriks), and stripe rust (*P. striiformis* Westend. f.sp. *tritici*). Of the three rusts, stem rust is widely distributed throughout the major wheat growing regions of Ethiopia, and may cause severe food shortages when it occurs in epidemic proportions (Admassu et al., 2012; Denbel et al., 2013). Yield losses from stem rust can reach up to 100% on susceptible cultivars (Park, 2007; Hodson, 2013).

In addition to diseases, grass weeds are among major wheat production constraints in Ethiopia. Continuous production of wheat especially in south-eastern highlands and repeated application of broad-leaf herbicides have increased the problem of grass weeds (Rezene and Yohannes, 2003; Bogale et al., 2011b). Reports indicated yield reduction up to 86%, depending upon weed type and density, crop variety and environmental conditions (Tessema and Tanner, 1997).

1.6 Stem rust of wheat

Wheat stem rust is caused by the fungal pathogen *Puccinia graminis* f.sp *tritici*. The fungus is an obligate parasite (Schumann and Leonard, 2000). The stem rust infects wheat, oats (*Avena sativa* L.), barley (*Hordihum vulgare* L.) and rye (*Secale cereale* L.), as well as wild grasses, but wheat is the only economic host (Leonard and Szabo, 2005). In Ethiopia, grass weed species such as *Lolium temolentum* and *Setaria pumila* were identified to be secondary hosts for wheat stem rust (Zerihun and Abdalla, 2000). The main alternate host for *P. graminis* is *Barberis vulgaris* (Roelfs et al., 1992). It is a major source of new combinations of genes for virulence and aggressiveness in the pathogen (McIntosh et al., 1995). Rust spores are wind-blown and can be spread over large areas in a short time. The pathogen is favoured by humid conditions and temperatures of 15 to 30℃ (Hollaway, 2011).

1.6.1 Life cycle of *Puccinia graminis*

The rust pathogen is heteroecious, requiring two unrelated host plants, wheat and barberry, to complete its life cycle (Singh et al., 2002). It is heterothallic, which involves both sexual and asexual stages (Kolmer, 2013). The sexual cycle of the pathogen needs alternate hosts. The fungus survives entirely in the asexual stage when there is no alternate host.

The stem rust pathogen produces five types of spores; teliospores, basidiospores, and urediniospores on cereal hosts, and pycniospores and aeciospores on the alternate hosts (Figure 1.1) (Singh et al., 2002). The disease cycle of wheat stem rust starts with the exposure of each new wheat crop to aeciospores or urediniospores of *P. graminis* f.sp. *tritici* (Xue et al., 2012). Urediniospore is the most important spore form because it is capable of cycling continuously on the cereal hosts and enables the disease to spread from field to field and survive from year to year. When the crop reaches maturity, teliospores develop. Teliospores usually remain covered by the epidermis of the host plant. Under favourable environment teliospores germinate and produce the basidiospores that are able to infect the alternate host. Once on the leaves of the alternate host, basidiospores give rise to the pycnial structures within which pycniospores will be produced (Kolmer, 2013). The production of pycniospores is important becouse it allows cross-fertilization. The pycniospores produce aeciospores, and establish primary inoculum for the new crop cycle.

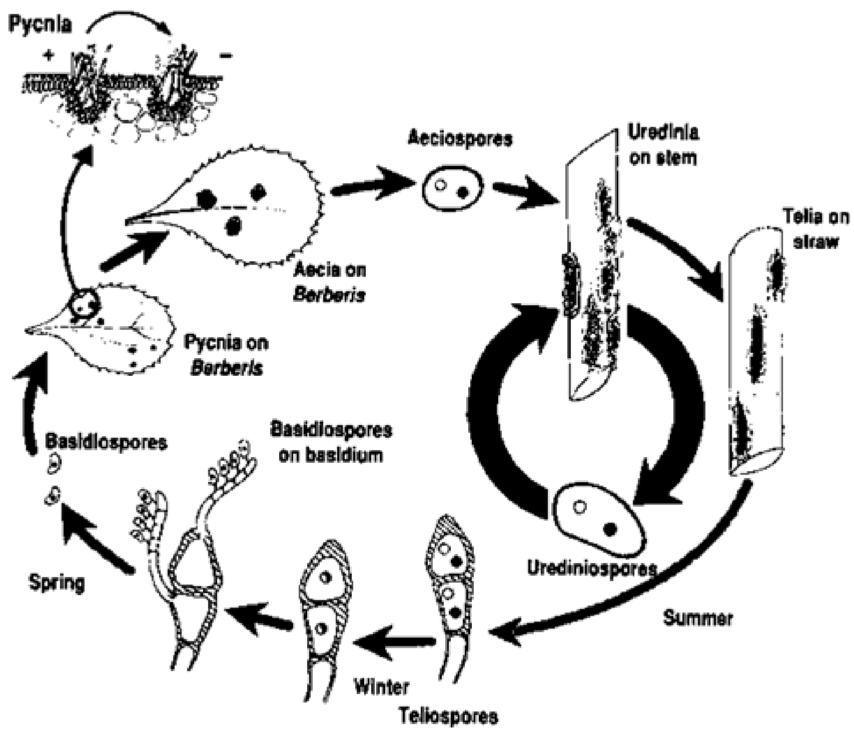

Figure 1.1. Life cycle of *P. graminis* f. sp *tritici*
Source: Courtesy V. Brewster.

1.6.2 Physiological races of stem rust

The wheat stem rust pathogen is known for its various physiologic specializations or races. Physiologic specializations of stem rust were first reported by Stakman and Piemeisal (1917). These races varied in their ability to infect different wheat varieties or differential hosts that carry distinct resistance genes or combinations. They develop by mutation, recombination, and selection for virulence against rust resistance genes in wheat (Singh and Rajaram, 2006). Since the discovery of pathogenic variability within stem rust, seasonal race surveys have been carried out to determine the range of variability gobally within the rust. Race surveys detect new and highly virulent pathogen phenotypes as they appear. The surveys also provide essential information to determine the gene combinations to be considered by breeding programs using major gene resistance (Huerta-Spino, 1994; Park et al., 2011).

Variable races of stem rust pathogen have been identified in wheat production areas in different continents. These new races have reduced the number of major rust resistance genes that are available for use (Kolmer, 2005). For example, Ug99, a virulent strain of stem rust, was first found in Uganda in 1999. The race Ug99 carries virulence to gene *Sr31*, which was known for its durability. Stem rust resistance in wheat cultivars with *Sr31* remained effective for more than thirty years (Wanyera et al., 2006). This race has evolved even further, accumulating additional virulence to important *Sr* genes, notably *Sr24* and *Sr36* (Jin et al., 2008, 2009). Currently, eight closely related variants of Ug99 are reported but slightly different in their avirulence/virulence patterns. The variants have spread to various wheat growing countries and their occurrence is known in eleven countries including Tanzania, Zambia, South Africa, Ethiopia, Kenya, Uganda, Zimbabwe, Eritrea, Yemen, Iran and Sudan (Mukoyi et al., 2011; Singh et al; 2011). Two variants of Ug99, PTKSK and PTKST, have been identified in Ethiopia (Hodson, 2014).

1.6.3 Symptoms and effects of *P. graminis* f.sp. *tritici* on wheat growth

Stem rust is marked by an eruption of elongated brown pustules on the leaves, stems, leaf sheaths, spikes, glumes, awns and occasionally grains of hosts. The wheat stem and leaf sheaths are the main tissues affected (Marsalis and Goldberg, 2006). The disease reduces foliage and root development and hence infected plants usually produce fewer tillers, set fewer seeds per head, and the kernels are shrivelled and smaller in size, with poor milling quality and food value (AFRD, 2014).

Severe infections under favourable environmental conditions can also result in death of tillers or entire plants. Under heavy infection the lesions of rust can occupy a significant portion of the host tissue and reduces the photosynthetic area, causing a loss of nutrient and water and disturbs the plant transport system (Berghaus and Reisener, 1985). The rupture of the plant epidermal cells by the fungus also results in a loss of water from the plant and disrupts transport of nutrients to the roots and cause premature death of the roots (Bushnell and Rowell, 1968). Hence, the disease hinders plants from expression of their full yield potential and causes poor crop performance and low wheat yields in many wheat growing areas. The fungus also absorbs the nutrients from the plant tissues that would be used for grain development and hence significantly affects grain filling and grain quality (Xue et al., 2012).

1.6.4 Economic importance of stem rust

The widespread nature of stem rust pathogen and its ability to mutate for virulence makes the disease important worldwide (Park, 2007). Under favourable conditions, yield losses up to 100% have been reported with susceptible cultivars (Leonard and Szabo, 2005; Park, 2007; Hodson, 2013). Ug99 has been reported to cause yield losses of more than 71% in experimental fields (CIMMYT, 2005). It is estimated that a one percent increase in stem rust severity results in approximately a one percent increase in yield loss (CIMMYT, 2005).

In Ethiopia, wheat stem rust is a significant problem in major wheat producing areas. Yield losses are estimated to reach up to 100% on susceptible cultivars in the country (Hodson, 2013). Severe epidemics of stem rust have been reported over the past 20 years in major wheat growing regions of Ethopia. In1993/94, a stem rust outbreak attacked the previously resistant bread wheat cultivar Enkoy, and resulted in yields losses of 65-100% (Shank, 1994). A 1998 epidemic attacked the high yielding variety Kubsa. Shina, released in 1999 for north western Ethiopia, succumbed to a stem rust epidemic in 2001. Since then there have been no severe stem rust epidemics. However, during the 2013 cropping season the disease caused

100% yield losses in cultivar Digelu, which is reportedly resistant to stem rust, in south-eastern Ethiopia (Hodson, 2013). The outbreak was recorded in high altitude areas (> 2200 masl), although it mainly occured in the low altitude areas of 1800 masl, extending rust incidence to low, medium and high altitudes production areas.

1.6.5 Management of the wheat stem rust

A number of methods are available to control stem rust, which include the use of fungicides, cultural practices and resistant cultivars.

1.6.5.1 Fungicides

Fungicides can play a vital role in stem rust management until new cultivars with genetic resistance are available (Loughman et al., 2005). They are usually considered where losses are expected to be very high, where grain prices are highly subsidized, and yield potential is high. Fungicides will give better control of stem rust when applied at the early stages of the epidemic (Hollaway, 2011). Thus, early disease detection and immediate application of fungicides should be considered in the control of stem rust with fungicides. A number of fungicides are highly effective against stem rust and have been used to successfully control the disease (Hollaway, 2011). However, the high costs of application, a lack of knowledge of the use of appropriate fungicides, and the unavailability of the fungicides are their main limitations, particularly for small-scale and resource poor farmers (Bishaw et al., 2010).

1.6.5.2 Cultural practices

Cultural methods largely depend on early planting and growing early maturing cultivars. These help to reduce the time of exposure of the crop to the pathogen and hence reduce yield loss. According to Schumann and Leonard (2000), use of early maturing wheat varieties reduce the threat of stem rust epidemics by limiting the length of time for stem rust epidemics to develop, as well as the numbers of urediniospores that can contribute to the spread of the disease to other areas. The date of disease onset is directly related to the development of an epidemic (Hamilton and Stakman, 1967) and is probably the single most important factor in determining the severity of the epidemic (Roelfs, 1985). The success of implementing this method depends on sufficient knowledge of the epidemiology of stem rust in a particular area (Roelfs et al., 1992). Moreover, early planting can lead to early heading and, in some environments, there is risk of head frosting that can be as damaging as the rust.

Eradication of alternate hosts and green bridges carrying inoculum from one crop to the next is a useful cultural practice. Eradication of the alternate hosts has a significant effect in reducing stem rust epidemics. It removes an early source of inoculum and eliminates the sexual cycle of the fungus, limiting the development of new races of the pathogen (Schumann and Leonard, 2000). Removing the green bridge, including volunteer cereals or wild accessory hosts, is essential to reduce the carryover of *P. graminis* f.sp. *tritici* to the next season wheat crop (Schumann and Leonard, 2000).

1.6.5.3 Use and development of resistant cultivars

Host plant resistance is the principal management strategy for the control of stem rust (Rouse and Jin, 2011). An ongoing struggle has been reported on identifying sources of resistance and developing resistant varieties against wheat stem rust for over 50 years (Singh et al., 2008a). The most successful control of stem rust appears to be when stem rust resistance genes from tetraploid sources, durum and emmer, were transferred into hexaploid wheat, which gave rise to the varieties Thatcher and Hope (Kolmer 2001). Thatcher and Hope have maintained some resistance to stem rust for many years. The most effective component of the resistance in these varieties is adult plant resistance conditioned by the *Sr2* gene (Singh et al., 2008b).

Two types of resistance to stem rust can be recognized, namely race-specific and race-non-specific resistance. Race-specific resistance is controlled by a few genes having major effects. Race non-specific resistance is governed by several to many genes each with a minor effect (Van der Plank, 1963). Resistance to stem rust in wheat has been largely based on race-specific resistance (Ayliffe et al., 2008). Many varieties with race-specific resistance have been released but they became susceptible shortly after their deployment, because virulent races that could overcome their resistance were found to be prevalent in the fungus population. Hence, for the long term, the main objective of the wheat breeder is to develop cultivars in which many additive genes are accumulated to express strong quantitative resistance to stem rust (Herrera-Fossel et al., 2007).

In wheat at least 50 *Sr* genes which confer resistance to different races of stem rust have been identified (McIntosh et al., 2011). Most of the designated *Sr* genes have proved to be qualitative and race-specific (Singh et al., 2011). According to Yu et al. (2014), five of the stem rust resistance genes, *Sr2, Sr55, Sr56, Sr57* and *Sr 58,* confer quantitative adult plant resistance. Of these, *Sr2* has proved to be durable in many parts of the world (Singh et al., 2010). It has remained effective against *P. graminis* f. sp *tritici* worldwide for more than 50 years (Hayden e al., 2004). The effectiveness of *Sr2* is that it forms a gene complex with other, unknown additive genes of a complementary nature (Singh and Rajaram, 2006). The resistance conferred by *Sr2* is characterized by a slow rusting response, and the resistance phenotype is expressed only at adult plant stage (Roelfs, 1988). According to McIntosh (1988) and Singh and Rajaram (2006) this resistance, in combination with other major or minor genes, provides a desirable genetic background for the deployment of other effective but less durable stem rust resistance genes. Thus, the use of *Sr2* remains important in the control of wheat stem rust in wheat breeding programs.

1.7 Slow rusting

Slow rusting is characterized by slow disease progress in the field, despite a compatible host-pathogen interaction (Caldwell, 1968; Parlevliet, 1975; Singh et al., 2005). Therefore, a cultivar that only has slow rusting resistance will display susceptible infection types both at the seedling and adult stages (Navabi et al., 2004; Singh et al., 2009). Slow rusting resistance is often described as partial resistance or adult plant resistance. According to Ahamed et al. (2004), slow rusting is a useful measure of resistance because it is the result of all factors that influence disease development such as differences in environment, cultivars and populations of the pathogen. This type of resistance is characterized by low infection frequency, increased latent period, reduced spore production, shorter sporulation period and reduced pustule expansion (Wilcoxson, 1981; Singh and Rajaram, 2006). These characteristics are known as slow rusting components and may act independently or complementarily to reduce fungal infection. The genetic control of slow rusting is predominantly additive and mostly inherited polygenically (Skovmand et al., 1978; Ahamed et al., 2004; Kumar et al., 2013). Slow rusting has been a stable trait over a relatively long period. This has been observed in wheat cultivars which rusted slowly when attacked by *P. garminis* spp. (Ahamed et al., 2004; Singh et al., 2008b).

1.8 Measurements of slow rusting

To select effectively for slow rusting resistance in the field, adequate disease epidemic and good disease measurements are necessary in order to distinguish susceptible lines from the slow rusting lines. In the field, slow rusting resistance have been described and estimated by means of final disease severity, the area under disease progress curve (AUDPC), apparent infection rate (inf-rate) and coefficient of infection (CI) (Pathan and Park, 2006; Ali et al., 2009; Shah et al., 2010; Tabassum, 2011; Safavi, 2012).

Wilcoxson et al. (1975) used the AUDPC and the inf-rate values to evaluate slow rusting resistance in wheat cultivars to stem rust. In 1978, Gavinlertvatana and

Wilcoxson found that AUDPC was a convenient and reliable parameter for comparing slow rusting capabilities, whereas inf-rates were limited in their utility to tag slow rusting because such estimates were made accurately only during the early phases of disease development. Shaner and Finney (1980), Wilcoxson (1981), Sawhney (1995), Shah et al. (2010), Tabassum (2011), Safavi (2012) and Safavi et al. (2013) also used AUDPC to identify slow rusting genotypes under field conditions. Harjit-Singh and Rao (1989) discussed AUDPC and inf-rate values, using theoretical models on the development of leaf rust. Their findings suggested that the AUDPC and the inf-rate measure different aspects of resistance and therefore the combination provide greater discrimination to evaluate the resistance of genotypes. They concluded that slow rusting is determined by comparing AUDPC values.

According to Singh et al. (2007), field selection of slow rusting resistance is preferable made by screening for using low AUDPC and terminal ratings, along with low CI. They argued this to be the most efficient approach in situations where greenhouse facilities are inadequate. Qamar et al. (2007) proposed that AUDPC and terminal disease ratings are reliable estimators for slow rusting resistance in wheat. Terminal rating was reported to be preferable because it is more economical, less labour-intensive and less time-consuming than measuring the AUDPC. Safavi (2012) also concluded that FRS and CI are the most appropriate and economical parameters for selection of slow rusting genotypes. Ali et al. (2008) suggested using CI for field based assessment of partial resistance.

In greenhouse experiments, latent period (days from inoculation until the first pustule erupted) and pustule size are important and widely used components for identifying slow rusting genotypes. Latent period, in particular, has been identified as an important component of partial resistance because it is measured and analysed easily and with the least possible error (Shaner and Finney, 1980; Ghannadha et al., 2005; Zahravi and Bihamta, 2010). Variation for latent period has been reported for rust diseases in various studies (Kolmer and Liub, 2001; Ghannadha et al., 2005; Zahravi and Bihamta, 2010). Pustule size is often used to measure spore production. Since measuring spore production is difficult, it can be estimated by pustule size, assuming a dose association between spore production and pustule size (Bakhshi et al., 2012).

1.9 Genetics of rust resistance

Understanding the nature and magnitude of genetic effects of resistance, and knowing the available resistance genes in the germplasm help wheat breeders to formulate an efficient breeding program for the achievement of durable resistance breeding (Hussain et al., 2011). Genetic procedures are used to obtain basic genetic information (Hussain et al., 2011). Griffing (1956) postulated a diallel technique for estimating the general combining ability (GCA) and specific combining ability (SCA) of lines and to characterize the nature and extent of gene action. GCA describes additive gene effects and SCA describe all the non-additive genetic effects. Usually only additive and dominance effects are assumed to be present and genetic analysis largely involves the use of F_1 progeny means from a set of crosses. However, to maximize genetic information regarding the presence of different kinds of genetic effects and the relative importance of these gene effects, means from other generations may be required. Generation mean analysis involves six basic generations (P_1, P_2, F_1, F_2, BC_1 and BC_2) and detects the presence or absence of epistasis, and when it is present, it measures it appropriately (Bernardo, 2002).

Considerable research has been conducted on the inheritance of rust resistance in wheat, and both diallel and generation mean analyses have been used to examine inheritance of rust resistance in wheat. Studies using combining ability analysis have reported both additive and non-additive gene effects for resistance to rusts in wheat (Navabi et al., 2003; Ahmed et al., 2004; Hasabnis and Kulkarni, 2004; Zahravi and Bihamta, 2010). Epistatic gene action have been reported for wheat when two or more race-specific resistance genes are effective against a race of stem rust, i.e., genes conditioning the highest level of resistance are epistatic to the gene(s) conditioning lower levels of resistance (Roelfs, 1988; Bhatiya et al., 2009; Irfaq etal., 2009; Zahravi and Bihamta, 2010). In durum wheat, however, several resistance genes interact additively. Combinations of two or more of these resistance genes resulted in a level of resistance greater than either resistance gene singly (Kurt and Les, 2005).

1.10 Farmers' preferences and their implications in wheat breeding

High yields and disease resistance have been the key objectives of most plant breeding programs. Accordingly, many high yielding and disease resistant varieties have been released. However, studies have showed that adoption of new varieties is often limited because the specific needs and preferences of farmers were not adequately considered by breeding programs (Weltzien et al., 2003; Witcombe et al., 2005). In Ethiopia, the national wheat improvement program has released more than 30 wheat varieties since 2003, when Ug99 was detected in the country. However, only a few varieties are being planted by farmers in the country (DRRW, 2010). One of the reasons for the poor adoption rate by farmers is the lack of preferred traits within the "improved" cultivars (Nelson, 2013; Shiferaw et al., 2013).

It is well known that development and transfer of modern varieties is not an end in itself. The goal of increased productivity and production of wheat will be realized if farmers adopt the varieties that are released by the plant breeders. Breeders must therefore consider farmers' preferences, the agronomic attributes of wheat varieties and the constraints reducing wheat production, and incorporat as criteria for developing, testing, and releasing varieties (Ceccarelli and Grando, 2009). This will enhance the level of adoption of a released variety, which takes a long time, and a high cost to develop. When farmers reject a newly released variety, this reprepersents a major waste of research resources.

1.11 Summary

Wheat is among the most important staple crops in Ethiopia. However, its production and productivity is low because of various yield limiting factors, including stem rust. This is the most prevalent and devastating wheat disease in nearly all wheat growing areas of the country. Attempts have been made to minimize or control stem rust losses through fungicides applications and different cultural methods. However, breeding for stem rust resistance remains the most economical, effective and practical method of stem rust management.

In recent times stem rust has been controlled by growing resistant varieties. However, the varieties succumbed to stem rust disease shortly after their introduction. In most cases, the failures have been due to the virulence present in the pathogen population and deployment of qualitative type of resistance in wide array of wheat cultivars. In view of this, it is important to identify and introduce resistant types that can be intrinsically durable like partial or slow rusting resistance. These types of resistances are characterized by slow epidemic build up despite a high infection type indicating a compatible host-pathogen interaction. Thus slow rusting resistance can be an alternative approach useful in developing wheat cultivars with durable resistance.

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CHAPTER 2

Appraisal of farmers' wheat production constraints and breeding priorities in rust prone agro-ecologies of Ethiopia

Abstract

Ethiopia is the second largest producer of wheat in sub-Saharan Africa although yields remain considerably below the global average due to several production constraints. The aim of the study was to identify the primary threats to wheat production, farmers' selection criteria for wheat varieties, and disease management practices with emphasis on wheat rusts in the Arsi, Bale and West Shewa administrative zones of Ethiopia. A total of 270 wheat growing households were surveyed in six districts of three administrative zones. Participatory rural appraisal tools, a semi-structured questionnaire and focus group discussions were used to engage with the farmers. Wheat rust diseases, the high costs of fertilizers, a shortage of improved seeds and high seed prices were the major constraints reported by 96%, 93%, 85% and 81% of respondents, respectively. The most important traits that farmers sought in wheat varieties were disease resistance (27.8%) and high grain yield (24.8%). Farmers estimated that in the Arsi, Bale and West Shewa zones losses due to stem rust disease were 70, 60 and 60%, respectively. Owing to the limited availability of stem rust resistant varieties, and the emergence of virulent pathotypes, fungicide application was the main disease management practice used by 60% of respondent farmers. To enhance wheat production and productivity, and to meet food security in Ethiopia, it is important to 1) develop stem rust resistant varieties considering farmers' preferences; 2) promote access to wheat production inputs; 3) strengthen seed multiplication and dissemination of improved varieties.

