Breeding investigations and validation of molecular markers linked with spot blotch disease resistance in wheat (*Triticum aestivum* L.) germplasm for the rain-fed conditions of Zambia

by

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Thesis Abstract

Wheat (*Triticum aestivum* L.) is an important cereal crop, second after maize in Zambia. Its production during summer rainy season is hampered by several abiotic and biotic constraints. Among the biotic constraints, spot blotch disease caused by *Bipolaris sorokiniana* (Sacc.) Shoem is the most devastating causing large wheat yield losses and grain quality deterioration. Under severe cases complete crop losses result. This is because resistance to spot blotch disease in most of the genotypes in Zambia is inadequate. Breeding for high yielding disease resistant genotypes is the most cost effective and sustainable way of increasing summer rain-fed wheat yields and achieving sustainable wheat production. The study was, therefore, undertaken to: a) determine farmers` preferences for rain-fed wheat cultivars and identify production constraints, b) assess genetic diversity using agro-morphological traits, c) screen germplasm for resistance to spot blotch, d) determine gene action controlling the inheritance of resistance to spot blotch disease, and e) validate simple sequence repeat (SSR) molecular markers previously reported to be linked with resistance to spot blotch disease.

A participatory rural appraisal established that wheat was an important crop among the small-scale farmers as it was a dual purpose crop used for home consumption and income generation. Coucal was the only wheat variety grown by the farmers under rain-fed conditions. The major constraints affecting summer wheat production in order of importance were; lack of good wheat seed, bird damage, weeds, termite damage, diseases (spot blotch being the most important), lack of markets and drought. High yielding cultivars with white coloured grain, combined with resistance to spot blotch disease, resistance to bird damage, termite damage and drought were the traits most preferred by the farmers.

The genetic diversity study revealed the existence of genetic variability amongst the genotypes. Principal component analysis identified plant height, tillers/m^2, peduncle length, days to heading, days to maturity and grain yield as the main traits that described the variability among the genotypes. The 150 genotypes tested were clustered into five groups based on Ward's method, indicating that they were from different genetic backgrounds. This suggests that superior genotype combinations could be obtained by crossing genotypes in the opposing groups. The study also established that hectolitre weight, tillers/plant, thousand grain weight (TGW), grains/spike, peduncle length, and tillers/m^2 could be effective selection criteria for high yield as they exhibited positive direct effects on yield and also significant and positive association with yield.

One hundred and fifty wheat genotypes from Zambia and CIMMYT-Mexico were screened for resistance to spot blotch disease. The study revealed significant variability among the

genotypes in their reaction to spot blotch disease. Genotypes were classified as resistant, moderately resistant, moderately susceptible, and susceptible. Genotypes 19HRWSN6 (Kenya Heroe), 19HRWSN7 (Prontia federal) were amongst the genotypes that were resistant across seasons. Most of the genotypes obtained from Zambia were moderately susceptible to susceptible across seasons. Nonetheless, eight genotypes with varying resistant reactions were selected for genetic analysis studies.

A genetic analysis using Hayman diallel approach of 8 × 8 mating design and generation mean (GMA) analysis of six generations (P1, P2, F1, F2, BCP1 and BCP2) of two cross combinations was conducted. The two biometrical methods revealed the importance of additive gene effects in controlling resistance to spot blotch disease. The absence of maternal and non-maternal reciprocal effects indicated that choice of female parent was not important in breeding for resistance to this disease. Epistatic gene effects were absent in the inheritance of resistance suggesting that selection would be effective in early generation. Resistance exhibited partial dominance. Both diallel and GMA revealed moderately narrow sense heritability of 56.0% and 55.5%, respectively, an indication that the trait could be improved through selection. The Wr/Vr graph showed that parents 30SAWSN10 (P1), 30SAWSN5 (P3) and Coucal (P4) displayed the maximum number of dominant genes hence can be used in breeding for resistance to spot blotch.

The molecular markers *Xgwm570*, *Xgwm544* and *Xgwm437* previously reported to be linked with resistance to *Bipolaris sorokiniana* were validated and their association with resistance confirmed. The markers amplified fragments in resistant parental genotypes that were similar to the F2 resistant and moderately resistant lines but not in susceptible ones. The significant relationship between the marker and resistance to *Bipolaris sorokiniana* was also established considering the significance of regression analysis (*Xgwm570*, P=0.003; *Xgwm544*, P=0.03 and *Xgwm437*, P=0.03). The adjusted R^2 values observed (*Xgwm570* =11.0%; *Xgwm544*=10.0% and *Xgwm437*=7.0%) further revealed the association between the marker and resistance. The study, therefore, shows that the markers can be useful in Zambia as they would increase the efficiency for the identification of resistant genotypes. This implies that screening of the genotypes could be done even in the absence of the disease epidemic.

Overall, the results from this study indicate that the opportunity of improving resistance to spot blotch disease exists by utilizing the information generated. This information could be important during planning and implementation of breeding for resistance.

Declaration

I, Batiseba Tembo, 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 The thesis does not contain any other persons' data, pictures, photographs 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 written sources have been quoted then:

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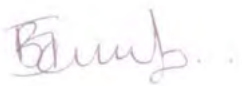
Signed  Date ……..

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

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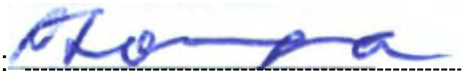
Dr Julia Sibiya (Supervisor)

Signed:  Date……..

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To God be the Glory and Honour.

Dedication

To

My dear husband Maliro Banda

Our sons Clement, Dalitso, Malie and Chiyamiko

My father Fredson Martin Tembo and my mother Matilda Tembo

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Introduction to thesis

1 Importance of wheat

Wheat *(Triticum aestivum* L.*)* is one of the most important and widely grown food crops in the world. It is consumed in more than 175 countries with an average consumption of 67 kg per capita (kg yr^{-1}) (van Ginkel and Ogbonnaya, 2007; Ortiz et al., 2008; FAO, 2009; Reynolds et al., 2009). It contributes 20% of the total calories consumed by the world population. In terms of world wheat production, Africa produces the least compared to other continents like Asia, Europe, and America (FAOSTAT, 2009) (Table 1). Africa produces about 22 million metric tonnes (MT) per year, against an annual consumption of 54 million MT (FAOSTAT, 2009) resulting in imports of more than 60%, to meet the demand (Curtis, 2002; Ortiz et al., 2008; Reynolds et al., 2009). In Africa, Zambia is amongst the top 10 wheat producers (Table 1) and is the second major producer after South Africa in southern Africa with a productivity of 5.7 t ha^{-1} (FAOSTAT, 2009).

2 Wheat production in Zambia

In Zambia, wheat is the second most widely grown cereal crop after maize, with an average annual production of 183 856 t yr^{-1} compared to millet (48 482 t yr^{-1}), rice paddy (32 976 t yr^{-1}) and sorghum (24 780 t yr^{-1}) (FAOSTAT, 2009). It is grown by both commercial and small-scale farmers. Commercial farmers grow wheat as a cash crop in the cool, dry season (April to September), under irrigation mainly in the following provinces: Southern, Lusaka, Central, Copperbelt and some parts of the Eastern province. Small-scale farmers on the other hand, grow wheat under the hot and humid summer rainy season (November to March) because of lower production costs when compared to growing the cool, dry season irrigated wheat (ZARI, 2008). These small-scale wheat farmers are located in all parts of the country, but mainly in the northern part. However, summer rain-fed wheat production in Zambia is still low due to high temperatures and a high prevalence of diseases. Once these problems are solved, summer rain-fed wheat production could expand and complement winter production.

Table 1: Wheat production and productivity in the world and Africa (2009)

Content	Area harvested (million ha)	Production (million t)	Productivity (t ha^{-1})
Africa	09.64	22.07	2.29
Asia	102.12	301.00	2.95
America	39.03	108.08	2.77
Europe	61.09	228.71	3.74
World Total	211.88	659.86	3.11
Africa			
Egypt	1.32	8.52	6.45
Morocco	2.98	6.40	2.15
Ethiopia	1.68	3.08	1.83
Algeria	1.85	2.95	1.60
South Africa	0.64	1.96	3.05
Tunisia	0.80	1.65	2.06
Sudan	0.40	0.64	1.66
Zambia	0.03	0.20	5.70
Kenya	0.13	0.13	1.01
Zimbabwe	0.01	0.04	3.08

Source: FAOSTAT, 2009.

Despite the low wheat productivity amongst small-scale farmers, the area under wheat production in Zambia has increased over the past six years. According to Central Statistical Office (CSO) (2006), Zambia produced 53 479 MT against an annual consumption of 200 000 MT in 2006. In 2007, wheat production rose to 115 843 MT as a result of an increased production area due to the anticipated, attractive prices in the 2007/2008 marketing season (Zinyama, 2009). Wheat production further increased to 195 456.36 MT in 2009 and 273 584.00 MT in 2013, again due to increased production area. Although there has been an increase in production over the years, there is still a shortfall of wheat required in the country. Zambia still imports the balance to meet the increasing demand of wheat. In April 2015, the Honorable Minister of Agricultural and Livestock announced a shortfall of 60 000 MT of wheat in the country and hence allowed the importation of 75 000 MT to cushion the wheat shortage (ZNBC, 2015). So, in order to become self-sufficient, Zambia needs to enhance its wheat production in the summer rainy season.

3 Constraints to wheat production in Zambia

There are several constraints limiting wheat production in Zambia. These include socio-economic factors such as the cost of pesticides, fertilizer, herbicides, fungicides and irrigation, abiotic factors, including soil acidity and high temperature; and biotic factors, mainly spot blotch, powdery mildew, stem rust and leaf rust diseases (ZARI, 2008).

3.1 Socio-economic constraints

Wheat farmers are faced with a fair share of socio-economic constraints which include; high cost of farm inputs such as pesticides, fertilizers, herbicides and fungicides, unstable electricity supply needed for irrigation, high electricity tariffs from the power utility company, and lack of affordable loans from the lending institutions (Zinyama, 2009). The cost of inputs coupled with high interest rates negatively affect wheat production. Most of the wheat cultivars grown require the use of expensive fertilizers, fungicides, and/or herbicides which most farmers cannot afford. These socio-economic constraints thus contribute to low wheat production in Zambia as they discourage wheat farmers from expanding their production.

3.2 Abiotic constraints

Soil acidity and high temperature are the major abiotic constraints affecting wheat production in Zambia. Soil acidity is a common problem in most parts of the country especially in the north. It causes poor yields due to a high concentration of aluminium ions (Al^{3+}), which inhibits root growth resulting in decreased nutrient uptake by plants (Ryan et al., 2011). Lime is used as a control measure but it is scarce and expensive. There is, therefore, a need to improve wheat cultivars on their tolerance to aluminium as most of the currently grown cultivars are sensitive to aluminium toxicity.

High temperature is another abiotic constraint limiting high yields in Zambia. It affects photosynthesis and physiological processes in the plant, thereby affecting plant growth and development (Wang et al., 2003; Mohammadi et al., 2004; Wahid et al., 2007; Ortiz et al., 2008). Mohammadi et al. (2004) and Rehman et al. (2009) indicated the optimal temperature for wheat growth to be 25°C, above which yields start to decrease. Wahid et al. (2007) reported that high temperatures (above 35^{o}C) before anthesis induced sterility in plants causing huge yield losses through non production of wheat spikes. Other studies have reported a reduction of 4% in grain weight for each degree increase in temperature (Mohammadi et al., 2004; Chauhan et al., 2010). Temperatures above 25^{o}C during wheat production cycle are not unusual in Zambia both in the rainy and cool, dry seasons. High temperature has also been reported in many wheat growing areas to be the primary cause of

poor yields and quality (Rehman et al., 2009). In view of the above, there is need to breed for heat tolerance to improve wheat production and productivity in Zambia as most of the varieties under cultivation are not heat tolerant.

3.3 Biotic constraints

Diseases are amongst the most important biotic constraints that reduce yield and quality of wheat in Zambia (ZARI, 2008). The diseases include: stem rust caused by *Puccinia graminis* sp *tritici,* powdery mildew caused by *Erisiphe graminis* sp. *tritici,* leaf rust caused by *Puccinia recondita* sp *tritici* and spot blotch (*Helminthosporium* leaf blight) caused by *Bipolaris sorokiniana* (Sacc.) Shoem. In Zambia, low incidences of wheat stem rust were reported at Golden Valley Agricultural Research Station (GART) in 2009, although the rust race is not yet known (FAO, 2010). The disease has been reported to be devastating where it has epidemic proportions resulting in huge crop losses (over 70%) and sometimes destruction of the entire crop (Dubin and Brennan, 2009; USDA, 2010; Vurro et al., 2010). Therefore, wheat stem rust presents a threat to wheat production if its incidence and severity are high. In addition, to Zambia, the disease is prevalent in east Africa (Kenya and Uganda) (Njau et al., 2009) and has been reported in Zimbabwe and South Africa (FAO, 2010).

Powdery mildew is common in irrigated wheat in the cool dry season in Zambia, while leaf rust occurs both in the cool dry season and summer rainy seasons. Both powdery mildew and leaf rust diseases require attention in terms of developing resistant germplasm because the resistance of most of the varieties is unsatisfactory. At present, most commercial farmers use fungicides as a control measure against the diseases, while small-scale farmers do not use chemicals. This implies that disease levels will always be high in the small-scale farmers' fields resulting in low yields and poor quality.

Of all the diseases presently affecting summer rain-fed wheat production in Zambia, spot blotch is the most serious and devastating (Muyanga, 1994). Khan and Chowdhury (2011) and Srivastava and Tewari (2002) showed that spot blotch was a major disease causing huge yield losses in the tropics and subtropics. Severe yield losses (about 7 to 100%) due to the fungus have been reported to occur in Zambia, Bangladesh, Brazil and Bolivia (Mehta, 1997; Chaurasia et al., 1999). In Bangladesh, for example, yield losses of about 80% have been reported in warmer areas (Duveiller and Gilchrist, 1994). The outbreak of spot blotch disease is favoured by wet, humid and warm temperatures (Alam et al., 1994; Khan and Chowdhury, 2011). These conditions are common in Zambia during the summer rainy season. As a result, spot blotch can be a devastating disease causing complete crop loss. The development of cultivars tolerant to spot blotch will boost sustainable summer rain-fed wheat production in Zambia as the crop is in high demand. The absence of resistant

genotypes to *Bipolaris sorokiniana* in Zambia has necessitated the search and identification of resistance sources to aid in the development of resistant genotypes. In this study, wheat germplasm was screened in several environments during the summer season to identify resistant genotypes.

On the other hand, screening for resistance to spot blotch resistance, requires a method that allows screening for resistance in both summer and winter seasons. The molecular markers reported to be linked with resistance to *Bipolaris sorokiniana* could accelerate the identification of resistance genotypes as they are independent of the environment effect. The markers could improve on selection efficiency of resistant germplasm, as germplasm evaluation could be done in any season as opposed to the current situation where screening for resistance is done in the summer season only. Before the markers could be used in marker assisted breeding, validation is required to determine their value in marker assisted selection. No research has been reported on validation of the markers previously reported to be linked with resistance genes to *Bipolaris sorokiniana*, hence the study.

4 Genetic diversity

Efficiency in breeding requires the understanding of the amount of genetic variation existing among the genotypes that can be used to manipulate plants to prevent crop destruction through biotic and abiotic stresses. According to Govindaraj et al. (2015), genetically diverse plants are important in breeding as they provide greater opportunities for cultivar development and improvement with desired characteristics; such as resistance to diseases, pests, high yield and tolerance to abiotic stresses. In wheat, genetic diversity has been studied using morphological and agronomic traits as well as using molecular markers. In Zambia there is no information on genetic diversity among wheat genotypes. There is, therefore, a need to conduct studies on genetic diversity to guide selection of genotypes with diverse genetic backgrounds with desired traits to be used in breeding process. In this study, agro-morphological traits were used to study genetic variability among the genotypes using a standard list of wheat descriptors (IBGR, 1978).

5 Genetic control of resistance to spot blotch

Genetic resistance is the best strategy of controlling spot blotch disease as it is environmentally friendly and cost effective. However, knowledge of gene action involved in the control of resistance is important as it helps to formulate strategies for selecting superior genotypes for improving the trait (Salama et al., 2006). There are contradicting reports on inheritance of resistance to spot blotch. Some studies have suggested qualitative inheritance (Duveiller and Dubin, 2002), while others have suggested polygenic inheritance (Kumar et

al., 2002; Duveiller and Sharma, 2009). Sharma et al. (2004) and Khan et al. (2010) reported that additive gene action played a major role in controlling resistance to spot blotch. A study by Joshi et al. (2004) indicated three additive genes controlling the inheritance of resistance to spot blotch disease in wheat. Duveiller and Sharma (2009) showed that dominant and recessive genes controlled the inheritance of resistance and in some cases epistasis has been reported. Sharma et al. (2006) found that resistance to spot blotch was conditioned by partially dominant genes and inherited quantitatively with moderately to high heritability estimates. Therefore, based on these reports there is need to ascertain the nature of inheritance and the gene action involved in controlling resistance to spot blotch. No information is available about the genetic basis of spot blotch resistance in rain-fed wheat material in Zambia. Hence, this study is designed to determine the type of gene action mode of inheritance of resistance to spot blotch disease in rain-fed wheat cultivars.

6 Farmers preferences in wheat breeding

The inclusion of farmers in the development of cultivars is very important as they are the main users of the varieties (Kudadjie et al., 2004). It has been reported that farmers do not normally adopt varieties when they lack their preferred traits, even if they were superior (Banziger and Cooper, 2001). Participatory research provides researchers with vital information and a better understanding of farmers' preferences and constraints to be considered during the breeding process (Islam et al., 2008). Banziger and Cooper (2001) reported that farmer's participation in breeding, through participatory research, encourages adoption and enhances production of new varieties. In Zambia, till now, research has been carried out in wheat without the knowledge of farmers preferred traits or production constraints. Thus, there is a need to investigate farmers' preferences to incorporate in the breeding to enhance the adoption of varieties and also understand factors that limit production. In this study, farmer preferred traits of rain-fed wheat cultivars and production constraints were studied.

7 Problem Statement

It has been observed that some farmers, both commercial and small-scale, are willing to grow rain-fed wheat due to low production costs involved. However, currently no deliberate effort has been put towards development of germplasm resistant to spot blotch disease, despite it being the major factor contributing to low yields. This can be attributed to the fact that more effort has been directed towards breeding high yielding cultivars, without the consideration of other survival traits such as resistance to diseases. In addition, there has been no active breeding in the National Wheat Programme for the past eight to ten years.

The programme has relied heavily on CIMMYT germplasm for screening and selection of high yielding cultivars for favourable climates. Nonetheless, the most cost effective and sustainable way of increasing summer rain-fed wheat yields and achieving sustainable wheat production amongst small-scale farmers in Zambia is through breeding cultivars that are resistant to spot blotch disease.

8 The overall goal

The overall goal of the study is to contribute to the improvement of wheat production in Zambia, in order to improve household food security and livelihoods amongst small-scale farmers through use of resistant cultivars.

8.1 Research objectives

The specific objectives of the study were:

1. To determine farmers` preferences for rain-fed wheat cultivars and identify production constraints.
2. To assess genetic diversity in wheat germplasm adapted to summer rain-fed conditions in Zambia.
3. To screen germplasm from Zambia and CIMMYT-Mexico for resistance to spot blotch.
4. To determine gene action controlling the inheritance of resistance to spot blotch disease caused by *Bipolaris sorokiniana*.
5. To validate three simple sequence repeat (SSR) *(Xgwm544*, *Xgwm570* and *Xgwm437*) markers previously reported to be linked with resistance to *Bipolaris sorokiniana* a pathogen causing spot blotch disease.

9 Research hypothesis

1. Farmers are aware of the summer wheat production constraints and have specific preferences regarding rain-fed wheat cultivars.
2. Adequate genetic diversity does exist in the germplasm used in the study.
3. Sources of resistance to spot blotch disease exist and can be exploited for breeding for resistance.
4. Resistance to spot blotch disease is controlled by additive gene action and its inheritance is polygenic.

5. The previously reported SSR markers for resistance are linked with resistance to *Bipolaris sorokiniana* causing spot blotch disease in Zambia, and can therefore be used for marker assisted breeding.

10 The thesis structure

This thesis is presented in seven different chapters. Chapters are independent of each other and the contents may overlap.

Introduction to thesis

Chapter 1 Literature review.

Chapter 2 Farmers` perception of constraints and preferences of rain-fed wheat varieties in Mpika district of Muchinga province of Zambia.

Chapter 3 Assessing genetic diversity in 150 wheat genotypes using agro-morphological traits and the association between traits.

Chapter 4 Genetic variability among wheat (*Triticum aestivum* L.) germplasm for resistance to spot blotch disease in Zambia.

Chapter 5 Genetic analysis of resistance to spot blotch disease in rain-fed wheat (*Triticum aestivum* L.).

Chapter 6 Validation of microsatellite molecular markers linked with resistance to *Bipolaris sorokiniana* in wheat (*Triticum aestivum* L.).

Chapter 7 Overview of the research findings.

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Chapter 1

Literature review

1.1 Introduction

This chapter will focus on reviewing literature covering relevant topics on breeding wheat genotypes for resistance to spot blotch but firstly it will give insights on (i) origin and history of wheat, (ii) rain-fed wheat production in Zambia, (iii) spot blotch disease and its distribution, (iv) the pathogen and its life cycle, (v) symptoms of spot blotch disease on wheat, (vi) effects of spot blotch on wheat, (vii) management of spot blotch, (viii) breeding for resistance to spot blotch, and (ix) participatory research for crop improvement.

1.2 Origin and history of wheat

Wheat is a self-pollinated plant that belongs to the *Poaceae* (*Gramineae*) family and tribe *Triticeae.* It is known to have been domesticated more than 10, 000 years ago (Marcussen et al., 2014) and originated from Southwest Asia (Poehlman, 1987). The cultivation was first done in Levant countries and Turkey, and the early farming utilized the wild diploid wheat species (DD, *Aegilops)* (Brenchley et al., 2012). Marcussen et al. (2014) reported that due to evolution in agriculture, the wild species were replaced with the domesticated diploid and polyploidy wheat varieties. The cultivated present day common wheat *(Triticum aestivum* L.*)* originated from hybridization between the cultivated tetraploid emmer (AABB, *Triticum turgidum*) and the diploid goat grass (DD, *Aegilops taushii*) (Poehlman, 1987). The common wheat is classified into winter or spring wheat types. Winter wheat is cultivated in the temperate climate as it requires vernalization for it to flower while spring wheat is cultivated in the tropics and subtropics and it does not require vernalization process (Curtis, 2002).

1.3 Rain-fed wheat production in Zambia

In Zambia, spring wheat is the type of wheat grown and can either be grown under irrigated conditions (cool dry season) from May-September, or under the summer rainy season (rain-fed) from November-April. Irrigated wheat is grown by commercial farmers as it is capital intensive. Summer wheat production on the other hand, is dominated by small-scale farmers due to its low production costs. However, the production of rain-fed wheat is low among the farmers and yields range between 1-2 t ha^{-1} (ZARI, 2008). Several factors contribute to

low yield levels of rain-fed wheat. Some of which include higher incidences of foliar diseases (*Helminthosprium* spp. and leaf rust) and aluminimum toxicity (Mooleki, 1997). The conditions during the rainy season such as, high humidity (85%), high night (18^{o}C) and day temperatures (32^{o}C), and dew which keep leaves wet for long periods, provide a perfect environment for the proliferation of a number of diseases (Negassa et al., 2012). Spot blotch (*Helminthosprium* spp.) is the most destructive amongst the diseases causing high yield losses of more than 85% (Raemaekers, 1988). Thus, the productivity of rain-fed wheat could be enhanced through use of cultivars resistant to spot blotch.

1.4 Spot blotch disease and its distribution

Spot blotch, also known as bipolaris leaf blight or helminthosporium leaf blight, is a foliar fungal disease caused by *Bipolaris sorokiniana* (Sacc.) Shoem (Maraite, 1997; Sharma and Duveiller, 2007). It occurs worldwide and is more prevalent in the warmer environments (Kumar et al. 2002; Khan and Chowdhury, 2011; Krishnendu et al., 2011). Warmer environments are often characterised by wet and humid conditions which favour the multiplication and spread of the spot blotch disease (Kumar et al., 2002). Duveiller and Gilchrist (1994) reported spot blotch disease to be more prevalent in areas where temperatures are higher than 17.5^{o}C during the coolest months. The disease has also in recent years been reported in cooler environments (Khan and Chowdhury, 2011).

In Zambia, spot blotch is the major disease limiting wheat production in summer rainy season (Raemaekers, 1988; Mukwavi et al., 1990; Muyanga et al., 1990). Mukwavi et al. (1990) reported that most locations in Zambia are `hot spots` for spot blotch disease. Other areas where spot blotch has been reported to be of economic importance include; Tanzania (Mgonja, 1990), Bangladesh, India (Joshi and Chand, 2002), South Asia (Khan and Chowdhury, 2011), Malawi, Kenya, South Africa, Zimbabwe, Sudan (Krishnendu et al., 2011), Brazil (Duveiller and Gilchrist, 1994), Paraguay, Bolivia (Mehta, 1997), and wheat growing areas of southern China (Van Ginkel and Rajaram, 1997). However, Zambia and Brazil have been identified as the two 'hot spots' for spot blotch disease (Raemaekers, 1988; Duveiller and Gilchrist, 1994).

1.5 The pathogen (*Bipolaris sorokiniana* (Sacc.) Shoem) and its life cycle

Bipolaris sorokiniana (Sacc.) Shoem is a non-specific, virulent pathogen and a causal agent of many diseases such as; spot blotch (leaf spot or leaf blight), head blight, black point, common root rot and crown rot, seedling blight and node cankers in wheat and barley

(Kumar et al., 2002). Apart from wheat and barley, the pathogen also attacks rice, which acts as a green-bridge that increases spore concentration in rice-wheat rotation growing areas (Van Ginkel and Rajaram, 1997). It also affects triticale and a variety of grasses and in some instances dicotyledonous crops like beans (Duveiller and Gilchrist, 1994) and several other crops as listed in Table 1.1 (Iftikhar et al., 2009). This pathogen has many alternative hosts which help to sustain the inoculum throughout the year.

Table 1.1: Other hosts of *Bipolaris sorokiniana* (Sacc.) Shoem

Number	Common name	Scientific name
1	Oats	Avena sativa
2	Peanuts	*Arachis hypogea*
3	Cabbage	*Brassica compestris*
4	Chickpea	*Cicer arientenum*
5	Soybean	*Glycine max*
6	Sunflower	*Halianthus annus*
7	Lentils	*Lens culinaris*
8	Millet	*Pennisetun amaricanum*
9	Sesam	*Sesamum indicum*
10	Sorghum	*Sorghum bicolor*
11	Mash	*Vigna radiate*
12	Mung bean	*Vigna mungo*
13	Maize	*Zea mays*

Source: Adapted from (Iftikhar et al., 2009)

Sources of inoculum for *Bipolaris sorokiniana* (Sacc.) Shoem reported by Malaker et al. (2008) and Reis et al. (1998) are infected seed, volunteer plants, infected crop residues, secondary hosts (Table 1.1) and free dormant conidia in the soil. Infected seed is the major mechanism of pathogen survival and the pathogen starts growing immediately after it comes into contact with moisture (Duveiller and Gilchrist, 1994). This means infected seed provides inoculum to the growing plant (Duveiller and Dubin, 2002; Malaker et al., 2008). Infected seed has been reported to reduce seedling emergence by about 38% (Manamgoda et al., 2011). Furthermore, Manamgoda et al. (2011) revealed that the pathogen can overwinter in the soil and crop residues and thus infect seedlings the following season (Figure 1.1). Researchers including Duveiller and Gilchrist (1994) and Reis et al. (1998) indicated that the spores for *Bipolaris sorokiniana* (Sacc.) Shoem could survive in the soil for about thirty seven months. The conidia are the major dispersal and survival propagules of the pathogen (Reis and WA, 1984). However, the sexual state of *Bipolaris sorokiniana* (Sacc.) Shoem where two compatible types produce perithecia and ascospores was only reported in Zambia (Raemaekers, 1991). Nonetheless, it is considered not important in the disease cycle as it does not contribute much to the genetic variation in the pathogen (Zhong and Steffenson, 2001; Leisova-Svobodova et al., 2012).

The conidia of the pathogen is transmitted from one leaf to another by rain splashes and wind thus increasing disease infection (Duveiller and Gilchrist, 1994) (Figure 1.1). Studies by Duveiller and Gilchrist (1994) and Duveiller and Dubin (2002) showed that leaves that remain moist for more than eighteen hours, at temperatures of 18^{o}C or higher encourage disease development. Areas with temperatures above 17^{o}C during the coolest months and high relative humidity are also reported to be at a very high risk of a spot blotch epidemic (Duveiller and Sharma, 2009). These climatic conditions are very common during the rainy season in Zambia. Frequent rain and dew coupled with high relative humidity of about 85% experienced in the rainy season in Zambia causes wheat foliage to remain wet for longer periods leading to increased fungal germination and sporulation (Raemaekers, 1988). The spore concentration on leaves increases as the crop matures (Alam et al., 1994; Chaurasia et al., 1999) and the severity is reported to be devastating after flowering (Duveiller et al., 2005; Duveiller and Sharma, 2009). The pathogen has also been reported to be more severe in stressed environments and in soils deficient in potassium (Duveiller and Sharma, 2009). According to Manamgoda et al. (2011) potassium reduces multiplication and survival of the pathogen by controlling plant metabolism, hence affecting food supplies to the pathogen. Duveiller and Dubin (2002) in their report indicated that spot blotch is the most aggressive disease caused by *Bipolaris sorokiniana* (Sacc.) Shoem and much effort was needed to breed for cultivar resistance in wheat to control the disease.

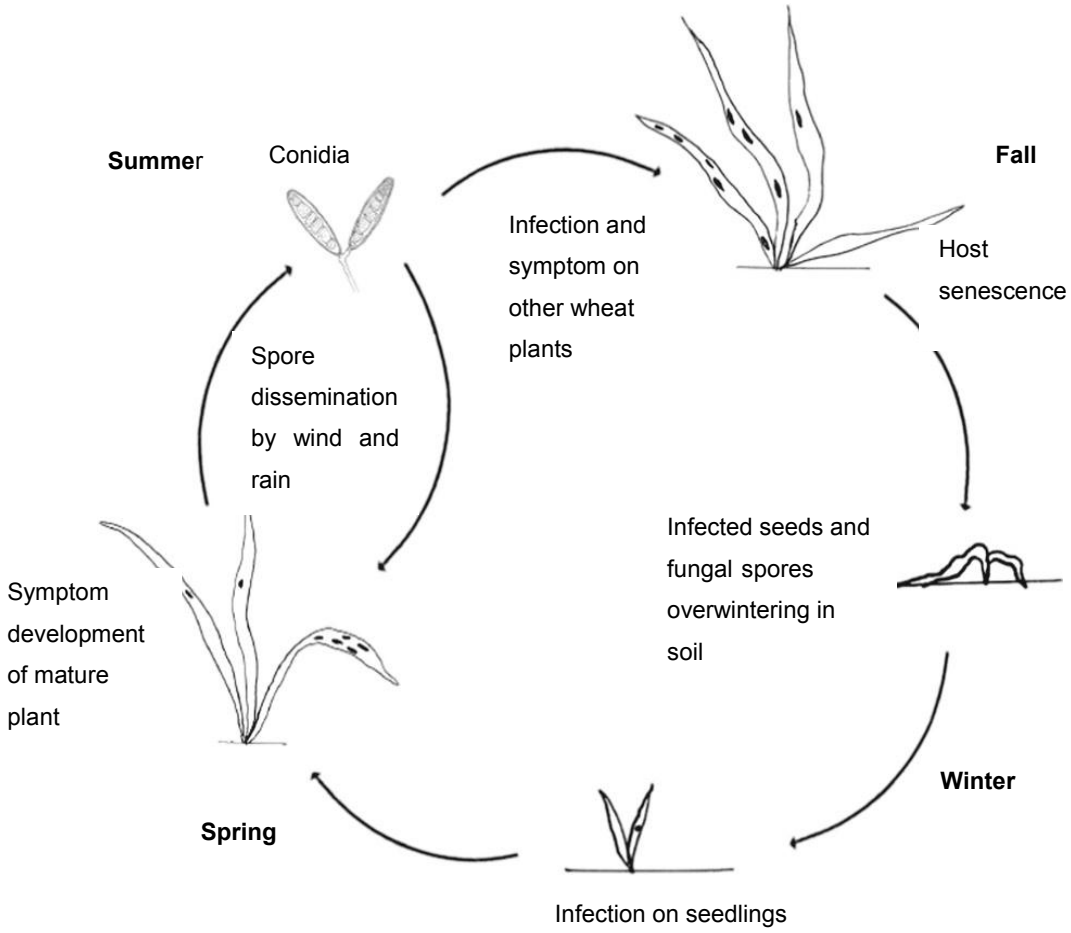

Figure 1.1: Schematic representation of life cycle of *Bipolaris sorokiniana*
Source: Manamgoda et al. (2011)

1.6 Symptoms of spot blotch on wheat

Spot blotch disease causes lesions on leaves that are usually elongated to oval in shape and have a dark brown colour, 1 to 2 mm long and without chlorotic margins on early lesions (Gilchrist-Saavedra et al., 1997; Krishnendu et al., 2011) (Figure 1.2a). Gilchrist-Saavedra al. (1997) and several other researchers (Raemaekers, 1988; Duveiller and Dubin, 2002) reported that as the lesions matures, the center of the lesion turns light brown to tan colour, surrounded by an irregular dark brown ring. As the disease progresses, the lesions coalesce to form large lesions covering the whole leaf causing a reduction in photosynthetic area (Figure 1.2b) (Sharma et al., 2004). Infection on the spikelet (Figure 1.2c) causes shriveled grain with black points (dark staining on the embryo end of the seed) ending up with poor quality grain and reduced yield (Figure 1.2d) (Duveiller and Gilchrist, 1994; Duveiller and Dubin, 2002; Kumar et al., 2002; Krishnendu et al., 2011).

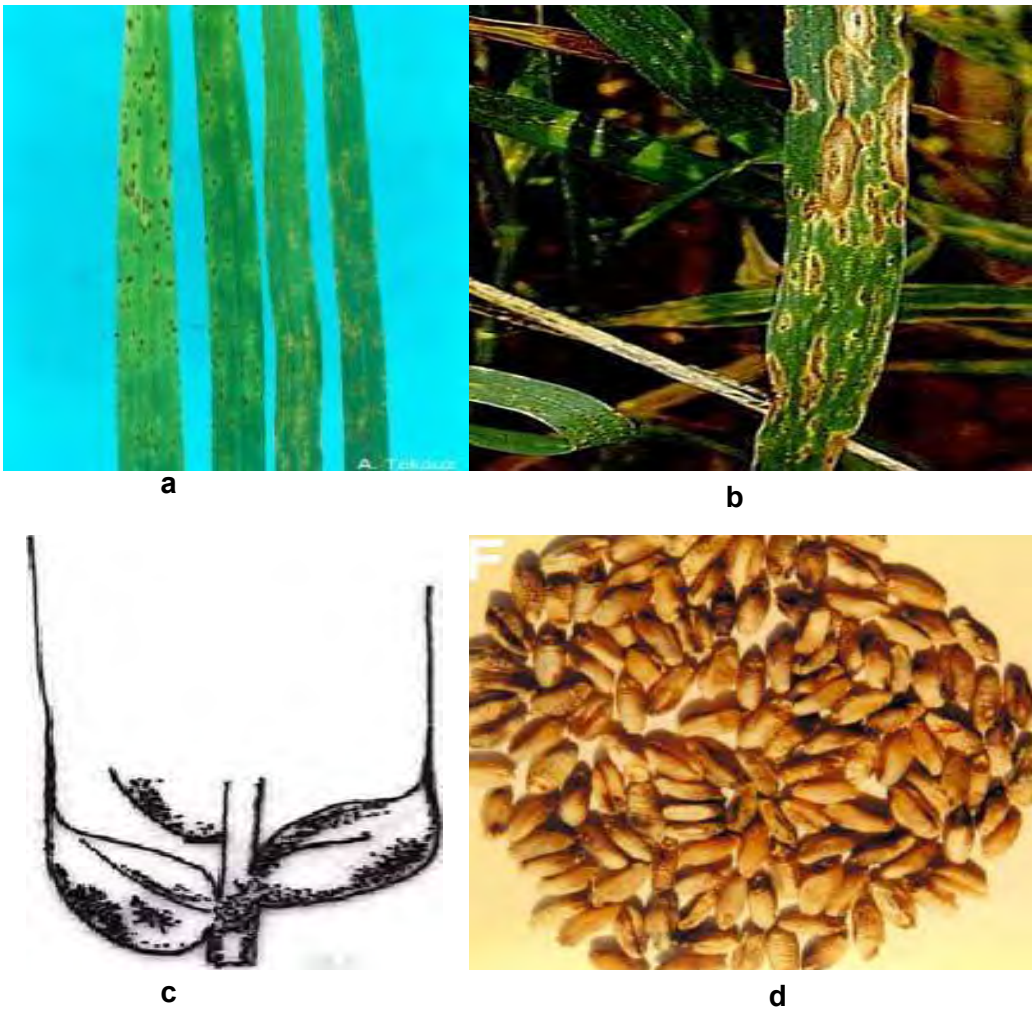

Figure 1.2: Spot blotch symptoms on wheat leaves (a and b), on the spike (c) and black point on wheat grain (d)

Source: (a) and (b) Duveiller et al. (1997), (c) Krishnendu et al. (2011), and (d) Kumar et al. (2002)

1.7 Effects of spot blotch on wheat

Spot blotch is one of the most destructive diseases in warmer and humid environments of the world. Over twenty five million hectares of wheat land have been reported to be infected with the disease causing yield losses ranging from 20-100% (Joshi and Chand, 2002). Spot blotch interferes with photosynthesis through secretion of toxins which inhibit photosynthetic electron transport and photophosphorylation (Duveiller and Gilchrist, 1994), thus affecting the production of sugars in the plant. In addition, the disease absorbs nutrients produced by the plant for its own use instead of being transported to the grain resulting in reduced grain weight and grain yield (Agrios, 1997).

Factors such as sowing time, host genotype and low soil fertility are responsible for increased spot blotch incidence hence high yield losses (Duveiller and Sharma, 2009). For example, in Zambia and Bolivia the effect of spot blotch is reported to be highest in early summer planting, whereas in southern parts of eastern India and Bangladesh, and South America early plantings result in a reduction of yield due to spot blotch (Duveiller et al., 2005; Duveiller and Sharma, 2009). Furthermore, studies done in South Asia revealed that the use of susceptible genotypes coupled with abiotic and soil nutrients stress increases disease severity, hence yield losses (Duveiller et al., 2005). In South Asia, yield loss of between 20 – 52% has been reported (Khan and Chowdhury, 2011) while in India, yield losses of 15.5% have been reported (Joshi and Chand, 2002). In Zambia yield losses of over 85% due to spot blotch have been reported during summer rainy season (Raemaekers, 1988). For this reason, spot blotch was identified in Zambia as a major disease hampering summer rain-fed wheat production both in small-scale and commercial wheat production systems (Namwila, 1983; Raemaekers, 1988; Mukwavi et al., 1990; Muyanga, 1994). In warmer areas of Bangladesh, yield losses of about 80% have been reported (Duveiller and Gilchrist, 1994). Thirty eight percent yield loss has also been reported in farmers' fields in Nepal (Duveiller and Sharma, 2009). Under severe infection, complete crop loss (100%) due to spot blotch is possible because the disease attacks all plant parts (Joshi and Chand, 2002; Duveiller et al., 2005).

1.8 Management of spot blotch disease

Several management methods have been used to prevent the infection and spread of the disease. Fungicides for the control of spot blotch have been reported to be effective more especially the triazole group (e.g. tebuconazole and propiconazole) (Duveiller and Dubin, 2002). Duveiller and Dubin (2002) recommended spraying wheat fields with the fungicide at one- to two-week intervals to keep the disease under control as it is an aggressive disease. However, the problem with fungicides is the cost and availability to the small-scale farmers. Therefore, it is not an economic and efficient way of controlling spot blotch amongst the resource poor small-scale farmers.

Another strategy of managing spot blotch is planting healthy seed; planting seed with less than 30% black point and seed treatment with fungicides (Duveiller and Gilchrist, 1994). Nonetheless, use of healthy seeds has been reported not to be effective unless inocula from the soil and secondary hosts are reduced (Duveiller and Dubin, 2002).

Cultural practices such as adequate fertilization, timely planting, reduced seed rate and sanitation are other methods important for managing spot blotch (Reis, 1990; Duveiller and

Dubin, 2002; Duveiller et al., 2007). Adequate fertilization, thus a balance of nitrogen, phosphorus and potassium application, was found to reduce disease severity by 15-20% (Duveiller and Sharma, 2009). Soils deficient in potassium have been reported to have high disease severity as the pathogen finds it easy to invade the system of the plant (Vergnes et al., 2007). Studies by Vergnes et al. (2007) showed that potassium was important for protecting the plant by directly affecting pathogen development, multiplication and survival, and also controlling internal metabolism, hence affecting food supply to the pathogen.

As for planting date, Duveiller and Sharma (2009) showed that it depended upon which part of hemisphere a country belonged to and the type of cropping system used. In Zambia for example, delayed summer planting would be important to reduce disease infection as early planting favours the development of spot blotch disease (Aulakh and Rimkus, 1988). Adjusting the planting date helps to avoid disease infection so that the period of flowering to milk stage does not coincide with warm and humid conditions conducive for disease development (Mathur and Cunfer, 1993). Duveiller et al. (2005) also reported that adjusting seeding rate could greatly help in reducing the effects of spot blotch disease in most wheat growing areas, as this reduces humidity within the plants micro-environment therefore restricting spread of the disease. Clearing the fields of volunteer crops, weeds, stubbles and secondary-host also reduces inoculum. However, the methods aforementioned have been ineffective in controlling the disease among small-scale farmers in Zambia.

Crop rotation is another important method for reducing amount of inoculum by separating wheat crops in time and space. Several researchers recommended rotation as a reliable method of controlling spot blotch in wheat fields (Naitao and Yousan, 1997; Reis et al., 1998). Rotation of wheat with non-host plants (oil seed rape and vetch) was reported to be effective in reducing soil inoculum (Duveiller and Sharma, 2009). However, Duveiller and Dubin (2002) revealed that rotation periods needed to be long enough to effectively reduce soil inoculum. On the other hand, Loughman et al. (1997) in their studies indicated that although crop rotation does reduce early inoculum on crops, it does not effectively control leaf disease due to movement of inoculum by wind from adjacent areas. The use of crop rotation among small-scale farmers is not a feasible control method due to small farm sizes.

Besides these methods discussed above, use of varietal mixtures has been reported as a potential alternative for reducing the development of spot blotch disease (Sharma and Dubin, 1996; Duveiller and Sharma, 2009). Studies by Sharma and Dubin (1996) showed that cultivar mixtures of different genetic backgrounds reduced the development of spot blotch disease and greatly increased wheat grain yield. However, Duveiller and Dubin (2002) and Joshi et al. (2004a) recommended an integrated approach as a much better way of

controlling spot blotch. Integrated management is a combination of different management control methods and it includes use of fungicides, crop rotation, proper crop management, with host resistance being the basis of the control measures. Studies by Mehta (1997) revealed that integrated management is complicated, moreover the programmes involved in integrated management are costly and not easily accessible to the majority of farmers. Breeding for resistant cultivars is thus the most environmentally friendly, economical, and effective control measure of spot blotch for sustainable agriculture (Naitao and Yousan, 1997).

1.9 Breeding for resistance to spot blotch disease of wheat.

1.9.1 Determination of genetic diversity in wheat

Selection of parents for hybridization is one of the important tasks faced by breeders and it involves exploitation of the greatest genetic variability among genotypes (Yoshida, 2004; Khodadadi et al., 2011). Breeders exploit genetic variability for creation of new improved genotypes with desired traits. Knowledge of genetic variability in the germplasm is critical for designing an effective breeding programme (Herrera et al., 2008). Several techniques have been used to assess genetic variability in wheat genotypes for use in hybridization. Such techniques include morphological and agronomic traits, and various molecular markers. For estimation of morphological and agronomic traits, a standard list of wheat descriptors is used (IBPGR, 1978). Yoshida (2004), reported use of morphological traits to be simple and cheaper than molecular markers though it might not be very reliable as the traits are usually influenced by the environment. Bibi et al. (2009) revealed that although the use of morphological characteristics was cheap, it was time consuming and laborious since extensive field evaluations were required. Nonetheless, phenotypic characterization is still widely used for estimation of genetic variability because it is easily accessible (Zeb et al., 2010).

On the other hand, several molecular markers have been used in wheat for the determination of genetic diversity and have been reported to be quick, efficient and reliable (Bibi et al., 2009; Zeb et al., 2010). Among them, random amplification of polymorphism DNA (RAPDs), restriction fragment length polymorphism (RFLPs), amplified fragment length polymorphism (AFLPs) and simple sequence repeats (SSRs) have been used. Each of these markers has a different principle, amount of polymorphism, application, requirements and cost (Hoisington et al., 2002; Herrera et al., 2008). However, SSR markers have been the mostly used in wheat for genetic diversity characterization because of their efficiency in detecting genetic polymorphism and discriminating among genotypes (Herrera et al., 2008; Salem et al., 2008; Khodadadi et al., 2011). Nonetheless, a combination of phenotypic and

genotypic data provides useful information on genetic diversity to increase efficiency in breeding programme (Yoshida, 2004).

In wheat, genetic diversity has over the year narrowed down due to modern breeding (Zeb et al., 2010). According to Zeb et al. (2010) narrow genetic base negatively affects breeding for biotic and abiotic resistance which require genotypes of different genetic background. Therefore, estimation of genetic diversity of wheat genotypes for hybridization programmes would enhance breeding for biotic resistance in germplasm of local wheat as only parents with higher genetic distance would normally be considered. For this study agro-morphological traits were used to assess genetic variability among 150 genotypes. According to Sammour (2011), morphological traits were the most appropriate and practical tools for assessing genetic diversity in a large number of genotypes.

1.9.2 Sources of resistance

According to Russell (1978), use of resistant varieties is the key to reducing fungal diseases. Several resistance sources from China (Yangmai-6, SW89-5193; SW89-5422, G162, Ning8319), South America (Ocepar7, BH1146; Maringa) and other derivatives of wild crosses such as *Thinopyrum curvifolium* (Chirya-3, Chirya-7) have been used in breeding for spot blotch resistance (Van Ginkel and Rajaram, 1997; Duveiller and Sharma, 2009). Sources of resistance have also been derived from synthetic hexaploid wheats of *Aegilops tauschii* (syn. *Triticum tauschii*) and durum wheats (Van Ginkel and Rajaram, 1997). Resistance to *Aegilops tauschii* has been reported on the D genome (Duveiller and Dubin, 2002). Despite the availability of sources of resistance, breeding for spot blotch resistance in wheat has been rather slow due to the quantitative nature of the inheritance, and variability and aggressiveness of the pathogen (Duveiller and Dubin, 2002; Joshi et al., 2004b; Jaiswal et al., 2007). In their studies, Duveiller et al. (2007) reported ineffectiveness of the selection methods in identifying multiple genes controlling resistance as reasons for the slow progress in breeding for resistance. In Zambia, there is no information about sources of resistance available which is critical in breeding for resistance to spot blotch, hence the study. Although sources of resistance to spot blotch have been identified in China, Mexico and South America, they cannot be used directly in the breeding programme in Zambia because they have not been evaluated under field conditions to establish their resistance. A variety may be resistant in China or South America but susceptible in Zambia due to differences in pathogen variability (Aggarwal et al., 2004). To identify resistant genotypes, there is need to screen genotypes in various environments in Zambia to expose them to existing pathogen variation.

1.9.3 Screening and rating of spot blotch disease

Screening for resistance to spot blotch in wheat has been done either in the field or greenhouse through visual assessment and use of molecular markers. In the field, screening for resistance has been conducted in hot spot areas where the disease is prevalent (Mujeeb-Kazi et al., 2007). Susceptible spreader-row plants have been planted between experimental plots to increase disease pressure in the field to ensure no disease escape (Sharma et al., 2004; Duveiller and Sharma, 2009). Another way of increasing disease pressure is by directly spraying the plants with the spores of the pathogen at different Zadok`s physiological growth stages (GS), GS29 (main shoot and 9 or more tillers) and GS40 booting stage (Zadoks et al., 1974; Duveiller and Sharma, 2009).

Several rating scales have been used to assess resistance in wheat germplasm (Nagarajan and Kumar, 1998). The disease rating scale based on symptoms that combine severity and lesions has been used (Duveiller and Sharma, 2009). However, Duveiller and Sharma (2009) reported that they are difficult to use and are not accurate. Disease incidence by estimating percentage of diseased plants per plot have also been used to assess severity of spot blotch (Iftikhar et al., 2010). Though this method has been reported to be useful in surveys, it might not be informative due to non-specificity of the fungus (Duveiller and Sharma, 2009). Nonetheless, the most common method for assessing foliar diseases other than rust in *Triticum* spp. is the Saari Prescott 0-9 scoring scale (Eyal et al., 1987). Nagarajan and Kumar (1998) indicated that the rating scale based on 0-9 for the mean disease expression on leaves, recorded on the genotype for the each plot of the genotypes under evaluation, was the best method for assessing severity for breeding for resistance to spot blotch. For this study, the Saari Prescott 0-9 scoring scale was employed for assessing the disease.