Key words: Ethiopia, participatory rural appraisal, seed source, stem rust, wheat

2.1 Introduction

Wheat (*Triticum aestivum* L.) is one of the world's leading cereal grains serving as a staple food for more than one-third of the global population. Globally, it is cultivated on approximately 218 million hectares of land (HGCA, 2014). Ethiopia is the second largest wheat producer in sub-Saharan Africa, after South Africa (GAIN, 2012). In Ethiopia wheat is cultivated on 1.6 million hectares of land, accounting for 13.25% of the total cropland, with an annual production of 3.4 million tons, contributing about 14.85% of the total cereal production (CSA, 2013). In terms of area of production, wheat ranks fourth after teff (*Eragrostis tef* Zucc.), maize (*Zea mays* L.) and Sorghum (*Sorghum bicolor* L.). In total grain production, wheat ranks third after teff and maize in the country.

Wheat is largely grown in the mid and highland areas of Ethiopia spanning at altitudes of 1500 to 3000 m above sea level (masl). However, it is mainly grown between 1800 to 2500 masl in the country (Winch, 2007). Arsi, Bale and Shewa administrative zones of the Oromia Regional State of Ethiopia are among the major wheat areas with 56% of the wheat produced in Ethiopia coming from these zones (CSA, 2013). The Arsi and Bale zones are included among the highest potential agro-ecologies in Eastern Africa for wheat production with 479, 290 ha under wheat (Jobie, 2007; CSA, 2013).

In Ethiopia wheat is predominantly grown by small scale farmers at a subsistence level, and these farmers experience a wide range of biotic, abiotic and socio-economic constraints. Wheat rusts, stem rust (*Puccinia graminis* Pers. f.sp. *tritici* Eriks and Hann), leaf rust (*P*. *triticina* Eriks) and stripe or yellow rust (*P*. *striiformis* Westend. f. sp. *tritici*) are the major biotic constraints in all wheat growing regions of the country. To combat yield losses due to wheat rusts and other abiotic constraints, the National Wheat Improvement Program has released more than 30 wheat varieties since 2003. However, only a few rust resistant wheat varieties are being planted by farmers in the country (DRRW, 2010). Poor adoption of varieties has mostly risen from the weak integration of variety requirements between breeders and farmers (Nelson, 2013). Therefore integration of farmers' perceptions and preferences will contribute greatly in improving wheat productivity.

Understanding farmers' preferences, attributes of wheat varieties and wheat production constraints enables breeders to set wheat breeding priorities (Weltzien and Christinck, 2009). Hence, the aim of the study was to identify wheat production threats, farmers' variety selection criteria, and disease management practices with special emphasis on wheat rusts in Arsi, Bale and West Shewa administrative zones of Ethiopia.

2.2 Research methodology

2.2.1 Description of the study areas

The study was carried out in three selected administrative zones: Arsi, Bale and West Shewa (Figure 2.1). The zones are situated in the Oromia Regional State of Ethiopia. The zones are all major wheat growing areas but differ in terms of agro-ecological diversity, and in the use of modern wheat production technologies. The Arsi and Bale Zones are situated in the south-eastern of Ethiopia while the West Shewa Zone is in the Central highlands of Ethiopia (Figure 2.1).

The Arsi Zone has three climatic zones, namely highlands (43.4%), midlands (27.5%) and lowlands (29.1%). Bale Zone has highlands, midlands and lowlands at 15%, 21% and 64% of the total land area, respectively. Likewise, three agro-ecological zones are distinguishable in West Shewa: highlands (57%), midlands (25%) and lowlands (18%) (CSA, 2013). Highlands are defined as those areas with altitudes of 2300-3200 masl, midlands with altitudes of 1500-2300 masl and lowlands with altitudes of 500-1500 masl.

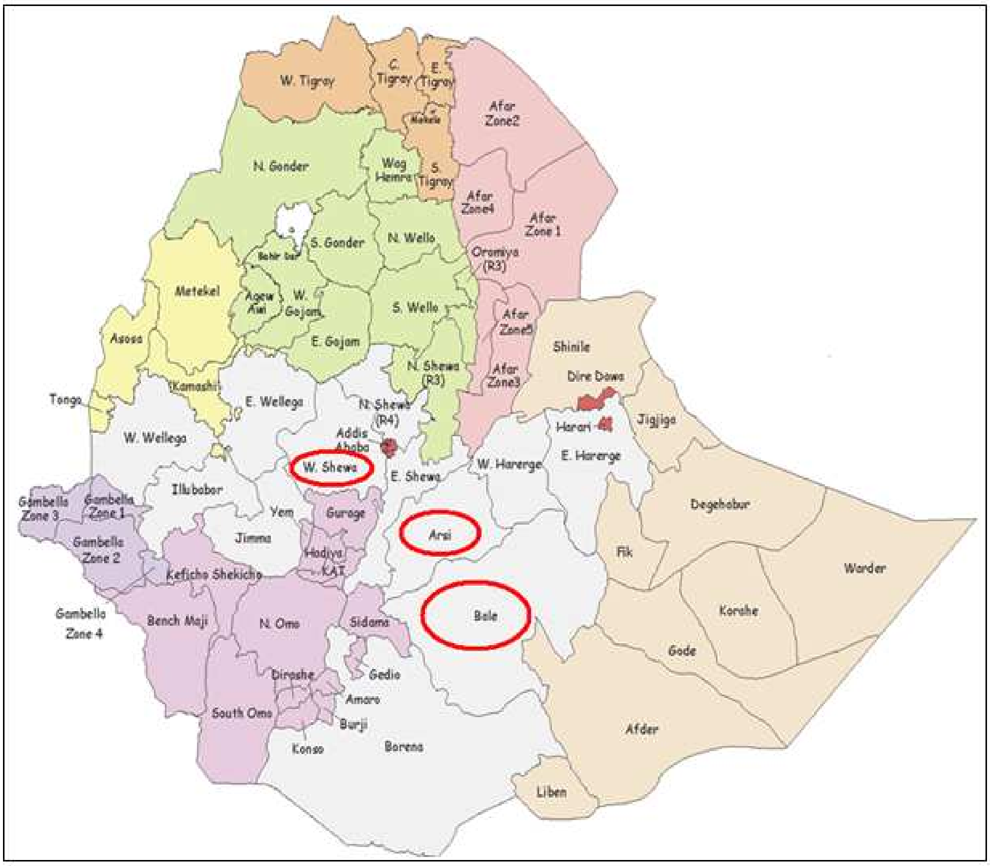

Figure 2.1 Map of Ethiopia showing the three study areas: Arsi, Bale and West Shewa zones.

2.2.2 Data source

Both qualitative and quantitative data were collected from primary and secondary sources. Primary data were collected through semi-structured interviews and focus group discussions. The secondary data were obtained from the zonal and district agricultural offices of the respective districts included in the study.

2.2.3 Sampling

A multi-stage sampling procedure was used involving the selection of zones, districts, villages and wheat farmers. A non-random purposive selection method was used to select from the zonal through the districts, villages and farmers levels. Fifteen farmers from each village were interviewed, providing a total of 270 farmers for the study. Individual farmers were selected from each village representing various socio-economic backgrounds (data not shown) and both gender (Table 2.1). Zone level agricultural experts and district agricultural development offices assisted with the identification of the sampled districts, villages and respondents.

Table 2.1. Study zones, districts and villages and number of sampled wheat farmers in Arsi, Bale and West Shewa administrative zones of the Oromia Regional State of Ethiopia

Zone	District	Village	Male	Female	Total
Arsi	Tiyo	Ketar-Genet	13	2	15
		Abusera	11	4	15
		Hamsa-Gasha	12	3	15
	Munisa	Gerenbo-Talole	12	3	15
		Didibe Yadola	13	2	15
		Gumguma	14	1	15
Bale	Sinana	Sambitu	13	2	15
		Nano-Robe	14	1	15
		Obora	15	0	15
	Gasera	Danbel	13	2	15
		Awsencho	14	1	15
		Naqe	14	1	15
West Shewa	Jeldu	Kolu-Gelan	12	3	15
		Seriti-Denko	13	2	15
		Tulu- Bultuma	14	1	15
	Dandi	Boda- Boseka	14	1	15
		Cheleleka- Bobe	13	2	15
		Serewa-Debisa	14	1	15
				Total	270

2.2.4 Data collection

Semi-structured questionnaire was designed on topics related to the general socio-economic characteristics of the household, wheat varieties grown, production constraints, wheat rust diseases and their management. Enumerators were recruited for data collection who lives in the area, fluent speakers of local language (Oromifa), well acquainted with local and cultural contexts, and working within the selected districts. They were trained on the contents of the interview schedule and data collection techniques. Pre-test on non-sample respondents was also made under supervision of the researcher. Finally, the formal survey was conducted on 270 households after necessary modification and adjustments were accommodated as per the result obtained from the pre-test. At the end of each day all questionnaires were checked with the enumerators and clarifications were made.

Focus group discussions were held in each district to understand farmers' varietal preferences and the specific traits that influence a farmer's decision to grow a wheat variety, and the major constraints affecting wheat production. Each group was composed of 10-15 wheat farmers (both male and female). Checklists were developed and used to guide focused group discussions with farmer groups and individual key informants. The farmers were encouraged to use their local language that they were most familiar with. The development agents most familiar with the local language facilitated the group discussions. During the discussion, the farmers were asked to list wheat varieties they grow and to identify the traits that they used in selection of the varieties, and list the main constraints limiting wheat production.

2.2.5 Data analysis

Data (both qualitative and quantitative) obtained from sample respondents were sorted, coded and subjected for statistical analyses using the Statistical Package for Social Sciences computer software (SPSS Inc., 2005). Both descriptive and inferential statistical procedures were used to analyze the data obtained from farm households.

2.3 Results

2.3.1 Demographic characteristics and socio-economic factors

The sample population contained 88.2% males and 11.8% females. Almost all the respondents (99%) who participated in the study were farmers in agricultural production. The mean family size of the sampled population was 5.1 and about 85% of interviewed farmers had family sizes greater than 3 persons per household. In the study areas, children were contributing to farm labour significantly. Farmers who were illiterate constituted 21%. Farmers educated up to primary and secondary level constituted 62% and 17%, respectively.

2.3.2. Farming system

Household total crop land in the study areas ranged from 0.5 to 15 hectares, with mean farm size of 2.5 ha (SD 2.43). The majority of the interviewed farmers allocate most of their land for wheat as the number one crop. Of the 2.5 hectares of mean farm size owned by individual farmers, a mean of 1.85 ha were dedicated to wheat production in the study areas. Farmers in the surveyed areas grow different assemblage of crops. These include cereals, pulses and oilseed crops. In addition to wheat, other major crops grown by majority of farmers in the Arsi Zone were barley (*Hordium vulgare* L.) (71%), maize (*Zea mays* L.) (51%), teff (*Eragrostis tef* (Zucc.) Trotter) (41%), faba bean (*Vicia faba* L.) (46%) and linseed (*Linum usitatissimum* L.) (18%). The three major cereal crops widely grown after wheat were barley (58%), maize (40%), and teff (39%) in the Bale Zone. In West Shewa most farmers grow maize (67%), teff (63%), barley (41%), faba bean (38%), grass pea (*Lathyrus sativus* L.) (24%), and noug (*Guzotia abysinica* Cass.) (15%).

Wheat is grown both in the main and short rainy seasons in the Sinana and Gasera Districts of Bale Zone. The main rainy season has long rains which start in June and end in September. It is the period when the largest wheat area is cultivated. In the short rainy season, the rain starts in February and ends in April. Seventy three percent of farmers in these districts grow wheat in both the main and the short rainy seasons, while 27% of them only utilize the main season to produce wheat. On the other hand, farmers in the Arsi and West Shewa Zones only grow wheat during the main rainy season. In the study areas, wheat is produced solely under rain fed conditions.

2.3.3. Wheat seed source

The sources of seed for farmers are presented in Table 2.1. The informal sector was the source of seed for 84.4% of the farmers in the area, where 68.1% respondents used farm-saved seeds, and 8.9% and 7.4% of respondents used seeds from other farmers and local markets, respectively. The formal sector provided for only 15.5%, where 12.2% of farm households sourced their seed from Agricultural Offices (AO) in the respective districts and 2.2% from producers' cooperatives (Table 2.1).

Table 2.1. Farmers' sources of wheat seed in the Arsi, Bale and West Shewa administrative Zones of the Oromia Regional State of Ethiopia

Seed source	Seed source in 2011 cropping season	
	Frequency	% response
Other farmers	24	8.9
Agricultural Offices	33	12.2
Research centers	3	1.1
Producer cooperatives	6	2.2
Local markets	20	7.4
Own stock	184	68.1
Total	270	100

2.3.4. Wheat varieties grown by farmers and genetic diversity

Table 2.2 shows the different wheat varieties grown by farmers in the study areas. Most farmers grow more than one variety, making the proportions above 100%. The most commonly grown wheat varieties in the Aris Zone were Digelu, Kubsa and Tusie at 88.75%, 53.35% and 39.75%, respectively. In the Bale Zone, Tusie (77.2%), Digelu (70.75%) and Madawalabu (46.2%) were the dominant wheat varieties grown by the majority of farm households. Digelu (40.2%) and Kubsa (38.9%) were popular varieties in West Shewa Zone. Variety Digelu was grown by 88.8%, 70.8% and 40% respondents in Arsi, Bale and West Shewa zones, respectively. This variety is still in high demand and is being rapidly multiplied. Fifty five percent of respondents in Arsi and 38% in West Shewa grow Kubsa on their farms.

The new bread wheat varieties, Kakaba and Danda'a that were released in 2010 were grown in Arsi by 15% and 10% of the farmers, respectively. Danda'a was grown only by 3.4% of farmers interviewed in Bale. None of the respondents in West Shewa grew these varieties, while 10.7% of farm household used local wheat varieties. In all surveyed areas farmers grew only bread wheat type.

Table 2.2. Wheat varieties grown, year of release and proportion of wheat farmers in Arsi, Bale and West Shewa administrative zones of Ethiopia

Variety	Year of release	Arsi	Bale	West Shewa
		% response	% response	% response
Kubsa	1995	53.35	10.15	38.90
Digelu	2005	88.75	70.75	40.20
Galama	1995	-	3.35	30.55
Dashen	1984	-	-	31.75
Kakaba	2010	15.85	3.40	1.10
Madawalabu	2000	6.80	46.20	1.15
Pavon 76	1982	11.10	1.15	-
Tusie	1997	39.75	77.20	-
Hawi	2000	1.10	-	-
Sofumer	2000	9.10	20.35	-
Danda'a	2010	10.25	-	-
Local	-	-	6.75	10.70

2.3.5. Farmers'-preferred traits

In all the study sites, farmers use a combination of criteria in selecting wheat varieties. The major and common reasons behind varietal preferences are given in Table 2.3. The most important criteria across the sites were disease resistance (27.8%), high yield (24.8%) and a combination of the two (27%). In Arsi 31.1% of respondents prefer a combined high yield and disease resistance as the key criteria for selecting wheat varieties. Disease resistance was a key criterion for 27.8% and 37.8% farmers in Bale and West Shewa, respectively.

Environmental adaptability was a criterion for 7.8% of farmers in Arsi and 8.9% in Bale and 2.2% of respondents in West Shewa. High market value in combination with other traits was also a major selection criterion in the study sites because wheat is a major source of income in the areas.

Table 2.3. Farmers'-preferred traits required of improved wheat varieties in the Arsi, Bale and West Shewa zones of Ethiopia

Farmers'-preferred traits	Zones							
	Arsi		Bale		West Shewa		All survey	
	Freq	%	Freq	%	Freq	%	Freq	%
Grain yield	25	27.8	15	16.7	27	30	67	24.8
Disease resistance	16	17.8	25	27.8	34	37.8	75	27.8
Grain yield and disease resistance	28	31.1	22	24.4	23	25.6	73	27.0
Environmental adaptability	7	7.8	8	8.9	2	2.2	6	6.3
Disease resistance and food quality	4	4.4	3	3.3	0	0	12	2.6
Grain yield and high market value	3	3.3	2	2.2	1	1	10	2.2
Grain yield, food quality and high market value	2	2.2	5	5.6	0	0	9	2.6
Grain yield, early maturity, disease resistance and food quality	1	1.1	4	4.4	2	2.2	7	2.6
Grain yield, disease resistance, high market value and food quality	4	4.4	6	6.7	1	1.1	11	4.1
Total	90	100	90	100	90	100	270	100

†Freq=frequency of respondents

Farmers in group discussions were also asked to associate a particular wheat variety they currently grow with its preferred and non-preferred traits. The most commonly grown varieties, along with their preferred traits, are summarized in Table 2.4. Farmers in the study areas selected wheat varieties Madawalabu, Sofumer, Danda'a and Kakaba for their disease resistance. Tusie is tolerant of rust and is preferred for its market value. Kubsa and Galama are disease susceptible varieties but are still grown for their high grain yield and biomass which is used for animal fodder, fuel and house roofing material. White seeded varieties such as Kubsa, Dashen, Digelu, Kakaba, Danda’a, Tusie and Pavon 76 are largely grown by farmers for sale because they are preferred by urban consumers.

Farmers in group discussions stated that Kakaba is tolerant to lodging because of its semi-dwarf nature. It is early maturing variety and was preferred by farmers who practiced double cropping. Kakaba was also preferred for its soft straw which makes it suitable for animal fodder. In contrast, Digelu has hard straw, making it little use for animal fodder. Farmers raised that variety Digelu is late maturing. However, they were convinced that this variety is high yielding and better in areas that receive extended rainfall. Danda’a was preferred by the farmers for its tillering capacity, resistance to disease and long spike. The farmers who grew Danad’a considered it as a substitute for the old susceptible wheat variety, Galama. The female famers who participated in the group discussions also stated that the variety has good bread making quality. However, the farmers indicated that Denda’a has less threshability and difficult to thresh using conventional harvesting. Hence, farmers obliged to use combine harvesting.

Table 2.4. Wheat varieties currently grown by farmers in the Arsi, Bale and West Shewa zones and their outstanding traits

Wheat varieties	Preferred traits	Non- Preferred traits
Kubsa	High grain yield, high biomass, multiple use at home, white seed, adaptable to environment	Susceptible to disease
Digelu	High grain yield, multiple use at home, white seed	Late maturity, hard straw
Galama	High biomass, multiple use at home, adaptable to environment	Susceptible to disease, late maturity
Dashen	White seed	Susceptible to disease
Kakaba	High grain yield, disease resistant, early maturity, white seed, tolerant to lodging, soft straw for animal fodder	-
Madawalabu	High grain yield, disease resistant, early maturity	-
Pavon 76	White seed, early maturity	Susceptible to disease
Tusie	White seed, tolerant to rust	-
Sofumer	High grain yield, disease resistant	Purple seed color
Danda'a	High grain yield, disease resistant, white seed, tillering capacity, bread making quality, long spike	Late maturing

2.3.6. Wheat production constraints

Almost all sampled farmers (96%) considered the wheat rusts as the most important production constraint. High input prices, especially of fertilizers (93%) and improved seeds (81%), were identified as important limiting factors of wheat production after rusts. Lack of access to seeds of improved wheat varieties followed by low market prices of wheat were also identified as important factors by 85% and 66% of the farmers, respectively (Table 2.5).

The farmers’ perceptions of wheat production constraints and their ranks between locations are summarized in Table 2.5. There was a marked agreement in the identified constraints in the three survey zones, with some variation in the ranking between the zones. High seed prices were ranked fourth following a lack of access to seeds of varieties in the Arsi and West Shewa zones, whereas farmers in the Bale Zone perceived high seed prices as equally important to the lack of access to seeds of improved varieties and both ranked third. Lack of credit access was perceived as an important constraint in West Shewa while it ranked lower in the Arsi and Bale zones.

Table 2.5. Major wheat production constraints and their ranks in Arsi, Bale and West Shewa zones of Ethiopia.

Constraints	Zones											
	Arsi			Bale			West Shewa			All Surveyed		
	†Freq.	%	Rank	Freq.	%	Rank	Freq.	%	Rank	Freq.	%	Rank
Rusts (yellow rust and stem rust)	87	96.7	1	86	95.6	1	86	95.6	1	259	96	1
Lack of seed of improved varieties	81	90.0	3	77	85.6	3	71	78.9	3	229	85	3
High seed price	78	86.7	4	77	85.6	3	65	72.2	4	220	81	4
High fertilizer price	85	94.4	2	82	91.1	2	84	93.3	2	251	93	2
Shortage of fertilizer	15	16.7	9	17	18.9	9	21	23.3	9	53	20	9
Low producer price	61	67.8	5	62	68.9	5	54	60.0	5	177	66	5
Weeds (grass weeds)	37	41.1	7	32	35.6	6	25	27.8	8	94	35	7
Poor soil fertility	11	12.2	10	10	11.1	11	12	13.3	11	33	12	11
Other diseases and pests	42	46.7	6	29	32.2	7	39	43.3	7	110	41	6
Unpredictable rain	18	20.0	8	13	14.4	10	16	17.8	10	61	17	10
Lack of access to credit	7	7.8	11	24	26.7	8	54	60.0	5	85	31	8

†Freq=frequency of respondents

2.3.7 Wheat stem rust and farmers' rust management methods

In the present study, yield losses were estimated based on the difference between yield from stem rust free wheat farms and diseased wheat farms in study areas (Table 2.6). Accordingly, yield losses of 70.7%, 60.5% and 60.0% were reported in the Arsi, Bale and West Shewa zones, respectively (Table 2.6).

Table 2.6. Yield losses due to stem rust in Arsi, Bale and West Shewa, Ethiopia

Zone	Mean wheat productivity		Loss (%)
	Under low/no rust infestation (t ha^{-1})	Under high rust infestation (t ha^{-1})	
Arsi	4.1	1.2	70.7
Bale	3.8	1.5	60.5
West Shewa	2.0	0.8	60.0

To reduce losses from rust infestations, fungicides are being used by most producers. More than 60% of interviewed farmers used fungicides for rust management (Table 2.7). Tilt@ (Propiconazol), Bayfidan@ (triadimenol), and Mancozeb were the major fungicides used by the farmers for rust control. Only 15% of the respondents had adopted new varieties for the control of rusts. Kakaba, Danda'a and Digelu were widely adopted resistant wheat varieties. On the other hand, a few farmers in Bale (6.7%) were planting early to avoid rust damage. In contrast, almost 20% of the farmers did not use any control measure to protect their wheat farms from rust infection.