Several scientists have recommended the use of molecular markers in the screening for resistance to spot blotch as they speed up the process of identifying resistance lines (Kuldeep et al., 2008; Duveiller and Sharma, 2009). Molecular markers associated with resistance to spot blotch include; *Xgwm67* on chromosome 5B, *Xgwm57*0 on chromosome 6A, *Xgwm469* on chromosome 6D (Sharma et al., 2007; Duveiller and Sharma, 2009), *Xgwm437* on chromosome 7D and *Xgwm544* on chromosome 5B (Kumar et al., 2005) and also four QTL on chromosome 2AL, 2BS, 5BL and 6BL (Kumar et al., 2009). Adhikari et al. (2012) identified four genomic regions (chromosome 1A, 3B, 7B and 7D) associated with resistance to spot blotch disease at seedling stage using Diversity Arrays Technology (DArT) markers. Das et al. (2002) identified 18 random amplified polymorphic DNA (RAPD) markers linked with resistance, while Ragiba and Prabhu (2009) identified four RAPD markers associated with resistance to spot blotch in wheat genotype 'Chirya 3'. However, these

previously identified markers require validation before using them in marker assisted breeding to test their usefulness. Awasthi and Lal (2014) reported that molecular markers were only suitable for use in a breeding programme if they constantly appeared in diverse genetic backgrounds. Information on the validation of markers linked with spot blotch resistance in Zambia is non-existent. Thus, the present study was also undertaken to validate *Xgwm544*, *Xgwm570* and *Xgwm437* molecular markers linked with resistance to spot blotch in wheat.

1.9.4 Characteristics associated with resistance to spot blotch

Selection for resistance to spot blotch has been reported to be difficult due to the absence of reliable phenotypic markers (Joshi and Chand, 2002). Several morphological characteristics such as erect leaf posture (Joshi and Chand, 2002), stay-green (Joshi et al., 2007) and leaf tip necrosis (*Ltn*) (Joshi et al., 2004a) have been associated with lower disease severity in wheat. Erect leaf posture has been reported by Joshi and Chand (2002) to be important in reducing spore germination of *Bipolaris sorokiniana* as dew and moisture, critical for spore germination, are not retained on the leaf surface for a long period of time. They also revealed that wheat cultivars with erect leaf posture yield more than those with recurved type of leaves. Stay-green is another characteristic associated with resistance to spot blotch. Plants that exhibit stay-green characteristics or delayed leaf senescence are regarded as resistant to spot blotch as they resist growth and development of pathogens (Joshi et al., 2007). According to Joshi et al. (2007), plants with stay-green characteristic carry out active photosynthesis even under stress conditions (biotic and abiotic). Hence it is an important characteristic for increased wheat productivity under stress environments (Joshi et al., 2007; Rehman et al., 2009). In addition, the trait has been reported by Ibrahim and Quick (2001) to be heritable. Leaf tip necrosis (*Ltn*) associated with *Lr34/Yr18* or slow mildew development has also been reported to be an important marker for durable resistance against spot blotch disease in wheat (Joshi et al., 2004a). However, Duveiller and Sharma (2009) indicated that the trait might not be useful under severe spot blotch epidemic.

Other traits that have been used for screening and are associated with resistance to spot blotch include a bright, golden peduncle (Van Ginkel and Rajaram, 1997), and greener and thinner leaves with less distance between vascular bundles (Rosyara et al., 2007), although it has been reported that the traits are complex for use in breeding (Duveiller and Sharma, 2009). In order to further determine potential parents that would produce superior progenies, knowledge of gene action and inheritance of resistance to spot blotch disease is vital.

1.9.5 Gene action and inheritance of resistance to spot blotch in summer rain-fed wheat

In any breeding programme, it is important to understand the mode of gene action and heritability of the trait to formulate an efficient programme to be followed for crop improvement of the desired character (Khattab et al., 2010). Information on gene action can be acquired by evaluating progenies obtained through hybridization between genetically diverse parents, and also from estimating combining ability (general and specific) effects (Joshi et al., 2004; Muthuramu et al., 2010). General combining ability (GCA) provides information mainly on additive gene action, while specific combining ability (SCA) provides information on non-additive gene action (Iqbal and Khan, 2006). Falconer and Mackay (1996) reported three types of genetic effects (components), namely additive, dominance and epistasis, for quantitative traits. According to Falconer and Mackay (1996) additive genetic variance is transmitted from parent to offspring and it is the main determinant of resemblance between relatives and of response to selection. Dominance gene action favours the production of hybrids, while additive gene action indicates that standard selection techniques would be effective in bringing beneficial change in the character in question (Azizi et al., 2006).

Salama et al. (2006) indicated that the knowledge of the nature of gene action is important as gene action provides a guideline for selecting superior parents for improving desired traits. Yang et al. (2002) and Singh et al. (2007) showed that if the additive gene effects were greater than non-additive gene effects then selection could be done in early segregating generations. With regard to spot blotch disease, studies by Sharma et al. (2004) showed that general and specific combining ability were both important in the expression of resistance to the disease, implying both additive and non-additive gene action were important in resistance to spot blotch disease. Nonetheless, they reported that additive gene mechanism played a larger role in controlling resistance to spot blotch disease than non-additive gene action. Similar findings were also reported by Khan et al. (2010a, b).

Studies on the inheritance of resistance to spot blotch disease in wheat have been limited (Joshi et al., 2004b) hindering progress in breeding for resistance to the disease (Joshi et al., 2004b; Goel et al., 2010). There are some contradicting reports on the inheritance of resistance to spot blotch disease. Some studies have indicated the existence of monogenic inheritance (Duveiller and Dubin, 2002), while several other researchers have suggested quantitative (polygenic) inheritance (Duveiller and Gilchrist, 1994; Kumar et al., 2002; Duveiller and Sharma, 2009) for resistance to spot blotch disease. Similarly, Kuldeep et al. (2008) indicated that polygenes were important in the inheritance of spot blotch disease resistance. A study by Dubin and Rajaram (1996) and Joshi et al. (2004b) indicated three

additive genes controlling the inheritance of resistance to spot blotch disease in wheat. In a field study in Nepal, Neupane et al. (2007) reported that spot blotch resistance was dominant and controlled by one major gene in wheat germplasm. However, seedling resistance to spot blotch disease was reported to be controlled by two complementary recessive genes (Singh et al., 2000; Ragiba et al., 2004). Sharma et al. (2006), and Duveiller and Sharma (2009) showed that dominant and recessive genes controlled the inheritance of resistance and in some instances epistasis has been reported. Sharma et al. (2006) found that resistance to spot blotch was conditioned by partially dominant genes and inherited quantitatively with moderate to high heritability estimates. Therefore, based on these reports, it seems that the inheritance depends on the genetic background of the material used. This shows why these studies would be important on Zambian wheat germplasm.

Development of resistance to spot blotch in wheat in Zambia still remains a challenge. Currently, there have been no studies in Zambia regarding gene effects or inheritance of resistance to spot blotch disease in summer rain-fed wheat cultivars. Genetic studies to obtain information on heritability of resistance to spot blotch disease are essential to make progress in breeding for resistant cultivars. This suggests the importance of investigating inheritance of resistance to spot blotch disease in summer rain-fed wheat cultivars.

In wheat, several mating designs have been used to understand the genetics of resistance to spot blotch disease. However, the most extensively used designs are the diallel Griffing (1956) approach, line x tester, generation mean analysis and to some extent the diallel Hayman (1954) approach. In this study, gene action responsible for controlling resistance to spot blotch disease resistance in rain-fed wheat was studied through the diallel mating design Hayman (1954a) approach and generation mean analysis.

1.9.6 Mating designs

Diallel analysis using Hayman approach (1954a) is one of the most preferred method for diallel analysis as it determines gene action as well as detecting the presence or absence of epistasis and maternal effects. Hayman approach uses two steps in analysing diallel crosses; the numerical and the graphical representation approach (Singh and Chaudhary, 1995; Sharma, 2006). Both the numerical and the graphical representation provides information on gene action. In this analysis, the following assumptions (Hayman, 1954a) are to be fulfilled; diploid segregation, no difference between reciprocals, independent action of non-allelic genes, no multiple allelism, homozygous parents and genes are to be independently distributed between the parents. Thus, information obtained from diallel analysis using Hayman approach could be of great value to breeders in designing effective selection methods. In wheat, Hayman approach has been used to estimate genetic effects of quantitative traits (Chowdhry et al., 2002) and also resistance to diseases such as stem rust

(Cheuiyot et al., 2014) and stripe rust (Dehani and Moghaddam, 2004). However, there are no reports on Hayman approach for studying the gene effects of resistance to spot blotch.

The generation mean analysis (GMA) has also been widely used in different crops including wheat to estimate genetic effects of quantitative traits including resistance to diseases (Checa et al., 2006; Hussain et al., 2011; Zaazaa et al., 2012, Tetteh et al., 2013). However, in wheat, reports on GMA for resistance to spot blotch are very few. In an experiment conducted by Goel et al. (2010), generation mean analysis revealed that resistance was controlled by two dominant genes with duplicate epistastic interaction in the cross involving UP338, PBW154 and Sonalika, while for the cross between UP338 x WH58 and Sonalika x WH58 they found complimentary gene interaction conditioning resistance to spot blotch disease. Thus, GMA was used in this study to attain a better understanding of resistance to spot blotch disease using six generations; P1, P2, F1, F2, two backcross generations (BCP1 and BCP2) and also to compliment the genetic information achieved from the diallel analysis.

Like in the diallel, GMA has been used in both cross- and self-pollinated plants. The advantage of using GMA is that the errors incurred using this technique are smaller as it works with means (first order statistics) rather than the variances (second order statistics) (Bernardo, 2002). Singh and Singh (1992) revealed that GMA, though a simple technique, provides opportunity to measure both the main gene effects (additive and dominance) and the epistatic gene interaction (additive x additive, additive x dominance and dominance x dominance) which diallel analysis cannot detect. They indicated a greater reliance could be placed on the genetic outcomes found when generation mean analysis was used in combination with other designs.

1.9.7 Maternal effects in the inheritance of resistance to diseases

Maternal effects can be defined as the contribution of the maternal parent to the phenotype of its offspring beyond the equal chromosomal contribution expected from each parent (Roach and Wulff, 1987; Chahal and Gosal, 2002). Maternal effects are regarded as a source of error and a nuisance that reduce the precision of genetic studies (Falconer and Mackay, 1996) in that they increase environmental error and inflate genetic variance thus reducing response to selection (Roach and Wulff, 1987; Falconer and Mackay, 1996). In genetic studies, maternal effects are investigated through reciprocal crosses (Roach and Wulff, 1987; Zhu and Weir, 1994). Roach and Wulff (1987) revealed that reciprocal pairs have comparable nuclear genetic contribution such that any disparity in performance between them will be due to maternal effects.

In wheat, maternal effects have been reported to greatly influence grain protein, grain weight (Millet et al., 1984; Barnard et al., 2002) and grains per spike, but not number of tillers per plant, peduncle length and thousand grain weight (Hussain et al., 2008). On the other hand Joshi et al. (2004c) reported that reciprocal differences were not significant in wheat. No studies on maternal influence on resistance to spot blotch have been conducted. Therefore, this study aims at investigating the contribution of maternal effects to resistance of spot blotch in wheat. The presence of maternal effects would influence the choice of females to be used in breeding for resistance to spot blotch while its absence suggest that they would be no genetic effects in using a parent as either male or female.

1.10 Participatory research for crop improvement

Participatory plant breeding involves farmers in breeding programmes and is necessary in addressing their problems as they get involved with selecting varieties of their choice (Ceccarelli and Grando, 2009). Participatory research could therefore facilitate identification of cultivars preferred by farmers. Ceccarelli and Grando (2007) and Witcombe et al. (1996) reported that in conventional breeding, breeders produce and release varieties without knowing and understanding farmers' preferences. For this reason most newly released varieties were often rejected or not adopted by the farmers (Witcombe et al., 1996; Danial et al., 2007). Ceccarelli and Grando (2009) found conventional breeding method to be limiting as it favoured farmers in high potential environments rather than those in marginal environments. This leads to unsuccessful plant breeding programmes in marginal areas. Further, breeders often bred high yielding varieties on-station that did not perform well in farmers` fields or they lacked qualities preferred by the farmers (Ceccarelli and Grando, 2009). Farmers are the main players in breeding as they decide on whether to adopt the new variety or not, depending on the traits of the new variety (Dawson et al., 2008). Therefore, participatory research is crucial in obtaining information from farmers regarding their preferences and constraints they face. This would assist breeders to breed crops which would easily be adopted and in turn help to increase food security at both household and national levels. Thus, participatory breeding is a critical approach for an effective breeding programme and for enhancing adoption of varieties by farmers. This study therefore investigated farmers` preferences and production constraints of the summer rain-fed wheat cultivars.

2.0 Summary

It has been established from the literature that wheat is an important cereal crop in Zambia, whose consumption comes second after maize. It is produced as a summer rain-fed and

irrigated crop in winter. Summer production is dominated by small-scale farmers whose yields range from 1-2.5 t ha^{-1}. Higher summer rain-fed yields are limited by a number of factors; high temperature, drought, aluminium toxicity and diseases. Spot blotch and leaf rust are the major disease but spot blotch was found to be the most devastating disease limiting high summer rain-fed wheat yields in Zambia and also in many warmer humid wheat growing areas in the world. In Zambia there are no cultivars resistant to spot blotch as much effort has been concentrated on breeding high yielding wheat cultivars for favourable environments. Use of resistant cultivars is the only practical, effective and sustainable solution of controlling spot blotch disease. Other methods of control such as fungicides, crop rotation and integrated management are too expensive and not feasible on small-scale farmers' fields.

Visual screening is the most common method used for identifying resistant phenotype and to some extent molecular markers. Morphological traits that have been associated with resistance to spot blotch and could be used for direct screening include stay-green, leaf-tip necrosis and erect leaf posture. Molecular markers have been suggested as an important tool for screening spot blotch resistance. Molecular markers linked with spot blotch resistance have been identified. However, these require validation in several genetic backgrounds to test their effectiveness before using them in marker assisted programme. Unfortunately, reports on validation of these markers are limited.

On inheritance of resistance to spot blotch, some reports indicated monogenic while others indicated polygenic. Dominant and recessive genes were reported to control inheritance of resistance to spot blotch and in some instances epistasis. Diallel mating design (Griffing, 1956) has been widely used in wheat to study genetic effects. Generation mean analysis (limited), line x tester cross and to some extent diallel Hayman (1954a) approach have been used.

It has been established that involving farmers in breeding programmes will likely improve farmers` adoption of the new cultivars. This is because farmers provide the necessary information about traits important in their local environment.

It is clear from the literature reviewed that information of resistance to spot blotch in wheat is available in Mexico, India, Bangladesh, Brazil, Australia, Pakistan, while limited information is available from Southern Africa. Although sources of resistance have been identified in the aforementioned countries, they cannot be used directly in the breeding programme in Zambia because they have to be evaluated under field conditions to establish their resistance. Moderate success in breeding for resistance to spot blotch has been reported in some countries, but not in Zambia, yet spot blotch greatly affects summer rain-fed wheat

yields. This literature reviewed has shown that spot blotch resistance is the major challenge that needs to be addressed to achieve anticipated high production levels of wheat yields in the summer rainfall productions. The challenge is to identify sources of resistance to spot blotch with different genetic background and breed resistant cultivars adapted to the local conditions and with farmer preferred traits to enhance adoption.

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Chapter 2

Farmers' perceptions of constraints and preferences of rain-fed wheat varieties in Mpika district of Muchinga province of Zambia

Abstract

Wheat production amongst the small-scale farmers in Zambia has declined over the years. To determine the causes of this decline, a participatory rural appraisal (PRA) was conducted in Mpika district of Muchinga Province of Zambia as a case study to assess farmers' perceived constraints and preferences of rain-fed wheat varieties. Focus group discussions, semi-structured questionnaires, scoring and ranking were used in the study involving 32 male and 27 female farmers. The results showed that both men and women were involved in wheat production. Only one wheat variety (Coucal, amber coloured variety) has been grown by small-scale farmers since 1988. Since then, no other rain-fed varieties have been introduced to the area. Most farmers indicated that wheat was a very important dual purpose crop in their area as it was used for home consumption and income generation. The average wheat fields were 0.48 ha with 1.5 t ha $^{-1}$ as the average grain yield. Family labour (64.4%) was the major source of human power in most farming activities in the study area. Major rain-fed wheat production constraints identified were lack of wheat seed reported by 86.0% of the respondents, bird damage reported by 72% of the respondents followed by termite attacks (32.2%), weeds (23%), diseases (spot blotch, smut and ergot) and lack of markets (16.4%) and dry spell (drought) (11.0%). The most important traits preferred by farmers in order of importance were grain colour (white coloured grain), high yielding and disease resistance. Other preferred attributes were resistance to termite attack, bird damage and drought. Increasing wheat production amongst small-scale farmers will greatly increase household income to improve livelihoods. In order to improve and sustain wheat production amongst small-scale farmers, it is thus imperative that wheat breeders in Zambia address attributes preferred by farmers in their breeding programmes.

2.1 Introduction

Wheat *(Triticum aestivum* L.*)* is the second most important cereal crop after maize in Zambia (FAOSTAT, 2009). It is produced both during the summer rainy season and cool dry season under irrigation. Summer wheat production is dominated by small-scale farmers whose yield ranges from 1 to 2 t ha^{-1} (ZARI, 2008). This low yield is attributed to rainy season wheat disease complexes and abiotic stresses such as high temperature and soil acidity (Raemaekers, 1988; Mooleki, 1997). Leaf spots and head blights are the common diseases during the rainy season due to wheat foliage being wet for long periods of time from dew and regular rains which encourage fungal germination and sporulation (Raemaekers, 1988; Mukwavi et al.,1990). The spot blotch disease which infects all plant parts, is the major problem that needs attention, for a successful rain-fed wheat production in Zambia (Raemaekers, 1981) as most of the wheat varieties grown do not have adequate resistance to the disease. Yield losses of over 85% due to spot blotch have been reported and occasionally, under severe attack, complete crop loss results (Raemaekers, 1988). The disease also lowers the grade and quality of the wheat grain.

Another problem contributing to a reduction of rain-fed wheat production potential was that production preceded research (Hurd, 1981). Plant breeders were mainly concerned with high yielding cultivars while overlooking other important traits vital to the farmer. This meant that wheat varieties were given to farmers without prior knowledge as to whether they performed well in farmers` fields or whether they had qualities preferred by the farmers. Therefore, once these problems are dealt with, summer rain-fed wheat production could expand and complement winter production. Hurd (1981) reported that rain-fed wheat was easier to manage by small-scale farmers and could play a vital role in increasing wheat production in Zambia. Accordingly, for any breeding programme to be successful, knowledge of farmers` perceived constraints and preferences should be considered (Odengo et al., 2002).

Participatory research is one of the strategies that enables researchers to identify problems faced by local communities and assist in finding solutions as the local people are actively involved in the process (Feldstein and Jiggins, 1994). It also enhances communication with farmers (De Groote et al., 2004). Ceccarelli and Grando (2007) reported that participatory research was a necessary tool in addressing farmers` problems as the farmers themselves actively participated in selecting varieties of their choice. Odengo et al. (2002) and Pungulani et al. (2012) reported that any technology that does not consider farmers' preferences and conditions has a reduced chance of being accepted and adopted. Sibiya et al. (2013) reported low adoption of modern maize hybrids and improved OPVs amongst the farmers in the Amazizi districts of KwaZulu-Natal, South Africa, as these maize varieties lacked farmer preferred characteristics. Joshi and Witcombe (2003) reported that farmers in Nepal chose

their own preferred local rice landraces which were then crossed with exotic cold tolerant parents. Their report also indicated that a new cultivar from the same cross was released in 1996 which according to Gyawali et al. (2007), became popular amongst farmers and was willingly adopted. A study conducted in Malawi (Pungulani et al., 2012), revealed that bambara groundnut genotype 2762, which was one of the top four high yielding, was not among the list of farmers` best four preferred cultivars due to lack of traits preferred by farmers.

Farmers are the main players in breeding as they decide on whether to adopt the new variety or not, depending on the traits of the new variety (Dawson et al., 2008). It is, therefore, important to include farmers' preferences at an initial stage in the breeding programme to enhance the likelihood of adoption of the varieties. This would in turn help to increase food security at both household and national levels. In Zambia, there is lack of information about summer rain-fed wheat production constraints and variety preferences from a farmers' point of view. The objectives of this study were, therefore, to investigate farmers' rain-fed wheat production constraints and variety preferences.

2.2 Materials and methods

2.2.1 Study area description

The study was conducted in Mpika district of Muchinga province of Zambia because of the district`s potential of rain-fed wheat production by small-scale farmers. In this district, two areas, Mufubushi; latitude 12º06.624' S and longitude 31º14.635' E, altitude of 1409 meters above sea level (masl) and Mpika Main; latitude 11º47.994' S and longitude 31º27.202' E., altitude of 1375 masl were used in the survey. These areas are located in Region III, a high rainfall region which normally receives over 1250 mm rain per annum from November to April (Aregheore, 2009) (Figure 2.1). The district has several soil types, but the dominant one is Acrisol (clay-rich) (Soils Research Team, 2002). Mpika district also has an annual average maximum temperature of 30.0ºC and an annual minimum temperature of 10.1ºC (Aregheore, 2009). The total population and number of farmers in Mpika district is shown in Table 2.1 below.

Table 2.1: Number of villages, farmers and total household in Mpika Central Block

Name of the camp	Number of famers males	females	Total number of farmers	Number of villages	Number of household	Total population
Mufubushi	1837	1464	3301	34	1550	10850
Mpika Main	2645	2005	4650	49	4221	25326
Total	4482	3469	7951	83	5771	36176

Source: Mpika block office, Mpika District, Muchinga Province, Zambia

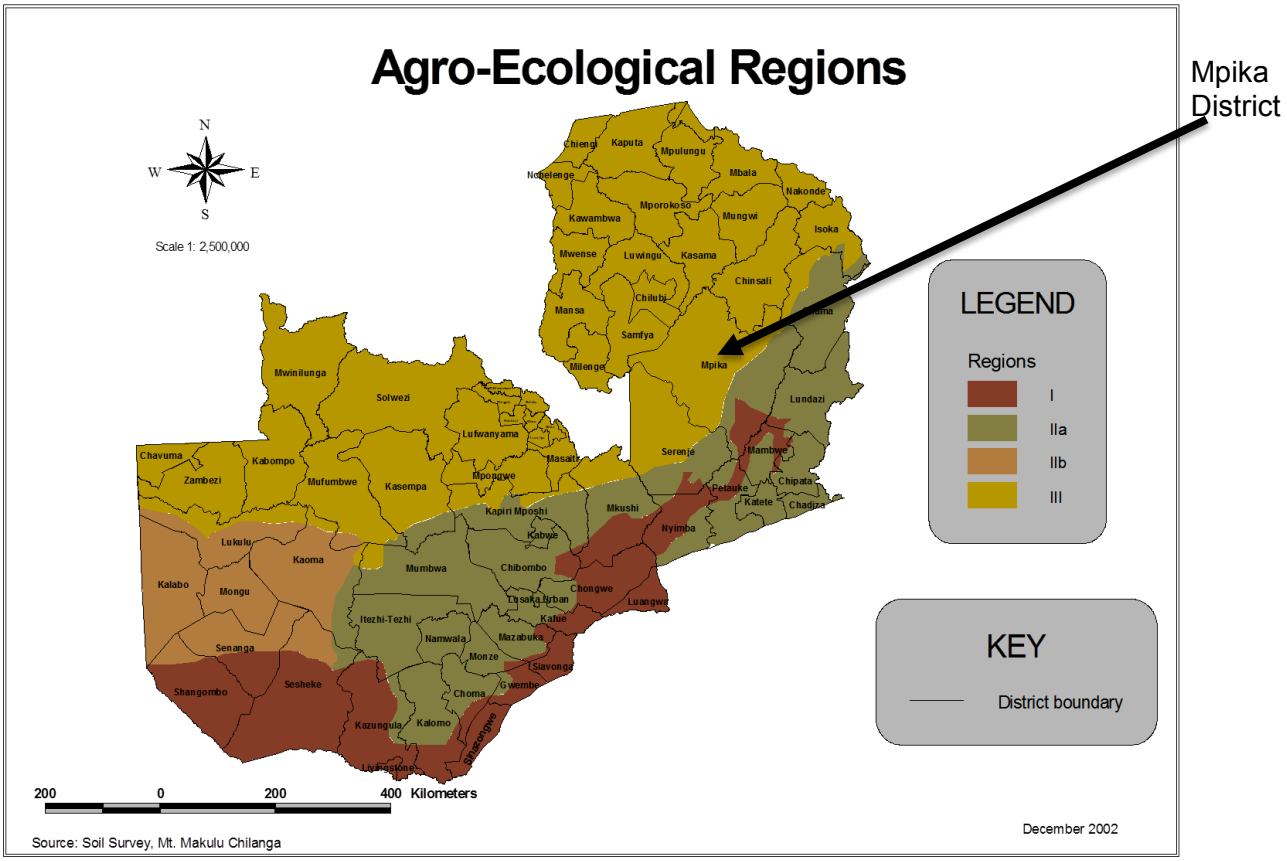

Figure 2.1: Map showing Agro-ecological zones of Zambia and Mpika district the study area
Source: Soil Survey, Mt. Makulu, (2002)

2.2.2 Selection of farmers

Fifty-nine farmers from 13 villages were involved in the formal and informal participatory rural appraisal (PRA) interviews. Of the 59 respondents (Table 2.2), 32 were male representing 54.2% of the respondents, while 27 were female representing 45.8%. The farmers were

selected with the help of the local agricultural extension officers based on the farmers' farming knowledge and also knowledge on wheat production. Farmers selected were of various ages ranging from 16 years old to above 55 years old.

Table 2.2: Total number of wheat farmers selected for the PRA in Mpika District

	Village	Females	Males	Total number of wheat farmers
1	Mufubushi Centre	4	8	12
2	Changilo	2	2	4
3	Kafuko	1	1	2
4	Kambale	2	2	4
5	Kapoko	0	1	1
6	Kawama	1	1	2
7	Kwacha	3	1	4
8	Lubanga	1	1	2
9	Mpika main	4	4	8
10	Mubabe	1	3	4
11	Mumbulu	2	4	6
12	Mwaisen	5	2	7
13	Sotambe	1	2	3
	Total number of wheat farmers	27	32	59

2.2.3 Data collection

The research team consisted of the principal investigator, a research scientist expert in participatory appraisals and two agricultural extension officers from the Ministry of Agricultural and Livestock of the extension branch. Before meeting the farmers, the research team brainstormed on how best to conduct the study in order to obtain the required information. During the meeting with the farmers, one member of the research team who was fluent in the farmers' local language explained the purpose of the research to be carried out in their areas.

The participatory rural appraisal was carried out in November 2013. Primary and secondary information data were obtained during the study. Primary data sources were obtained using PRA techniques such as focus group discussions, semi-structured questionnaires, ranking and scoring (Davis, 2001). Focus group discussion was used to identify common problems facing the rain-fed wheat small-scale farmers in these areas as described by Cavestro (2003). All 59 farmers were involved in focus group discussions and groups comprised both men and women (Figure 2.2). The discussion was guided by open ended and semi-structured questions which covered issues related to wheat production constraints, solutions

on how to manage wheat production constraints, characteristics preferred by farmers in summer rain-fed wheat cultivars, type of wheat they would like to grow, availability of wheat markets and other general information. During the focus group discussions farmers also scored and ranked major crops grown in their area and wheat production constraints. According to Adebo (2000), PRA scoring and ranking assists to identify group and individual priorities. During the group discussions, women spoke freely in the presence of their male folks.

Figure 2.2: Focus group discussion in Mpika district, November 2013

The individual interviews enabled farmers to express themselves freely without the influence of the group (Figure 2.3). In addition, it helped to identify individual farmers' priorities. Secondary data was obtained from the district extension offices of Mpika.

Figure 2.3: Individual discussions at Mufubushi and Mpika-Main areas, November 2013

2.2.4 Data analyses

Data obtained from the group discussions, scoring and ranking, and semi-structured interviews was used for analysis. The data was coded and analysed using SPSS version 16.0. Simple descriptive statistics such as frequency distributions, percentages and means were used to determine relationships among the data.

2.3 Results

2.3.1 Social economic and agricultural information of Mufubushi and Mpika-Main areas

2.3.1.1 Household and demographic information

About 84.7% of the farmers interviewed were married. Additionally, 20.3% of the respondents were below 35 years old. Those between the age of 36 to 55 years and above 55 years were 55.9% and 23.0%, respectively. In terms of education, most of the farmers (98.3%) attended formal education. It was observed that the majority (59.3%) of the farmers in the study areas attended primary level education with Mufubushi having the highest number (60.8%) than Mpika-Main (50.0%). Those who attended junior and senior secondary level education were 18.6% and 22.0%, respectively, across the study areas.

2.3.1.2 Land size and preparation, and agricultural information access

Farming in these areas was basically at small-scale level with a small range of crops. The average land size per household was 15.6 hectares (ha) with 1.5 ha and 68 ha as minimum

and maximum land sizes, respectively. However, the average land size used for farming was 4.83 ha with 0.75 ha as minimum and 5.5 ha as maximum. The most common farming implements owned by most household were the hand hoes, axes and ox drawn ploughs. Hoes were owned by 94.9% of the households, axes by 61.0% while 23.7% of the households owned ox drawn ploughs.

In terms of method of land preparation, 64.4% of the households in Mufubushi and Mpika-Main areas prepared their land for farming activities using hand hoes while 16.9% used ox drawn ploughs and 18.6% use both hand hoes and ox drawn ploughs. Furthermore, 98.3% of the households indicated that they produced enough food through to the next harvest season. Ninety-three percent of farmers accessed agricultural information through agricultural extension workers.

2.3.1.3 Food and cash crops grown by farmers

Ninety-six percent of the respondents identified maize as the major crop grown because it was their staple food (Figure 2.4). In Mufubushi, the second major crop was beans reported by 80.4% of the farmers and was ranked second after maize (Table 2.3) followed by soybeans, groundnuts, sunflower, cassava and then finger millet. In Mpika-Main the second major important crop was groundnuts reported by all the respondents (100%) and was ranked second to maize, followed by soybeans, sunflower, beans, cassava and finger millet. Bambara groundnuts, paprika, rice, sugarcane, sweet potatoes, Irish potatoes, bananas, vegetables, green maize and star grass were grown as minor crops in both areas. Sunflower and soybeans were grown as cash crops while beans and groundnuts were mainly grown as intercrops in maize, cassava and finger millet fields. This confirmed the ranking during group discussions.

The study established that although wheat was not listed amongst the top seven major crops, farmers disclosed that it was the most desired crop in almost all the households in the study area. This was confirmed by the number of people who approached the research team for wheat seed after the group discussion. Out of the 59 respondents 39 (66.1%) were involved in wheat production. These farmers indicated that wheat was a dual purpose crop, that is, it was used mainly as a cash crop as well as for home consumption.

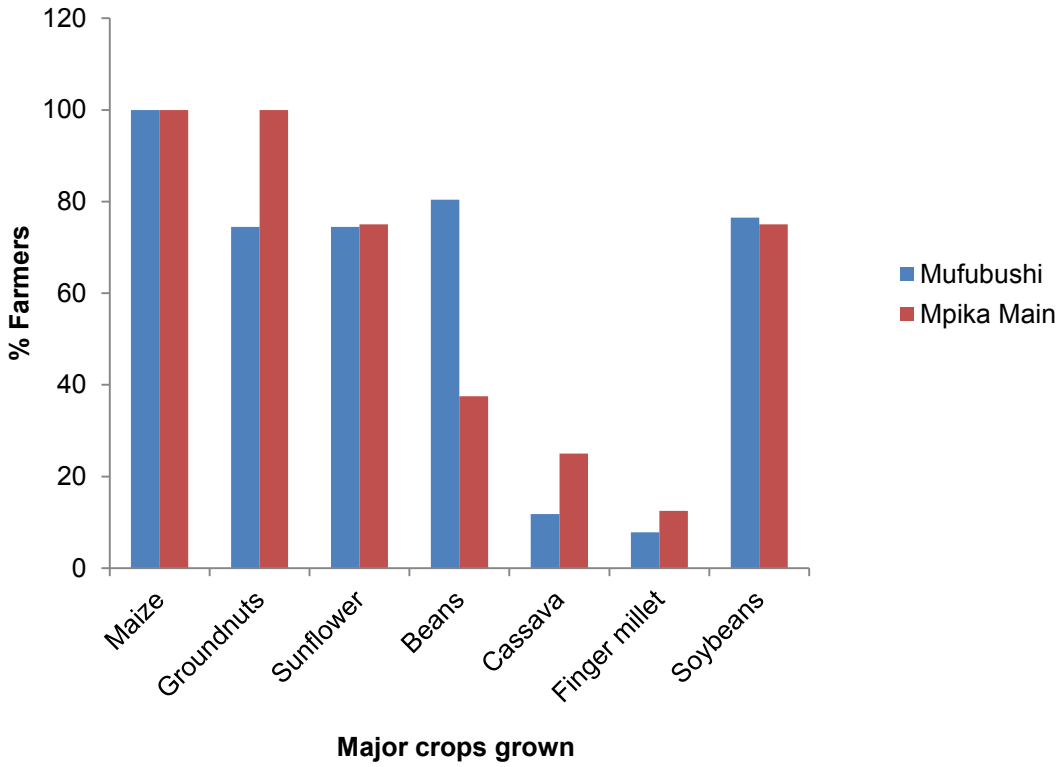

Figure 2.4: Percentage of farmers indicating the major crops in the study areas

Table 2.3: Mean values of farmers' ranking of the major crops

Crop	Mufubushi (n=51)	Mpika Main(n=8)
Maize	1	1
Groundnuts	4	2
Sunflower	5	3
Beans	2	6
Cassava	7	3
Finger millet	3	7
Soybeans	5	5

2.3.2 Wheat production in Mufubushi and Mpika -Main

In Mufubushi and Mpika-Main, wheat was mainly grown during the summer season as a rain-fed crop. Both men and women were involved in wheat production. A wheat variety named Coucal (amber coloured variety) introduced to the area in the late 1980s by Zambia-Canada wheat project, was the only variety grown. The wheat fields ranged from 0.25 ha to 3.0 ha, with an average of 0.48 ha. Farmers reported yields of 0.2 - 4.0 t ha^{-1} with an overall mean of 1.25 t ha^{-1}. Most farmers (69.2%) (27 of the 39 wheat farmers) planted wheat in

December and the rest planted in January and November. The crop was planted in rows and hand drilling was the most popular sowing method in the study area. More than 53.8% of the farmers used hand hoes to control weeds in their fields while 46.2% used both chemicals and hand hoe weeding. Basal and top dressing fertilizer were applied to enhance wheat growth and production. The amount of fertilizer applied per hectare ranged from 100 kg to 300 kg ha^{-1}. Farmers followed the recommendations made by Zambia-Canada (Zam-Can) wheat projects and Catholic projects that had operated in the area. These projects were largely the source of fertilizers. Less than 10% of the farmers bought fertilizer on their own.

Over the past 25 years, there has been a reduction in wheat production in these two areas. Many farmers (86.0%) attributed this reduction to lack of availability of improved wheat seed in the area. This was confirmed by the extension workers. Lack of readily available local markets for wheat was another cause for the reduction in wheat production.

2.3.3 Wheat production constraints as perceived by farmers

Wheat production constraints identified by farmers are presented in Table 2.4. On average most of the farmers interviewed ranked bird damage as the number one limiting factor to wheat production. They revealed that bird damage was serious during the milk dough stage of wheat seed production and hence required constant scaring to protect it from damage. The majority of farmers, 96.6%, indicated that they resorted to physical bird scaring as there was no other way of protecting their wheat from birds. The investigations showed that farmers found bird scaring increasingly onerous as it required constant scaring for a minimum of three weeks per season. Termites were ranked as second most important factor affecting rain-fed wheat production. Although termites were a problem, 61.3% of the farmers did not apply chemicals nor other protective measures to control them, while 25.8% and 12.9% applied chemicals and wood ash, respectively, to reduce termite damage. It was observed that farmers believed that poor soils and dry spells were the main causes of termite damage.

Weeds were ranked the third most important constraint. Most of the farmers (53.8%) relied on hand hoe weeding while 46.2% used both chemicals and hand hoe weeding. The farmers that depended on hand hoe weeding reported weeding to be time consuming, tedious and unpleasant as it required several weedings to control the weeds. Further investigation revealed that weeding in wheat fields required skill to differentiate wheat from weeds especially during early stages of wheat growth. Any slight mistake in the differentiation of wheat from weeds led to the removal of the wheat crop by mistake. Lack of reliable markets was ranked fourth as a wheat production constraint. Table 2.5 shows farmers views on how wheat marketing could improve in their area. Other important production constraints identified by farmers were diseases, bad weather (dry spells/drought), labour involved in

harvesting and threshing of wheat, and red ants (they believed that red ants sucked sap from wheat plants hence weakening the plant).

Table 2.4: Wheat production constraints as viewed by farmers in the study areas

Constraints	% of respondents	rank values
Birds	72.0	1
Weeds	23.0	3
Lack of markets	16.4	4
Termites	32.3	2
Diseases	16.2	5
Drought	11.1	6
Labour	10.0	7

Table 2.5: Farmers proposed views on how wheat marketing could improve

	Proposed views on improving marketing of wheat.
1	Advertising wheat markets so that farmers are aware of them
2	Government commitment to rain-fed wheat production by buying their wheat as they do with crops such as maize
3	Government linking farmers to markets
4	Farmers forming agricultural co-operatives so that it is easier for buyers to buy their wheat

2.3.4 Farmers' perception of spot blotch disease and management strategies

During group discussions, farmers used descriptive rather than specific names to identify diseases affecting their rain-fed wheat crop. However, with the help of pictures during discussions, they were able to identify various diseases affecting the wheat crop in their area. Generally, results from the formal survey indicated leaf spot (spot blotch disease) (Figure 2.5a), black powdery heads (smut) (Figure 2.5b) and sticky heads (ergot) as the most common diseases. During the same group discussions, farmers disclosed that leaf spots were seen earlier in the growth stage of wheat compared to black powdery heads and sticky heads which came in later during heading. About 90.9% farmers mentioned leaf spot to be the most prevalent disease affecting the wheat crop during the rainy season. About 81.1% of the farmers indicated that spot blotch disease appeared in their fields during the flowering stage, while 18.1% witnessed the disease at the maturity stage. Fifty-four percent

of the farmers indicated soil type to be the major cause of spot blotch disease while 45.5% considered dry spells to be the cause. They further pointed out that after the dry spells, more leaf spots were observed on leaves and sometimes stems.

(a) Leaf spot (spot blotch)

(b) Black powdery heads (smut)

Figure 2.5: Prevalent diseases during rainy season as identified by farmers

Farmers adopted some control measures to reduce the effect of spot blotch. These included uprooting diseased plants practised by 63.6% of the farmers. They viewed that removing diseased plants was a better way of minimizing the spread of the disease to other plants. However, uprooting diseased plants was reported to be tedious and time consuming. About 9.0% of the farmers attempted to apply lime to the soil with a view of reducing the amount of disease inoculum in the soil. Eighteen percent tried early planting to minimize disease pressure while 9.1% removed infected leaves to try and reduce spot blotch disease. Overall, results showed that farmers did not apply any chemicals to manage the disease as they lacked resources to purchase the chemicals. Nevertheless, no clear effective spot blotch management strategies emerged during the formal interviews and during the focus group discussions.

When farmers were asked to indicate the management option they thought would be effective to control spot blotch disease, they believed that practices such as crop rotation, early planting and applying chemicals could help control the disease and increase wheat yield. Most farmers pointed out that they thought these methods could be effective as they had tried other methods but with no success. Furthermore, some farmers disclosed that

yields in the presence of spot blotch were much lower compared to yields in the absence of the disease (Figure 2.6).

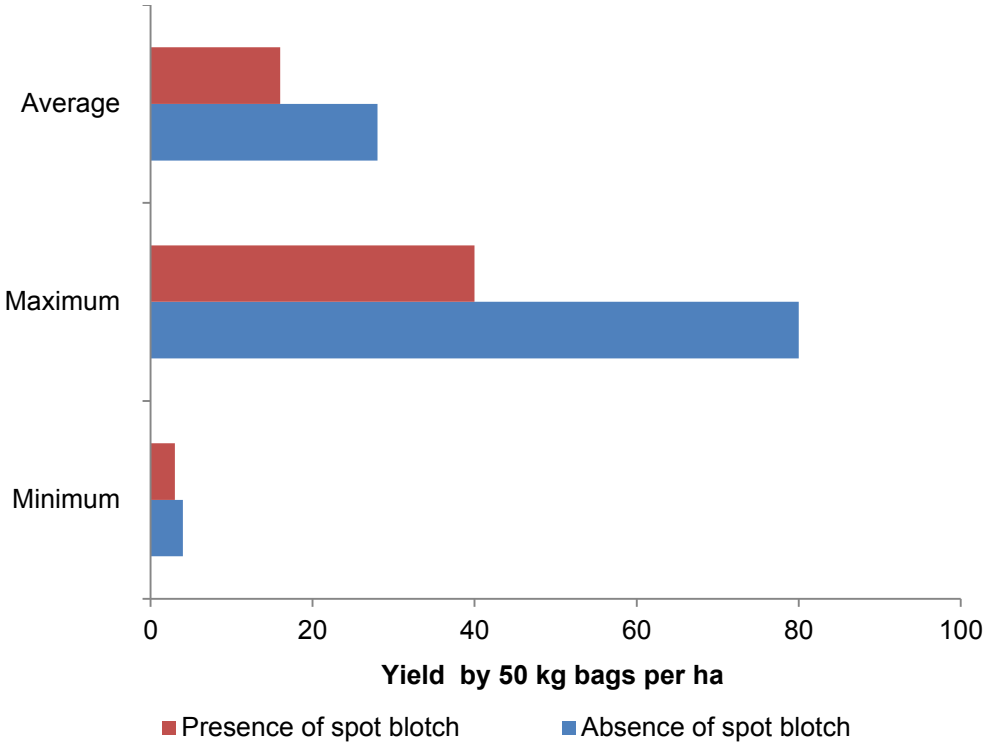

Figure 2.6: Wheat yield estimates in 50 kg bags in the presence and absence of spot blotch disease

2.3.5 Wheat cultivar preferences

Coucal (amber coloured variety) was the only wheat variety introduced to the farmers in Mufubushi and Mpika-Main in the past fifteen years. Despite having one variety, farmers had their own preferred traits of an 'ideal' wheat variety. White colour was the most preferred characteristic by the majority of the farmers (61.6%) (Figure 2.7). It was observed that the only wheat variety which was available in the study areas was amber coloured and did not coincide with the majority of farmers' preferences. A visit to the local market also revealed that the white coloured grain type was the most preferred grain colour. Farmers gave several reasons why they preferred white type of wheat among them; they consumed wheat as a whole grain hence preferred the white type to amber (red), while others took wheat to local gridding mill to make wheat flour for making local buns. They indicated that the flour colour from the local millers depended upon the colour of wheat grain taken there, as no further processing was done. However, less than 30% of the farmers preferred amber type of wheat. It is understood that these were sensitized that the amber type was highly nutritious

and good for prevention of cancer. The second most preferred traits were high yield and disease resistance. Other traits preferred by farmers included resistance to termite and bird damage, and drought tolerance (Figure 2.7).

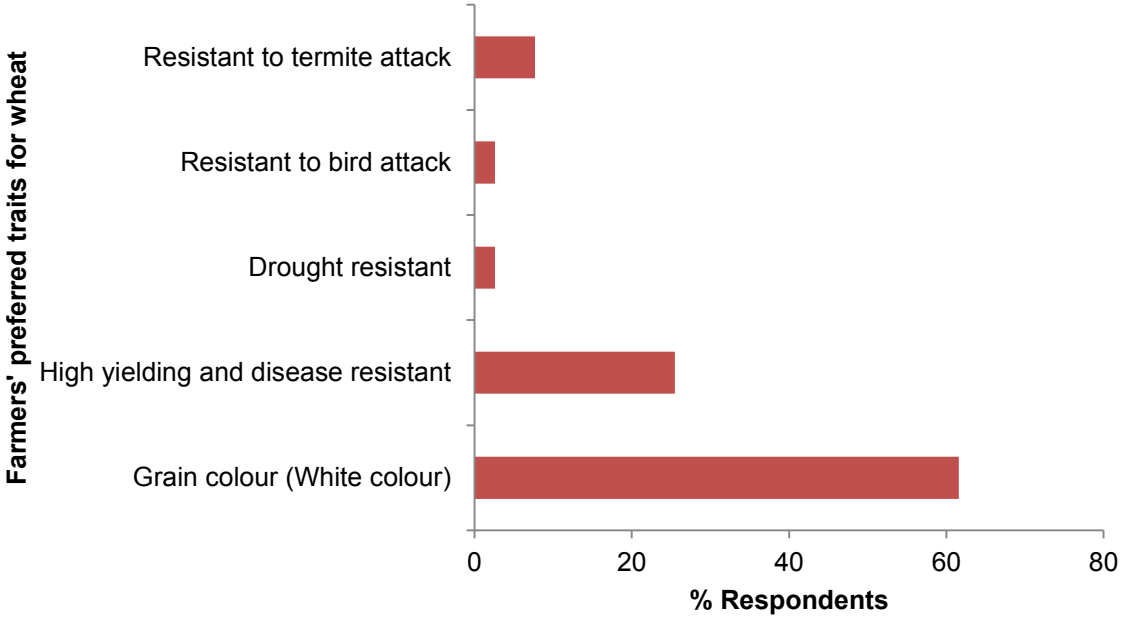

Figure 2.7: Farmers' preferred traits for wheat

2.4 Discussion

2.4.1 Social economic and agricultural information of Mufubushi and Mpika-Main areas

The farmers relied principally on family labour for all farm activities in Mufubushi and Mpika-Main as it was cheap and readily available. These findings are in line with findings of Adeogun et al. (2010) who reported that families were a key component of labour amongst small-scale farmers. There were no gender differences regarding agricultural activities as both men and women participated. As a family they were actively involved in agriculture and no marital responsibilities associated with culture, especially for women, barred them from working together as a family. This had a positive effect on agricultural production as the entire family provided labour required for farming. The majority of famers were in the age group between 16 and 55 years old. According to Adeogun et al. (2010), younger farmers are generally keener to learn and adopt new technologies as compared to older farmers. In terms of education, most farmers had received some formal education. Their ability to read and write makes it easier for this group of farmers to learn and understand skills for

increased wheat production. Hojo (2002), reported that literacy in farming households was essential as it reduced risk aversion. In addition, it increases comprehension and chances of adoption of new agricultural technologies. Furthermore, education influences an individual's willingness to learn and acquire new technologies and adopt them (Feder et al., 1985; Adeogun et al., 2010).

The study revealed that most households did not utilize all their available land. This could be attributed to a number of things such as the types of implements they owned and the methods of land preparation used on their farms. It was evident from the ownership of implements that the principal source of farm power for most household was human power. The contribution of human power to land preparation was more than draught animals. The reliance on human labour for land preparation activities contributed to limited use of farm land. Traditionally, farmers in these areas did not keep cattle hence the reliance on human labour. However, in recent years cattle have been introduced to these areas which could lead to increased use of draught animals. The findings revealed that farmers needed draught animals to improve their agricultural productivity including wheat.

It was found that the majority of households produced enough food to see them through to the next cropping season as they had access to production information from extension workers. This implied that farmers' agricultural information needs were met as the extension workers provided them with relevant information that helped them in their farming operations and hence produced enough food. This is in line with what Fashola et al. (2007) who reported that the force to increase crop productivity depended upon the linkages between the research workers, extension workers and the farmers. In addition, Roling (1990) revealed that extension workers were vital in the flow of information to farmers to increase crop productivity.

2.4.2 Wheat production and constraints as perceived by farmers

The number of farmers involved in wheat production and the amount of land that was allocated to wheat crop revealed that wheat production in Mufubushi and Mpika-Main is on a small scale. The area under production has the potential to increase if research institutions (wheat team) can train extension workers on wheat production who in turn will assist the farmers to improve production of the rain-fed wheat in their areas. In addition, on-farm and on station wheat field days could significantly help in promoting rain-fed wheat production as wheat farmers could be given an opportunity to assess the crop in the field. Akinsorotan (2009) revealed that field days were important in helping to learn by seeing what other farmers were doing and also convince them to go into production. Nonetheless, the study areas have high wheat production potential. This was evident from the number of farmers who were requesting for wheat seed after the focus group discussion. Besides, both men

and women participated in the production of wheat. These farmers should be encouraged to remain in wheat production because they have the experience in wheat farming. Moreover, the effective participation of both men and women would influence wheat production positively at household and community level. These observations are in close agreement with Kifle (2013) and Raney et al. (2011) who reported that both men and women in farming households play an important role in agricultural production. Farmers planted wheat in rows and hand drilling was the common sowing method practiced by the farmers. Row planting is a good method as it helps in identifying the weeds during early growth stages of wheat. Moreover, hand hoe weeding becomes much easier and faster than when not planted in rows.