Table 2.7. Wheat rust control measures practiced in the study areas

Control measures	Zone							
	Arsi		Bale		West Shewa		All surveyed	
	†Freq	%	Freq	%	Freq	%	Freq	%
None	14	15.6	15	16.7	24	26.7	53	19.6
Chemical	62	68.9	60	66.7	57	63.3	179	66.3
Resistant variety	14	15.6	9	10.0	8	8.9	31	11.5
Cultural practice (early planting)	0	0	6	6.7	1	1.1	7	2.6
Total	90	100	90	100	90	100	270	100

†Freq= frequency of respondents

2.4 Discussion

In Ethiopia wheat research programs to develop improved wheat varieties were initiated during the 1950s. Despite 60 years of wheat breeding in the country, most of the released cultivars had been poorly adopted by small-scale farmers (Zegeye et al., 2001; DRRW, 2010). Majority of farmers in the study areas continue to grow older varieties such as Kubsa and Galama that are often susceptible to diseases. The reseaons for the persistence of older varieties were lack of farmers' preferred traits in the new cultivars, unavailability of sufficient quantity of new seed or its poor distribution in the study areas and the risk avoidance adopted by farmers who grow a mixture of varieties to spread their risks.

In the past, durum wheat was the most widely grown wheat type in the major wheat growing areas of Ethiopia. Ethiopian durum wheat land races are valuable sources of resistance to rust diseases (Denbel and Badebo, 2012). To date bread wheat has become predominant in most wheat areas of the country. Farmers in the study areas shifted to bread wheat production owing to its productivity per unit area relative to durum wheat. However, this may seriously threaten the existence of local durum wheat land races in the country if strategic seed conservation is not undertaken on a national scale.

Wheat rusts have been major threats to wheat production in Ethiopia. In recent years, novel pathotypes of the rusts have overcome resistant wheat varieties (ICARDA, 2011). The study sites are among the most rust prone areas of Ethiopia and the wheat farmers in these areas frequently suffer serious losses from rusts epidemics (Hodson et al., 2013; Periyannan et al., 2013). The yellow rust outbreak in 2010 significantly reduced the national wheat annual production. The major wheat producing regions including the study zones were seriously affected of the epidemics with losses up to 70% (Hunde et al., 2012; Yami et al., 2013). Hence, farmers in all the study areas were in agreement that wheat rusts are the most important production constraints. High prices of chemical fertilizers and improved seeds were also important production limiting factors in the study areas. An increase in fertilizer prices due to the removal of government subsidies has decreased fertilizer use in the study areas. Consequently, farmers apply chemical fertilizers below the recommended rates. Under such circumstance, it is difficult to increase the wheat yields on small scale farms. Bishaw et al. (2010) reported a serious gap between the recommended rate and the actual amount applied by the farmers.

Farmers in the study areas were well aware of the benefit of resistant varieties for the control of rust diseases. However, majority of the respondents grow old varieties for the reasons described earlier and due to high prices of seeds of improved varieties, and doubts about the level of resistance provided by these new varieties to rust diseases. Hence, farmers use fungicides for the control of the rusts. The producers applied fungicides at early growth stages but the application rates were below the optimum rates to get the desired level of benefits. Reasons given for the use of lower fungicide rates included a lack of awareness of the recommended rates, and the shortage and high price of chemicals. Early planting is another important rust control measure. It reduces the time of exposure of the crop to the pathogen and hence reduces yield loss (Tolessa et al., 2014). However, early planting is not a widely adopted disease control measure by the farmers in the PRA zones.

Although farmers in the study zones had a range of preferences regarding wheat varieties and specific traits, they were in agreement that disease resistance is the most important trait compared to all other traits. This indicated that farmers were concerned about the susceptibility of the existing varieties to rust diseases. The farmers in the study areas also indicated grain yield as a key criterion for selecting wheat varieties after rust resistance. In general, to ensure a high level of variety adoption and therefore the high productivity of the crop, the wheat breeding programme in the country should put more emphasis on solving the problems of wheat farmers, increase the frequency with which it releases new varieties that resist diseases and yield well. Besides, the seeds of newly developed varieties must be produced in sufficient quantities in the study areas to make the research efforts more successful.

2.5 Conclusions

Records from the current survey revealed that bread wheat was the most widely grown wheat type, and indigenous durum wheat varieties had been completely replaced with modern bread wheat varieties in the study areas. The main constraints identified were diseases (wheat rusts) (96%), the high cost of fertilizers (93%), lack of access to seeds of new improved varieties (85%) and high seed prices (81%). Based on the findings of the survey and discussions with farmers, disease resistance and high grain yield appeared to be the most farmers'-preferred traits of new wheat varieties. Yield losses reaching up to 70% were recorded due to wheat stem rust in the study areas. The majority of farmers in the study areas grow rust susceptible wheat varieties. The study also revealed that fungicides were widely used to control wheat rusts, although application rates were mostly lower than optimal.

Given the strong demand for high yielding and disease resistant wheat varieties, future research should be directed to develop new varieties with high yields, acceptable agronomic characters and disease resistance. In addition, the current seed multiplication and dissemination pathways should be speeded up and seeds should be delivered to farmers at affordable prices, to ensure adoption of modern wheat varieties and increase wheat productivity. Efforts should also be made to conserve the indigenous durum wheat landraces and make use of them in developing modern wheat varieties.

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CHAPTER THREE

Assessment of Ethiopian wheat lines for slow rusting resistance to stem rust of wheat caused by *Puccinia graminis* f.sp. *tritici*

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CHAPTER 3

Assessment of Ethiopian wheat lines for slow rusting resistance to stem rust of wheat caused by *Puccinia graminis* f.sp. *tritici*

Abstract

The emergence and rapid spread of virulent races of wheat stem rust has driven a search for sources of resistance for durable resistance breeding. This study was carried out to identify possible sources of stem rust resistance among Ethiopian wheat lines. Two hundred fifty two wheat accessions and a universal suscept, cultivar Morocco were evaluated for their resistance at the seedling stage to the stem rust isolate Ug99 in a controlled environment. Ninety one lines that exhibited intermediate and susceptible seedling reactions were further field tested in 2012 main season for their slow rusting characteristics. Among the ninety one, thirty eight genotypes that had high to moderate level of slow rusting were advanced to a 2013 off-season field evaluation. Slow rusting resistance at the adult plant stage was assessed through the determination of final disease severity (FRS), coefficient of infection (CI), and relative area under disease progressive curve (rAUDPC). The results revealed that wheat lines H04-2, 204408-3, 214551-1, 231545-1, 7041-1, 7514-1, 226385-1, 226815-1, 7579-1, and 222495-1 had low values of FRS, CI, and rAUDPC and were regarded as good slow rusting lines. Of these 231545-1, 7041-1, 226815-1 and 7579-1 exhibited complete susceptibility at the seedling stage, with infection types ranging from 3- to 3+, which suggests that they possess true slow rusting resistance. Lines 237886-1, 227059-1, 203763-1, 226275-1, 227068-2, 226278-1 and 7994-1 had moderate values for the stem rust resistance parameters and were identified as possessing a moderate level of slow rusting. High correlation coeficients were observed between different parameters of slow rusting. Among the slow rusting lines 231545-1, H04-2 and 222495-1 had high yields and kernel weight in both seasons. The slow rusting lines identified from this study can be used to breed for stem rust resistance in wheat.

Keywords: durable resistance, resistance breeding, slow rusting, stem rust, wheat

3.1 Introduction

Wheat (*Triticum aestivum* L.) is the most important food grain for billions of people worldwide, being cultivated on 15.4% of the arable land. It accounts for around 30% of global grain production and 44% of cereals used as food (Khanzada et al., 2012). Wheat is one of the most important cereal crops produced in Ethiopia. It ranks fourth in area under cultivation after teff (*Eragrostis tef* (Zucc.) Trotter), maize (*Zea mays* L.) and sorghum (*Sorghum bicolar* (L.) Moench). In total grain production, wheat ranks third after teff and maize (CSA, 2013). However, its production and productivity is severely constrained by the wheat rust diseases namely stem rust, leaf rust and stripe rust caused by the fungi *Puccinia graminis* Pers. f.sp. *tritici* Eriks and Hann, *P*. *triticina* Eriks and *P*. *striiformis* Westend. f.sp. *tritici*, respectively. Stem rust is the major production constraint in most wheat growing areas of the country (Denbel et al., 2013) which often causes yield losses reaching up to 100% on susceptible cultivars (Park, 2007).

The stem rust pathogen is known to rapidly develop new virulence to resistance genes owing to mutation events and genetic recombination. In recent years, new races of *P. graminis* f.sp. *tritici* (*Pgt*) have been reported in wheat production areas globally (Singh et al., 2008). Wheat growing environments such as the East African highlands, with continual wheat production and favorable microclimates, are known hot spots for the rapid evolution and spread of new rust races**.** The occurrence and spread of virulent stem rust races in and out of the region have threatened wheat production globally (Periyannan et al., 2013).

In 1999 a new virulent race of *Pgt* known as Ug99 was found in Uganda (Pretorius et al., 2000). The race carries virulence to stem rust resistance gene *Sr31*, which was derived from rye and remained effective for more than thirty years (Wanyera et al., 2006). Ug99 has since spread through this region and to Middle East and South Asia (Nazari et al., 2009; Singh et al., 2009). Ug99 was first reported in Ethiopia in 2003 (Singh et al., 2008). Globally Ug99 has evolved even further, accumulating additional virulence to important *Sr* genes, notably *Sr24* and *Sr36* (Jin et al., 2008, 2009).

Various control options are available to minimize losses caused by stem rust. The cheapest and most environmentally friendly management strategy to reduce losses to stem rust disease would be the use of resistant wheat cultivars. To date more than 50 *Sr* genes that confer resistance to different races of stem rust have been identified (McIntosh et al., 2011). Several of the designated genes are qualitative and race-specific (Singh et al., 2011). Of these genes and alleles, at least 27 are effective or partially effective to the Ug99 race group including *Sr33*, introgressed from the wild relatives *Aegilops tauschii* and *Sr35*, transferred from *Triticum monococcum* to bread wheat (Periyannan et al., 2013; Saintenac et al., 2013; Yu et al., 2014). A major risk associated with the utilization of such race-specific genes is the ability of pathogens to defeat the genes when they are deployed alone in wheat cultivars as has been demonstrated by the Ug99 defeating *Sr24*, *Sr36* (Jin et al. 2008, 2009). Therefore, efforts to identify and incorporate genes that confer durable resistance are crucial (McDonald and Linde 2002; Ayliffe et al., 2008).

Slow rusting resistance is a type of resistance that is both race non-specific and durable (Sawhney, 1995). Slow rusting resistance is often described as partial resistance or adult plant resistance. Such slow rusting resistances are polygenic and reduce the infection efficiency and retard growth and development of the pathogen, especially in adult plants. According to Yu et al. (2014) a total of five designated wheat stem rust resistance genes confer quantitative adult plant resistance: *Sr2, Sr55, Sr56, Sr57* and *Sr58*. The effects of race non-specific genes are pronounced post seedling growth stages (Nzuve et al., 2012). These genes are also characterized by non-hypersensitive responses (Navabi et al., 2004; Singh et al., 2009).

Wheat varieties resistant to rusts have been developed through the national wheat improvement research program and in collaboration with the International Maize and Wheat Improvement Center (CIMMYT) in Ethiopia. However, most of the varieties do not possess durable resistance and became susceptible shortly after their introduction. In most cases, the failures were due to new virulent pathotypes and deployment of the same R-gene(s) in wide array of wheat cultivars (Admassu et al., 2012). According to Admassu et al. (2012) most stem rust resistance genes present in wheat cultivars and breeding lines of Ethiopia are race-specific and ineffective to most of the prevalent races of *Pgt.* The epidemic proportion of stem rust on wheat variety Digelu (HAR 3116) in Bale Zone during the 2013 cropping season is a classical example. Digelu is a recently promoted bread wheat variety with major gene resistance. It was resistant to stem rust at the time of its release, but new virulent races were present in 2013, even before its cultivation was in substantial areas. Digelu developed extremely high levels of stem rust, which led to 100% yield losses during the season (Hodson, 2013). The failures of many promising cultivars such as Digelu, which were reportedly resistant to stem rust, indicate the importance of breeding for durable resistance using polygenes.

In view of the rapid evolution and spread of new virulent races of stem rust, the frequent failure of new varieties with stem rust resistance and the limited availability of sources of durable resistance, it is imperative to develop new wheat cultivars using different sources of resistance. The objective of this study was to identify adult plant, slow rusting resistance in Ethiopian wheat lines.

3.2 Materials and methods

The study was subdivided into two experiments: seedling reaction test conducted in a controlled greenhouse; and a field test to identify adult plant resistance in the wheat lines.

3.2.1 Seedling infection response

The goal of this experiment was to eliminate lines with major gene resistance, which is expressed in seedlings. Two hundred fifty two wheat lines were tested in a greenhouse for their stem rust resistance at the seedling stage. Accessions used in this study were bread wheat (*T. aestivum*) lines obtained from the Institute of Biodiversity Conservation and Research (IBCR) in Ethiopia. True-to-type lines were identified over two selection cycles, in the 2011 off-season (January to May) and the 2011 main season (June to October). These lines, along with a standard set of 20 differential varieties and the susceptible check cultivar Morocco were raised in 10 cm plastic pots in environmentally controlled greenhouse at the Ambo Plant Protection Research Center, Ambo, Ethiopia.

Five to six seeds per line were sown in each pot. Each pot was filled with compost, light soil and sand at a 1:1:1 ratio (v/v/v). The wheat lines were tested against race TTKSK (Ug99). Race TTKSK represents a broad spectrum of virulence in the Ethiopian stem rust population. The isolate was derived from single pustules, increased in isolation, and maintained on cultivar, Morocco, during the off-season of 2012. Seedlings were inoculated at the 2-3 leaf stage using spore suspension adjusted to 4 X 10^6 spores ml^{-1} using spore inoculators. Spores were suspended in a light mineral oil, Soltrol 170 (Chevron Phillips Chemical company, The woodlands, Texsas, United States).

Inoculated seedlings were placed in a dew chamber in darkness for 18 hours at 18-22^oC and 98-100% relative humidity. Upon removal from chamber, plants were exposed to 3 h of fluorescent light to dry dew on the leaves. Inoculated plants were then transferred to greenhouse benches where the temperatures were kept between 18 and 25^oC and the relative humidity at 60-70% (Stubbs et al., 1986).

Seedling infection types were scored 14 days after inoculation using a 0 to 4 scale (Stakman et al., 1962). The IT readings of 3 (medium-size uredia with/without chlorosis) and 4 (large uredia without chlorosis or necrosis) were regarded as compatible reactions. Other readings, i.e. 0 (immune or fleck), 1 (small uredia with necrosis), and 2 (small to medium uredia with chlorosis or necrosis) were incompatible. The variations were refined by modifying characters as follows: -, uredinia somewhat smaller than normal for the infection type; +, uredinia somewhat larger than normal for the infection type.

3.2.2 Field evaluation

For the field experiment the test materials comprised 91 wheat lines which showed susceptible and mixed (intermediate and susceptible) infection types in the seedling test. In order to evaluate the lines for their slow rusting resistance, a field evaluation was conducted during the 2012 cropping season at the Debre-Zeit Agricultural Research Center which is an internationally known hot spot for stem rust and is therefore suitable for screening of wheat for resistance to stem rust. Thirty eight lines were then selected for their good slow rusting characteristics and planted at the

Debre-Zeit Agricultural Research Center during the 2013 off-season for verification of the initial results. The Debre-Zeit Agricultural Research Center is found at altitude of 1900 m above sea level. The center receives mean annual rainfall of 851 mm. The average annual minimum and maximum temperatures are 8.9^{o}C and 28.3^{o}C, respectively (Denbel et al, 2013). A susceptible check, Morocco, was used as a comparative control in the experiments.

The lines were planted in plots consisting of double rows of 1 m long with 20 cm row spacing. Each line was planted manually at a rate of 2 g of seed per two rows. Experiments were established using an alpha lattice design of 10 x 9 with two replicates during the 2012 main season and 8 x 5 with two replicates during the 2013 off-season. The spacing between plots was 40 cm. A mixture of two susceptible checks, Morocco and a standard cultivar PBW343, were planted perpendicular to the experimental blocks one week before the experimental plots to serve as spreader rows.

Epidemics of stem rust were initiated by the inoculation of spreader rows with *Pgt* race TTKSK using urediniospores maintained at the Ambo Plant Protection Research Center. A water suspension of these urediniospores of the stem rust race was inoculated onto spreader rows using an ultra low volume sprayer to generate fine mist. This took place twice when most plants were at the stem elongation. Fertilizers and other agronomic practices were applied according to the recommended practices for wheat production in the area.

Slow rusting behaviour among the wheat genotypes was assessed through host response and epidemiological parameters: final rust severity (FRS), coefficient of infection (CI), area under disease progress curve (AUDPC) and infection rate (r). Stem rust severity, estimated visually as a proportion of the plant stem affected, was recorded according to the modified Cobb scale (Peterson et al., 1948). Severity was assessed three times at twenty days interval from ten randomly pre-tagged plants of each entry, starting when stem rust levels on Morocco reached 50% severity. The host plant response to infection was also scored using the description of Roelfs et al. (1992). If a line displayed multiple infection responses to stem rust, they were all recorded (example: MR-MS, MS-S). The coefficient of infection (CI) was calculated by multiplying the level of disease severity and the constant value of infection type. The constant values for infection types were used based on; R=0.2, MR=0.4, MR-MS =0.6, MS=0.8, MS-S= 0.9, S=1 (Stubbs et al., 1986).

Estimation of the area under disease progress curve (AUDPC), which is a better indicator of disease expression over time (Vanderplank, 1963), was performed for each experimental unit with the formula of Wilcoxson et al. (1975):

$$AUDPC = \sum_{i=1}^{n} [0.5\,(x_i + x_{i+1})]\,[t_{i+1} - t_i].$$

Where xi = stem rust severity on the i^{th} date, t_i = the time in days after appearance of the disease, and n = number of dates on which stem rust was recorded. The infection rate (inf-rate), was also estimated in terms of disease severities recorded on wheat lines in different times (Vanderplank, 1968). The infection rate (inf-rate) per time unit (t) for each line was calculated as the regression coefficient of ln [X/(100 – X)], where X is average coefficient infection plotted against time in days (Vanderplank, 1968).

A thousand kernel weight was taken from randomly sampled kernels and recorded in grams for each plot. The two rows of each entry were harvested and their grains weighed using an electronic balance and for conversion to tonnes per hectare.

3.2.3 Data analysis

Relative forms of the epidemiological parameters were generated by comparing the respective values of each entry with the susceptible variety Morocco. A standard analysis of variance was conducted to identify significance differences among the wheat lines for grain yield and thousand kernel weight. Fisher’s least significant differences test (P = 0.05) was used to compare the means. Correlation analysis was used to determine the relationships among slow rusting parameters. The spearman’s rank correlation coefficients were estimated to test the change in ranking of wheat lines over the two seasons in terms of disease parameters, grain yield and thousand kernel weight. The data were analysed using SAS and SPSS softwares (SAS, 2002; SPSS, 2005).

3.3 Results

3.3.1Seedling reaction

The greenhouse experiment revealed that the bread wheat lines differed in their reaction to the stem rust isolate TTKSK. Out of 252 wheat lines tested in a greenhouse 161 showed resistance reactions (0; to 2), 72 had susceptible reactions (3- to 3) and 19 had mixed reactions (2+ and 3-) at the seedling stage (data not shown). The susceptible check, Morocco, displayed infection type 3+ at the seedling stage. Among the 91 entries that showed susceptible and mixed reactions, 38 were advanced and evaluated for slow rusting resistance. The result of seedling assessment for 38 selected lines is presented in Table 3.1.

3.3.2 Field assessment

The selected lines were evaluated in the field using parameters such as disease severity, area under disease progress curve or the measurement of apparent infection rates and coefficients of infection values.

3.3.2.1 Final rust severity (FRS)

There was wide variation in the stem rust severities ranging from 1 to 70% during the 2012 cropping season, and from 1 to 85% during the 2013 off-season at the Debre-Zeit Agricultural Research Center. Diverse field reactions ranging from moderately resistant and moderately susceptible (MR-MS) to susceptible (S) responses were observed at the Debre-Zeit trials.

Among the 91 accessions evaluated during the 2012 main season, 13 accessions (14%) showed disease severities of up to 30%, with field responses varying from MR-MS to MS-S. Twenty five lines had severities ranging from 31 to 50% while the remaining 53 accessions displayed more than 50% final rust severities. Out of the 13 accessions in the first group, seven: H04-2, 204408-3, 214551-1, 7514-1, 226385-1, 222495-1 and 237886-1, had mixed seedling reactions (2+ and 3-) while six: 231545-1, 7315-1, 7041-1, 7516-1, 226815-1 and 7579-1, showed susceptible (3- to 3) infection types. The susceptible check, Morocco, displayed the highest disease severity of 70% with a completely susceptible (S) response. The final rust severities and their infection types of the lines in the first and second group are presented in Table 3.1.

Thirty eight genotypes, 13 in the first group and 25 in the second group, together with the susceptible check Morocco, were planted at the Debre-Zeit Agricultural Research Center during the 2013 off-season for further tests. Despite the heavy stem rust disease pressure in the field during the season (Figure 3.1), 10 wheat lines remained in the first group, exhibiting final rust severities ranging from 1 to 30%, with moderately resistant to moderately susceptible (MR-MS) to moderately susceptible to susceptible (MS-S) resistance responses. On the other hand, the remaining 3 accessions 7315-1, 7516-1, and 237886-1 showed above 40% disease severities, with MS or MS-S field responses (Table 3.1). The few resistant lines identified could serve as potential sources of new resistance for introgression into currently grown susceptible cultivars. Among the 25 wheat genotypes which displayed disease severities between 31 to 50% during main season, only six: 227059-1, 203763-1, 226275-1, 227068-2, 226278-1 and 7994-1, showed disease severities between 31 to 50% during the off-season of 2013. In the remaining 19 lines the final disease severities observed were more than 50%. Cultivar Morocco developed disease severity of 85% during the season.

Figure 3.1. Wheat stem rust epidemics at Debre-Zeit Agricultural Research Center, Ethiopia, during the 2013 off-season

Although disease pressure during the off-season was higher compared to the main season, some genotypes such as 7041-1, 7579-1, 227059-1, 203763-1, 227068-1, 226275-1, 227068-2 and 7994-1 showed lower disease severities during the off-season than during the main season. Since these lines were surrounded by susceptible lines during the main season, the conditions for spread of the disease would have been more favorable and the disease severities observed for these genotypes would have been higher during the main season.