Coucal was the only variety known to the farmers for the past fifteen years. This implies that there has been no progress in breeding for new rain-fed wheat varieties. The lack of good varieties has caused many small scale-farmers to abandon summer wheat production, which clearly shows the need for wheat breeders to develop new improved rain-fed varieties so that farmers could have a wider choice to improve the production of rain-fed wheat. Disease and weed pressures were also highlighted by farmers as important constraints for summer wheat production which emphasizes the need for breeding and introducing appropriate cultivars tolerant to different disease complexes and weed pressures. According to Naitao and Yousan (1997) a more feasible, sustainable and effective way to control diseases on small-scale farmers' fields is breeding for resistance.

Lack of a good seed source was also cited as a limiting factor to rain-fed wheat production and forced farmers to save seed or source it from neighbours. A reliable supply of rain-fed wheat seed from both private and public sectors is required for sustainable summer wheat production amongst small-scale farmers. Drought, bird and termite damage were other factors limiting rain-fed wheat production amongst small-scale farmers. Farmers failed to combat these limiting factors causing low yield. The provision of drought tolerant varieties, varieties resistant to bird and termite damaged would help provide a solution to these problems.

Lack of readily available local markets was also an important constraint for farmers involved in wheat production. Increased markets could greatly transform wheat production as farmers would be able to purchase wheat seed and sell their harvest without problems. Furthermore, it is suggested that small-scale wheat farmers form an association as marketing of their produce becomes easier through associations. Additionally, the Government of the Republic of Zambia (GRZ) through the Ministry of Agriculture and Livestock (MAL) should put deliberate policies aimed at improving summer wheat production such as buying the crop from the farmers as they do with crops such as maize and rice through the Food Reserve

Agency (FRA). Readily available local markets could be an incentive for farmers to increase the area under wheat production as more land would be used for production (Shepard and Prowse, 2009). Furthermore, Huang (2014) indicated that availability of markets is one of the major drivers for agricultural growth as it helps farmers to get cheaper inputs and higher output prices.

2.4.3 Farmers' perception of spot blotch disease and management strategies

Leaf spots (spot blotch) disease was cited by farmers as one of the important diseases during the rainy season. This is in agreement with the findings of Raemakers (1988), Mukwavi et al. (1990) and Mooleki (1997) that spot blotch was very common during the rainy season. However, it was found that farmers were not aware of the causes of spot blotch disease. This implies that there is a need to educate farmers on the causes of spot blotch disease as this would help to reduce the disease in the area as the use of recycled infected seed, containing black points, would diminish. Infected seeds are a source of contamination as they provide pathogen inoculum to the growing plant (Duveiller and Dubin, 2002; Malaker et al., 2008).

Some farmers were aware of the effect of the disease on wheat grain yield but lacked information on effective management strategies. The farmers opted to adopt control measures that they thought would help them manage the disease, such as removing infected leaves, use of agricultural lime and also uprooting diseased plants. None of the methods they adopted helped in controlling the disease. Other farmers were not aware of the effects of the disease hence never adopted any control measures to combat the disease. Consequently, there is need to strengthen agricultural extension so that information on effective and sustainable management practices can reach the farmers.

From the survey, it was observed that farmers were informed on the wheat farming practices, such as land preparation, sowing and also harvesting but lacked information on crop protection. According to Glendenning et al. (2010), farmers require a wide range of information to sustain their farm activities. Nonetheless, the study established that a more sustainable spot blotch disease management strategy, including the use of resistant varieties, is required to help combat the disease.

2.4.4 Wheat cultivar preferences

Generally, farmers preferred a wheat variety that would be high yielding, disease resistant, resistant to termite and bird damage, drought tolerant and with white coloured grain. They believed that having a variety with resistant to the aforementioned constraints would help them obtain good yields which would in turn bring them more income to their families. Farmers also preferred to have a wide range of wheat varieties to choose from other than

Coucal. This therefore, confirms the importance of developing new wheat varieties that incorporate farmers' preferred traits for the study areas.

2.5 Conclusion

The findings of the study showed that farmers are engaged in crop production as a wide range of crops are grown on their small pieces of land. Wheat is one the crops grown by a few farmers (66.1% of the respondents), mainly due to lack of good varieties, with only one variety (Coucal) introduced in 1988 being grown. Furthermore, the variety lacks preferred characteristics. There has been no active breeding for rain-fed varieties for the past fifteen years. Farmers desired a wide range of rain-fed wheat cultivars other than the current one to boost rain-fed wheat production. Besides that, lack of good seed sources, lack of readily available markets, drought, bird and termite damage, and disease complexes also made most farmers to abandon summer wheat production. Farmers identified leaf spots (spot blotch), smut and ergot diseases as the most important diseases. However, no sustainable control method was used to control the diseases. It is, therefore, important that breeders develop cultivars with resistance to these diseases.

The study also established that wheat was a desired crop in almost all the households in the study area as it is a dual purpose crop. The characteristics most preferred by farmers in wheat were grain colour (white type), high yield, resistance to disease, tolerance to termite attack and tolerance to drought and bird damage. To enhance rain-fed wheat production amongst small-scale farmers, it is essential to develop rain-fed wheat cultivars incorporating farmers` preferred traits.

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Chapter 3

Assessing genetic diversity in 150 wheat genotypes using agro-morphological traits and the association between traits

Abstract

Knowledge of the genetic variability in germplasm is critical for effective selection in breeding. To this effect, an experiment was conducted in 2013 and 2014 to assess genetic diversity of 150 wheat genotypes to be used in the Zambian breeding programme based on agro-morphological traits. Highly significant differences ($P<0.001$) were observed among the genotypes in most of the traits studied. The PCA showed four principal components (PC) which accounted for 69.1% of the total variation. First principal component (PC1) accounted 30.6%, PC2 16.5%, PC3 11.3% and PC4 10.6% of the variation. Hectolitre weight, peduncle length, tiller/m^2 and grain yield were important traits for classifying genotypes on PC1. For PC2, days to heading, days to maturity and plant height were important for classifying genotypes. Cluster analysis of the traits based on Ward's method and squared Euclidean distance grouped genotypes into five clusters. Genotypes within the same cluster displayed similarity in the traits studied. Clusters IA consisted only of exotic genotypes from the International Maize and wheat Improvement Centre (CIMMYT) in Mexico. All clusters except cluster IA had a mixture of both local and exotic genotypes. Genotype 94, another local genotype, was grouped in cluster IB among the genotypes that were intermediate in days to heading and in yield. Short and early maturing locally adapted genotypes 95 and 97 were in cluster II. Genotypes 91 and 92, locally adapted genotypes in cluster III, were among the tall, high yielding and high tillering genotypes. Genotypes 93, 98 and 149 were among the low tillering with intermediate hectolitre weight and plant height in cluster V. The grouping of the genotypes into different clusters indicated that the 150 genotypes had different genetic backgrounds which provides a great opportunity for genetic improvement. Furthermore, traits such as hectolitre weight, tiller/plant, thousand grain weight, grains/spike, peduncle length, and tillers/m^2 could be effective selection criteria for high yield as they exhibited positive direct effects on yield and also positive and significant association with yield.

3.1 Introduction

Genetic diversity assessment is the foundation for crop improvement in a wide range of crop species including wheat (Salem et al., 2008). Breeders rely on genetic variability for identification of diverse parental genotypes for variety development and selection of genotypes for different breeding purposes (Eivazi et al., 2008; Kalimullah, 2012). However, modern intensive breeding narrowed the genetic diversity in wheat (Warburton et al., 2006; Wang et al., 2007; Abouzied et al., 2013). The narrow genetic base presents a problem in case of any disaster (disease or pest), as the entire crop could be lost as it becomes vulnerable to diseases or pests hence threatening food supplies (Huang et al., 2002; Mir et al., 2006; Wang et al., 2007). Moreover, it presents difficulties in breeding crops for adaptation to biotic and abiotic stresses (Dodig et al., 2010). In Zambia, there is no information regarding genetic diversity of wheat genotypes as no comprehensive studies have been done. Exploring genetic diversity among wheat genotypes could help to increase knowledge of the extent of genetic variability amongst the genotypes. In addition, this could assist in the development of plants resistant to abiotic and biotic stresses and adapted to various agro-climatic conditions (Zhu et al., 2000; Wang et al., 2007; Nawaz et al., 2009; Khodadadi et al., 2011).

Morphological and agronomic traits have been used to assess genetic diversity in a wide range of crop species. According to Li et al. (2009) and Anas and Yoshida (2004), morphological and agronomic traits provide a simple and direct way of determining genetic variations among genotypes while at the same time assessing their performance under normal growing conditions. Furthermore, the method is cheaper compared to use of molecular markers (Li et al., 2009). Cui et al. (2001) reported that morphological data could effectively be used in estimating genetic diversity as morphological differences in plants were a result of genes controlling the trait. Additionally, Sammour (2011) reported that morphological traits were the most appropriate and practical tools for assessing genetic diversity in a large number of genotypes. However, use of morphological and agronomic traits has been reported to be unreliable as they are usually influenced by environment in the field, have low heritability, low polymorphism, late expression (Nagahvi et al., 2009; Zeb et al., 2009), may be controlled by epistatic and pleiotropic gene effects (Fufa et al., 2005) and are limited in number (Ahmed et al., 2010; Zarkti et al., 2010). In addition, the method is time consuming and requires extensive field trials making it more expensive than molecular markers (Bibi et al., 2009; Mondini et al., 2009). Despite that, morphological traits have been used successfully for genetic diversity assessments and development of cultivars (Fufa et al., 2005). Benesi et al. (2013) revealed that using detailed morphological descriptors for classification of genotypes was significant even in the presence of more precise DNA markers.

Essential morphological and phenological descriptors for evaluating and classifying wheat genotypes include plant height, spike length, tillers per plant, peduncle length, thousand grain weight, days to heading , days to maturity and yield (International Board for Plant Genetic Resources (IBPGR)(IBPGR, 1978). Autrique et al. (1996) successfully used morphological traits to examine and select genetically diverse genotypes for breeding purposes from a large pool of genotypes. Efficiency in selection for genetically diverse superior genotypes also requires knowledge of the relationships between traits. Trait associations in wheat have been studied using simple correlation and path analysis (Nukasani et al., 2013). Correlation coefficient measures the degree and direction of linear relationship between traits. The path coefficient measures direct effects of one trait upon another trait and also the indirect effect of the one trait via another trait (Salehi et al., 2010). The information on association between traits are important as they would help breeders in formulating effective selection strategies for breeding desired genotypes. The present study therefore, was conducted to assess genetic variability of 150 wheat genotypes using agro-morphological traits. The association between traits using correlation and path analysis is also reported. This information is not available to wheat breeders in Zambia.

3.2 Materials and methods.

3.2.1 Experimental sites

The study was conducted during two consecutive years in summer season of 2013 (2012/13) and 2014 (2013/14) at three sites in each year. In 2012/13 season, the study was carried out at Mutanda Research Station located at 12º25.959' S and 26º12.620' E (Environment 1), Mt. Makulu Research Station at 15º32.946' S and 28º15.078' E (Environment 2) and Golden valley Agricultural Research Trust (GART) at 14º58.185' S and 28º06.134' E (Environment 3). For 2013/14 season the experiment was evaluated at Mpongwe Seed-Co Research Farm located at 12º06.622' S and 3º114.660' E (Environment 4), Mt. Makulu Research Station at 13º32.831' S and 28º03'.626 E (Environment 5) and GART at 14º58.056' S and 28º05.875' E (Environment 6).

3.2.2 Experimental material, layout of the experiment and crop management

One hundred and fifty wheat genotypes were used in the study. The materials comprised nine genotypes from Zambia Agricultural Research Institute (ZARI), one from Seed-Co, two from the University of Zambia (UNZA) and 138 (advanced lines and nurseries) from International Maize and Wheat Improvement Centre (CIMMYT), Mexico. The list of genotypes used for genetic diversity study is presented in Appendix 3.1.

The experimental field was laid out in a 10 × 15 alpha lattice design. Each genotype was planted in 2.5 meters long plot of two rows, 20 cm between rows with a plant to plant distance of 10 cm. Spacing of 40 cm between plots was used. Standard agronomic practices were followed for good crop management. Weeding was done by hand.

3.2.3 Measurements

Evaluation of morphological characteristics was done using descriptors recommended by the International Board for Plant Genetic Resources (IBPGR)(IBPGR, 1978). Observations were recorded on five plants per plot. Means for each trait were used for further statistical analysis. Data recorded was as given below.

1. **Growth habit**
    a. Plant height (cm) – was recorded as height of plant at maturity, excluding awns.
    b. Number of tillers per square meter – determined by counting number of tillers bearing ear spikes at the time of harvest per meter length of each row.
    c. Tillers per plant – determined by counting number of tillers bearing spikes per plant based on an average of five plants.
2. **Maturity**
    a. Days to heading (flowering) – recorded as number of days from sowing to the date when the spike completely emerged from the flag-leaf sheath on 50% of the plants in the plot.
    b. Days to maturity – recorded as number of days from sowing to the date when 50% of the glumes have lost their green colour.
3. **Yield and yield components**
    a. Spike (ear) length (cm) – was measured from the base to the tip of the spike, an average of five spikes.
    b. Number of grains per spike – was determined by counting the number of grains per spike from the central portion of the spike; an average of five spikes.
    c. Grain yield per plot (g/plot) – was measured by harvesting plants in a plot, threshing them and record grain weight.
    d. Thousand grain weight – one thousand grains were counted from the bulk of grains of each entry and weighed on an electronic balance to determine its weight (g).
    e. Peduncle length (cm) – was measured from the highest node to the base of the spike.

f. Hectolitre weight (kg $h^{-1}l$) – measures the weight of hundred litres of wheat and was measured from the grain density bulk of grains of each entry using a hectolitre (hl) device.

3.2.4 Data analysis

Data obtained was subjected to analysis of variance using general linear model procedure (PROC GLM) in SAS version 9.3 (SAS Institute, 2011) to test significant differences among the genotypes. Analysis of variance (ANOVA) was performed separately on individual experiment of each environment and combined across environments. A combined ANOVA was conducted to determine the effect of genotypes, environment (location, year, and year x location) and the interaction. Genotypes and sites were considered fixed while replications and years were considered as random effects.

The following linear statistical model for combined analysis was used (Annicchiarico, 2002):
Yijkr =μ + gi + lj + (gl) ij + yk + br (ljyk) + (gy) ik + (ly) jk + (gly) ijk + eijkr)
Where Yijkr = observation of genotype i in location j in year k and block r, μ = overall mean, gi = effect of genotype i, lj = effect of location j, yk = effect of year k, br (ljyk) effect of block r within location j and year k, (gy) ik = genotype i x year k interaction, (ly) jk = location j x year k interaction, (gly) ijk = genotype i x location l x year k interaction and eijkr= residual effect

The association for all the traits was estimated using simple linear correlation coefficient to determine the degree of association between the traits. Path analysis was also performed using the correlation values to assess the direct and indirect effects of different traits on grain yield following the method in Singh and Chaudhary (1995). Path coefficient values proposed by Lenka and Mishra (1973) as cited by Lule and Mengistu (2014) were used in this study. Path coefficients of < 0.09 were considered as having negligible direct effects, 0.10 to 0.19 as low, 0.20 to 0.29 as moderate and 0.30 to 0.99 as high direct effect on grain yield. Residual effects which determine how the causal factor (independent variable) accounts for variability of the dependent factor (yield) were estimated using the formula below (Singh and Chaudhary, 1995);

Residual effect (h) = $\sqrt{1-\sum Piyriy}$

Where, Piy is the component of direct effect of independent i^{th} factor and the dependent factor y (yield) as determined by path analysis, and riy is the correlation coefficient of i^{th} factor with y (yield) as measured by correlation.

Based on the mean values for each trait, the principal component analysis was performed in GenStat version 14 (Payne et al., 2011) to detect traits that explained the most variability in the data set and also to cluster genotypes based on the similarities. In this study, the trait

with the coefficient equal to or greater than 0.3 was considered to discriminate the genotypes more than those with coefficient less than 0.3 (Badu-Apraku et al., 2006; Sanni et al., 2012). Cluster analysis based on Ward's method (Ward, 1963) using squared Euclidean distance was used to group genotypes in to clusters using Statistical Package for Social Scientists (SPSS) 16.0 version for windows (SPSS, 2007).

3.3 Results

3.3.1 Variation among the genotypes

The analysis analysis indicated significant differences (P< 0.001) (Table 3.1) among the 150 genotypes for the eleven characters studied viz. days to heading, days to maturity, spike length, grains per spike, hectolitre weight, plant height, thousand grain weight (TGW), tillers/plant, tillers/m^2 and grain yield. Days to heading ranged between 49 to 86 days with the earliest being Sonalika (SB 50) from Mexico with 49 days. Sonalika was also the earliest genotype to mature (68 days) (Figure 3.1). Genotype 19HRWSN7 showed the highest number of days to head (86 days) as well as days to maturity (110 days) (Figure 3.1). In addition, it also exhibited the lowest grain yield (0.1 t ha^{-1}) and TGW (23.42 g). The highest yield was recorded in genotype 30SAWSN10 (2.0 t ha^{-1}). Genotype 30SAWSN5 was second highest in grain yield per hactare (1.8 t ha^{-1}) and recorded the highest hectolitre weight. Spike length was high in genotype SB9 (14.0 cm) with SB1 having shortest spike length (6.0 cm). The highest number of grains per spike was recorded in genotype 20HRWYT7. Coucal a locally adapted genotype was the tallest (83.6 cm) among all the genotypes followed by Kwale also a locally adapted genotype. Number of tillers/plant was high in genotype 19HRSWN21 while the highest number of tillers/m^2 was recorded in genotype 19HWSN22 and Kwale. The longest peduncle length was recorded in genotypes 19HRWSN21, Coucal and Kwale. Among all the genotypes, genotype SB34 had the highest TGW compared to the others. Location effect was significant for all the traits. The year effect was significant for all traits except for tiller/m^2. Genotype × location interactions were significant for all traits except for spike length and grains/spike (Table 3.1). Genotype × year interaction were significant for all traits except for spike length, tillers/plant and hectolitre weight. Genotype × year × location interactions were not signicant for spike length, tillers/plant and hectolitre weight but significant for all other traits.

Figure 3.1: Wheat genotypes during 2012/13 season

Table 3.1: Combined analysis of variance for 150 wheat genotypes evaluated in 2013 and 2014

		Mean squares										
Source	Df	Days to heading	Days to maturity	Spike length (cm)	Grains/spike	Hectolitre weight (kg hl^{-1})	Plant height (cm)	Peduncle length(cm)	Thousand Grain weight (g)	Tillers/ plant	Tillers/m^2	Grain yield (t ha^{-1})
Year (Y)	1	26719.01**	6268.27**	275.52**	6131.97***	165.01*	240.48***	1266.67***	1109340.38***	587.67*	255.16^{ns}	28344.69***
Location (L)	2	39588.56*	27835.79**	307.17*	14724.47*	453.62**	86361.17**	1459.13***	762116.64**	2479.56*	296000.5*	325280.60*
L x Y	2	35349.97**	16695.87**	642.57*	11593.9**	294.87*	95175.56*	1622.78*	559310.48*	4349.28**	2702355.35*	155182.23**
Rep (Y x L)	6	28.48	929.02	11.13	212.56	3.74	561.79	11.88	1041.12	43.91	15756.72	876.75
Genotype (G)	149	232.46***	302.72**	7.14**	178.36**	4.95***	233.45***	45.03***	597.46***	9.06**	2710.50***	2236.41***
G x Y	149	41.17**	103.16**	5.87^{ns}	71.18**	2.92^{ns}	70.49***	7.00**	318.34**	3.16^{ns}	2007.17***	591.56***
G x L	298	41.42**	95.17***	5.85^{ns}	81.18^{ns}	3.26**	77.27***	5.78**	328.78**	5.00**	1977.79**	1056.50**
G x Y x L	298	36.89**	81.27**	5.83^{ns}	83.53*	2.82^{ns}	64.53**	5.52**	306.29**	4.58^{ns}	2021.14**	697.98
Error	894	18.12	49.15	5.17	63.12	2.62	49.37	4.6	136.96	4.00	1533.45	463.10
Corrected total	1799											
CV		7.35	7.89	28.0	25.0	29.0	10.89	23.40	24.00	28.06	29.0	30
R^2 (%)		93.70	82.96	62.39	72.17	67.0	91.14	81.91	97.10	84.28	90.66	82.40
Mean		58	89	8	32	1	65	9	48	7	60	0.8
Maximum		86	110	14	40	7	84	17	79	10	140	2.0
Minimum		49	68	6	20	0	54	6	23	5	60	0.1

***, **, * indicate significance at P< 0.001, P< 0.01 and P< 0.05, respectively, ns= non-significant, Df: Degree of freedom, Rep = replication

3.3.2 Correlations and path coefficient analysis

Negative and significant correlation coefficients were detected between days to heading with peduncle length (P< 0.001), thousand grain weight (TGW) (P< 0.001) and grain yield (P< 0.01) (Table 3.2). Negative and non-significant correlations were observed between days to heading with hectolitre weight, tillers/m^2, and tillers/plant while days to heading showed positive and non-significant correlations with spike length, grains/spike and plant height. Days to maturity were negatively and significantly correlated with hectolitre weight, peduncle length, TGW and grain yield. Positive and highly significant correlation (P< 0.001) was observed between grain yield with grains/ spike, hectoliter weight, thousand grain weight, peduncle length, tiller/m^2 and tillers/plant. Thousand grain weight was highly significantly and positively correlated with plant height, hectoliter weight and grain yield. Plant height was highly significant and positively associated with peduncle length, grains per spike, tillers/m^2 and grain yield (P< 0.001). Significant association (P<0.01) was also observed between plant height and spike length, hectolitre weight, days to maturity and tillers/plant.

From the path analysis results (Table 3.3), hectolitre weight (0.46) and tillers/plant (0.34) exhibited the highest positive direct effect on grain yield, while moderate direct effects on grain yield were observed through TGW (0.21). Grains/spike (0.19) showed a low positive direct effect on grain yield. The direct effect of peduncle length and tillers/m^2 on grain yield was positive though not so pronounced. Direct effects of days to heading, days to maturity, plant height and spike length was negative and negligible <0.09. The residual effect was 0.48.

Table 3.2: The Pearson correlation coefficient matrix for agro-morphological traits evaluated in 150 wheat genotypes in six environments

	DH	DM	SL	GS	HL	PH	PL	TGW	TP	TM^2	GYD
DH	1.00										
DM	0.62***	1.00									
SL	0.05^{ns}	0.09^{ns}	1.00								
GS	0.01^{ns}	0.00^{ns}	-0.01^{ns}	1.00							
HL	-0.16^{ns}	-0.17*	-0.06^{ns}	0.16*	1.00						
PH	0.11^{ns}	0.19*	0.26***	0.21**	0.21**	1.00					
PL	-0.32***	-0.35***	0.03^{ns}	0.13^{ns}	0.35***	0.42***	1.00				
TGW	-0.28***	-0.21**	-0.06^{ns}	-0.10^{ns}	0.37***	0.16*	0.22**	1.00			
TP	-0.09^{ns}	-0.09^{ns}	-0.10^{ns}	0.21**	0.45***	0.29***	0.47***	0.11^{ns}	1.00		
TM^2	-0.02^{ns}	-0.01^{ns}	-0.03^{ns}	-0.04^{ns}	0.19*	0.10^{ns}	0.34***	-0.07^{ns}	0.53***	1.00	
GYD	-0.22**	-0.26***	-0.15^{ns}	0.32***	0.75***	0.24***	0.46***	0.43***	0.65***	0.27***	1.00

***, **, * indicate significance at P< 0.001, P< 0.01, P< 0.05 respectively, ns= non-significant, DH=days to heading, DM=days to maturity, SL=spike length, GS=grains/spike, HL=hectolitre weight, PH=plant height, PL=peduncle length, TGW=thousand grain weight, TM^2=tillers/meter^2 square, TP=Tillers/plant, GYD=grain yield.