Table 3.1. Seedling reaction, adult plant infection type, CI and final rust sverity in wheat lines to stem rust at the Debre-Zeit Agriculture Research Center, Ethiopia

Lines	Seedling reaction	Main season (2012)		Off-season (2013)	
		FRS	CI	FRS	CI
H04-2	2+3-[a]	1 MR-MS[b]	0.6	2 MR-MS	1.2
204408-3	2+ 3-	1 MR-MS	0.6	5 MS	4.0
231545-1	3	3.5 MS	2.8	10 MS	4.0
214551-1	2+ 3-	5 MR-MS	2..0	20 MS	16.0
7315-1	3-	19 MS	15.2	60 MS	48.0
7514-1	2+ 3-	21 MS	16.8	30 MS	24.0
7041-1	3-	24 MS	19.2	20 MS	16.0
7516-1	3-	24.5 MS-S	22.1	40 MSS	36.0
226385-1	2+ 3-	25.2 MS-S	22.7	30 MSS	27.0
226815-1	3-	28.2 MS	22.6	30 MS	24.0
7579-1	3-	30 MS-S	27.0	20 MSS	18.0
222495-1	2+ 3-	30 MS-S	27.0	30 MSS	27.0
237886-1	2+3-	30 MS-S	27.0	42 MSS	33.6
204408-2	3-	31 MS	24.8	60 MS	48.0
7312-1	2+ 3-	31.5 MS	25.2	70 MS	56.0
226899-1	3-	32 MS	25.6	70 S	70.0
204408-1	3	34 MS	27.2	70 MS	56.0
214520-1	3-	34.6 MS	27.7	60 MS	48.0
203881-2	3-	37 MS-S	33.3	60 MSS	54.0
227067-1	3-	40 MS	32.0	70 MS	56.0
226925-1	3-	40.2 MS	32.2	70 S	70.0
227059-1	3-	40.5 MS	32.4	40 MS	32.0
230084-1	3-	42 MS	33.6	70 MS	56.0
203763-1	3+	42 MS-S	37.8	40 MSS	36.0
227068-1	2+ 3-	42.5 MS	34.0	20 S	20.0
7489	2+ 3-	43 MS	34.4	60 MS	48.0
7491-1	3-	43 MS-S	38.7	60 MSS	54.0
5397-1	3-	45 MS	36.0	70 MS	56.0
7312-1	3-	46 MS	36.8	60 MSS	54.0
7502-1	3-	47 MS	37.6	70 MS	56.0
226275-1	3-	49 MS	39.2	40 S	32.0
227068-2	3-	49 MS	39.2	40 MS	32.0
203881-1	3-	49 MS	39.2	70 S	70.0
226278-1	3-	50 MS	40.0	50 MS	40.0
7994-1	3-	50 MS	40.0	40 MS	32.0
7487-1	3-	50 MS	40.0	70 MS	56.0
226278-2	3-	50 MS	40.0	60 S	60.0
7847-1	3- 2+	50 MS	40.0	60 MS	48.0
Morocco	3+	70 S	70.0	85 S	85.0

[a] Segregating for reaction, the commonest infection type is placed first.

[b] MR = moderately resistant, MS = moderately susceptible, MR-MS = moderately resistant to moderately susceptible, MS-S = moderately susceptible to susceptible and S= susceptible; FRS = final rust severity; CI = coefficient of infection

3.3.2.2 Coefficient of infection (CI)

The data on disease severity and host reaction was combined to calculate a coefficient of infection (CI). In the present study, during the main season of 2012, seven wheat genotypes: H04-2, 204408-3, 231545-1, 214551-1, 7315-1, 7514-1 and 7041-1, showed CI values between 0-20. Thirty one lines had CI values of 21-40 (Table 3.1). The remaining 53 wheat genotypes had CI values above 40, designated as having low levels of slow rusting (data not shown). In the main season only the susceptible check had a CI value of more than 60.

Table 3.1 shows that disease pressure was extremely high during the off-season as indicated by the CI of 85 of the susceptible check. However, seven wheat lines (H04-2, 204408-3, 231545-1, 214551-1, 7041-1, 7579-1 and 227068-1) responded with the CI values of less than 20 during the season and were therefore designated as having high level of slow rusting. Twelve wheat lines displayed CI value between 21 and 40 in the season while 19 genotypes had CI values more than 40.

3.3.2.3 Area under disease progress curve (AUDPC) and apparent infection rate (inf-rate)

During the main season, the highest AUDPC (1050) and inf-rate (0.160) were generated by the susceptible check variety, Morocco. Similarly, Morocco had the highest AUDPC value (1150) and inf-rate (0.229) during the 2013 off-season. In contrast, Lines H04-2, 204408-3, 214551-1, and 231545-1 had the lowest AUDPC and inf-rates in both seasons.

Among the 91 lines tested during the main season, nine accessions (H04-2, 204408-3, 214551-1, 231545-1, 7041-1, 237886-1, 226385-1, 7315-1 and 7514-1), showed rAUDPC values up to 30% of the check (Table 3.2). These lines showed MR-MS to MS-S types of infection in the field and were considered to have good levels of partial resistance. Twenty nine genotypes exhibited rAUDPC values up to 70% of Morocco while the remaining 53 had rAUDPC greater than 70%. Data for wheat lines showing AUDPC values up to 70% of the check are presented in Table 3.2.

During the 2013 off-season, 10 wheat genotypes: H04-2, 204408-3, 214551-1, 231545-1, 7041-1, 226385-1, 7514-1, 227068-1, 203763-1 and 7579-1, exhibited rAUDPC values less than 30% of Morocco. These lines had variable responses: MR-MS to S in the field evaluation. On the other hand, 28 lines showed relative AUDPC values up to 70% of the susceptible check with MS to S field responses. Despite high infection types (MS and S) exhibited on moderately slow rusting lines, stem rust developed slowly as indicated by their AUDPC values. Seven lines: H04-2, 204408-3, 214551-1, 231545-1, 7041-1, 226385-1 and 7514-1, showed low levels of disease severity (1-30%) with lower rAUDPC values (1-30%) in both seasons and these lines are considered as the best slow rusting lines under test (Table 3.2).

Infection rates of all genotypes were less than Morocco in both seasons (data not shown). Most of the wheat lines were observed to have lower apparent infection rates during the 2012 cropping season than in the 2013 off-season. The highest mean inf-rate value of 0.164 was recorded for Line 226899-1 followed by Line 7487-1 (Inf-rate=0.161) during the off-season. Lines H04-2 and 204408-3 showed a constant disease severity, thus showing no increase per unit time with an inf-rate of 0 in both seasons (Table 3.2).

Table 3.2. Area under disease progress curve (AUDPC), relative area under disease progress curve (rAUDPC) and infection rate (Inf-rate) of wheat lines when infected by stem rust.

	Main season (2012)			Off-season (2013)		
Lines	AUDPC	r-audpc	Inf-rate	AUDPC	r-audpc	Inf-rate
H04-2	1.0	0.10	0	7.0	0.61	0
204408-3	26.6	2.53	0	37.5	3.26	0
214551-1	39.0	3.71	0	84.0	7.30	0.063
231545-1	59.1	5.63	0.001	57.5	5.00	0.002
7041-1	168.0	16.00	0.056	132.0	11.48	0.060
237886-1	282.5	26.90	0.020	355.5	30.91	0.027
226385-1	299.1	28.49	0.038	300.0	26.09	0.003
7315-1	302.0	28.76	0.037	371.4	32.30	0.072
7514-1	304.6	29.01	0.092	329.4	28.64	0.034
7516-1	325.8	31.02	0.115	349.0	30.35	0.142
227068-1	353.0	33.62	0.081	100.0	8.70	0.030
222495-1	355.4	33.85	0.044	367.4	31.95	0.047
203881-2	359.0	34.19	0.065	400.0	34.78	0.097
203763-1	365.0	34.76	0.087	255.0	22.17	0.087
226815-1	370.6	35.30	0.078	375.0	32.61	0.056
7579-1	379.5	36.14	0.035	120.0	10.43	0.004
226899-1	388.6	37.01	0.090	499.6	43.44	0.164
204408-2	412.6	39.30	0.083	600.0	52.17	0.084
7312-1	441.4	42.04	0.051	764.0	66.43	0.103
204408-1	456.0	43.43	0.033	758.0	65.91	0.096
226278-2	476.0	45.33	0.057	490.0	42.61	0.095
227068-2	503.0	47.90	0.088	470.0	40.87	0.020
214520-1	507.9	48.37	0.090	538.0	46.78	0.084
203881-1	516.0	49.14	0.073	580.0	50.43	0.127
226275-1	518.0	49.33	0.075	444.0	38.61	0.069
226925-1	556.2	52.97	0.102	700.0	60.87	0.100
227059-1	568.0	54.10	0.084	385.2	33.50	0.082
226278-1	570.0	54.29	0.123	560.0	48.70	0.095
7491-1	580.4	55.28	0.087	584.6	50.83	0.094
7312-1	586.0	55.81	0.060	557.5	48.48	0.037
7489	598.0	56.95	0.036	532.0	46.26	0.054
230084-1	602.0	57.33	0.117	770.6	67.01	0.138
7847-1	603.0	57.43	0.061	480.0	41.74	0.118
227067-1	612.8	58.36	0.106	624.6	54.31	0.109
5397-1	622.8	59.31	0.092	728.0	65.30	0.096
7502-1	656.9	62.56	0.091	762.2	66.28	0.101
7994-1	686.5	65.38	0.073	500.0	43.48	0.055
7487-1	714.6	68.06	0.114	790.0	68.75	0.161
Morocco	1050.0	100.00	0.160	1150.0	100.00	0.229

[a] AUDPC = area under disease progress curve; r-audpc = relative area under disease progress curve; Inf-rate = infection rate

3.3.2.4 Correlation between slow rusting parameters of wheat stem rust

The correlations among the field based slow rusting parameters were significant (Table 3.3). A positive and highly significant correlation of CI with final rust severity (r = 0.983) and AUDPC (r = 0.919) was found during the main season. Strong correlation coefficients of 0.985 and 0.925 were also observed between CI with FRS and AUDPC during the off-season, respectively.

The high correlation coefficient was also observed between AUDPC and final rust severity in both seasons; r = 0.923 during the 2012 main season and r = 0.928 during the off-season of 2013. Relatively low correlations were observed during the off-season between infection rate and the other slow rusting parameters (Table 3.3). This indicated that although severity or the AUDPC was increasing, the rate of infection slowed down over time because as the epidemic progressed less plant tissue was available for further infection and the rate of epidemic development reduced (Freedman and Mackenzie, 1992).

Table 3.3 Linear correlation coefficients of pair-wise relationships between slow rusting parameters for stem rust of wheat evaluated over two seasons at Debre-Zeit Agricultural Research Center, Ethiopia

	Main season (2012)[a]			Off-season (2013)		
Parameters	Inf-rate	AUDPC	FRS	Inf-rate	AUDPC	FRS
Inf-rate	-			-		
AUDPC	0.760**	-		0.516**	-	
FRS	0.725**	0.923**	-	0.598**	0.928**	-
CI	0.726**	0.919**	0.983**	0.580**	0.925**	0.985**

[a] FRS = final rust severity; rAUDPC = relative area under disease progress curve; Inf-rate = infection rate; CI - coefficients of infection; **Correlation is significant at p=0.01

Therefore, selection of lines having low rAUDPC values (below 30% of the check), final disease scores of less than 30 MS and CI between 0-20 provides a sound basis for identifying slow rusting resistance, which is one of the durable resistance breeding strategies. Accordingly, wheat lines H04-2, 204408-3, 214551-1, 231545-1 and 7041-1, with highly slow rusting resistance characteristics AUDPC < 30%, CI 0-20 and FRS 0-30% in both seasons were selected for further resistance breeding. Five lines 7514-1, 226385-1, 226815-1, 7579-1 and 222495-1 had FRS score between 20 MS and 30 MS, CI value between 15 and 30 with rAUDPC value ranging from 10 to 35% of the check in both seasons and were regarded slow rusting. Of the above listed partial resistant lines, 231545-1, 7041-1, 226815-1 and 7579-1 showed characteristics of true race non-specific slow rusting resistance as they exhibited low level of slow rusting parameters during both seasons and complete susceptibility (3- to 3) at the seedling stage (Tables 3.1 and 3.2).

The spearman's rank correlation coefficients for slow rusting parameters over the two seasons were highly significant (Table 3.4). A high spearman correlation would mean that the genotypes ranked fairly similar in both seasons, and the effects due to the environment were minimal. The two season data had greater correlations of 0.649, 0.570, 0.835 and 0.642 for FRS, CI, AUDPC and inf-rate, respectively.

Table 3.4 Spearman's rank correlation coefficients of slow rusting and yield variables over two seasons at Debre-Zeit Agricultural Research Center, Ethiopia

Parameters[$]	Inf-rate	AUDPC	FRS	CI	GY	TKW
Correlation coefficients	0.642**	0.835**	0.649**	0.570*	0.702**	0.746**

[$] Inf-rate = infection rate; FRS = final rust severity; AUDPC = area under disease progress curve; CI = coefficients of infection; GY = grain yield; TKW = thousand kernel weight;
**Correlation is significant at p=0.01; *Correlation is significant at p=0.05; n=38

3.3.2.5 Grain yield and thousand kernel weight

There was a highly significant difference (P < 0.001) between entries for grain yield (Table 3.5). From the outset, it should be emphasized that the differences in grain yield among the entries could be explained not only by differences in the levels of disease attack but also in the yield potential of the varieties.

During 2012, the highest grain yield, 4.73 t ha^{-1}, was obtained from Line 7502-1 whereas the lowest, 1.18 t ha^{-1}, was displayed by Line 227068-1. In the off-season, the highest yield was displayed by Line 231545-1 (3.81 t ha^{-1}) while the lowest (1.24

t ha^{-1}) was from Line 226385-1. The disease severity recorded on Line 7502-1 was 47% during the main season but the yield obtained from the line was higher than some lines that had low disease severities, such as H04-2 (1%) and 231545-1 (3.5%). The ranking of the genotypes for yield changed when exposed to higher disease pressure during 2013 off-season. For example, Line 7489 had greater yield (3.52 t. ha^{-1}) as compared to Line 7491-1 (3.23 t ha^{-1}) during the main season, under low disease pressure. However, the yield obtained from Line 7491-1 (2.79 t ha^{-1}) was higher than the yield of Line 7489 (2.50 t ha^{-1}) under higher disease pressure during 2013. This can be due to varying levels of tolerance expressed by different lines.

Among the slow rusting lines identified, 231545-1, H04-2 and 222495-1 had the highest yields in both seasons (>3 t ha^{-1}). Their comparatively better yields make them superior candidates as donor parent for the incorporation of durable resistance in the bread wheat improvement program. The yields obtained from some of local wheat lines such as 227068-1, 226385-1 and 7847-1 were below the yield of the susceptible variety Morocco. This would be due to their lower genetic potential for yield.

Although there were variations in grain yields among the entries, there was no protected check plot established for each genotype to obtain information to calculate yield loss. Nevertheless, when the means and ranges obtained from 2012 main season are compared with the mean yields and ranges of 2013 off-season, these statistics were higher for both grain yield and thousand kernel weight (TKW), suggesting that the rust disease reduced the grain yield and TKW as the disease pressure increased in the season.

The wheat lines also showed variation in thousand kernel weight ($p < 0.01$). The highest TKW was recorded from Line H04-2 in the main and off-seasons at 38.5 g and 35.0 g, respectively. The lowest was obtained from 7994-1 (10 g) in the main season and from 7312-1 (9 g) in the off-season. Among the slow rusting genotypes identified 231545-1, H04-2, and 222495-1 had high TKW values in both seasons.

The spearman's rank correlation coefficients for yield variables over the two seasons were highly significant (Table 3.4). Grain yield and TKW had correlations of 0.702** and 0.746**, respectively.

Table 3.5 Grain yield and thousand kernel weights (TKW) of 38 wheat lines

Lines	Main season (2012)		Off-season (2013)	
	Grain yield (t ha^{-1})[a]	TKW (g)	Grain yield (t ha^{-1})	TKW (g)
H04-2	3.30efgh	38.5a	3.31b	35.0a
204408-3	2.75ijkl	31.0bcd	2.75c	26.0cde
231545-1	3.82bcd	30.0cd	3.81a	31.0b
214551-1	2.64klmno	23.0ghijk	2.43de	23.0efg
7315-1	4.06b	22.0hijk	2.05ghij	16.0ijkl
7514-1	2.38lmnop	22.0hijk	1.55mnop	18.0hij
7041-1	2.34lmnop	25.0efghi	2.43de	25.0def
7516-1	4.03bc	22.0hijk	3.54b	12.0mno
226385-1	1.31tu	28.0cdef	1.24q	27.0cd
226815-1	2.21opq	28.5cde	2.18fghi	22.0fg
7579-1	1.61rstu	26.5defgh	1.95ijk	28.0bcd
222495-1	3.13fghi	31.0bcd	3.44b	28.0bcd
237886-1	2.47klmno	28.0cdef	2.45de	19.5ghi
204408-2	2.30lmnop	23.0ghijk	2.03hij	18.0hij
7312-1	3.41defg	32.0bc	2.35def	22.0fg
226899-1	3.03ghij	28.0cdef	2.02hij	27.5bcd
204408-1	2.95hij	27.0defg	2.23efgh	18.0hij
214520-1	2.20opq	27.5cdefg	1.51nop	14.0klm
203881-2	2.69ijklmn	28.5cde	2.30def	17.0hijk
227067-1	4.70a	22.0hijk	2.39def	16.0ijkl
226925-1	3.62bcde	25.0efghi	2.02hij	23.0efg
227059-1	2.33lmnop	23.5fghij	2.28defg	18.0hij
230084-1	2.29mnop	14.5opq	1.46opq	10.0no
203763-1	1.70rst	20.0jklm	1.82jkl	16.0ijkl
227068-1	1.18u	14.0opq	1.43opq	20.5gh
7489	3.52def	17.0lmnop	2.50d	15.0jklm
7491-1	3.23efgh	18.5klmno	2.79c	14.0klm
5397-1	2.27nopq	18.5klmno	1.50nop	12.0mno
7312-1	3.58cdef	16.5mnop	1.95ijk	9.0o
7502-1	4.73a	14.0opq	3.38b	10.0no
226275-1	2.86hijk	20.0jklm	2.92c	23.0efg
227068-2	1.82qrs	17.0lmnop	1.84jkl	17.0hijk
203881-1	2.73ijklm	21.5ijkl	1.63lmno	13.0lmn
226278-1	1.81qrs	15.0nop	1.61lmnop	20.0gh
7994-1	1.94pqr	10.0q	2.37def	15.0jklm
7487-1	2.68jklmno	22.0hijk	1.75klm	17.0hijk
226278-2	1.94pqr	19.5jklmn	1.42opq	15.0jklm
7847-1	1.37stu	12.5pq	1.37pq	12.0mno
226899-1	3.03ghij	28.0cdef	2.02hij	27.5bcd
Morocco	2.00pqr	35.0ab	1.72klmn	29.0bc
CV (%)	8.38	10.47	5.32	9.54
$LSD_{.01}$	4.56	4.88	2.35	3.72

[a] Means within a column followed by the same letter are not significantly different at p=0.01.; TKW= thousand kernels weights

3.4 Discussion

Field assessment for slow rusting resistance was carried out using 91 wheat lines exhibiting intermediate and susceptible reactions at seedling stage. Wheat lines that exhibit intermediate (2+) and/or susceptible (>3-) infection types may possess race non-specific resistance (Parlevliet, 1988; Sawhney, 1995) and these lines may provide durable resistance when their field assessment results confirm their slow rusting character. Hence, candidates for source of slow rusting resistance were those lines that exhibited susceptible and mixed (2+ and 3-) reaction types.

Slow rusting resistance was assessed through the infection type, final rust severity (FRS), coefficient of infection (CI) and relative area under rust progress curve (rAUDPC). In many cereal rust pathosystems, slow rusting characteristics of cultivars have been described and estimated by means of disease severity at a certain crop development stage, the area under disease progress curve or the measurement of the apparent infection rates and coefficients of infection values (Broers et al., 1996; Pathan and Park, 2006).

The present study found considerable variation in the final rust severities of the accessions tested that could be attributed to differences in the number of resistance genes present and mode of gene action. Safavi (2012) proposed that wheat lines with FRS values of 1-30%, 31-50% and 51-70% were regarded as possessing high, moderate, and low levels of slow rusting resistance, respectively. Lines with a low final disease severity under high disease pressure may possess more additive genes (Singh et al., 2005). FRS represents the cumulative result of all resistance factors during the progress of epidemics. Many earlier researchers such as Ali et al. (2009), Shah et al. (2010), Tabassum (2011) and Safavi and Afshari (2012) also used final severity as a parameter to assess slow rusting behaviour of wheat lines. Previously Ali et al. (2009) considered that lines with CI values of 0-20, 21-40, 41-60 could possess high, moderate and low levels of slow rusting resistance, respectively. Based on the AUDPC values, Ali et al. (2009) categorised the wheat lines into two distinct groups. One group included lines exhibiting AUDPC values up to 30% of the check and the second group included lines showing AUDPC values up to 70% of the check. The genotypes in group I were regarded as expressing good levels of slow rusting, and that of group II were expressing moderate slow rusting resistance. According to Parlevliet (1988) wheat lines with variable field infection responses of MR-MS to S are expected to possess genes that confer partial resistance.

Infection rate in the present study showed more variation among the tested lines than disease severity and AUDPC, and it did not distinguish lines displaying different level of slow rusting resistance with regard to other parameters. For example, Line 7041-1 has FRS, CI, and rAUDPC less than Line 226385-1 but its infection rate is higher in both seasons. Similar results were found for stem rust and leaf rust of wheat (Ali et al*.,* 2009; Safavi, 2012; Safavi and Afshari, 2012).

This study established the presence of strong correlation between slow rusting parameters (Table 3.3). The positive correlations between the parameters observed were in agreement with the results of other researchers on cereal rust pathosystems (Safavi, 2012; Shah et al. 2010). All disease parameters were highly correlated in the present study, suggesting that FRS and CI are considered as preferable selection parameters. Qamar et al. (2007) and Safavi et al. (2013) reported higher selection gains of slow rusting reistance using low final ratings and CI under field condition. Further, the present study found high Spearman's rank correlations for infection parameters and yield variables suggesting that the ranking of the wheat lines for these variables did not change significantly over the seasons (Table 3.4).

Wheat lines H04-2, 204408-3, 214551-1, 231545-1, 7041-1, 226385-1, 7514-1, 226815-1, 7579-1 and 222495-1 had high slow rusting resistance with low levels of disease severity (1-30%) while Lines 237886-1, 227059-1, 203763-1, 226275-1, 227068-2, 226278-1 and 7994-1 had moderate levels of slow rusting resistance with FRS of 30-50% and CI values ranging from 21-40. According to Singh et al. (2004) genotypes in both group I and II could have durable resistance which can serve as good parents for breeding. Hence, lines in both groups could be utilized in wheat improvement programs.