Table 3.3: Estimates of direct (diagonal bold) and indirect effect of 10 traits under study on grain yield

	DH	DM	SL	GS	HL	PH	PL	TGW	TP	T/M^2	GYD
DH	**-0.02**	-0.05	0.00	0.02	-0.07	0.00	-0.01	-0.06	-0.03	0.00	-0.22
DM	-0.01	**-0.08**	-0.01	0.00	-0.08	0.00	-0.01	-0.04	-0.03	0.00	-0.26
SL	0.00	-0.01	**-0.06**	0.00	-0.03	0.00	0.00	-0.01	-0.03	0.00	-0.15
GS	0.00	0.00	0.00	**0.19**	0.07	0.00	0.00	-0.02	0.07	0.00	0.32
HL	0.00	0.01	0.00	0.03	**0.46**	0.00	0.01	0.08	0.15	0.00	0.75
PH	0.00	-0.01	-0.02	0.04	0.09	**-0.01**	0.02	0.03	0.10	0.00	0.24
PL	0.01	0.03	0.00	0.02	0.16	-0.01	**0.04**	0.05	0.16	0.00	0.46
TGW	0.00	0.02	0.00	-0.02	0.17	0.00	0.01	**0.21**	0.04	0.00	0.43
TP	0.00	0.01	0.01	0.04	0.21	0.00	0.02	0.02	**0.34**	0.00	0.65
T/M^2	0.00	0.00	0.00	-0.01	0.09	0.00	0.01	-0.02	0.18	**0.01**	0.27

DH=days to heading, DM=days to maturity, SL=spike length, GS=grains/spike, HL=hectolitre weight, PH=plant height, PL=peduncle length, TGW=thousand grain weight, TM^2=tillers/meter^2 square, TP=Tillers/plant, GYD=grain yield. ‡Residual effect= 0.48

3.3.3 Principal component analysis for agro-morphological traits

Results from the principal component analysis for the two years combined, showed that the four principal components (PC1, PC2, PC3 and PC4) accounted for 69.1% of the total variation in the phenological and morphological traits (Table 3.4). The first principal component (PC1) contributed 30.6%, PC2 contributed 16.5%, PC3 and PC4 contributed 11.3% and 10.6%, respectively, of the total variation. The Eigen values (Table 3.4) showed that the relative discriminating power of the principal components was high for PC1 (3.37) followed by PC2 then PC3 and least for PC4. Table 3.4 and Figure 3.2 shows that much of the variation in PC1 was contributed by grain yield (0.45), peduncle length (0.40), tillers /m^2 (0.41) and hectolitre weight (0.41). Thousand grain weight (TGW), grains/spike and tillers / plant contributed less to PC1. Days to heading, days to maturity and spike length contributed negatively and less to PC1. The traits which contributed more to PC2 were days to heading (0.54), days to maturity (0.53) and plant height (0.41). All other traits, except TGW, contributed positively but less to PC2. Spike length (0.57), peduncle length (0.45) and TGW (0.37) contributed more to PC3 while hectolitre weight (0.30), grains/spike (0.36), spike length (-0.43), peduncle length (-0.35) and tillers/plant (-0.46) contributed more to PC4. The traits that loaded more on PC1 and PC2 were used to cluster the 150 genotypes into closely related groups.

Table 3.4: Eigenvectors of the first four principal components (PC1, PC2, PC3 and PC4) axes for 150 wheat genotypes evaluated in 2013 and 2014

Trait	PC1	PC2	PC3	PC4
Days to heading	-0.22	**0.54**	-0.10	0.22
Days to maturity	-0.22	**0.53**	-0.01	0.16
Spike length	-0.05	0.19	0.57	**-0.43**
Grains/spike	0.14	0.27	0.04	**0.36**
Hectolitre weight	**0.41**	0.04	0.04	**0.30**
Plant height	0.21	**0.41**	0.45	-0.14
Peduncle length	**0.40**	0.01	0.11	-**0.35**
TGW	0.25	-0.23	0.37	0.28
Tiller/m^2	**0.41**	0.23	-0.29	-0.10
Tillers/plant	0.24	0.20	-0.48	**-0.46**
Grain yield	**0.48**	0.04	-0.04	0.28
Eigen value	3.37	1.82	1.25	1.17
Percent variation	30.63	16.52	11.33	10.65
Cumulative	30.63	47.15	58.48	69.13

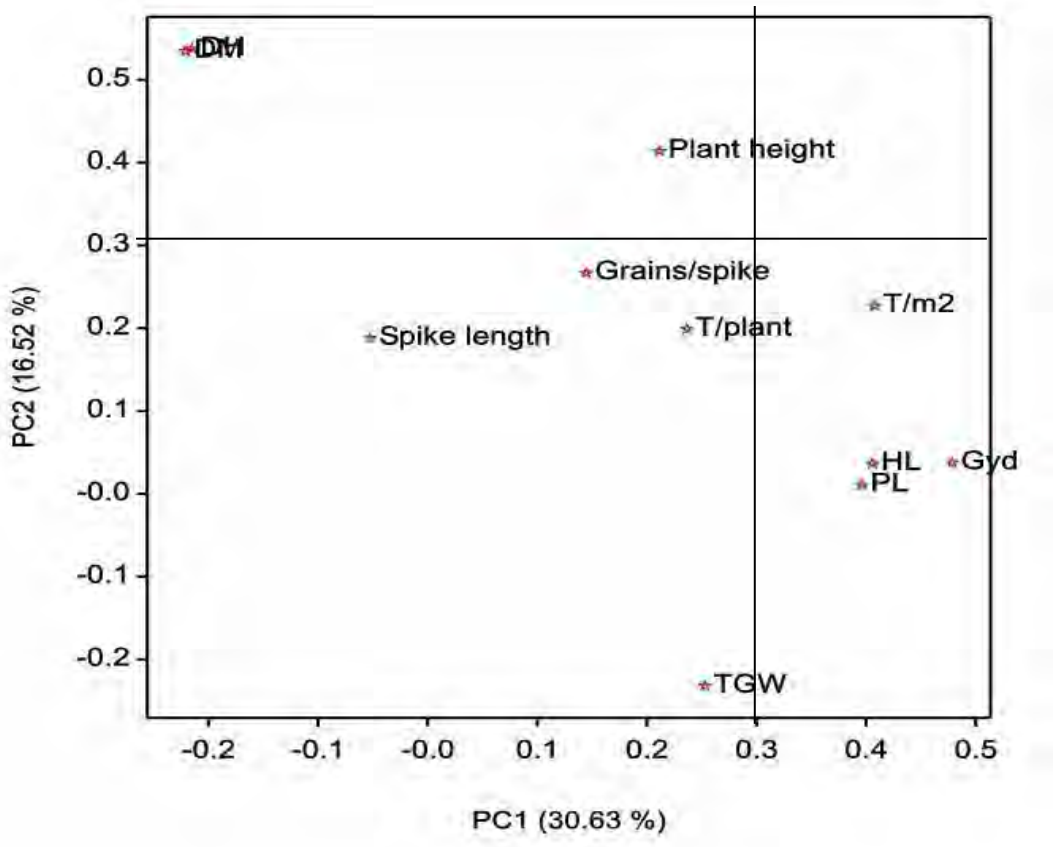

Figure 3.2: Loading plot of agro-morphological traits across environments.

Where, DH=days to heading, DM=days to maturity, HL=hectolitre weight, PL=peduncle length, TGW=thousand grain weight, T/m2=tillers/meter^2, T/plant=Tillers/plant, Gyd=grain yield

3.3.4 Cluster analysis

The dendogram (Figure 3.3) revealed five major cluster I, II, III, IV and V. Cluster I had two sub groups A and B. Members of each cluster are presented in Table 3.5. Sub-cluster I A consisted of sixteen genotypes (10.7%) mostly from Mexico-CIMMYT. The genotypes in this cluster were late maturing with average grain yield. The date of maturity for this group ranged between 91–99 days. Sub-cluster I B grouped thirty-five genotypes (23.3%) that were intermediate in days to maturity and grain yield. It had one local genotype 94 (Mampolyo). Cluster II grouped twelve genotypes (8.0%) which were short, early heading and early maturing. The shortest being genotype 147 from CIMMYT-Mexico with 53.6 cm while the earliest was genotype 144 (Sonalika) also from CIMMYT-Mexico with 68 days to maturity. Cluster II contained two local genotypes 95 (Nkhanga) and 97 (Pwele). Cluster III

contained fourteen genotypes (9.3%) of which two were local genotypes, 91 (Coucal) and 92 (Kwale). The genotypes in this cluster were high yielding, high tillering, had long peduncle length, high TGW, tall as well as late maturing. The yield ranged between 1.5 t ha^{-1}-2.0 t ha^{-1}. The tallest genotype in this cluster was genotype 91 (Coucal) from Zambia with 83.6 cm. High tillering genotypes included genotype 15, 92, 77 and 73. Number of tillers/m^2 for these genotype ranged between 131 and 140 tillers. Fourty-three genotypes in cluster IV were characterized by being intermediate in plant height, hectolitre weight and peduncle length. Among the fourty-three, genotype 150 (UNZAWV2) and 96 (Nseba) were local genotypes. Cluster V grouped thirty genotypes (20%). This cluster included three local genotypes, 98 (Sahai), 93 (LoerrieII) and 149 (UNZAWV1). The wheat genotypes in this cluster were low tillering with with short peduncle length, low TGW and low yielding. Number of tillers ranged between 60 and 72 tiller/m^2. The hectolitre weight ranged between 0.5 to 1.23 kg hl^{-1}.

Table 3.5: Grouping genotypes based on cluster analysis and the members present in each cluster based on Ward's method

Cluster	Frequency	Cluster membership
IA	16	56, 134, 31, 42, 50, 59, 24 82, 3, 47, 118, 106, 123, 142, 7,
IB	35	32, 66, 29, 89, 25, 83, 57, 113, 126, 8, 6, 64, 30, 26, 78, 62, 74, 107, 61, 72, 43, 138, 120, 41, 79, 39, 94, 99, 13, 21, 9, 44, 69, 130, 80
II	12	81, 84, 14, 88, 148, 95, 97, 147, 67, 71, 144, 5
III	14	15, 16, 73, 55, 75, 11, 19, 70, 77, 90, 91, 92, 86
IV	43	10, 63, 129, 103, 1, 150, 115, 11, 96, 111, 140, 141, 143, 2, 45, 135, 33, 12, 34, 105, 116, 137, 51, 133, 22, 20, 85, 37, 124, 128, 122, 87, 108, 136, 109, 117, 18, 56, 112, 27, 28, 76, 46
V	30	98, 104, 132, 100, 125, 35, 36, 93, 52, 68, 53, 114, 60, 102, 145, 149, 49, 38, 65, 4, 40, 127, 54, 139, 101, 146, 131, 119, 23

Names of the genotypes are given in Appendix 3.1

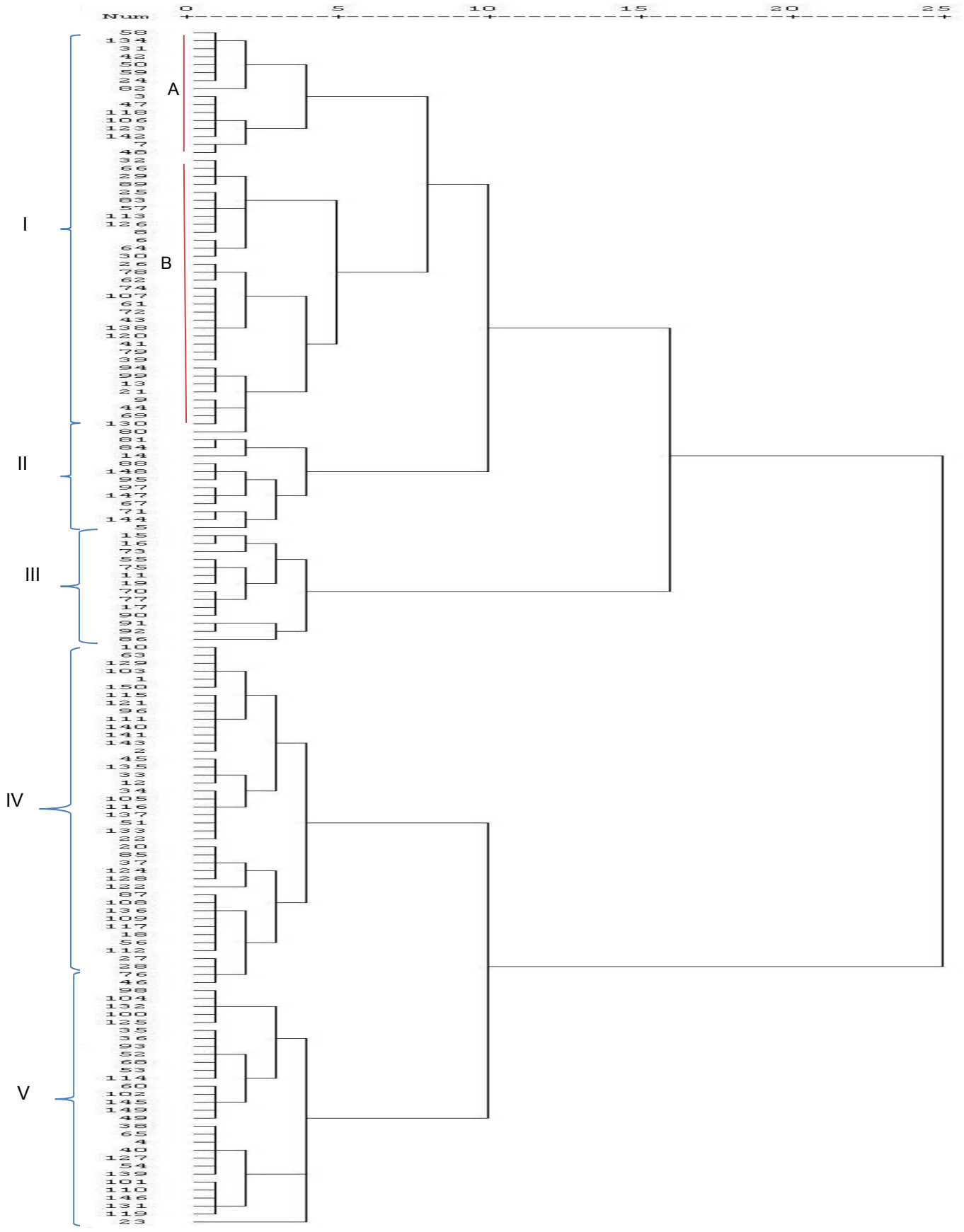
Num
0
5
10
15
20
25
A
B
I
II
III
IV
V

Figure 3.3: Dendrogram of 150 wheat genotypes based on agro-morphological traits using hierarchical cluster analysis (Ward's method and squared Euclidean distance). Genotypes are labelled 1 to 150. Names of genotypes are given in Appendix 3.1

3.4 Discussion

Variations existed amongst the 150 wheat genotypes with respect to all the traits under study. This will enable selection and hybridization of genotypes with desired traits to develop new genotypes and improve on the existing varieties. The significant genotype × location interaction (GLI) on most traits suggests the differential expression of genotypes across locations, hence confirming the presence of genetic differences among the genotypes. Nonetheless, the existence of GLI complicates selection of superior genotypes (Farshadaf et al., 2012). The non-significant GLI on traits such as spike length and grains/spike implies that these traits were non responsive to changes in the environment. The genotype × year interaction on traits such as spike length, grains/spike and hectolitre weight were not significant indicating that the performance of these traits was consistent over the years (Gomez and Gomez, 1984). Significant year × location and genotype × year × location interactions on most traits shows that there was inconsistency in the performance of the genotypes in different locations in both years. Similar findings were reported by Sial et al. (2000).

The negative correlation observed in this study between days to heading with grain yield suggests that selection for very early heading would give lower yields. Similar findings were reported by Gashaw et al. (2007). The negative and highly significant association between days to maturity with yield implies that selection for very late maturing varieties (>95 days) could result in decrease grain yield. Gashaw et al. (2007) found negative and non-significant correlation between days to maturity with grain yield. Wallace (1985) reported that the complex modulation of days to heading and days to maturity of genotypes in response to photoperiod and temperatures under diverse environments, usually bring about the opposite effect observed on grain yield. Number of tillers/m^2, tillers/plant, grains per spike, peduncle length, thousand grain weight (TGW), plant height, peduncle length and hectoliter weight had a significant and positive association with grain yield. This shows that grain yield potential can efficiently be improved by selecting for these traits. Selecting for high number of tillers indicates that there could be more spikes and grains resulting in increased yield. These results are in agreement with Leila and Al-Khateeb (2005), Khan et al. (2010) and Siahbidi et al. (2013). The negative and non-significant correlation between spike length with grain yield obtained in this study means that selection of this character may not be helpful in yield improvement. However, indirect selection for this trait for yield improvement could be through plant height.

The highly significant and positive association observed between tillers/m^2 with grains per spike, hectolitre weight, plant height and peduncle length indicates that the improvement on

tillers/m^2 may hasten the per se performance of aforementioned characters. The highly significant positive association between plant height with peduncle length, spike length, grains per spike and TGW means that while selecting for plant height, spike length, grains per spike TGW and the length of the peduncle should also be considered. This suggests that the improvement of plant height would see the improvement in performance of these other characters. This is in agreement with the study by Daoura et al. (2013). Besides, the results revealed that peduncle length was not only an important component of grain yield for providing photosynthates to the developing grain but also a major contributor to the height of wheat plants a desired character for high straw yield (Zafarnaderi et al., 2013). Similar results were reported by Nukasani et al. (2013). Negative and non-significant correlation, which was observed between grains/spike with thousand grain weight, implies that grains/spike had negligible effects on TGW. This indicates that the selection for this character may not be helpful in improving TGW. Ali et al. (2006) and Khan et al. (2010) also reported negative and significant correlations between grains per spike with TGW.

The positive direct effects exhibited by hectolitre weight, tiller/plant and TGW to yield, entails that direct selection of these traits could increase the grain yield per hectare. Nukasani et al. (2013) revealed that during selection for yield increases all traits with positive effects on yield though with less correlation magnitude should not be ignored. As such in this study, traits like grains/spike, tillers/m^2 and peduncle length with positive but low direct effects should be considered during selection for high yield. The residual factor value was found to be 0.48. This explains that the traits used in this study explained only 52% of the variability observed in the yield (Singh and Chaudhary, 1995), which implies that there are some other factors not included in this study which were causing variation in grain yield.

From the principal component analysis, traits which were responsible for the separation of genotypes for PC1 included grain yield, tillers/m^2, hectolitre weight and peduncle length; implying that PC1 was related to yield and its contributing components. This component reflected on yield potential of genotypes through some yield components. For PC2, days to heading, days to maturity and plant height were identified as major traits for genotype separation. This axis therefore could be named as phenological and plant height axis. Two principal components (PC1 and PC2) were used to cluster genotypes to observe the relationship that existed between genotypes since they contributed more than half of the total variation (Ajmal et al., 2013). Furthermore, the traits that loaded more on PC1 and PC2 showed the strongest discriminatory power in separating genotypes hence were used to classify genotypes (Badu-Apraku et al., 2006). In this study, five clusters were identified and

these clusters showed a clear separation among themselves. The improvement of any trait of importance among genotypes could easily be done by sampling and utilizing genotypes from appropriate contrasting clusters. For example, the early maturing genotypes (cluster II) could be selected to breed for early maturing type of genotype (68 days). Genotypes such as 91 and 92, both locally adapted genotypes were in the same cluster indicating that they were closely related in terms of the studied traits. Genotypes 93, 98 and 149 belonged to the same cluster revealing some similarities among them. Genotypes 95 and 97 were grouped in the same cluster. All in all, the results of this study showed that a high level of variability existed among genotypes which could further be exploited and used in wheat breeding programme. According to Furat and Uzun (2010), genetic improvements largely depend on the presence of genetic diversity in the genotypes.

3.5 Conclusion

Overall the study showed that high genetic variability existed in the material under study which provides an opportunity for further genetic improvement. The principal component analysis grouped 150 genotypes into seven clusters. Hectolitre weight, peduncle length, tiller/m^2, grain yield, days to heading, days to maturity and plant height contributed huge amount of variation that exist among the clusters. Genotypes in cluster II, early maturing (68 days) and short genotypes (54 cm) could be used in improving maturity and plant height; whilst genotype in cluster III such as number 73 (30SAWSN10) and number 86 (30SAWSN5) could be used for yield improvement. On the other hand, hectolitre weight, tiller/plant, TGW, grains/spike, peduncle length, and tillers/m^2 had positive and highly significant correlation with yield and also exhibited positive directs effects on yield, suggesting that selection of these traits for high grain yield could be effective.

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Appendix 3.1

Genotype No.	Genotype name	Genotype No.	Genotype name	Genotype No.	Genotype name	Genotype No.	Genotype name
1	16HRWYT13	44	20HRWYT3	87	30SAWSN6	130	SB38
2	16HRWYT14	45	20HRWYT30	88	30SAWSN7	131	SB39
3	16HRWYT18	46	20HRWYT31	89	30SAWSN8	132	SB4
4	16HRWYT19	47	20HRWYT32	90	30SAWSN9	133	SB40
5	16HRWYT20	48	20HRWYT33	91	Coucal	134	SB41
6	16HRWYT5	49	20HRWYT34	92	Kwale	135	SB42
7	16HRWYT7	50	20HRWYT35	93	Loerrie II	136	SB43
8	16HRWYT9	51	20HRWYT36	94	Mampolyo	137	SB44
9	19HRWSN15	52	20HRWYT37	95	Nkhanga	138	SB45
10	19HRWSN16	53	20HRWYT38	96	Nseba	139	SB46
11	19HRWSN19	54	20HRWYT39	97	Pwele	140	SB47
12	19HRWSN2	55	20HRWYT4	98	Sahai	141	SB48
13	19HRWSN20	56	20HRWYT40	99	SB1	142	SB49
14	19HRWSN21	57	20HRWYT41	100	SB10	143	SB5
15	19HRWSN22	58	20HRWYT42	101	SB11	144	SB50
16	19HRWSN23	59	20HRWYT43	102	SB12	145	SB6
17	19HRWSN24	60	20HRWYT44	103	SB13	146	SB7
18	19HRWSN25	61	20HRWYT45	104	SB14	147	SB8
19	19HRWSN26	62	20HRWYT46	105	SB15	148	SB9
20	19HRWSN27	63	20HRWYT47	106	SB16	149	UNZAWV1
21	19HRWSN3	64	20HRWYT48	107	SB17	150	UNZAWV2
22	19HRWSN6	65	20HRWYT49	108	SB18		
23	19HRWSN7	66	20HRWYT5	109	SB19		
24	20HRWYT10	67	20HRWYT50	110	SB2		
25	20HRWYT11	68	20HRWYT51	111	SB20		
26	20HRWYT12	69	20HRWYT6	112	SB21		
27	20HRWYT13	70	20HRWYT7	113	SB22		
28	20HRWYT14	71	20HRWYT8	114	SB23		
29	20HRWYT15	72	20HRWYT9	115	SB24		
30	20HRWYT16	73	30SAWSN10	116	SB25		
31	20HRWYT17	74	30SAWSN11	117	SB26		
32	20HRWYT18	75	30SAWSN12	118	SB27		
33	20HRWYT2	76	30SAWSN13	119	SB28		
34	20HRWYT20	77	30SAWSN14	120	SB29		
35	20HRWYT21	78	30SAWSN15	121	SB3		
36	20HRWYT22	79	30SAWSN16	122	SB30		
37	20HRWYT23	80	30SAWSN18	123	SB31		
38	20HRWYT24	81	30SAWSN19	124	SB32		
39	20HRWYT25	82	30SAWSN2	125	SB33		
40	20HRWYT26	83	30SAWSN21	126	SB34		
41	20HRWYT27	84	30SAWSN3	127	SB35		
42	20HRWYT28	85	30SAWSN4	128	SB36		
43	20HRWYT29	86	30SAWSN5	129	SB37		

Chapter 4

Genetic variability among wheat (*Triticum aestivum* L.) germplasm for resistance to spot blotch disease in Zambia.

Abstract.

Spot blotch caused by *Bipolaris sorokiniana* (Sacc.) Shoem is the most devastating disease limiting summer wheat productivity in Zambia because most of the varieties grown are susceptible. A study was, therefore, conducted to identify sources of resistance to spot blotch disease. One hundred and fifty genotypes were evaluated in a 10 x 15 α- lattice design with two replications under field conditions in 2013 and 2014. The 150 genotypes showed different levels of resistance to spot blotch disease. The disease severity was higher in 2014 compared to 2013. None of the 150 wheat genotypes was immune to the disease. Overall across environments, 13.3% of the genotypes from CIMMYT-Mexico were resistant and moderately resistant. Some of the most resistant lines across environments were 19HRWSN6 (Kenya Heroe), 19HRWSN7 (Prontia federal) and 19HRWSN15 (BRBT2/METSO). These lines could be used to improve spot blotch resistance in the locally adapted genotypes. Eighty-seven percent of the genotypes were moderately susceptible to susceptible and include local genotypes. Sonalika was the most susceptible in Mpongwe, Mt. Makulu Research Station and Golden Valley Agricultural Research Trust (GART), while 20HRWYT33 was the most susceptible at Mutanda Research Station. The genotype plus genotype by environment (GGE) biplot was used to visualize patterns amongst genotypes in terms of resistance and susceptibility and also determine relationships among test locations. The GGE biplot grouped the six environments into three mega-environments (ME). Mega-environment I had GART environment 6. Mpongwe (E4), Mt. Makulu environments (5 and 2) and GART environment 3 formed ME II, while ME III had only Mutanda (E1). Genotypes 16HRWYT5, SB50 and 20HRWSN33 were the most susceptible genotypes in MEs I, II and III, respectively. Genotype 19HRWSN7 was the most resistant across test locations. The relationship between the test locations in ME III was highly correlated indicating that they provided similar information on genotypes. This suggests that one location could be chosen among the locations in the same ME for screening spot blotch resistance each year. This could aid in reducing the cost of genotype evaluation and improve efficiency as genotypes would be handled in fewer environments.