Both grain yield and kernel weight of the tested lines were significantly affected during 2013 off-season. The difference in yields between the two seasons could be attributed to either difference in environmental conditions or due to differences in stem rust infection. Environmental conditions such as temperature and moisture considerably affect disease expressions and consequently of yield. Several researchers have reported stem rust reducing grain yields of wheat cultivars (Pretorius et al., 2007; Singh et al., 2008). The effect of rust on grain yield is due to the great injury to the photosynthetic surface of the plant (Berghaus and Reisener, 1985) and the energy expenditure in plant defence mechanisms rather than for growth and grain formation (Smedegaard-Petersen and Tolstrup, 1985). According to Craigie (1957) and Bushnell and Rowell (1968) the fungus also reduces the food and water supply within the plants. The fungus needs food and water for spore production that would otherwise be used in the formation of well developed kernels. Further, there is a loss of water by evaporation through the numerous ruptures caused by the fungal pustules. The yield from heavily rusted plants is, therefore, much reduced and the quality of the grain is lowered.

It is worth noting that stem rust caused kernel weight reduction in the genotypes (Table 3.5). Nzuve et al. (2012) also reported that stem rust significantly reduce TKW in wheat. It is well-established that the significant effect of stem rust on TKW is brought about by its effect on photosynthesis and subsequent grain filling. According to Agrios (1987) the competition of rust fungi for photosynthate at grain filling would have increased importance in reduction of number and size of seeds on plants.

3.5 Conclusions

In this study, the Lines H04-2, 204408-3, 214551-1, 231545-1, 7041-1, 7514-1, 226385-1, 226815-1, 7579-1, and 222495-1 exhibited lower levels of FRS (< 30 MS-S) and coefficient of infection (< 27), indicating a high level of partial resistance. Of these 231545-1, 7041-1, 226815-1 and 7579-1 were identified to have true slow rusting resistance because they had seedling reactions that ranged from 3- to 3. Seven lines: 237886-1, 227059-1, 203763-1, 226275-1, 227068-2, 226278-1 and 7994-1, expressed moderate level of slow rusting resistance in both seasons while the remaining 21 lines were susceptible. The correlations among the field based slow rusting parameters were highly significant. Among the slow rusting lines comparatively better TKW and grain yields were produced by 231545-1, H04-2 and

222495-1. The slow rusting lines identified from this study can be used for durable stem rust resistance breeding.

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CHAPTER 4

Heterosis and combining ability analysis of stem rust resistance and grain yield and related traits in bread wheat

Abstract

Six selected bread wheat (*Triticum aestivum* L.) genotypes were crossed in a half diallel mating design to identify the best parents and crosses for breeding for stem rust reistance, high grain yield and desirable agronomic traits. The 15 F1's and their parents were evaluated under field inoculation of the stem rust pathogen using a randomized complete block design with two replications at the Debre-Zeit Agricultural Research Center of Ethiopia during the 2014 off-season. The results indicated that sufficient genetic variability was observed among tested genotypes for all characters studied. Grain yield revealed maximum heterosis over the mid-parent (31.45%) followed by thousand kernel weight (28.85%) and tillers per plant (15.40%). The maximum negative heterosis values of -11.01% and -8.02% were observed for plant height and days to maturity, respectively, which were in the desired direction. The majority of the crosses expressed negative heterosis over the mid-parent for area under disease progress curve (AUDPC), indicating these crosses manifested tolerance against stem rust. The maximum better-parent heterosis was recorded for grain yield (25.38%). The significance of general combining ability (GCA) effects for all characters and specific combining ability (SCA) effects for most of the traits indicated that the contribution of additive and non-additive genes to the genetic factors controlling these traits, respectively. However, the ratio of σ^2gca/σ^2sca were less than unity for grain yield, thousand kernel weight and plant height, suggesting the preponderance of non-additive gene action at the F1 for these traits. Additive gene action was predominant in the inheritance of AUDPC, kernels per spike, number of tillers per plant and days to maturity. Wheat genotypes 231545-1, 7041-1, H04-2 and Danda'a had significantly negative GCA effects for AUDPC, suggesting their suitability for use in wheat breeding programs to improve resistance to stem rust. Lines H04-2, Digelu and Danda'a showed good GCA effects for most of the characters investigated. Kubsa was good general combiner for kernels per spike, tillers per plant and plant height. Crosses 231545-1 x H04-2, 7041-1 x H04-2, Digelu x Kubsa and Danda'a x Kubsa were good combinations for AUDPC. H04-2 x Danda'a, Digelu x Kubsa and Danda'a x Kubsa were good combinations for grain yield and thousand kernel weight. Crosses Digelu x Kubsa and H04-2 x Danda'a also showed significant SCA effects for tillers per plant. The maximum negative SCA effects for plant height and days to maturity were exhibited by the crosses Digelu x Kubsa and 7041-1 x H04-2, respectively. Overall, H04-2 and Danda'a were good general combiners, and crosses involved these lines performed well for most of the traits. Hence, lines H04-2 and Danda'a may be used in breeding programs to develop stem rust resistant and high yielding wheat cultivars.

Key words: diallel analysis, heterosis, general combining ability, specific combining ability, wheat

4.1 Introduction

Wheat (*Triticum aestivum* L.) is an important food grain for billions of people worldwide, being cultivated on approximately 218 million hectares of land (HGCA, 2014). It accounts for around 30% of global grain production and 44% of cereals used as food (Khanzada et al., 2012). Ethiopia is the second largest wheat producer in sub-Saharan Africa, after South Africa (GAIN, 2012). In Ethiopia wheat is cultivated on 1.6 million hectares of land, with a mean yield of 2.1 t ha^{-1}, far less than potential yields of 8-10 t ha^{-1}, (CSA, 2013). The low productivity is partially attributed to the prevalence of wheat rust diseases and lack of durable resistant variety. Stem rust caused by the fungi *Puccinia graminis* Pers. f.sp. *tritici* Eriks and Hann (designated as *pgt* here after), is the major production constraint in most wheat growing areas of the country (Denbel et al., 2013). It is the most devastating type of rust, which causes yield losses up to 100% on susceptible cultivars (Park, 2007; Hodson, 2013). Various control options are available to minimize yield losses caused by stem rust, including the use of resistance cultivars. Breeding for stem rust resistance requires knowledge on the genetics of resistance in wheat genotypes.

The major emphasis in wheat breeding is on the development of disease resistant and high yielding varieties. This requires information on combining ability of parents available for use in a hybridization program, and also the nature of gene action involved in the expression of desirable traits in their progenies. In a breeding program, selection of parents showing good general combining ability (GCA) effects and their progenies with high specific combining ability (SCA) effects for desirable traits are essential (Desale et al., 2014). The GCA and SCA estimates will help in formulating efficient and effective breeding procedure to bring about rapid improvement in a crop (Desale et al., 2014).

Estimation of heterosis is an important aspect in hybrid breeding programs (Seboka et al., 2011). Heterosis breeding provides a way to overcome the yield barriers. It has been largely used in cross-pollinated crops. In self-pollinated crops there is some evidence reflecting the value of breeding for increased heterosis such as in wheat (Haq and Laila, 1991). Several studies also indicated the presence of considerable heterosis in bread wheat cultivars of diverse genetic bases. Rasul et al.

(2002) reported maximum heterosis over the mid-parent at 31.65% for grain yield. Akbar et al. (2010) reported heterosis values of 29.70% for grain yield per plant, 26.17% for thousand kernel weight and 12.95% for tillers per plant. Zaazaa et al. (2012) found heterosis over mid- and better-parents for grain yield ranging from 28.15 to 50.14% and -5.38 to 42.07%, respectively. Effective use of heterosis largely depends on its direction and magnitude (Kumar et al., 2011; Zaazaa et al., 2012). Two types of heterosis are distinguished: mid-parent or better-parent heterosis. The mid-parent heterosis is an increase in a given character of the hybrid compared to the mean of the parents. Better-parent heterosis is an increase in the character of the hybrid compared to that of the better-parent for the character (Falconer and Mackay, 1996).

Several researchers (Griffing, 1956; Hayman, 1954; Mather and Jinks, 1982) have developed techniques to analyse for the GCA effects of parents and the SCA effects of their crosses. Combining ability analysis through diallel crosses were developed by Griffing (1956), an approach that has been widely used to study the ability of parents to transfer their desirable traits to their progenies and to compare the performance of lines in hybrid combinations. Diallel analysis helps to identify the gene actions that control different traits and combinations of different genotypes with respect to their general and specific combining ability effects (Zeeshan et al., 2013).

Information on GCA and SCA effects for the desired traits among the wheat genotypes is important to identify the best combiners for successful wheat hybridization. General combining ability is defined as the mean performance of a line in hybrid combinations. Specific combining ability is referred to as the deviation in performance of a particular cross from its theoretical performance predicted on the basis of general combining ability (Schlegel, 2010). General combining ability is attributed to additive gene effects while specific combining ability is attributed to non-additive gene actions (Nazim-Uddin et al., 2009). During pure line or pedigree breeding of self-fertilizing crops such as wheat, individual plant selection commences at the F_2 followed by line selection at the F_3 and later generations to exploit the additive genetic effects. In wheat continued selfing and selection after crosses will enssure success in isolating superior genotypes. Selection is less effective in isolating and fixing superior genotypes if there are dominance and epistasis genetic effects. The dominance gene action would favour the production of hybrids; additive gene action would indicate that standard selection procedures would be effective (Girma, 2006).

The present study was undertaken: i) to quantify the magnitude and direction of heterosis in wheat hybrids for stem rust resistance and yield and yield components; ii) to identify the best combining parents and their hybrids on the basis of their general and specific combining ability effects, respectively, for stem rust resistance, and yield and yield related traits.

4.2 Materials and methods

4.2.1. Plant materials

The experimental materials consisted of 21 wheat genotypes, which comprised of six parents and their 15 F1's obtained from a 6 x 6 half diallel. Table 4.1 presents details of the selected parents. Three parents, 231545-1, 7041-1 and H04-2, were identified as being slow rusting wheat lines against *Pgt* among local wheat accessions in previous evaluation studies (Chapter 3). The remaining three genotypes, Digelu, Danda'a and Kubsa, originated from the International Wheat and Maize Improvement Center (CIMMYT)/Ethiopia and which were released as high yielding wheat varieties (Table 4.1). Of these Danda'a is an improved variety with slow rusting resistance. All the wheat lines used in the cross are stable and homozygous, descended from controlled selfing and selection. The six wheat genotypes have genetic variability for yield, disease resistance as well as for various yield components.

Table 4.1 Wheat parents used for a half diallel cross

No	Parent	Pedigree	Resistance to stem rust	Farmers'- preferred agronomic features
1	231545-1	Local	Resistant	Adaptable to environment
2	7041-1	Local	Resistant	"
3	H04-2	Local	Resistant	"
4	HAR 3116 (Digelu)	SHA7/KAUZ	Susceptible	High yield, white seed
5	Danphe #1 (Danda'a)	Kiritati//2*PBW65/2*Seri.1B	Moderately Resistant	High grain yield, high biomass, long spike, white seed, high bread making quality
6	HAR 1685 (Kubsa)	ND G9144//KAL/BB/3 /YACO''S''/4VEE#5''S''	Susceptible	High grain yield, high biomass, white seed

4.2.2 Crosses and mating design

The six parental lines (Table 4.1) were grown and crossed in a field during the 2013 main season at the Ambo Plant Protection Research Center in Ethiopia using a half-diallel mating design to produce 15 F_1 populations. Parental lines were planted at three different dates with one week intervals inorder to synchronize floral anthesis for crosses.

4.2.3 Experimental design, stem rust inoculation and field management

The 15 F1 populations together with the six parental lines were planted at the Debre-Zeit Agricultural Research Center during the 2014 off-season to evaluate their stem rust reaction, and yield and yield related traits. The Debre-Zeit Research Center is situated at an altitude of 1900 m above sea level. The center receives a mean annual rainfall of 851 mm. The mean annual minimum and maximum temperatures are 8.9^{o}C and 28.3^{o}C, respectively (Denbel et al., 2013).

The lines were planted in plots consisting of double rows of 1 m long with 20 cm row spacing, in a randomized complete blocks arrangement with two replications. The border spacing between plots was 40 cm. Experimental blocks were bordered with two rows of a susceptible check wheat variety, Morocco, as a spreader of stem rust. An artificial stem rust epidemic was created in the field by inoculation of the spreader rows at stem elongation stage by uniformly spraying a spore suspension containing urediospores of *pgt* race TTKSK, using an ultra-low volume sprayer. Urediospores of *Pgt* race TTKSK were obtained from the Ambo Plant Protection Research Center, Ethiopia. Fertilizers and other agronomic practices were applied according to the recommended practices for wheat production in the area.

4.2.4 Disease assessment

Stem rust severity was estimated visually as a proportion of the plant stem affected following a modified Cobb scale (Peterson et al., 1948). Severity was assessed three times at twenty days interval from ten randomly pre-tagged plants of each entry, starting when stem rust levels on Morocco reached 50% severity. The host plant response to infection was also scored using the description of Roelfs et al. (1992) as, R = resistant (flecks and small uredinia), MR = moderately resistant (flecks and small to moderate uredinia), MS = moderately susceptible (moderate to large uredinia), S = susceptible (large uredinia).

Genetic effects controlling stem rust resistance were determined using Area Under Disease Progress Curve (AUDPC) as a measure of stem rust resistance. Estimation of AUDPC was performed for each experimental unit from the rust severity data using the formula of Wilcoxson et al. (1975):

$$AUDPC = \sum_{i=1}^{n} [0.5 (x_i + x_{i+1})] [t_{i+1} - t_i]$$

Where xi = stem rust severity on the i^{th} date, t_i = the time in days after appearance of the disease, and n = number of dates on which stem rust was recorded.

4.2.5. Data collection for yield and yield related traits

Days to maturity (DM) was recorded as the number of days from emergence to when 95% of the plants in a plot were physiologically mature. Plant height (PHT) was measured (in centimetres) from five randomly selected plants in a plot, measuring from the base to the tip of the panicle after flowering. The number of kernels per spike (KPS) was counted from ten randomly selected spikes per genotype. Grain yield (GY) was determined from the two rows of each entry and later converted to tonnes per hectare (t ha^{-1}). Thousand kernel weight (TKW) (in grams) was measured using a randomly sampled thousand kernels adjusted to 12% moisture content.

4.2.6 Data analysis

The data from the F1 crosses and parents were subjected to analysis of variance to determine significant differences between genotypes. Genetic analyses were carried out for characters that showed significant differences among the genotypes using SAS computer software (SAS, 2002).

4.2.7 Estimation of heterosis

The percent increase or decrease of F1 hybrids over mid-parent, as well as better-parent, was calculated to estimate possible heterotic effects using the method of Falconer and Mackay (1996):

MPH= (F1-MP)/MP X 100

Where MPH is mid-parent heterosis; MP is mid-parent value; F1 is the mean value of F1 progenies, MP = (P1+P2)/2 in which P1 and P2 are the means of Parents 1 and 2, respectively

BPH = (F1-BP)/BP x 100

Where, BPH is better-parent heterosis, BP is the better-parent value and F1 is the mean value of F1 progenies. The minimum values were considered as better parent in the case of AUDPC, plant height and days to maturity. Significance of mid- and better-parent heterosis values were tested with t-test as suggested by Wynne et al. (1970).

4.2.8 Estimation of combining ability effects

General combining ability (GCA) and specific combining ability (SCA) effects were estimated according to the Model I, Method II of Griffing (1956) using SAS computer software (SAS, 2002). This model involves parents and one set of F1 hybrids, excluding the reciprocals, providing P(P+1)/2 cross combinations. Data were analysed using the general linear model:

$$Y_{ijk} = \mu + g_i + g_j + s_{ij} + e_{ijk}$$

where Y_{ijk} is the observed measurement for the ij^{th} cross grown in the k^{th} replication or environment; μ is the population mean; g_i, and g_j are the GCA effects; s_{ij} the SCA effect; and e_{ijk} the error term associated with the ij^{th} cross evaluated in the k^{th} replication. The restrictions imposed on the combining ability effects are $\sum g_i = 0$, and $\sum s_{ij} = 0$ for each j (Griffing, 1956).

The ratio of additive versus non-additive gene action in the expression of the character was compared from the ratio of components of GCA variance to SCA variance. When the ratio is greater than unity it indicates the predominance of additive gene action, while a value less than unity indicates the predominance of non-additive gene action for the trait (Singh and Chaudhary, 1985).

4.3 Results and discussion

4.3.1 Analysis of variance and mean performance of genotypes

The analysis of variance revealed highly significant (P<0.01) differences among the 21 genotypes for all traits studied (data not shown), wich indicated the presence of inherent variation among the materials. The mean performance of parents and F1 crosses for stem rust reaction, yield and yield related traits is presented in Table 4.2. Kubsa with an AUDPC value of 582.0 showed the highest susceptible response. H04-2 developed the lowest AUDPC value (1.0) followed by Lines 231545-1 (48.0), 7041-1 (148.0), Danda'a (204.0) and Digelu (287.0) in ascending order. The AUDPC values in crosses involving slow rusting parents 231545-1 and 7041-1 were less than their respective mid-parental values. This indicated the partial dominant nature of AUDPC in these crosses. Different levels of intermediate disease reaction in most of slow ruster x slow ruster, and slow ruster x susceptible crosses, suggested a polygenic mode of inheritance of rust resistance, which is in agreement with reports on the genetic analysis of rust resistance (Ahamed et al., 2004; Irfaq et al., 2009). The AUDPC value of 625.0 in fast ruster x fast ruster was higher than the AUDPC value of 582.0 in its susceptible parent. Among the crosses 231545-1 x H04-2 had the lowest AUDPC value (12.0) (Table 4.2).

In this study, both the crosses and the parents showed high levels of variation in their mean performances for most yield related traits (Table 4.2). Among parental genotypes, Danda'a yielded the highest at 3.9 t ha^{-1} while 7041-1 had the lowest grain yield (2.5 t ha^{-1}). Kubsa grew more tillers per plant and took a longer time to mature. It provided higher mean values in kernels per spike but the lowest thousand kernel weight. Line 7041-1 expressed the lowest mean values for most of the traits recorded (Table 4.2).

Among crosses, H04-2 x Danda'a gave the highest mean grain yield and thousand kernel weight of 4.89 t ha^{-1} and 42.0 g, respectively. This cross also showed moderate to good performances in most of the traits. Most of the hybrids involving Danda'a or Digelu as one parent recorded high mean values for grain yield, thousand kernel weight and kernels per spike. Similarly, H04-2 contributed earliness to maturity in most of its progeny and negatively contributed for plant height. The shortest plant height of 88.5 cm was recorded in cross 7041-1 x H04-2. Most crosses involving H04-2 recorded short plant height and higher productive tillers; while 231545-1 x 7041-1 produced the tallest plant height of 110.0 cm, which is an undesirable trait for wheat improvement. This cross also exhibited poor performance for most of the traits except for AUDPC.

Generally, progenies from crosses involving Danda'a or Digelu performed better in most of the agronomic traits, followed by crosses that involved H04-2. The overall mean of the crosses exceeded that of the parental genotypes for grain yield and TKW. Whereas for kernels per spike, tillers per plant, plant height and days to maturity, the overall mean of crosses were lower. Thus, most F1 hybrids were shorter in plant height and earlier in maturity than parental genotypes. The *per se* performance of parents were better in the kernels per spike and tillers per plant when compared to their F1 hybrid progeny.

Considering individual crosses, all of the 15 hybrids, except crosses 231545-1 x 7041-1 and 231545-1 x Kubsa, performed as well or better than their respective best parents for one to three of the traits studied. Those crosses that had mean values equal or better than the best parents for the traits under study clearly showed the potential to generate good F1 hybrids combining many desirable traits, and would allow selection of superior transgressive segregants at the F_2. In agreement with the present results, a number of studies have reported that at least one hybrid performing better than the best parent for most of the yield and related characters they selected (Joshi et al., 2004; Seboka et al., 2011). Even though crosses exhibited significant differences, none of the hybrids had mean values greater or equal to the best performing parents for number of kernels per spike.

Table 4.2. Mean performance of parents and their hybrids for AUDPC, grain yield and yield related traits

	Traits‡						
Genotypes[a]	AUDPC	GY (t ha^{-1})	TKW (g)	KPS	TPP	PLH (cm)	DM
P1	48.00	3.42	28.00	36.80	17.68	110.50	112.00
P2	148.00	2.50	24.00	27.50	17.64	90.36	115.00
P3	1.00	3.54	39.00	34.25	20.00	96.50	97.00
P4	287.00	3.72	29.00	49.00	18.33	98.80	109.00
P5	204.00	3.90	28.00	49.25	13.50	102.50	109.00
P6	582.00	2.93	24.00	52.82	22.00	99.60	130.00
P1XP2	81.10	2.29	26.50	28.50	12.70	110.00	118.50
P1XP3	12.00	2.99	31.00	36.50	18.00	92.10	99.50
P1XP4	137.50	4.10	33.00	44.00	16.70	98.00	110.00
P1XP5	117.90	3.51	29.00	38.50	16.33	101.65	110.50
P1XP6	291.70	1.99	21.00	36.00	16.67	105.50	126.00
P2XP3	59.00	2.10	31.00	24.50	18.00	88.50	97.50
P2XP4	214.00	3.80	31.50	30.00	16.50	98.04	107.50
P2XP5	96.30	4.07	29.00	33.75	14.70	99.40	111.00
P2XP6	362.00	2.74	26.00	25.00	15.64	100.50	120.50
P3XP4	157.00	3.88	31.00	38.00	20.64	95.02	100.50
P3XP5	197.00	4.89	42.00	42.00	19.33	94.60	99.50
P3XP6	625.00	3.04	29.00	32.00	19.33	91.03	117.50
P4XP5	219.00	4.60	32.50	48.50	16.67	104.75	107.50
P4XP6	625.00	4.10	32.50	50.00	22.33	91.60	122.50
P5XP6	201.00	4.30	33.50	50.00	17.00	101.00	115.00
Parents' Mean	211.67	3.34	28.67	41.60	18.19	99.71	112.00
Crosses' mean	226.37	3.49	30.57	37.15	17.37	98.11	110.90
Grand mean	222.17	3.45	30.02	38.42	17.60	98.57	111.21
R^2	0.96	0.81	0.78	0.92	0.82	0.97	0.96
CV (%)	17.44	14.44	13.55	9.65	9.86	1.49	2.84

[a] See Table 4.1 for codes of genotypes

‡AUDPC = area under disease progress curve, GY= grain yield, TKW = thousand kernel weight, KPS = number of kernels per spike, TPP = number of tillers per plant, PLH = plant height, DM = days to maturity

4.3.2. Magnitude of heterosis

The estimates of heterosis of F1 hybrids over mid- and better-parent were computed for all characters studied as they showed significant differences between genotypes (Table 4.3). In the case of stem rust disease reaction, the majority of the crosses expressed negative heterosis over the mid-parent, of which only 7041-1 x Danda’a and Danda’a x Kubsa revealed significantly negative mid-parent heterosis for the trait, indicating these crosses manifested tolerance against stem rust disease as compared to their parents. The mid-parent heterosis values ranged from -51.02% for 231545-1 x H04-2 to 114.41% for H04-2 x Kubsa. The better- parent heterosis values ranged from -34.93% for 7041-1 x Danda’a to 62400% for H04-2 x Kubsa.

Significant positive mid-parent heterosis values for grain yield were obtained from six of the 15 cross combinations (Table 4.3). H04-2 x Danda’a was the best cross which showed significantly positive heterosis values over mid-and better-parent. Yield heterosis relative to mid- and better-parent ranged from -37.32 (231545-1 x Kubsa) to 31.45% (H04-2 x Danda’a) and -41.81% (231545-1 x Kubsa) to 25.38% (H04-2 x Danda’a), respectively. In this study, the lowest and highest levels of heterosis over better-parent were recorded on the same hybrids that showed similar trend in case of mid-parent heterosis for grain yield. The results indicated that high yielding varieties, Digelu and Danda’a, when involved in the crosses were predominantly responsible for enhancing yield potential. Similar findings were reported by Saini and Prakash (2005), Kumar et al. (2011) and Jain and Sastry (2012). The expression of grain yield heterosis above the mid- and better-parent was reported by several investigators in bread wheat (Sharma et al., 2003; Hussain et al., 2007; Jatoi et al., 2014).

For thousand kernel weight, an important yield component, 60% of the hybrids showed positive heterosis over mid- and better-parent, indicating that majority of the hybrids showed better performance than their respective parents in the desirable direction. Three hybrids Danda’a x Kubsa, H04-2 x Danda’a and Digelu x Kubsa showed significant positive mid-parent heterosis values of 28.85%, 25.37% and 22.64% for the trait, respectively. In the present study the best mid-parent heterosis was observed in grain yield followed by thousand kernel weight. These results were in agreement with those of Singh et al. (2004), Inamullah et al. (2006), Ilker et al. (2010) and Seboka et al. (2011), who all reported a considerable degree of positive heterosis over mid- and better-parent for thousand kernel weight.

Number of kernels per spike is an essential component of grain yield in wheat. Spikes with more seeds contribute to higher grain yield of the crop. However, in the present study, most of the hybrids, except three, showed negative heterosis over mid-parent for the trait. All hybrids showed negative heterosis over better-parent. The cross 231545-1 x H04-2 recorded the highest mid-parent heterosis value of 2.74% while the lowest heterosis of -37.75% was found in 7041-1 x Kubsa. Results of the study conducted by Baric et al., (2004) and Ullah et al. (2011) are in agreement with the present results. Contrary to these findings, Inamullah et al. (2006), Ilker et al. (2010), Khan and Ali (2011), Seboka et al. (2011) and Jatoi et al. (2014) observed positive mid- and better-parent heterosis for number of kernels per spike in wheat.

The number of tillers per plant is another important yield component. Plants with more tillers contribute positively to grain yield per plant and its positive heterosis is useful in a wheat breeding program. However, in this study, 11 and 13 crosses exhibited negative mid- and better-parent heterosis, respectively. Significant positive mid-parent heterosis was obtained only from H04-2 x Danda'a (15.40%), while none exhibited significant better-parent heterosis for this trait. These indicated that most crosses failed to produce more tillers than their respective parents with higher tillers. This is in agreement with Farooq and Khaliq (2004), Inamullah et al. (2006) and Seboka et al. (2011) who reported negative heterosis in most of the crosses for the number of productive tillers per plant. Rasul et al. (2002) also reported negative estimates for hetrosis for number of tillers per plant in all the crosses they studied. Conversely, Hussian et al. (2004), and Khan and Ali (2011) reported many hybrids that had positive mid- and better-parent heterosis. Akbar et al. (2010) also reported positive mid-parent heterosis in all the crosses they studied.

Plant height is important yield trait, directly affecting yield. Tall varieties have low harvest indices and low grain yields (Ahmad et al. 2013). The negative estimates of mid- and better-parent heterosis for plant height are preferred in varietal development, because short plant height is desirable for high harvest index, resistance to lodging and more responsive to fertilizer (Hammad et al. 2013). In this study 6 and 7 crosses showed positive heterosis over mid- and better-parent for plant height, respectively (Table 4.3). The remaining 9 and 8 crosses exhibited negative heterosis over the mid- and better-parent, respectively, indicating reduced plant height in these crosses. Of these, 8 and 3 crosses showed significantly negative mid- and better-parent heterosis for plant height. The cross 231545-1 x 7041-1 had the highest mid- and better-parent heterosis of 9.53 and 21.74%, respectively, while 231545-1 x H04-2 and Digelu x Kubsa exhibited the lowest mid- and better-parent heterosis of -11.01 and -7.29%, respectively. These results were in agreement with earlier research findings in which negative mid- and better-parent heterosis for plant height was reported in most of the hybrids (Akbar et al., 2010; Khan and Ali, 2011). The result showed that heterotic interaction improves genetic diversity and provides ample chances to select for the desired genetic combinations (Inamullah et al., 2006; Khan and Ali, 2011). Contrary to these results, Singh et al. (2004) and Seboka et al. (2011) reported positive heterosis for plant height, which conflicted with the general breeding objective of reducing the straw length of wheat.

Early maturing genotypes are desirable for breeding (Hammad et al. 2013). In the present study 10 and 2 crosses showed negative mid- and better-parent heterosis for days to maturity, respectively. This suggested that heterosis resulted in early maturity. Crosses 7041-1 x H04-2 (-8.02%), 231545-1 x H04-2 (-4.78%), 7041-1 x Digelu (-4.02%) and Danda'a x Kubsa (-3.77%) exhibited significant negative mid-parent heterosis for this trait. This indicated that these hybrids were earlier than their respective late parents. Akbar et al. (2010) also observed that heterosis studies could be effectively used for incorporating early maturity in wheat. This result was also in agreement with Inamullah et al. (2006), Akbar et al. (2010) and Seboka et al. (2011) who reported negative mid-parent heterosis for maturity in some crosses they studied.

Table 4.3. Percentage heterosis over the mid-parent (MPH) and better-parent (BPH) for AUDPC of stem rust, yield and yield related traits in 6 x 6 half diallel cross of bread wheat at Debre-Zeit Agricultural Research Center during 2014.

Cross[a]	AUDPC[b]		GY (t ha^{-1})		TKW (g/1000 seed)		KPS		TPP		PLH (cm)		DM	
	MPH[c]	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH
P1XP2	-17.24	68.96	-22.64*	-33.04**	1.92	-5.36	-11.35	-22.55**	-28.09**	-28.17**	9.53**	21.74**	4.41*	5.80**
P1XP3	-51.02	1100.00	-14.08	-15.54	-7.46	-20.51*	2.74	-0.82	-4.46	-10.00	-11.01**	-4.56**	-4.78*	2.58
P1XP4	-17.91	186.46**	14.85	10.22	15.79	13.79	2.56	-10.20	-7.25	-8.89	-6.35**	-0.81	-0.45	0.92
P1XP5	-6.43	145.63*	-4.10	-10.00	3.57	3.57	-10.52	-21.83**	4.75	-7.64	-4.55**	-0.83	0.00	1.38
P1XP6	-7.40	507.71**	-37.32**	-41.81**	-19.23	-25.00*	-19.66**	-31.84**	-15.98**	-24.23**	0.43	5.92**	4.13*	12.50**
P2XP3	-20.81	5800.00*	-30.46**	-40.68**	-1.59	-20.51*	-20.65*	-28.47**	-4.36	-10.00	-5.28**	-2.06	-8.02**	0.52
P2XP4	-1.61	44.59**	22.19**	2.15	18.87	8.62	-21.57**	-38.78**	-8.26	-9.98	3.66**	8.50**	-4.02*	-1.38
P2XP5	-45.28**	-34.93	27.19**	4.36	11.54	3.57	-12.05**	-31.47**	-5.59	-16.67*	3.08**	10.00**	-0.89	1.83
P2XP6	-0.82	144.59**	0.92	-6.48	8.33	8.33	-37.75**	-52.67**	-21.09**	-28.91**	5.81**	11.22**	-1.63	4.78*
P3XP4	9.03	15600.00**	6.89	4.30	-8.82	-20.51*	-8.71	-22.45**	7.70	3.20	-2.69**	-1.53	-2.43	3.61
P3XP5	92.20*	19600.00**	31.45**	25.38*	25.37**	7.69	0.60	-14.72*	15.40*	-3.35	-4.92**	-1.97	-3.40	2.58
P3XP6	114.41**	62400.00**	-6.03	-14.12	-7.94	-25.64**	-26.50**	-39.42**	-7.95	-12.14*	-7.16**	-5.67**	3.52	21.13**
P4XP5	-10.79	7.35	20.73*	17.95	14.04	12.07	-1.27	-1.52	4.74	-9.06	4.07**	6.02**	-1.38	-1.38
P4XP6	43.84**	117.77**	23.31*	10.22	22.64*	12.07	-1.79	-5.34	10.74	1.50	-7.66**	-7.29**	2.51	12.39**
P5XP6	-48.85**	-1.47	25.92**	10.26	28.85**	19.64	-2.03	-5.34	-4.23	-22.73**	-0.05	1.41	-3.77*	5.50*

[a] See Table 4.1 for codes of genotypes

[b]AUDPC = area under disease progress curve, GY= grain yield, TKW = thousand kernel weight, KPS = number of kernels per spike, TPP = number of tillers per plant, PLH = plant height, DM = days to maturity

[c] MPH = percent mid-parent heterosis, BHP = percent better-parent heterosis,

* = significant at 0.05 and **= Significant at 0.01

4.3.3. Combining ability analysis

Analysis of variance for combining ability revealed that GCA mean squares were highly significant for all the traits. SCA mean squares were also significant for all the characters except for number of tillers per plant (Table 4.4). This indicates the involvement of both additive and non-additive gene actions in determining the inheritance of studied characters. Results of this study are in accordance with the findings of Ahamed et al. (2004), Kumar et al. (2011), Jain and Sastry (2012), Adel and Ali (2013) and Fellah et al. (2013), who reported significant differences for both GCA and SCA for these traits. The components of variance indicated more GCA variance (1051.82) than SCA variance (139.45) for AUDPC of stem rust (Table 4.4). The higher values for the GCA variance over the SCA variance indicated predominance of the additive component over the non-additive component for this trait. Additive gene effects have also been reported by Ahamed et al. (2004) and Lohithaswa et al. (2013) for leaf rust resistance and Irfaq et al. (2009) for stripe rust resistance in wheat. A greater portion of the variance in the crosses could be attributed to the differences in the parental genotypes, which resulted from the diverse breeding history of the varieties. In this study, genes with additive effects largely determined the inheritance of resistance to stem rust, which is useful in a durable resistance breeding programs in wheat.

On the other hand, further variance ratio analysis showed that non-additive gene action was primarily responsible for the inheritance of grain yield, thousand kernel weight and plant height as the ratios were less than unity (Table 4.4). The present findings thus supported the results of Jain and Sastry (2012), Fellahi et al. (2013) and Zeeshan et al. (2013), who reported non-additive genetic variance as the main component of genetic variance of these traits. However, a predominance of additive gene effects was reported by Houshmand and Vanda (2008), El-Awady and Abo-El-Ela (2011) and Kamaluddin et al (2011) for the yield contributing characters in wheat. Kamaluddin et al. (2007), Rashid et al. (2012), Aslam et al. (2014), Zare-Kohan and Heidari (2014) reported the importance of both additive and non-additive gene actions for grain yield and its components. The difference in the results of various studies may be attributed to differences of the breeding materials and to genotype x environment interactions. In the present study the preponderance of non-additive gene action indicated the possibility of hybrid breeding, and selection for superior homozygous individuals for grain yield, thousand kernel weight and plant height should be delayed to advanced selfing generations.

Table 4.4 Analysis of variance and components of genetic variance for GCA and SCA and $\sigma^2_{gca}/\sigma^2_{sca}$ ratio for AUDPC of stem rust, yield and yield related traits from 6 x 6 half diallel cross of bread wheat.

Traits[‡]	Mean squares		Variance components		
	GCA[†]	SCA	σ^2_{gca}	σ^2_{sca}	$\sigma^2_{gca}/\sigma^2_{sca}$
AUDPC	147283.04**	21174.77**	1051.82	139.45	7.54
GY	0.75*	3.96**	0.02	0.12	0.17
TKW	42.04*	88.62**	0.86	6.45	0.13
KPS	539.53**	31.54*	0.54	0.35	1.54
TPP	33.28**	5.50ns	0.16	1.18	0.14
PLH	41.10**	182.53**	0.10	0.78	0.13
DM	712.43**	52.04**	0.67	0.47	1.43

[†]**= Significant at 0.01, * = significant at 0.05 and ns= non-significant, GCA= general combining ability, SCA= specific combining ability, σ^2_{gca} = variance of general combining ability, σ^2_{sca} = variance of specific combining ability, $\sigma^2_{gca}/\sigma^2_{sca}$ = ratio of variance of general combining ability to variance of specific combining ability
[‡]AUDPC = area under disease progress curve, GY = grain yield (t ha^{-1}), TKW = thousand kernel weight (g), KPS = number of kernels per spike, TPP = number of tillers per plant, PLH = plant height (cm), DM = days to maturity

In the case of number of tillers per plant, the mean squares for GCA were highly significant while SCA mean squares were non-significant, indicating the greater importance of additive gene action in controlling the inheritance of this character. As indicated by various authors (Kamaluddin et al., 2007; El-Awady and Abo-El-Ela, 2011; Yao et al., 2012; Zare-Kohan and Heidar, 2014), when GCA is important, especially in self-pollinated crops, pure line selection is the best method of breeding to improve the character in question. This is because additive effects are readily transmissible from one generation to another. Chowdhry et al. (2005) and Hammad et al. (2013) also reported that the number of tillers per plant is controlled by additive genes. Contrary to the present observations, Rashid et al. (2012) reported the importance of both additive and non-additive gene action for tillers per plant, while

Khan et al. (2007) and Fellahi et al. (2013) detected the predominant effect of non-additive variances in tillers per plant.

For days to maturity and kernels per spike additive gene action was found to be more important than non-additive gene actions. Iqbal et al. (2006) and Sener (2009) also reported the importance of additive gene action for days to maturity and kernels per spike, respectively. However, Rashid et al. (2012) and Ahmad et al. (2013) indicated that non-additive gene action was important for the inheritance of days to maturity.

4.3.4. General combining ability (GCA) effects

To identify parents with good GCA it is necessary to determine the GCA effects of parents for the traits of interest. Following analysis of variance for combining ability, the GCA effects for all the characters were estimated (Table 4.5). For AUDPC, negative values for GCA or SCA effects indicate a contribution towards stem rust resistance while positive values represent that of susceptibility. Five parents revealed significant GCA effects, out of which the slow rusting parents, 231545-1, 7041-1, H04-2 and Danda'a, gave desirable significant negative GCA effects and were the best general combiners for AUDPC. This indicated that the parents contributed resistance genes in their respective cross combinations, reflecting their potential in the development of slow rusting genotypes or varieties. Among the slow rusting parents, 231545-1 expressed the highest negative GCA effects for AUDPC (-69.31) followed by 7041-1 (-52.12), H04-2 (-46.6) and Danda'a (-39.0). Digelu (7.18) and Kubsa (199.71) had positive GCA effects. Kubsa had the highest positive GCA effect (199.17), which indicated that it was a poor combiner for stem rust resistance. This result is in accordance with Ahamed et al. (2004), Irfaq et al. (2009) and Lohithaswa et al. (20013), who reported significant negative GCA effects for wheat rust resistance.

For yield, three parents, H04-2, Digelu and Danda'a, exhibited positive and significant GCA effects while the parents 231545-1, 7041-1 and Kubsa showed significant and negative effects, indicating their poor combining ability for the trait. Danda'a was the best general combiner while 7041-1 was the poorest for grain yield.

Therefore, from this study, H04-2, Digelu and Danda'a could be utilized in bread wheat grain yield improvement programs. Cultivars with positive GCA could be used in hybridization programs in order to accelerate the pace of genetic improvement of wheat grain yield (Zare-Kohan and Heidari, 2014). This outcome was in agreement with the findings of Anwar et al. (2011) and Zare-Kohan and Heidari (2014) who reported greater and positive GCA effects for grain yield. In this study parents with higher GCA effects generated higher SCA effects for grain yield.

Table 4.5. Estimates of general combining ability for AUDPC of stem rust, grain yield and related traits of six parental genotypes of bread wheat

Parent	Traits‡						
	AUDPC†	GY(t ha^{-1})	TKW (g)	KPS	TPP	PLH (cm)	DM
231545-1	-69.31**	-0.35*	-2.74*	-2.37*	-0.94*	6.54**	0.39ns
7041-1	-52.12**	-0.70**	-1.99ns	-8.38**	-1.86**	-1.19**	0.22ns
H04-2	-46.50**	0.07*	3.18**	-4.55**	1.49**	-5.46**	-10.23**
Digelu	7.18ns	0.55**	0.76ns	7.30**	1.02*	-1.57**	-1.19ns
Danda'a	-39.0**	0.73**	2.43*	5.66**	-1.25**	2.59**	-1.36*
Kubsa	199.71**	-0.30*	-1.65ns	2.33*	1.54**	-0.92*	11.97**
SE (gj)	16.70	0.18	1.31	1.21	0.56	0.46	0.98

†*= Significant at P < 0.05, **= significant at P< 0.01, ns=non-significant
‡AUDPC = area under disease progress curve, GY= grain yield, TKW = thousand kernel weight, KPS = number of kernels per spike, TPP = number of tillers per plant, PLH = plant height, DM = days to maturity

For thousand kernel weight three parents, H04-2, Digelu and Danda'a, showed positive GCA effects. The crosses involving the two parents with high GCA effect were among the crosses with higher mean value for thousand kernel weight. Three other parents, 231545-1, 7041-1 and Kubsa, showed negative GCA effects for the same trait. Parent H04-2 was the best general combiner, while 231545-1 was the poorest general combiner for this trait. This result was in conformity with the findings of Nazir et al. (2005), Zeeshan et al. (2013) and Desale et al. (2014) who found both positive and negative GCA effects for thousand kernel weight of parents involved in their studies.

Parents including Digelu, Danda'a and Kubsa had significant positive GCA effects for kernels per spike while the remaining parents showed significant negative GCA effects for the trait. The highest positive GCA effect of 7.3 was observed from Digelu while the lowest was from 7041-1 (-8.38). In the present study, even though H04-2 exhibited highly significant negative GCA effects for kernels per spike, it was the best general combiner for increased thousand kernel weight and tillers per plant, which would contribute to positive GCA effect for grain yield.

Three parents, H04-2, Digelu and Kubsa, had positive and significant GCA effects for number of tillers per plant. The remaining parents showed negatively significant GCA effects, indicating their poor combining ability. Similar findings were reported by Hammad et al. (2013).

All six parents were observed to have significant GCA effects for plant height, of which four (7041-1, H04-2, Digelu and Kubsa) had negative and two (231545-1 and Danda'a) had positive effects. These parents with significant positive GCA effects were good combiners for increased tallness, while those with significant negative GCA effects were good combiners for decreased plant height. The latter is an important requirement in wheat breeding. The result showed that there is a direct relationship between GCA effects of parents and mean plant height of parents and crosses. This result corroborated with that Javaid et al. (2001), Nazir et al. (2005) and Hammad et al., (2013), who reported significant negative GCA effects for plant height.

Two parents, H04-2 and Danda'a, had negative and significant GCA effects for days to maturity, refelecting the contribution of these parents for earliness in crosses which they were involved. A positive and significant GCA effect was observed in Kubsa (11.97) for the same trait. This parent increased lateness in its crosses. Ahmad et al. (2013) also reported considerable levels of negative GCA effects for days to maturity.

4.3.5. Specific combining ability (SCA) effects

The magnitude of SCA effects is important in selecting cross combinations with a higher probability of generating transgressive segregants. The results of the specific combining ability analysis are presented in Table 4.6.

Crosses with negative and significant SCA effects for AUDPC were: 231545-1 x H04-2, 7041-1 x H04-2, Digelu x Kubsa and Danda’a x Kubsa. Four crosses: 231545-1 x Digelu, 7041-1 x Danda’a, 7041-1 x Kubsa and H04-2 x Digelu, exhibited negative but non-significant SCA effects for the stem rust resistance reaction. These crosses reduced the disease severity but non-significantly. The GCA effects of the slow rusting parents involved in these crosses were significant in the desirable direction. One slow ruster x susceptible cross, Danda’a x Kubsa, showed significant negative SCA effects, indicating their ability to transmit the characters in the progenies though in varying degree. Seven crosses showed positive SCA effects for AUDPC, of which four crosses: 231545-1 x 7041-1, 7041-1 x Digelu, H04-2 x Danda’a and Digelu x Danda’a, were non-significant, indicating their moderate combining ability for rust resistance.

Six crosses exhibited significant and positive SCA effects for grain yield. Danda’a x Kubsa (1.43) recorded the highest SCA effect, followed by Digelu x Kubsa (1.23) and H04-2 x Danda’a (0.75). These hybrids were rated as good specific cross combinations for grain yield. Significant yield performances in the specific crosses were due to the involvement of best general combiners in the crosses. Similar results were also reported by Desale and Mehta (2013). Good SCA effects for grain yield were observed in only a few crosses because the slow rusting parents used in this experiment were local lines with low yield potential. However, Jain and Sastry (2012), Lohithaswa et al. (2013) and Yao et al. (2014) reported the significance of SCA effects in considerable number of crosses for yield in bread wheat.

Positive SCA effects for thousand kernel weight were found for eight of the 15 crosses, out of which four, 231545-1 x Digelu, H04-2 x Danda’a, Digelu x Kubsa and Danda’a x Kubsa, showed highly significant SCA effects. The cross Danda’a x

Kubsa had the highest SCA effect (13.08) among all 15 crosses, while the lowest estimate of SCA was found in cross 231545-1 x Kubsa (-10.08) (Table 4.6). This indicated that Danda'a x Kubsa could be selected for its specific combining ability to improve thousand kernel weight. Moreover, it was observed that actual thousand kernel weights recorded for the crosses with significant positive SCA effects were superior to other crosses (Table 4.2). Thus, there was a close agreement between crosses selected on the basis of their SCA effects and *per se* performance for thousand kernel weight in the present study. Similarly, positive significant value of SCA effects for thousand and hundred kernel weight in considerable number of crosses were reported by Kumar et al. (2011) and Desale et al. (2014), respectively.

Specific combining ability analysis revealed that six crosses (231545-1 x 7041-1, 231545-1 x Digelu, 7041-1 x Digelu, H04-2 x Danda'a, Digelu x Kubsa and Danda'a x Kubsa) showed positive but non-significant SCA effects for the number of kernels per spike. Crosses of these parents contributed for higher kernel number per spike but not significant. The remaining nine crosses had negative SCA for the trait, of which two crosses 7041-1 x Kubsa and H04-2 x Kubsa exhibited significant effects. Except for the cross Digelu x Danda'a, these crosses involved poor combiners for the trait.