4.1 Introduction

Spot blotch caused by *Bipolaris sorokiniana* (Sacc.) Shoem is the most important disease limiting high wheat yields in warm and humid environments (Srivastava and Tewari, 2002; Mikhailova et al. 2003; Khan and Chowdhury, 2011). It occurs worldwide especially in areas with high relative humidity (Mikhailova et al. 2003; Acharya et al., 2011). In Africa, the disease has been reported to occur in Kenya, Malawi, Sudan, South Africa, Zimbabwe (Acharya et al., 2011), Madagascar (Rakotondrmanana, 1981), and Zambia (Raemaekers 1988; Mukwavi et al., 1990).

In Zambia, spot blotch is the most prevalent and destructive disease in wheat during the rainy season (Raemaekers, 1988; Mukwavi et al., 1990; Muyanga, 1994). High temperatures and high humidity play a critical role in spot blotch disease problem. According to Duveiller and Gilchrist (1994), and Mehta (1997), the disease is most severe and damaging under optimum temperatures of between 18ºC and 32ºC, high relative humidity and free water. These weather conditions are common during the rainy season in Zambia. Frequent rain and dew coupled with high relative humidity of about 85% experienced in the rainy season in Zambia causes wheat foliage to remain wet for longer periods leading to increased fungal germination and sporulation (Raemaekers, 1988). This is similar to the findings of Mikhailova et al. (2003), who reported that the disease is most aggressive in areas where relative humidity is high.

Spot blotch attacks all plant parts and can cause large yield losses. Yield losses due to spot blotch disease range from 25-43% in South Asia, 18-22% in India, 70-100% in Nepal,15% in Bangladesh and 15-85% in Zambia (Raemaekers, 1988). Under severe infections the disease spreads to the spikes resulting in shrivelled grains with low grain weight, black points and can cause premature plant death (Raemaekers, 1988; Gubis et al., 2010). Apart from these effects, spot blotch disease also reduces the grade and quality of wheat (Kumar et al., 2002).

The management of spot blotch disease involving the use of fungicides is not only costly for small-scale farmers, but also difficult in its application and is not environmentally friendly (Iftikhar et al., 2009; Eisa et al., 2013). Use of proper crop rotation is also not feasible amongst small-scale farmers due to small farm sizes. Use of resistant cultivars is considered the most economical, cheap, sustainable and environmentally safe method of controlling the disease (Duveiller and Sharma, 2009; Iftikhar et al., 2009; Iftikhar et al., 2012), highlighting the need for the screening of wheat germplasm to identify sources of resistance for use in the breeding programme.

In Zambia, there is limited information available regarding sources of resistance to spot blotch disease. Hence, screening of wheat germplasm to identify sources of resistance that can be used in breeding for resistance is important. The objectives of this study, was thus to screen wheat germplasm in different environments to identify sources of resistance that could be used in breeding for resistance against spot blotch disease.

4.2 Materials and methods

4.2.1 Experimental sites and location

The study was conducted over two years, 2013 and 2014 summer seasons, at three sites in each year. In 2013 (2012/13 season), the study was carried out at Mutanda Research Station, Mt. Makulu Research Station and Golden Valley Agricultural Research Trust (GART). For 2014 (2013/14 season) the germplasm was evaluated at Mpongwe Seed-Co Research Farm, Mt. Makulu Research Station and at GART. Mean climatic data for each site and season is presented in Table 4.1.

Table 4.1: Mean climatic conditions for the six environments in 2013 and 2014

Location	Environment	Temperature		Relative humidity (%)	Rainfall (mm)	Latitude (South)	Longitude (East)
		Max (ºC)	Min(ºC)				
2013							
Mutanda	1	26.0	17.0	86.6	805.7	12º25.959'	26º12.620'
Mt.Makulu	2	28.0	17.0	80.0	722.6	15º32.946'	28º15.078'
GART	3	26.0	17.0	88.5	653.6	14º58.185'	28º06.134'
2014							
Mpongwe	4	25.8	20.4	77.5	1280.0	12º06.622'	3º114.660'
Mt. Makulu	5	27.7	17.5	78.5	725.6	13º32.831'	28º03.626'
GART	6	27.1	17.2	86.0	695.8	14º58.056'	28º05.875'

Source: Temperature, Relative humidity and Rainfall, Meteorological Station Lusaka

4.2.2 Wheat germplasm

One hundred and fifty wheat genotypes were used in the study and these are presented in Appendix 4.1. The materials comprised seven genotypes from Zambia Agricultural Research Institute (ZARI), one from Seed-Co, two from the University of Zambia (UNZA) and seventy-

two (advanced lines and nurseries) adapted to high rainfall conditions, eighteen adapted to semi-arid regions and fifty from the 2nd Cereal Systems Initiative of Asia (CSISA)-spot blotch nursery, from the International Maize and Wheat Improvement Centre (CIMMYT) Mexico.

4.2.3 Experimental design and crop management

Screening of genotypes was done under natural conditions in 'hot spot' areas. Screening of genotypes in hot spot sites increases chances of identifying genotypes resistant to the disease (Duveiller and Sharma, 2013). The experimental field was laid out in a 10 × 15 alpha lattice design. Each genotype was planted in a 2.5 m long plot of two rows, 20 cm inter row spacing with a plant to plant distance of 10 cm. Standard agronomic practices were followed for good crop management. Fertilizer application involved basal fertilizer (8% N, 24 % P_2O_5, 16 % K_2O, 0.5 % Zn, 5 % S and 0.1 % B) applied at planting at a rate of 300 kg ha^{-1} and four weeks after planting urea (46% N) was applied as topdressing to all plots at 150 kg ha^{-1}. Weeding was done by hand whenever necessary to eliminate any possible weed competition with the crop.

4.2.4 Disease assessment

Disease presence was evaluated based on foliar symptoms. Five plants were tagged at the onset of infection and were checked for disease throughout the experiment. The disease severity score was based on Saari and Prescott's scale for assessing foliar disease at seven days intervals (Eyal et.al., 1987). The severity score on the last day of scoring was used for analysis. Disease severity of each plot was found by averaging the severity ratings of the tagged plants (Nagarajan and Kumar, 1998). The scores range from 0 – 9. Zero was scored on leaves with no symptoms, 1 was scored on leaves having one or two necrotic spots to score of 9 on leaves having many extensive necrotic spots with pronounced chlorosis (Table 4.2 and Figure 4.1). Genotypes falling in the1-3 category were considered as resistant, 4 as moderately resistant, 5-6 as moderately susceptible and 7-9 as susceptible (Chaurasia et al., 1999) (Table 4.2).

Table 4.2: Modified scale to score spot blotch disease

Score	Rating scale %	Symptom description	Disease reaction
0	0	No symptoms	Immune
1	< 1%	One or two small necrotic spots without chlorosis	Resistant
2	1-3	Few small necrotic spots without chlorosis	Resistant
3	4-6	Few small necrotic spots with chlorosis	Resistant
4	7-12	Medium size necrotic spots with distinct but restricted chlorotic margin	Moderate resistant
5	13-24	Medium to large size necrotic spots with distinct but restricted chlorotic margin	Moderate susceptible
6	25-48	Large abundant necrotic spots with distinct chlorotic margin	Moderate susceptible
7	49-60	Large necrotic spots linked together with pronounced chlorosis	Susceptible
8	61-75	Extensive necrotic spots fully merge expanding longituditionally with pronounced chlorosis	Susceptible
9	76-100	Extensive necrotic spots almost covering the entire leaf area expanding longitudinally with pronounced chlorosis	Susceptible

Source: Adapted from Fetch Jr and Steffenson (1999)

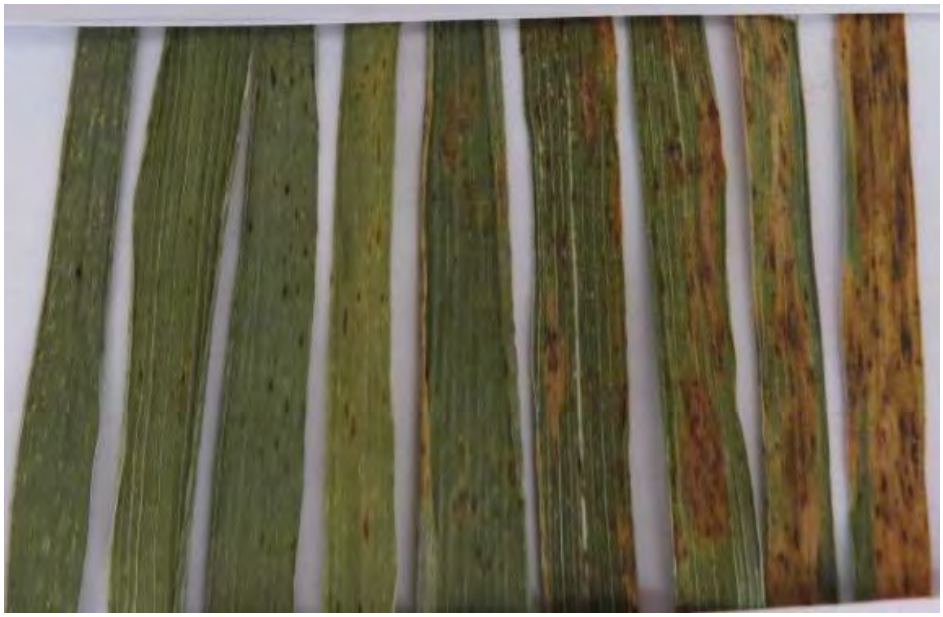


Figure 4.1: Visual rating scale for assessment of the severity of spot blotch disease on wheat (Photo: B. Tembo)

4.2.5 Data analysis

Data collected was subjected to analysis of variance (ANOVA) using individual plot data for each location separately. A combined analysis of variance was performed using the general linear model procedure (PROC GLM) in SAS version 9.3 (SAS Institute, 2011). A combined ANOVA was conducted to determine the effect of genotypes, environment (location, year, and year x location) and the interaction. Disease severity of each plot was found by averaging the severity ratings of the plants. Average disease severity scores for each plot were used for analysis (Nagarajan and Kumar, 1998). Genotypes and sites were considered fixed as they were purposely selected for the study while replications and years were considered as random effects.

The following linear statistical model for combined analysis was used (Annicchiarico, 2002):
Yijkr =μ + gi + lj + (gl) ij + yk + br (ljyk) + (gy) ik + (ly) jk + (gly) ijk + eijkr)
Where Yijkr = observation of genotype i in location j in year k and block r, μ = overall mean, gi= effect of genotype i, lj = effect of location j, yk = effect of year k, br (ljyk) effect of block r within location j and year k, (gy) ik = genotype i x year k interaction, (ly) jk = location j x year k interaction, (gly) ijk = genotype i x location l x year k interaction and eijkr = residual effect.

A genotype main effect (G) plus Genotype x Environment interaction (GE) (GGE) biplot was used to visualize patterns amongst genotypes (resistant and/or susceptible) in each

environment and to distinguish mega-environments. According to Yan and Tinker (2006), a biplot presents the best way of visualizing genotypes and environment interaction patterns and also to visualize presence or absence of cross-over genotype × environment interactions (GEI). The GGE biplot analysis was also used to explore relationships among test environments in their ranking of genotypes. Angles of < 90^o between test environments indicate positive correlation between them while right angles show no correlation. Test environments with angles > 90^o indicate negative correlation (Yan and Tinker, 2006). The discriminating ability of the test environment was also determined by the length of the vector. The length of the environment vector measures the discriminating ability of the test environment. Test environment with long vectors have more discriminating ability compared to those with shorter ones (Badu-Apraku et al., 2013). The GGE biplots were computed in Genstat version 14 (Payne et al., 2011). The GGE biplot analysis model equation was:

Yij- μj= $\lambda_1 \xi i_1 \eta j_1 + \lambda_2 \xi i_2 \eta j_2 + \varepsilon ij$; (Yan, 2001).

where Yij is the average yield of ith genotype in jth environment; μj is the average yield across all genotypes in jth environment; λ_1 and λ_2 are the singular values for principal component 1 (PC1) and PC2, respectively; ξi_1 and ηj_2 are the PC1 and PC2 scores, respectively, for ith genotype; ηj_1 and ηj_2 are the PC1 and PC2 scores, respectively, for jth environment; εij is the residual of the model associated with the ith genotype in jth environment.

4.4 Results

4.4.1 Combined analysis of variance

The analysis of of variance is presented in Table 4.3. The genotypes responded differently in different locations and years as shown by the significant (P< 0.001) genotype (G) × location (L), genotype (G) × year (Y) and G × L × Y.

Table 4.3. Analysis of variance for 150 wheat genotypes for spot blotch disease severity score tested in 2013 and 2014

Source	Degree of freedom	Mean square
Year (Y)	1	500.56***
Location (L)	2	303.19***
Y × L	2	17.13***
Replication (Y × L)	6	645.64
Genotype (G)	149	2.65***
G × Y	149	1.38***
G × L	298	1.69***
G × Y × L	298	1.54**
Error	894	0.67
Corrected total	1799	
CV (%)	15.40	
Mean	5.32	
R^2	93.43	

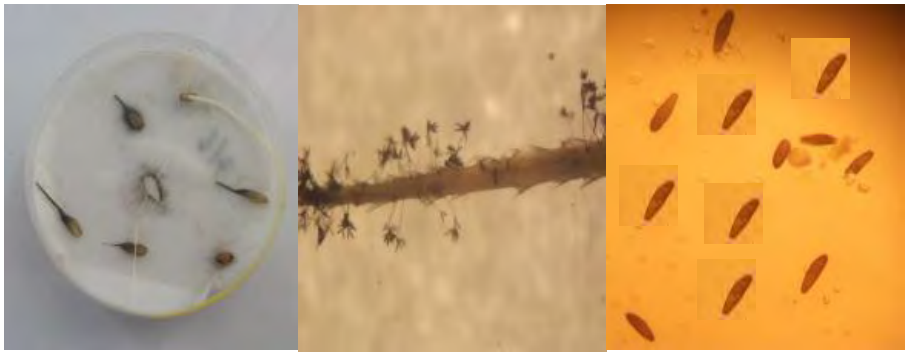
***, **, * indicate significance at P< 0.001, P< 0.01 and P< 0.05, respectively, ns= non-significant

4.4.2 Reaction of the wheat genotypes to spot blotch disease across years

The means of spot blotch disease severity scores for 150 genotypes for 2013 and 2014 and combined across environments are presented in Appendix 4.2.

During 2013 (2012/13 season) the 150 genotypes screened for spot blotch disease had a mean severity score of 4.3 with the range of between 2.0 and 8.0 (Appendix 4.2). Mutanda (E1) had a mean severity score of 3.0 (E1), Mt. Makulu (E2) had a mean score of 4.5 and GART (E3) had a mean severity score of 5.0. In 2014 the disease severity score ranged between 3.0 and 8.0 with the mean of 7.0. Mpongwe (E4) had a mean severity score of 7.3, Mt. Makulu (E5) 7.0 and GART (E6) 6.7. The mean disease severity score was higher in 2013/14 season than in 2012/13 season. During both years, disease symptoms were first observed on the lower leaves and progressed upwards as the season advanced. The symptoms were visibly uniform on most plant parts at flowering stage (Figure 4.2). Diseased leaves and glumes were sampled for the isolation of the fungus in the laboratory to confirm the symptoms of the pathogen. Isolates from the infected wheat glumes clearly showed the conidia of *Bipolaris sorokiniana* (Figure 4.3 (a), (b) and (c)).

Figure 4.2: Symptoms of spot blotch disease on wheat leaves at flowering stage



(a (b) (c)

Figure 4.3: (a) Wheat glumes with spores of *Bipolaris sorokiniana* on a petri dish, (b) Growth of *Bipolaris sorokiniana* on wheat glumes (c) Conidia of *Bipolaris sorokiniana* from the infected wheat glume observed at Mt. Makulu laboratory, 2013 (Magnification= x500)

The frequency distribution for spot blotch disease score for the year 2012/13 and 2013/14 season is presented in Figure 4.4. Based on 0-9 scale, none of the genotypes were symptomless during both seasons. In 2012/13 season, 14.0% were found to be resistant and 55.3% moderately resistant. Moderately susceptible and susceptible groups represented 24.0% and 6.7% of the genotypes respectively. During 2013/14 season, 8.7% of the genotypes were found to be resistant and 7.3% moderately resistant. Moderately susceptible and susceptible made up 36.7% and 47.3% of the genotypes respectively. Across seasons, 13.3% were resistant to moderately resistant, 86.7% moderately susceptible to susceptible.

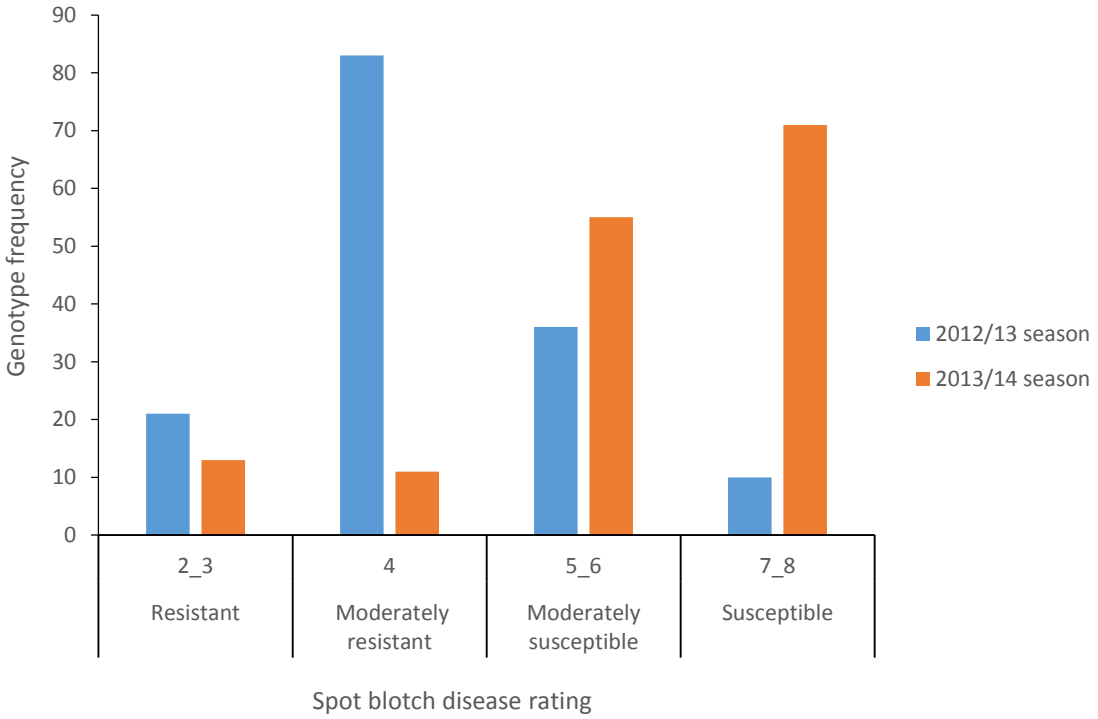


Figure 4.4: Frequency distribution for spot blotch disease severity during 2012/13 and 2013/14 seasons

In 2013 (2012/13season), the ten genotypes obtained from Zambia had a mean disease severity score of 5.0 ranging from 4.0 to 7.0. Seventy percent of these genotypes were moderately resistant while 30% were susceptible and no genotypes were found to be resistant (Figure 4.5). The disease severity score of the CIMMYT lines (72 advanced lines) adapted to high rainfall regions (HRWT) ranged from 2.0 to 5.0 with an average of 4.3. Eight percent were resistant while 49% and 43% were moderately resistant and susceptible, respectively. The eighteen genotypes adapted to semi-arid regions (SAWSN) had a mean disease score of 4.5 with a range of 3.0 to 7.0. Of the fifty spot blotch screening nursery (2nd CSISA-spot blotch) lines from CIMMYT, 26% were resistant while 64% were moderately resistant and 10% were susceptible. The disease score for these genotypes ranged between 2.0 to 8.0 with an average of 4.0. Some examples of the resistant genotypes in 2012/13 season were SB5, SB1 (Chirya-3), 19HRWSN6 (Kenya Heroe), SB4 and SB 33.

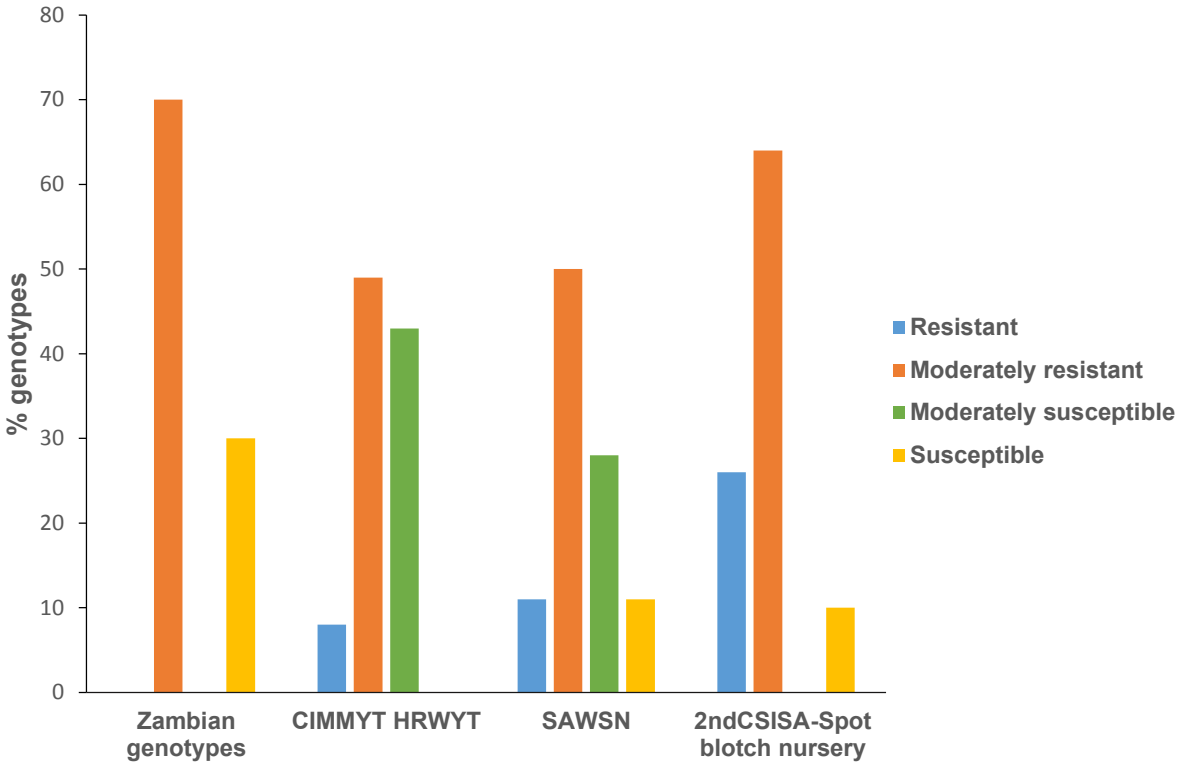


Figure 4.5: Reaction of genotypes from Zambia and CIMMYT-Mexico to spot blotch disease in 2012/13 season

In 2013/14 season, Zambian genotypes had a disease score ranging between 6.0 and 7.0 with an average score of 6.4. During this season 60% were moderately susceptible while 40% were susceptible (Figure 4.6). The mean disease severity score of the CIMMYT-Mexico genotypes adapted to high rainfall areas was 5.6 and the score ranged from 3.0 to 7.0. A majority (55%) of these genotypes were susceptible. Those adapted to semi-arid regions had a mean sore of 6.3 and the severity score ranged between 5.0 and 7.0. The 2nd CSISA-spot blotch nursery had the highest number (61%) of susceptible genotypes during this season. A few examples of resistant genotypes included 19HRWSN6 (Kenya Heroe), SB2 and 19HRWSN15.