For number of tillers per plant, six crosses showed positive SCA effects, of which only Digelu x Kubsa revealed a significant positive SCA effect (4.48) for the trait, indicating hybrid combinations of these parents contributed to more number of tillers per plant. Desale et al. (2014) also found that only a few crosses exhibiting significant and positive SCA effects among the hybrids they studied, for tiller numbers.

Crosses including 231545-1 x H04-2 (-5.56), 231545-1 x Danda'a (-4.68), 7041-1 x H04-2 (-3.27), H04-2 x Kubsa (-7.86) and Digelu x Kubsa (-8.38) that had significant negative SCA effects for plant height were identified as good cross combinations to decrease this trait (Table 4.4). Most of the crosses with negative SCA effects for plant height had at least one parent with a significant negative GCA effect for this trait. Four crosses, 231545-1 x 7041-1 (9.41), 7041-1 x Kubsa (9.39), Digelu x Danda'a (6.65) and Danda'a x Kubsa (3.73), had significant positive SCA effects for plant height (Table 4.6). It can be concluded that combinations of these parents resulted in taller plants. Furthermore, there was a close agreement between crosses selected on the basis of their SCA effects and *per se* performance for dwarfness. Similarly, considerable negative and significant SCA effects for plant height and its importance for wheat improvement were reported by Ahmad et al. (2013) and Aslam et al. (2014).

Table 4.6. Estimates of specific combining ability effects for AUDPC of stem rust, yield and its related traits of 15 crosses of bread wheat

Cross[a]	Traits[‡]						
	AUDPC[†]	GY (t ha^{-1})	TKW (g)	KPS	TPP	PLH (cm)	DM
P1XP2	17.77ns	-0.28ns	0.24ns	4.25ns	-2.00ns	9.41**	4.61*
P1XP3	-93.85**	-0.06ns	-1.43ns	-1.09ns	-0.05ns	-5.56**	-7.35*
P1XP4	-22.03ns	0.42ns	4.99*	4.06ns	-0.88ns	0.79ns	0.03ns
P1XP5	61.52*	-0.35ns	-3.18ns	-1.80ns	2.02ns	-4.68**	1.19ns
P1XP6	101.67*	-1.97**	-10.08*	-3.50ns	-2.13ns	1.54ns	2.32ns
P2XP3	-64.05*	-0.75*	-2.18ns	-0.58ns	-0.47ns	-3.27**	-8.99**
P2XP4	57.27ns	0.33ns	2.24ns	4.57ns	-0.17ns	-1.02	5.09**
P2XP5	-34.28ns	0.42ns	1.07ns	-1.54ns	0.30ns	-0.43	1.76ns
P2XP6	-37.83ns	-0.03ns	0.67ns	-11.21**	-5.44**	9.39**	-5.16ns
P3XP4	-25.35ns	-0.08 ns	-5.45ns	-1.76ns	0.62ns	-0.36ns	-1.56ns
P3XP5	60.8ns	0.75*	7.40**	5.13ns	2.59ns	-0.95ns	-2.39ns
P3XP6	332.78**	-0.27ns	-7.17ns	-7.13*	-0.73ns	-7.86**	8.50*
P4XP5	29.12ns	-0.17ns	-7.18ns	-2.47ns	-0.61ns	6.65**	-0.22ns
P4XP6	-140.93*	1.23*	7.92**	5.88ns	4.48*	-8.38**	0.33ns
P5XP6	-241.68**	1.43*	13.08**	7.08ns	1.51ns	3.73*	2.57ns
SE (Sij)	45.87	0.49	3.59	3.33	1.54	1.25	2.70

[a] See Table 4.1 for codes of genotypes

Significant at P < 0.05, **= significant at P< 0.01, ns=non-significant

[‡]AUDPC = area under disease progress curve, GY= grain yield, TKW = thousand kernel weight, KPS = number of kernels per spike, TPP = number of tillers per plant, PLH = plant height, DM = days to maturity

Three crosses, 231545-1 x 7041-1 (4.61), 7041-1 x Digelu (5.09), H04-2 x Kubsa (8.50), had significant positive SCA effects for days to maturity, indicating their later maturity than what would have been predicted based on their parental performances. Crosses that recorded maximum negative SCA effects for maturity, such as 231545-1 x H04-2 (-7.35) and 7041-1 x H04-2 (-8.99), were good specific combiners and may be used as good sources for earliness. Most of the crosses involving H04-2 as a parent exhibited negative SCA effect for the days to maturity (Table 4.6). Some crosses showed desirable SCA effects for more than one characters. Cross combination Danda'a x Kubsa showed significant and desirable SCA effects for AUDPC, grain yield and thousand kernel weight. Digelu x Kubsa recorded significant and desirable SCA effects for AUDPC, grain yield, thousand kernel weight, tillers per plant and plant height.

In this study, the GCA effects of the parents and SCA effect of their crosses indicated that the crosses between two strong general combiners were not necessarily the best specific combiners. The best specific combinations for different traits were either good x good, poor x good, poor x average and *vise versa* for general combiners. The genetic interaction involved in the crosses of good general combiners might be additive x additive, which is fixable in further selection generations (Gorjanovia and Kraljevia-Balalia, 2007) and can be exploited by using pedigree selection in early generations for improvement of the characters (Jadoon, 2011). Crosses that exhibited strong SCA effects as a result of poor x good or good x poor general combiners indicated dominance x additive or additive x dominance genetic effects. Kamaluddin et al. (2007), Joshi and Sharma (1984) and Singh et al. (1986) proposed that intermating between F1's, followed by selection, is a useful strategy for obtaining desirable segregants in crosses from good x poor GCA parents. Significant SCA effects from crosses between parents with poor x average GCA effects indicated the predominance of non-additive gene effects in the combinations. It would be worthwhile to resort to other breeding methodologies, such as bi-parental mating, recurrent selection and modified diallel mating for the exploitation of non-additive gene actions (Jadoon, 2011; Desale et al., 2014). The importance of non-additive variances implies that postponing selection of superior plants to advanced selfed generations is more effective (Fellahi et al., 2013, Farooq et al., 2014).

Most of the crosses identified as desirable, with high SCA effects on the basis of their grain yield and thousand kernel weight, had at least one of the parents with strong GCA effects for the trait. Hence selection from the transgressive segregating generations of these crosses is expected to lead to substantial genetic improvement for AUDPC, grain yield and thousand kernel weight in bread wheat. It is therefore suggested that information on GCA effects should be supplemented by SCA effects and hybrid performance of cross combinations to predict and exploit the transgressive nature of segregation (Desale et al., 2014).

4.4 Conclusions

In this study, significantly negative mid-parent heterosis values for AUDPC were -48.85 (Danda'a x Kubsa) and -45.28 (7041-1 x Danda'a). The cross H04-2 x Danda'a generated the significant mid-parent (31.45%) and better-parent (25.38%) heterosis for grain yield. The highest mid-parent (28.85%) and better-parent (19.64%) heterosis for thousand kernel weight were obtained from the cross Danda'a x Kubsa. Significant mid-parent heterosis value (15.5%) for tillers per plant was recorded by H04-2 x Danda'a. 231545-1 x H04-2 (-11.01%) and Digelu x Kubsa (-7.29%) gave the highest negative mid- and better-parent heterosis for plant height, respectively and 7041-1 x H04-2 gave the highest negative mid-parent heterosis value of -8.02% for days to maturity. These crosses may provide transgressive segregants for selection to develop a wheat variety with superior agronomic performances and stem rust resistance.

The mean squares due to GCA were significant for all the traits and SCA mean squares were significant for all the traits except for number of tillers per plant. This indicated that both additive and non-additive genetic variances roles in the inheritance of these traits. The GCA and SCA ratio (σ^2GCA/σ^2SCA) was less than unity for grain yield, thousand kernel weight and plant height indicating that the non-additive component played a relatively greater role in the inheritance of these traits. The preponderance of non-additive type of gene actions in these traits clearly indicated that selection of superior homozygous true breeding individuals should be postponed to advanced selfed generation. Additive gene action was predominant in the inheritance of AUDPC, kernels per spike, tillers per plant and days to maturity.

The greater magnitude of additive gene effects for these traits suggested that selection after continued selfing would enable fixing desirable genotypes.

Parents 231545-1, 7041-1, H04-2 and Danda'a were identified as the best general combiners for slow rusting. Lines H04-2, Digelu and Danda'a appeared to be good general combiners for most of the traits, including grain yield. Kubsa was a good general combiner for kernels per spike, tillers per plant and plant height. Crosses 231545-1 x H04-2, 7041-1 x H04-2, Digelu x Kubsa and Danda'a x Kubsa were good specific combiners with reduced AUDPC. Danda'a x Kubsa, Digelu x Kubsa and H04-2 x Danda'a were good specific cross combinations for grain yield, while the crosses H04-2 x Danda'a, Digelu x Kubsa and Danda'a x Kubsa were good for TKW; 231545-1 x H04-2, 231545-1 x Danda'a, 7041-1 x H04-2, H04-2 x Kubsa and Digelu x Kubsa for reduced plant height and 231545-1 x H04-2 and 7041-1 x H04-2 for reduced days to maturity. The parents H04-2 and Danda'a were good general combiners for reduced AUDPC and most of the important yield contributing traits in this study. Crosses involving H04-2 and Danda'a proved to have better SCA effects and mean performances for most of the characters. Hence these parents may be used in breeding programs to develop stem rust resistant and high yielding wheat cultivars. The study also revealed the potential to exploit heterosis breeding to improve yield and related traits in bread wheat in Ethiopia.

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CHAPTER 5

Introgression of durable resistance genes into farmers'-preferred and locally adapted stem rust susceptible wheat varieties

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Abstract

Wheat (*Triticum aestivum* L) suffers significant yield losses due to stem rust disease caused by *Puccinia graminis* Pers. f. sp. *tritici* Eriks. and Henn owing to the susceptibility of farmers'-preferred and popular wheat varieties in Ethiopia. Incorporation of durable resistance genes from known sources into these varieties could boost stem rust resistance and productivity. The objective of the study was to transfer adult plant resistance genes from two donor parents, Pavon 76 and Kenya Plume, into two Ethiopian grown and high yielding but stem rust susceptible wheat varieties, Kubsa (HAR1685) and Galama (HAR604). The single backcross-selected bulk approach was employed to introgress candidate genes. Crosses and backcrosses were performed at the Ambo Plant Protection Research Center to obtain F1 and BC1 seeds. The BC1 populations were selfed until the F3 generation. Selections were made during the BC1 through F3 for stem rust resistance and agronomic traits under stem rust epidemics at the Debre-Zeit Agricultural Research Center in Ethiopia. Selections of the BC1 and F2 populations were carried out from un-replicated plots while the F3 populations were established and selected using a randomized complete block design with three replications to determine genetic gain. Results showed reduced stem rust severities of 4.0-20.8% in F3 progenies. The maximum reduction (20.8%) in stem rust severity was obtained from the cross Kubsa x Pavon 76. All F3 populations except the cross of Galama x Kenya Plume had increased spike length (1.5-3.7%), number of kernels per spike (3.4-6.5%), thousand kernel weight (2.9-9.7%) and grain weights per spike (2.1-5.9%) compared to their respective recurrent parents. Days to heading and maturity were lower than the recurrent parents at the F3 populations by up to 7.8% and 9.2%, respectively. Among the F3 populations, progenies derived from the cross Kubsa x Pavon 76 had better mean performances with the best genetic gains in most of the characters studied. These progenies will be advanced and selected in subsequent generations to develop locally adapted pure line wheat varieties with improved stem rust resistance and farmers'-preferred agronomic traits.

Key words: adult plant resistance, genetic gain, single backcross, stem rust, wheat

5.1 Introduction

Wheat (*Triticum aestivum* L) is the most widely grown cereal crop in the world, encompassing approximately 218 million hectares of land (HGCA, 2014). It is the second most important food crop in the developing world after rice (ICARDA, 2014). Ethiopia is the second largest wheat producer in sub-Saharan Africa, following South Africa (GAIN, 2012). Wheat is an important staple food used in a wide variety of products in the country. However, its productivity is threatened by various biotic and abiotic constraints. Stem rust disease caused by *Puccinia graminis* Pers. f. sp. *tritici* Eriks and Henn (*Pgt*) is one of the widespread biotic factors that severely affect wheat productivity in the country (Abebe et al., 2012). Disease control is possible with fungicides but they are unaffordable for resource poor farmers in developing countries like Ethiopia (Rehman et al., 2013). Use of wheat cultivars with durable resistance genes is the most economic, effective and ecologically sustainable method of stem rust control.

More than 50 resistance genes have been described for stem rust (McIntosh et al. 2011). Most of these genes are race-specific and function in a gene-for-gene fashion (Singh et al., 2006). Virulence in the pathogen population has been evolving rapidly following the deployment of race-specific resistance genes, often associated with a boom and bust cycle (Burdon et al., 2014). Use of race non-specific resistance genes is the best strategy for breeding towards durable stem rust resistance. Race non-specific resistance is governed by polygenes each with minor effect. This form of resistance is considered to be durable and effective against a broad range of stem rust races with an optimal level of expression at the adult plant stages (Parlevliet, 1985; McIntosh et al., 1995).

The stem rust resistance gene *Sr2* is considered to be one example of a gene contributing to adult plant resistance, based up on partial resistance (McIntosh et al., 1995; Bansal et al., 2008). The gene is recessive and is closely linked to pseudo-black chaff (‘pbc’), characterized by stem and head melanism in wheat. It has provided durable resistance against stem rust worldwide for more than 50 years (Bhardwaj et al., 2014). Development and deployment of wheat cultivars with the *Sr2* gene could provide a long term genetic solution to rust control. Accordingly incorporating adult plant resistance genes into locally adapted but disease susceptible germplasm is a major goal in most wheat breeding programs (Vanzetti et al., 2011).

Several widely adapted wheat varieties grown in Ethiopia have succumbed to stem rust disease and dropped out of production despite desirable agronomic attributes. According to Admassu et al. (2012), most stem rust resistance genes present in these varieties are race-specific. To reduce the susceptibility of adapted wheat varieties it is essential to incorporate resistance genes from known and diverse sources through crosses or repeated backcrosses (Singh et al., 2008). Backcross breeding is an effective method to transfer few resistance genes into locally adapted but susceptible wheat varieties from selected donor parents (Robbins, 2012). The donor parent provides resistance gene and may not possess all the desirable agronomic traits found in the recurrent parent. Agronomically superior variety which lacks resistance gene(s) serves as the recipient or recurrent parent. The goal of backcrossing in wheat is to obtain a pure line as close as possible to the recurrent parent with the addition of the resistance gene (Ye et al., 2009).

A single backcross-selected bulk method is one approach to breed for adult plant resistance (APR) (Singh et al. 2006). It is believed that selecting for resistance based on additively inherited minor genes is difficult (Singh et al., 2005). However, single backcross-selected bulk method allows incorporating resistance to rust diseases based on multiple additive or minor genes into well-adapted but susceptible wheat cultivars (Singh and Huerta-Espino, 2004). A single backcross approach also favours selection of genotypes with increased yield potential and other agronomic traits (Wang et al., 2009). Wang et al. (2009) noted that a single backcrossing shifted the progeny mean towards the higher side by favouring retention of most of the desired additive genes from the recurrent parent while simultaneously allowing for the incorporation and selection of additional useful genes with small effects from the donor parent.

The process followed in a single backcross-selected bulk method are: backcross sources of APR to elite lines for one generation, then bulk phenotypically desirable backcross progeny in the F2, F3 and F4, select individual stem rust resistant lines from the F5 generation, and continue selections for desirable traits and disease resistance until the F6 generation (Velu and Singh, 2013). Wang et al. (2009) investigated the efficiency of this breeding strategy compared to other crossing and selection strategies through computer simulations for many parameters, such as the number of genes to be transferred and frequency of favorable alleles in donor and recurrent parents. Previous reports indicated that this breeding strategy has advantages in improving the adaptation of the recurrent parents and transferring most (more than 60%) of the desired genes. Todorovska et al. (2013) supported the computer simulated data of Wang et al. (2009) showing that the single backcrossing breeding strategy allows a considerable amount of favorable genes from donor parents to be transferred, and simultaneously, the local adaptation of the recurrent cultivars could be improved. Studies at the International Maize and Wheat Improvement Center (CIMMYT) have also proved that minor genes can be incorporated successfully into a cultivar by using a single backcross-selected bulk breeding scheme (Singh et al., 2004). In addition, with the single backcross-bulk selection method there are significant savings in time, labor, and costs such as during nursery preparation and planting (Van Ginkel et al. 2002). According to Wang et al. (2003), the savings in resources did not result in a penalty in genetic gains for yield.

The objective of this study was to introgress durable stem rust resistance genes from known resistance sources into farmers'-preferred and locally adapted but stem rust susceptible, improved wheat varieties using a single backcross-selected bulk method.

5.2 Materials and methods

5.2.1 Plant material

Two stem rust susceptible wheat varieties, Kubsa (HAR1685) and Galama (HAR604), were used as recurrent parents to transfer adult plant resistance gene into their genetic background (Table 5.1). Both are semi-dwarf bread wheat types that are widely adapted, high yielding and farmers'-preferred varieties in Ethiopia (Yami et al., 2013). The two varieties originated from the International Wheat and

Maize Improvement Center (CIMMYT) and were released for cultivation in Ethiopia during 1994 and 1995, respectively (Hussein and Pretorius. 2005). Kubsa is early maturing whereas Galama is late maturing.

Pavon 76 and Kenya Plume, which carry high levels of adult plant resistance to stem rust (Singh and McIntosh, 1986; Singh et al., 2013), were selected as the pollen or donor parents. Pavon 76 is a semi-dwarf CIMMYT derived bread wheat variety released in Ethiopia during 1982 (Khan et al., 2013). It also carries a slow-rusting resistance gene (*Lr46*) to leaf rust (Singh et al., 1998). Kenya Plume is an early maturing, old and tall Kenyan wheat cultivar released in 1965 that has maintained its resistance over fifty years (Singh and McIntosh, 1986) (Table 5.1).

Table 5.1 Name, year of release, origin, and pedigree of donor and recurrent wheat parents used in the study.

Parents	Year of release	Origin	Pedigree	*Sr* genes
Recurrent parent				
Kubsa (HAR1685)	1994	CIMMYT	NDG9144//KAL/BB/3/ YACO''S''/4VEE#5''S''	-
Galama (HAR604)	1995	CIMMYT	4777(2)//FKN/GB/3 /PVN"S"	Sr9e, Sr11 (Admassu et al., 2012)
Donor parent				
Pavon 76	1982	CIMMYT	VCM/CNO "S"/ 7C/3/KAL/BB	*Sr2, Sr8a, Sr9g,Sr30* (McIntosh et al., 1995) *Sr9e, Sr11* (Admassu et al., 2012)
Kenya Plume	1965	Kenya	Mida/McMurachy//Exchange/3/Kenya 184p	*Sr2, Sr5, Sr6, Sr7a, Sr8a, Sr9b, Sr12, Sr17* (McIntosh et al., 1995)

5.2.2 Population development

The study used a single backcross-selected bulk approach to transfer resistance genes and develop superior progenies. The breeding scheme is outlined in Figure 5.1. Pavon 76 and Kenya Plume were crossed with Kubsa and Galama to obtain F1 seeds. The resultant F1 seeds were planted and 40-50 spikes from each F1 populations were backcrossed to their recurrent parents to obtain 400 BC1 seeds.

The BC1 seeds were planted and 80 plants per cross showing intermediate stem rust resistance with close resemblance to their recurrent parents were selected. One spike from each selected plant was harvested and the seeds bulked. The bulked seeds represented the F2 generation. One thousand five hundred plants per cross were planted in the F2 and 300 plants were selected for showing desirable agronomic features and good to intermediate stem rust resistance levels. A single spike was harvested from each selected F2 plant and the seeds were bulked. Five hundred F3 plants from each cross were then evaluated in the field and 200 plants per cross were retained to establish the F4. The bulked plants in the BC1 and F2 generations were sown in fields in non-replicated field trials. The F3 progenies and their recurrent parents were established in plots consisting of 6 rows of 1.5 m long with 20 cm inter-row spacing, using a randomised complete block design with three replications. Evaluation and selection at the F4 and later generations will be carried out in the next selection cycles following the scheme described in Section 5.5.

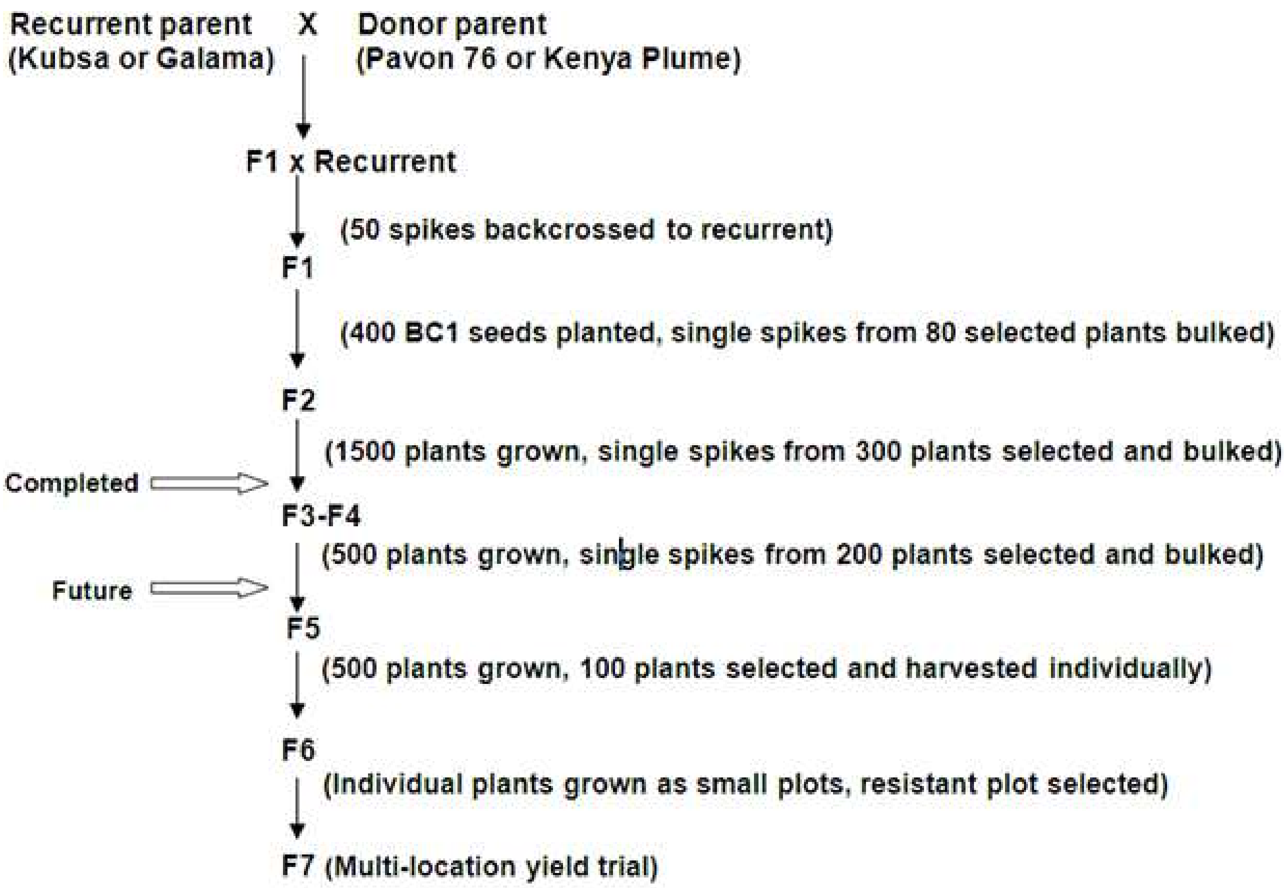

Figure 5.1. Schematic presentation of single backcross-selected bulk scheme showing the procedure of progeny selection

5.2.3. Experimental sites

Crosses and backcrosses were conducted during the off- and main seasons, respectively, in 2012 at Ambo Plant Protection Research Center in Ethiopia. The center is located at an altitude of 2200 m above sea level (masl) and receives a mean annual rainfall of 1100 mm. The mean annual minimum and maximum temperatures are 10.3^{o}C and 25.6^{o}C, respectively (Setotaw et al., 2014). Selections for resistance and other agronomic features in the BC1, F2 and F3 generations were carried out during the off- and main seasons of 2013, and off-season of 2014 at the Debre-Zeit Agricultural Research Center, where epidemics of *pgt* are prevalent. The Debre-Zeit Agricultural Research Center is found at an altitude of 1900 (masl). The center receives a mean annual rainfall of 851 mm. The mean annual minimum and maximum temperatures are 8.9^{o}C and 28.3^{o}C, respectively (Denbel et al, 2013).

5.2.4. Disease assessment

Terminal stem rust severity, estimated visually as a proportion of the plant stem affected, was recorded according to a modified Cobb scale (Peterson et al., 1948). Severity was assessed from twenty randomly taken plants of each plot when the plants were in the mid-dough stage and the mean of all stems was considered as the value for a plot. The host plant response to infection was also scored using the description of Roelfs et al. (1992) as R = resistant (flecks and small uredinia), MR = moderately resistant (flecks and small to moderate uredinia), MS = moderately susceptible (moderate to large uredinia), S = susceptible (large uredinia). The constant values for infection types were used based on; R=0.2, MR=0.4, MS=0.8, S=1 (Stubbs et al., 1986).

5.2.5. Data collection for agronomic traits

Spike length (SL) was measured in centimetres from the base of the rachis to the tip of the upper most spikelet from twenty randomly selected plants per cross. Twenty randomly sampled spikes per cross were threshed individually to determine the number of kernels per spike (KPS). Thousand kernel weight (TKW) (in gram) was measured using a random sample of thousand kernels adjusted to 12% moisture content. Grain weight per spike (GWPS) was determined from twenty random spikes per cross in gram. Plant height (PHT) was measured (in centimetres) from ten randomly selected plants in a plot measured from the base to tip of the panicle after flowering. Days to heading (DH) was recorded as the number of days from planting to 50% heading in each plot. Days to maturity (DM) was recorded as the number of days from emergence to when 95% of the plants in a plot were physiologically matured.

5.2.6. Statistical analysis

Disease severity and the agronomic data were subjected to analysis of variance to determine significant differences between single backcross derived F3 crosses and recurrent parents for disease resistance and agronomic traits. Data were analysed using the GLM procedure of SAS computer software (SAS, 2002). Genetic gains for all traits were calculated following Nyquist (1991) and expressed in percentage:

$$GG = [(\mu 1 - \mu 0)/\mu 0] x\ 100$$

where, GG = genetic gain; μ_0 is mean of initial population (recurrent parents) and μ_1 is mean of F3 population.

5.3. Results and discussion

5.3.1. Phenotypic evaluation

The field evaluation of single backcross derived F3 progenies showed significant ($P \leq 0.05$) variation for the agronomic traits and stem rust resistance (data not shown). This indicated the existence of genetic variability among the genotypes for the studied characters. Similarly, Menon et al. (2007) and Azam et al. (2013) evaluated F3 wheat progenies along with their parental lines and found significant differences at $P \leq 0.01$ for all the characters studied, including plant height, number of grains per spike, and grain yield per plant. The results of the phenotypic evaluations of F3 generations and their recurrent bread wheat parents are presented in Table 5.2.

Development of crop cultivars with disease resistance and superior agronomic performance has been the prime objectives of many plant breeding programs. Lower mean final rust severities were identified in all F3 populations in comparison with their recurrent parents (Table 5.2). F3 progenies derived from the cross Kubsa x Pavon 76 had the lowest mean terminal rust severity of 38% followed by Kubsa x Kenya Plume (42%). The rust severity of the cross Kubsa x Pavo 76 was significantly lower than that of the recurrent parent Kubsa. However, the final rust severities of the F3 progenies of the remaining crosses were not significantly different from their recurrent parents.

Table 5.2. Mean terminal stem rust severities and agronomic performances of the F3 populations and their recurrent parents at Debre-Zeit Agricultural Research Center, Ethiopia during 2014 offseason

F3 populations	FRS†	SL (cm)	KPS	TKW (g)	GWPS (g)	PLH (cm)	DH	DM
Gal x Pav‡	46.0ab*	9.25a	49.0a	32.0bc	1.98ab	85.3d	87.6a	123.0bc
Gal x K. plu	46.0ab	9.0b	37.5 b	31.5bc	1.79b	91.0bc	92.0a	129.0ab
K x Pav	38.0c	8.5c	50.0a	37.3a	2.15a	88.8bcd	59.0b	117.0c
K x K. plu	42.0bc	8.2d	45.0ab	35.0ab	1.96ab	95.5a	61.0b	120.5bc
Galama	50.0a	9.11ab	46.0a	31.0c	1.87b	87.35cd	95.0a	135.5a
Kubsa	48.0ab	8.2d	43.5ab	34.0abc	1.92b	92.75ab	64.0b	126.5abc
CV	6.44	0.80	7.05	4.20	3.95	1.90	5.42	3.13
R^2	0.83	0.98	0.84	0.93	0.85	0.90	0.97	0.85
LSD	7.51	0.18	8.18	3.61	0.20	4.40	10.64	10.10

†Gal x Pav= Galama x Pavon 76, Gal x K.pl = Galama x Kenya Plume, K x Pav = Kubsa x Pavon 76, K x K.pl = Kubsa x Kenya Plume

* Means in a column followed by the same letter are not significantly different

†FRS= final rust severity, SL= spike length, KPS = number of kernels per spike, TKW = thousand kernel weight, GWPS = grain weight per spike, PLH = plant height, DH = days to heading, DM = days to maturity

Spike related traits are important for grain yield formation. The F3 plants from the crosses Galama x Pavon 76 and Kubsa x Pavon 76 developed longer spikes than their corresponding recurrent parents. Individuals of the cross Galama x Kenya Plume had shorter spike lengths than the recurrent parent Galama. However, only the cross Kubsa x Pavon 76 had a significantly longer spike length than its recurrent parent Kubsa. The F2 plants from the cross Kubsa x Kenya Plume had mean spike length equal to the recurrent parent Kubsa (Table 5.2). The number of kernels per spike ranged from 37-50 in the F3 populations, while the variation in this parameter was lower in the recurrent wheat parents (46 for Galama and 43 for Kubsa) (Table 5.2). The number of kernels per spike was higher in F3 plants of the cross Kubsa x Pavon 76 (50), but not significantly different from its recurrent parent. Galama x Kenya Plume had a significantly lower number of kernels per spike than its recurrent parent.

All F3 populations had higher thousand kernel weights than their recurrent parents However, none of the crosses differed significantly from their corresponding recurrent parents (Table 5.2). The highest mean thousand grain weight (37.3 g) was recorded from the F3 population derived from the cross Kubsa x Pavon 76. The grain weight per spike was observed to vary within a narrow range in recurrent cultivars (1.87-1.92g) while the range was relatively wider for F3 populations (1.79-2.15). Among the F3 populations of the crosses, the F3 population from the cross Kubsa x Pavon 76 was distinguished by a significantly greater mean grain weight per spike than its recurrent parent, whereas none of the other populations differed significantly from their recurrent parents (Tables 5.2). F3 progenies of Kubsa x Pavon 76 had the mean value of 2.15 g for this trait (Table 5.2).

None of the F3 populations differed significantly from their recurrent parents for plant height and days to heading. However, F3 progenies of the crosses Galama x Pavon 76 and Kubsa x Pavon 76 had shorter plant heights than the corresponding recurrent parents. Days to heading for the F3 populations were 3 to 8 days earlier than their respective recurrent parents. The cross Galama x Pavon 76 matured significantly earlier than its recurrent parent, but none of the other three populations had significantly different maturity than their corresponding recurrent parents (Tables 5.2).

The study showed that all the F3 progenies of the crosses except Galama x Kenya Plume exhibited better performances in most of the desired characters in comparison to the recurrent parents: better stem rust resistance; higher kernel number per spike; higher thousand kernel weight and shorter plant height. This indicated that the single backcross-selected bulk method was effective way to improve stem rust resistance and other desirable traits in commercial wheat cultivars. This was in agreement with the findings of Singh et al. (2004) and Wang et al. (2009). Progenies from the cross Kubsa x Pavon 76 possessed the best combination of agronomic traits and disease resistance. Hence, progenies of this cross could be exploited in advanced segregating generations and yield trials.

5.3.2 Genetic gains in stem rust resistance and agronomic traits at the F3

Stem rust severity decreased in all the F3 populations of the crosses. The most resistant F3 progenies were the ones derived from the cross Kubsa x Pavon 76 followed by Kubsa x Kenya Plume. Genetic gains for stem rust resistance for these populations were 20.83 and 12.50%, respectively (Table 5.3).

The genetic gain for spike length was 3.66% of the initial population mean for Kubsa x Pavon 76 while the mean spike length in the F3 progenies of Kubsa x Kenya Plume did not differ from the original population mean. Mean number of kernels per spike ranged from 43.5 for the source population of the crosses Kubsa x Pavon 76 to 50 for F3 of the cross with a corresponding genetic gain of 14.96%, which was the maximum genetic gain obtained among the populations (Table 5.3). There was a clear response to selection for thousand kernel weights exhibited by the populations. The maximum genetic gain in thousand kernel weight was 9.71% at the F3 of Kubsa x Pavon 76. There was an improvement in the grain weight per spike from the initial generation to F3, with the genetic gains ranging from 2.08% for Kubsa x Kenya Plume plants to 11.98% for Kubsa x Pavon 76 plants (Table 5.3). The present findings corroborated with those of Memon et al. (2007), Ajmal et al. (2009) and Bilgin et al. (2011), who also observed good genetic advances for quantitative traits such as thousand kernel weight, kernels per spike and plant height. According to Memon et al. (2005), high genetic advances for major quantitative traits in wheat offers better scope for selection of genotypes in early segregating generations. The low genetic advance as observed for some of the characters in some of the progenies was also similar to the findings of Eid (2009) and Bilgin et al. (2011).

The F3 populations improved for stem rust resistance through selection were also equally good for spike related traits. This indicated that improvements in these traits from the source to F3 populations were associated with the decreases in the stem rust severities (Table 5.3). Many researchers have suggested that grain yield was significantly associated with stem rust resistance (CIMMYT, 2005; Wanyera et al., 2009; Macharia et al., 2013).

Days to heading and maturity decreased in all the F3 populations in response to selection. The high genetic gain observed for stem rust resistance, number of kernels per spike, thousand kernel weight and kernel weigh per spike indicated that there is good scope for improvement of adapted wheat cultivars by using a single backcross selection scheme.

Table 5.3. Genetic gains of stem rust resistance and agronomic traits of single backcross derived F3 populations evaluated under high stem rust pressure at the Debre-Zeit Agricultural Research Center, Ethiopia during the 2014 off-season.

Traits	Populations			
	Galama x Pavon 76	Galama x Kenya Plume	Kubsa x Pavon 76	Kubsa x Kenya Plume
FRS†	-8.00	-4.00	-20.83	-12.50
SL	1.54	-1.21	3.66	0.00
KPS	6.52	-18.48	14.94	3.45
TKW	3.23	1.61	9.71	2.94
GWPS	5.88	-4.28	11.98	2.08
PLH	-2.35	4.18	-4.26	2.96
DH	-7.79	-3.16	-7.81	-4.69
DM	-9.22	-5.53	-5.53	-7.51

†FRS= Final rust severity, KPS = Number of kernels per spike,
TKW = thousand kernel weight, GWPS = grain weight per spike

5.4 Conclusions

The single backcross-selected bulk approach was applied in order to incorporate durable stem rust resistance genes from resistance sources, Pavon 76 and Kenya Plume, into locally adapted, high yielding but stem rust susceptible wheat varieties, Kubsa and Galama. Single backcross derived F3 generations of the crosses were evaluated at the Debre-Zeit Agricultural Research Center under high disease pressure to determine genetic advances for stem rust resistance and agronomic traits. The most resistant plants were the F3 progenies of the cross Kubsa x Pavon 76, with terminal stem rust severity of 38% and a genetic gain of 20.83% over the recurrent parent, Kubsa. The F3 progenies of this cross also showed high genetic gains for most of the agronomic traits including number of kernels per spike (14.96%), thousand kernel weight (9.71) and grain weight per spike (11.98). The progenies of Kubsa x Pavon 76 will be advanced and selected in subsequent generations to develop locally adapted, pure line wheat varieties with improved stem rust resistance and farmers'-preferred agronomic traits. The improved performance of F3 progenies of the crosses for stem rust resistance and most of the agronomic traits over the recurrent cultivars indicated that the single backcross-selected bulk method can be used to incorporate durable resistance into adapted wheat cultivars to improve their resistance to stem rust and agronomic traits.

5.5. Future research direction

Bulk selection will be repeated through the production of the F_5 generation. By this stage, a high level of homozygosity will be established for the desired traits. Consequently 100 plants per cross with a high resistance level and good agronomic features will be selected and harvested individually. The selected plants will then be promoted to the F6 generation and grown in small plots (3-4 rows) to evaluate agronomic features and resistance. Plants with good grains are likely to be the ones possessing better genes for resistance, because stem rust will not have interfered with their development. Shrivelled seeds would suggest insufficient stem rust resistance. Finally the resistant F6 plots will be harvested for conducting multi-location yield trials in the following cropping season. The F4 to F7 generations will be planted as replicated trials. Inheritance studies will be conducted to understand the number and type of resistance genes involved in these lines. This could be followed by molecular mapping to determine genomic locations of minor, additive resistance genes contributing to adult plant resistance. Such information will be useful to establish and enhance genetic diversity for minor genes. At the end of the program superior wheat lines will be released that will provide farmers an option to grow durably resistant versions of their favourite wheat cultivars.

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An overview of the research findings

Introduction and objectives of the study

Wheat (*Triticum aestivum* L.) is the most widely grown crop around the world, serving as a staple food for one-third of the human population. Ethiopia is the second largest wheat producer in sub-Saharan Africa after South Africa. Wheat stem rust caused by the fungus *Puccinia graminis* f.sp. *tritici* is a major production constraint in most wheat growing regions in Ethiopia. It causes yield losses of up to 100% in epidemic situations. It is important to minimize losses incurred by the disease so that the yield potentials of improved wheat varieties can be realized. The available options for controlling stem rust, such as chemical and cultural controls, have not been effective in reducing yield losses. The principal management strategy for stem rust control has been resistant cultivars, which are effective and affordable to small-scale farmers. However, most of the currently grown, locally adapted and farmers'-preferred wheat varieties are susceptible to the disease. The major cause of the susceptibility of wheat varieties to stem rust is the narrow genetic base used in wheat lines that have been included in the wheat breeding programs of Ethiopia, and emergence of new and virulent races of the pathogen. Hence, there is a need for durable resistance that can withstand different races of the pathogen. The objectives of this study were initially established as:

- To determine the wheat production constraints, farmers' varietal preferences, and disease management practices, with special emphasis on wheat rusts
- To identify slow rusting resistance to stem rust in Ethiopian wheat lines
- To determine the levels of heterosis and combining ability, and to identify the best parents and crosses for breeding to stem rust resistance, high grain yield and desirable agronomic traits
- To introgress durable resistance genes from known durable resistance sources into farmers'-preferred and locally adapted but stem rust susceptible, improved wheat varieties

Research findings in brief

Appraisal of farmers' wheat production constraints and breeding priorities in stem rust prone agro-ecologies of Ethiopia

- A participatory rural appraisal (PRA) research was conducted involving 270 farmers in six districts of three administrative zones in Ethiopia.

- Important wheat production constraints were identified and prioritized, of which wheat rust diseases, the high costs of fertilizers, a shortage of improved seeds and high seed prices were the most significant factors limiting wheat production and productivity.

- The most important traits that farmers sought in wheat varieties were disease resistance and high grain yield.
- Estimated yield losses due to stem rust disease were more than 60% in all the surveyed areas.
- Fungicide application was the main disease management practice used by the majority of respondent farmers but they realised that this method was not affordable for stem rust management and that they needed a better control option.
- Indigenous durum wheat varieties were being completely replaced with modern bread wheat varieties in the study areas.

Assessment of Ethiopian wheat lines for slow rusting resistance to stem rust of wheat caused by *Puccinia graminis* f.sp. *tritici*

- Two hundred fifty two wheat genotypes were evaluated for their slow rusting resistance to stem rust under greenhouse and field experiments at Ambo and Debre-Zeit Agricultural Research Centers, Ethiopia, respectively.

- Wheat genotypes such as H04-2, 204408-3, 214551-1, 231545-1, 7041-1, 7514-1, 226385-1, 226815-1, 7579-1, and 222495-1 were identified as good slow rusting lines. Of these 231545-1, 7041-1, 226815-1 and 7579-1 exhibited complete susceptibility at the seedling stage (3- to 3+) and were regarded as true slow rusting lines.

- Wheat genotypes including 237886-1, 227059-1, 203763-1, 226275-1, 227068-2, 226278-1 and 7994-1 were identified as moderately slow rusting lines.
- Both highly slow rusting and moderately slow rusting lines identified from this study can be used for future manipulation in durable resistance breeding.

Heterosis and combining ability analysis of stem rust resistance and grain yield and related traits in bread wheat

- Fifteen F1 wheat genotypes were developed using a half diallel mating design, involving six parents. The 15 hybrids and 6 parents were used to estimate heterosis and combining ability at the Debrezeit-Agricultural Research Center, a known hot spot for stem rust disease, in Ethiopia.
- The maximum positive mid-parent (31.45%) and better-parent heterosis (25.38%) were observed for grain yield. The maximum negative heterosis were observed for plant height (-11.01%) and days to maturity (-8.02%).
- The majority of the crosses expressed negative heterosis over the mid-parent for AUDPC, indicating these crosses manifested enhanced resistance to the disease. Significantly negative mid-parent heterosis values for AUDPC were -48.85 (Danda’a x Kubsa) and -45.28 (7041-1 x Danda’a). The maximum better-parent heterosis value for the trait was -34.93.
- Significant ($p<0.05$) general combining ability (GCA) effects were detected for all the characters studied and specific combining ability (SCA) effects were significant ($p<0.05$) for all the traits except for the number of tillers per plant, indicating the contribution of additive and non-additive genes to total genetic effects controlling the traits.
- The relative magnitude of GCA and SCA variances, however, revealed that non-additive gene actions were more important for grain yield, thousand

kernel weight and plant height while additive gene action played a greater role in the inheritance of AUDPC, kernels per spike, tillers per plant and days to maturity.

- The study identified parental lines H04-2, Digelu and Danda'a as good general combiners for most of the characters studied.
- Wheat lines 231545-1, 7041-1, H04-2 and Danda'a expressed significant negative GCA effects for AUDPC, suggesting these lines can be used as parents in wheat breeding for stem rust resistance.
- Crosses 231545-1 x H04-2, 7041-1 x H04-2, Digelu x Kubsa and Danda'a x Kubsa had significantly negative SCA effects for AUDPC. These crosses will be selected in the stem rust resistance breeding program.
- In this study, wheat lines: H04-2 and Danda'a were good general combiners for most of the important studied characters and crosses involved these lines performed well for most of the traits. Hence, selection from the transgressive segregating generations of crosses of H04-2 and Danda'a could be expected to lead to substantial genetic improvement for these traits.

Introgression of durable resistance genes into farmers'-preferred and locally adapted stem rust susceptible wheat varieties

- Introgression of durable resistance genes from resistance sources, Pavon 76 and Kenya Plume, into two locally adapted wheat varieties, Kubsa (HAR 1685) and Galama (HAR 604), was conducted using a single backcross-selected bulk breeding approach.
- Single backcross derived F3 populations, along with the recurrent parents, were evaluated under high disease pressure at Debre-Zeit Agricultural Reasrch Center to determine the genetic gains for stem rust resistance and agronomic traits.

- The F3 progenies in all crosses except Galama x Kenya Plume were better performing for stem rust resistance and most agronomic traits than the recurrent parents.
- The F3 progenies of Kubsa x Pavon 76 displayed exceptional performances with high genetic gains for most of the characters studied. These progenies will be advanced and selected in subsequent generations to develop locally adapted pure line wheat varieties with improved stem rust resistance and farmers'-preferred agronomic traits.

Implications of the research findings to breeding wheat for stem rust resistance and higher yield

- Farmers' participation in wheat breeding programs is important for better adoption, acceptance and impact of improved varieties. Moreover, participatory research increases the job efficiency of the scientists and reduces research costs. The results of the participatory rural appraisal study were useful to identify the existing wheat production constraints and farmers' preferred traits in wheat genotypes. Their views and priorities will be considered by the wheat breeding program in Ethiopia.
- Wheat genotypes with slow rusting resistance were identified among the wheat accessions of Ethiopia, using greenhouse and field evaluations. They provide potential for breeding towards stem rust resistance. The 10 genotypes that displayed high levels of slow rusting resistance and 7 genotypes with moderate levels of slow rusting resistance are of great importance to achieving effective breeding for durable resistance to stem rust.
- The levels of mid-parent and better-parent heterosis detected in the present study showed the potential to commercially exploitate heterosis among the progenies of F1 for improvement of grain yield and stem rust resistance in bread wheat.
- Best parents and cross combinations for stem rust resistance and yield traits were identified that could be effectively utilized in wheat breeding for the

improvement of stem rust resistance and yield components, and therefore their incorporation in a future wheat breeding program is recommended.

- Both additive and non-additive variances were found to be important in the genetic control of stem rust resistance and agronomic traits, which suggests use of integrated breeding strategies to efficiently utilize the additive as well as non-additive genetic variability. Thus, the use of diallel mating with recurrent selection could provide the better conditions for recombination and accumulation of desirable genes.

- Incorporating durable polygenic resistance into the widely adapted wheat varieties, Kubsa and Galama, by using a single backcross-selected bulk method will improve the resistance and yield potential of the cultivars while maintaining their original characteristics. These could enhance wheat productivity and profitability of resource poor farmers in Ethiopia.