However, the most resistant genotypes across environments were from CIMMYT-Mexico and included 19HRWYT6 (Kenya Heroe), 19HRWSN7 (Prontia federal) and 19HRWSN15 (Appendix II). Some of the most susceptible across environement were genotypes, Sonalika from CIMMYT-Mexico, UNZAWV2, Pwele and Loerrie II from Zambia. Most of the Zambian genotypes evaluated had disease scores ranging between 5.0 and 8.0 (moderately susceptible and susceptible, respectively) across environments. No genotype from Zambia was resistant across environments.

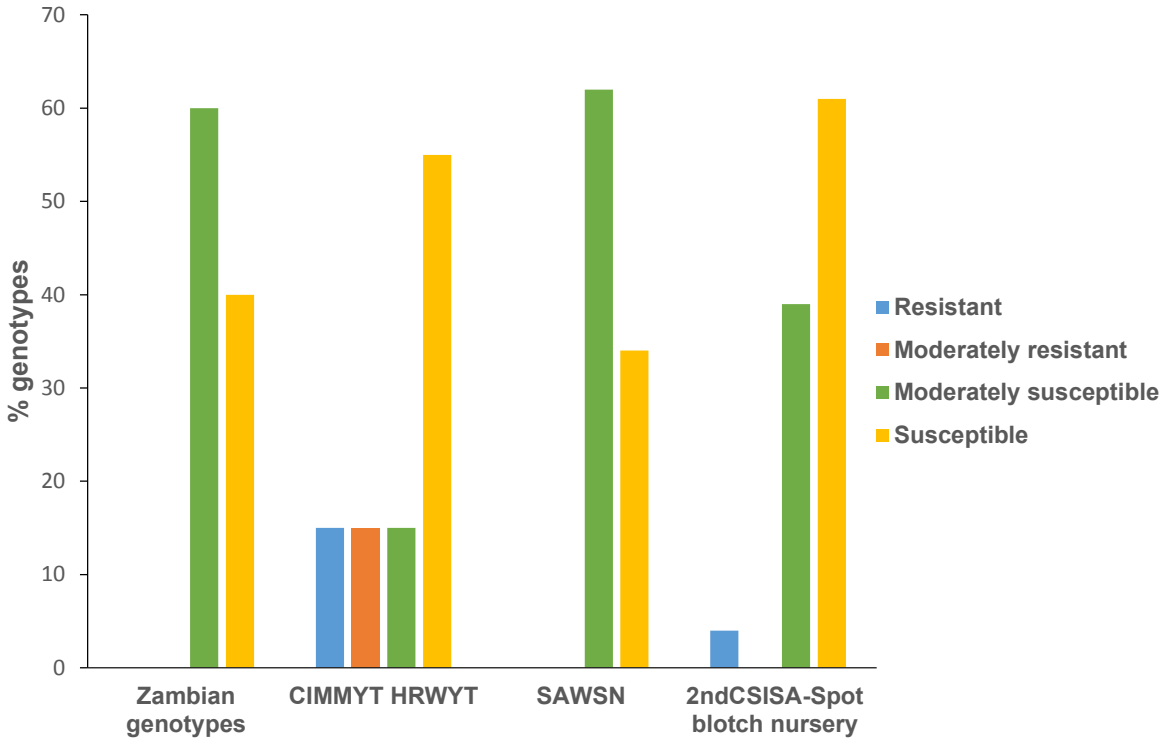


Figure 4.6: Reaction of genotypes from Zambia and CIMMYT-Mexico to spot blotch disease in 2013/14 season

4.4.3 GGE biplot analysis of environments and genotypes on spot blotch severity

The biplot (Figure 4.7) explained 51.0% (PC1=31.8% and PC2= 19.2%) of the total genotype (G) and genotype × Environment (GE) variation. The polygon view presented in Figure 4.7, was divided by the rays into five sectors. The genotypes fell into all the sectors but the locations fell in three of them. This shows that the environments comprised of three different mega environments (I, II and III). The mega- environment (ME) I consisted of environment 6. Mega-environment II had four environments (E) 2, 3, 4, and 5 while environment 1 appeared in mega-environment III. The vertex genotype in mega-environment I was genotype number 6 (16HRWYT5). The vertex genotypes in mega-environment II and III were genotype number 50 (Sonalika) and 52 (20HRWYT3), respectively. Genotype number 103 (19HRWSN7) and 45 (20HRWYT30) were the vertex genotype in a sector where there was no environment. However, genotype number 103 was located very far away from the test locations.

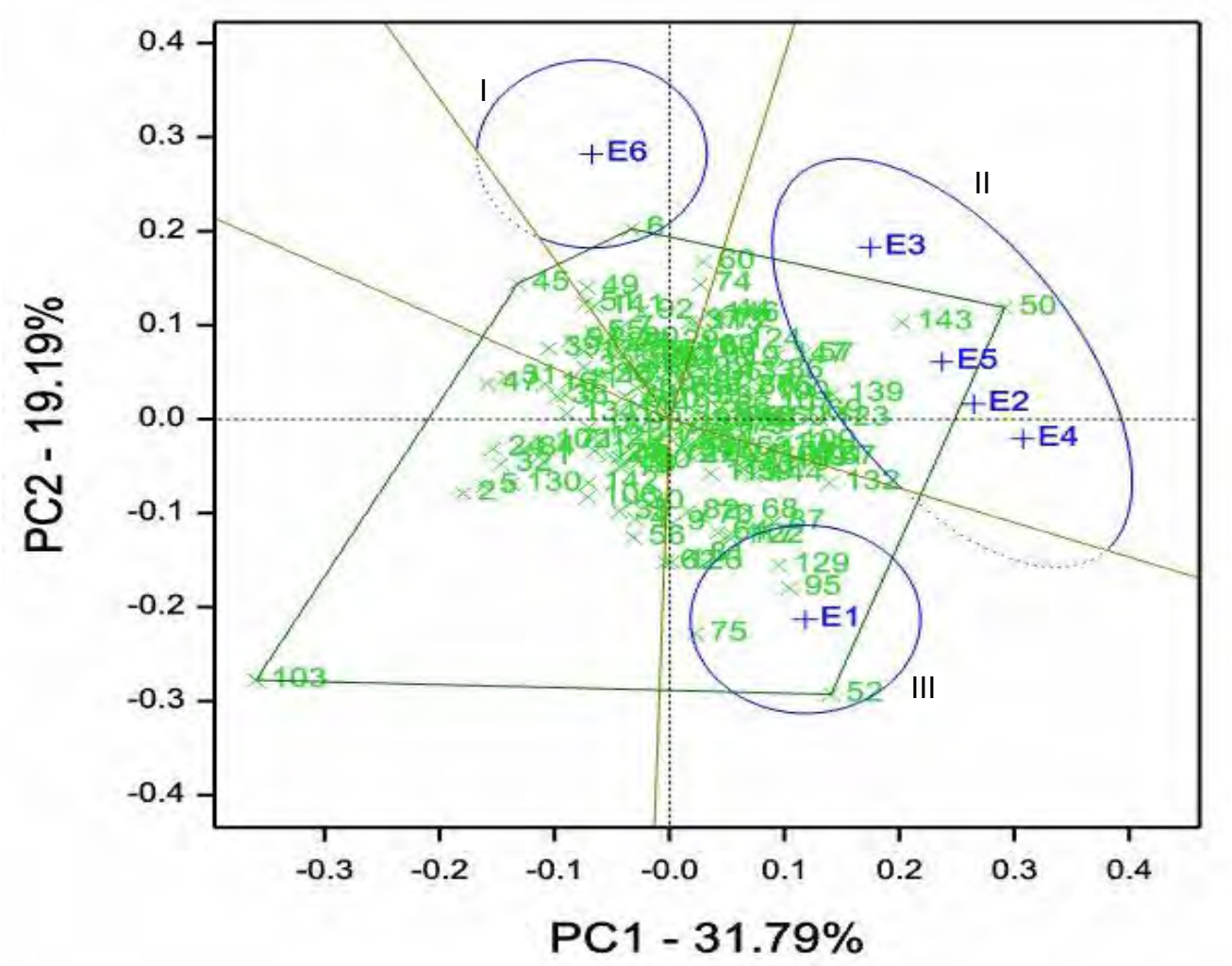


Figure 4.7: Polygon view of the GGE biplot showing which wheat genotype wins where and the mega-environments. Genotypes are labelled 1 to 150. Mega-environments are labelled I, II and III. Details for genotypes are given in Appendix 4.2

In this study all environments except E6 had positive PC1 scores. Environment 6 had a negative PC1 but close to the origin. Environments 2, 4, and 5 had positive PC2 values close to zero. Environments 6 and 3 had large positive PC2 values while environment 1 had negative PC2 scores (Figure 4.8). The angle between E2, E3, E4 and E5 was less than 90^{o}. The largest angle (> 90^{o}) was between E6 and E1 followed by the angle between E4 and E6. With respect to vector length from the origin of the biplot E4 had the longest vector. This was followed by E6, E1, E3, E2, and E5.

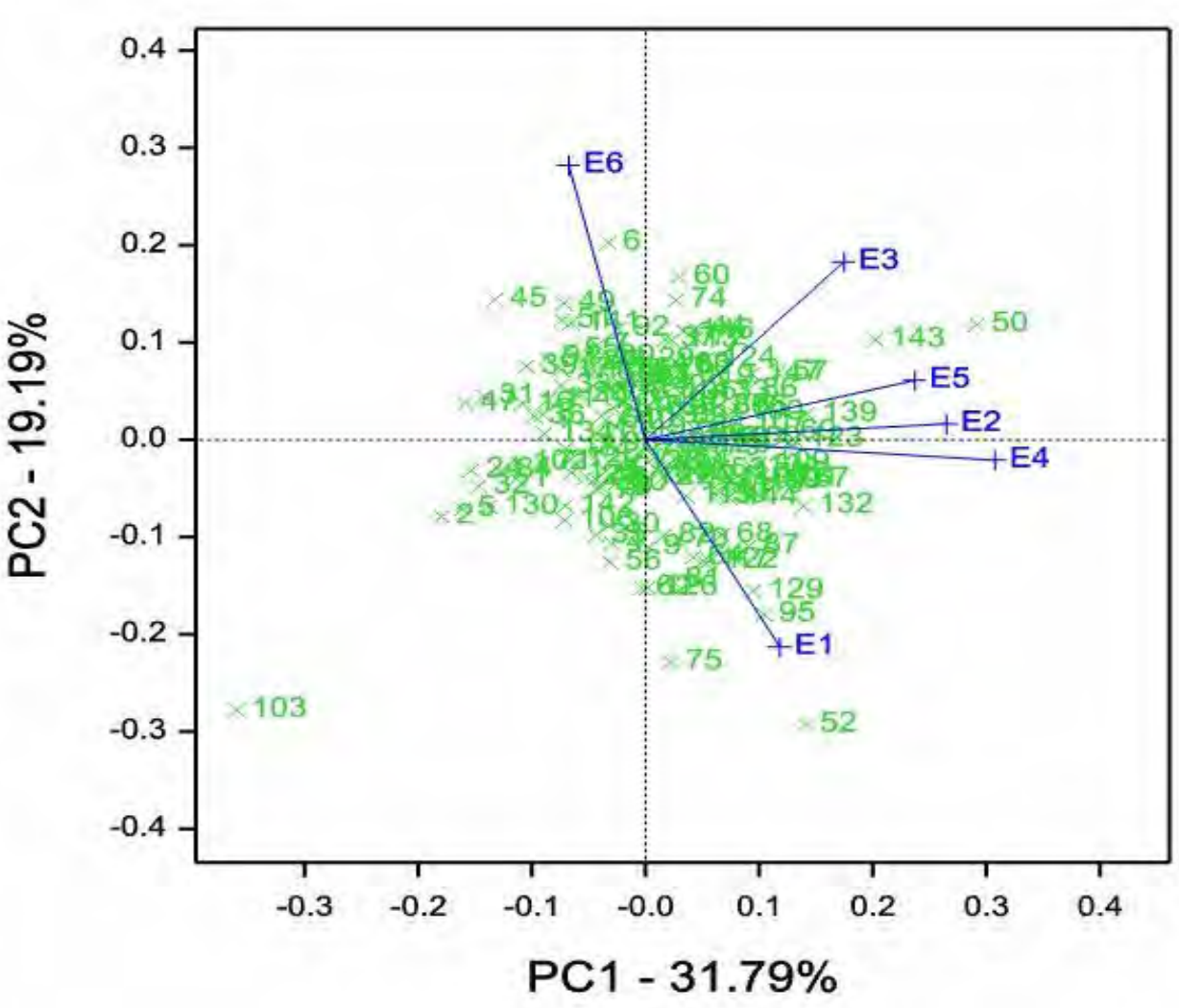


Figure 4.8: GGE biplot showing relationships among test environments in discriminating the genotypes. Environments are labelled E1 to E6. Details for environments are given in Table 1

4.5 Discussion

The analysis of variance revealed variability for resistance to spot blotch disease among the 150 genotypes across study environments. The variation in resistance could be attributed to the different weather conditions in different locations as well as the genetic background of the genotypes. The significance of years, locations, genotype × location interaction (GLI) suggests that genotypes responded differently to locations and years. Significant genotype (G) × year (Y), G × L × Y interactions indicate that the performance of genotypes was inconsistency over years (Gomez and Gomez, 1984). Therefore, screening of genotypes

over locations and years is worthwhile to identify genotypes with stable resistance to spot blotch disease.

Overall across years (seasons), some of the most resistant genotypes were 19HRWSN6 (Kenya Heroe), 19HRWSN7 (Prontia federal) and 19HRWSN15. No genotype was immune to spot blotch disease across seasons. Most of the genotypes obtained from Zambia were moderately susceptible to susceptible across seasons. This implies that the few genotypes that were resistant from CIMMYT-Mexico lines (19HRWSN6, 19HRWSN15, 30SAWSN10) could be utilized in wheat breeding programme to improve spot blotch resistance in Zambia, in the development of new genotypes resistant to spot blotch disease or used as wheat cultivars.

The mean disease severity scores for spot blotch disease for 2012/13 and 2013/14 season were 4.3 and 7.0, respectively. This difference could be attributed to highly conducive climatic conditions such as favourable temperatures, leaves remaining wet for quite a long period of time due to frequent rainfall (Table 4.1) and dew which favoured sporulation, multiplication and spread of the disease in 2013/14 than in 2012/13 season. The results are in line with the work done by several scientists who reported a close association between weather conditions and spot blotch disease severity (Kumar et al., 2002; Sharma and Duveiller, 2007; Duveiller et al., 2007; Acharya et al., 2011). This further emphasizes the need of breeding genotypes resistant to spot blotch to help reduce the disease.

In this study, three mega-environments (ME) were identified for spot blotch disease evaluation. A mega-environment refers to a group of environments that consistently share the best genotypes (Yan et al., 2007). In this study, genotype 6 (16HRWYT5) was the most susceptible in ME I (E6) as it is located at the vertex of the polygon. Genotype 50 (Sonalika) was the most susceptible genotypes in mega-environment II (E2, E3, E4 and E5), whereas genotype 52 was the most susceptible in mega-environment III (E1). The grouping of these genotypes in separate mega-environments was very consistent with their mean performance to spot blotch disease in the aforementioned environments. Genotype 103 (19HRWSN7) fell in a sector without any environment indicating that it exhibited high levels of resistance to spot blotch disease across all test environments (Yan et al., 2001). Genotypes on the vertex of the polygon in each sector are either the best or worst performing as they are further from the biplot origin (Yan and Tinker, 2006). Additionally, they are the most responsive compared to those located within the polygon (Adu et al., 2013). However, those within the polygon but close to the origin, show average reaction across all environments (Yan and Falk, 2002). In this case, genotype 12, 58, 120 and 134 were some examples of genotypes that showed average reaction to spot blotch severity across all locations.

In terms of environmental correlations, environments within ME II were highly correlated in their ranking of genotypes as indicated by the angle between them which was less than 90^{o} (Yan et al., 2007). This indicates that similar information about genotypes was obtained from these environment, suggesting that one location in this mega-environment could be chosen for genotype evaluation in each year. This would help to reduce on the cost of evaluating genotypes and improving efficiency of screening for resistance. The angle between environments E6 and E1, and between E6 and E4 was quite large showing that the environments were not correlated.

In terms of location versus season relationships, Mt. Makulu environments (2 and 5) were grouped in the same mega- environment II, an indication that the seasons were highly correlated. This shows that genotypic differences observed in this location was repeated across years, implying that, it could be a good location for genotype evaluation due to its repeatability. Repeatability is very essential for assessing a test location that is representative of all test locations over years (Bradu-Apraku et al., 2013). Thus a location is considered highly representative if its genotypic rankings are repeated cross years, so that genotypes selected in one year will have greater performance in forthcoming years (Yan et al., 2011). GART environments (E3 and E6) fell in different sectors both years, suggesting that there was no repeatability of genotypes in this location.

All locations except environment 6 had positive PC1 scores, an indication that they were discriminating of genotypes. However, environments 1 (Mutanda), 4 (Mpongwe) and GART (E6) were considered highly discriminating among genotypes as shown by the length of their vectors from the biplot origin. The length of a vector of a test environment estimates the discriminating ability of genotypes (Badu-Apraku et al., 2013). The longer the vector the higher the ability to discriminate genotypes and the shorter the vector the lesser the discriminating ability (Yan and Tinker, 2006). Mt. Makulu environments (E2 and E5) had short vectors indicating that they had the least discriminating ability of genotypes. Yan et al. (2010) indicated that environments with shorter vectors could be considered as independent test environments, treated as unique and essential test environment.

The GGE biplot showed that environments 1 (Mutanda), 3 (GART2012/13) and 6 (GART2013/14) contributed most of the GEI variability in terms of genotype reaction to spot blotch disease as these were located further apart in the biplot (Joshi et al., 2007). This implies that a genotype could have huge positive interaction with some environments while having large negative interactions with some other environments (Yan and Hunt, 2001). The GEI could affect the efficiency of breeding for resistance. Pinnschmidt and Hovmøller (2002) reported that GEI affects breeding for high levels of resistance due to inconsistency in the

phenotypic expression of the disease. Moreover, it complicates selection of desirable genotypes (Farshadaf et al., 2012).

4.6 Conclusion

There was large variation in spot blotch disease severity among the 150 wheat genotypes screened for resistance to spot blotch disease in different environments over years during the study period. This could be attributed to differences in environmental conditions in different locations and also the genetic background of the genotypes. However, some genotypes that possessed resistance to spot blotch disease were identified. Most of the resistant and moderately resistant genotypes were identified among CIMMYT-Mexico lines and none was found among the Zambian genotypes across seasons. The result reveals the problem of growing rain-fed wheat in Zambia and hence confirms the need to develop genotypes resistant to the disease. Some of the resistant genotypes identified across locations included 19HRWSN6, 19HRWSN7 and 19HRWSN15. These resistant genotypes could be utilized to enhance resistance in the locally adapted Zambian genotypes. The GGE biplot analysis identified genotype 19HRWSN7 as the most resistant across all test environments. Furthermore, three mega-environments were identified. Mega-environment I had GART environment E6. Mpongwe (E4), Mt. Makulu environments (E5 and E2) and GART environment E3 formed ME II, while ME III had only Mutanda (E1).The test locations within the mega-environment II were highly correlated. This implies that they discriminated the genotypes similarly hence one location within the mega environment could be chosen for genotype evaluations in each year. This would reduce the cost of evaluating genotypes and improve the efficiency of screening for resistance.

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Appendix 4.1

Pedigrees of 150 wheat genotypes wheat germplasm assessed for spot blotch disease reaction during 2012/13 and 2013/14 seasons

Variety	Name	Pedigree	Source
Loerrie II	KAVKAZ/BUHO´S'//KALVANSONA/BLUEBIRD	CM33027-F-15M-500Y-0M-87B-0Y	Mt.Makulu Research
Nkhanga	BUC'S'/PVN'S'	CM58766-18Y-3M-5Y-2M-OY	Mt.Makulu Research
Nseba	CHIL/2*STAR	CM112793-0TOPY-7M-020Y-010M-2Y-010M-0Y	Mt.Makulu Research
Pwele	CNO79/PRL'S'	CM83271-2Y-8B-1Y-2B-OY	Mt.Makulu Research
UNZAWV1		CMBW90MY3058-74M-015Y-015M-1Y-BATTILA/3*BCN	University of Zambia
UNZAWV2		CMBW90Y4399-OTOPM-1Y-010-01OY-8M	University of Zambia
Coucal		CM70377-3MB-0MM-1MB0MM-2MM-0MM	Mt.Makulu Research
Mampolyo			Mt.Makulu Research
Sahai			SeedCo, Zambia
Kwale			Mt.Makulu Research
SB1	CHIRYA.3	CIGM87.116-3Y-2M-1PR-3M-2PR-4B-0PR-1Y-0M	CIMMYT- Mexico
SB2	TILHI/4/CROC_1/AE.SQUARROSA(23)//PGO/3/CMH81.38/…	CMSS04Y0092S-099Y-099ZTM-099Y-099M-3WGY-0B	CIMMYT- Mexico
SB3	CROC_1AE.SQUARROSA(205)//KAUZ/3/SASIA/4/TROST	CMSS04Y00467S-099Y-099ZTM-099Y-099M-2WGY-0B	CIMMYT- Mexico
SB4	SERI.1B*2/3/KAUZ*2/BOW//KAUZ*2/4/KRONSTAD F2004	CGSS04Y00012T-099M-099ZTM-099Y-099M-7WGY-0B	CIMMYT- Mexico
SB5	CROC_1AE.SQUARROSA(205)//KAUZ/3/SASIA/4/TROST	CMSS04Y00467S-099Y-099ZTM-099Y-099M-1WGY-0B	CIMMYT- Mexico
SB6	PBW343*2/KUKUNA//KRONSTAD F2004/3/PBW343*2/KUKUNA	CGSS04Y00026T-099M-099Y-099ZTM-099Y-099M-8WGY-0B	CIMMYT- Mexico
SB7	SERI.1B*2/3/KAUZ*2/BOW//KAUZ*2/5/CN079//PF70354/MUS/3/…	CGSS04Y00058T-099M-099Y-099M-099Y-099M-1WGY-0B	CIMMYT- Mexico
SB8	TILHI / PALMERIN F2004	CMSS04Y00100S-099Y-099ZTM-099Y-099M-6WGY-0B	CIMMYT- Mexico
SB9	TILHI / PALMERIN F2004	CMSS04Y00100S-099Y-099ZTM-099Y-099M-5WGY-0B	CIMMYT- Mexico
SB10	CROC_1AE.SQUARROSA(205)//KAUZ/3/SASIA/4/TROST	CMSS04Y00467S-099Y-099ZTM-099Y-099M-7WGY-0B	CIMMYT- Mexico
SB11	PFAU/SERI.1B/ /AMAD/3/2*HUW234+LR34/PRINIA	CGSS04Y00053T-099M-099Y-099M-099Y-099M-3WGY-0B	CIMMYT- Mexico
SB12	MUNIA/CHTO/3/PFAU/BOW//VEE#9/4/CHEN/…	CMSS04Y00155S-099Y-099TZM-099Y-099M-2WGY-0B	CIMMYT- Mexico
SB13	PFAU/MILAN//PBW343*2/TUKURU	CMSS04Y00070S-099Y-099ZTM-099Y-099M-4WGY-0B	CIMMYT- Mexico
SB14	SERI.1B*2/3/KAUZ*2/BOW//KAUZ*2/4/KRONSTAD F2004	CGSS04Y00012T-099M-099Y-099ZTM-099Y-099M-5WGY-0B	CIMMYT- Mexico
SB15	SERI.1B*2/3/KAUZ*2/BOW//KAUZ/4/PBW343*2/KHVAKI/5/…	CGSS04Y00052T-099M-099Y-099M-099Y-099M-8WGY-0B	CIMMYT- Mexico

Appendix 4.1 continued

Variety	Name	Pedigree	Source
SB16	PRL/2*PASTOR/4/URES/JUN//KAUZ/3/BAV92	CMSS04Y00185S-009Y-099ZTM-099Y-099M-3WGY-0B	CIMMYT- Mexico
SB17	TIMBA/FILIN/MILAN/4/BCN/3/FGO/USA2111//…	CMSS04Y01341T-0TOPM-099Y-099ZTM-099Y-099M-4WGY-0B	CIMMYT- Mexico
SB18	MESIA//PBW343*2/KUKUNA	CMSS04Y00262S-099Y-099ZTM-099Y-099M-3GWY-0B	CIMMYT- Mexico
SB19	WHEAR/3/PBW343/PASTOR//ATTILA/3*BCN	CMSS04M00341S-0Y-099ZTM-099Y-099M-9WGY-0B	CIMMYT- Mexico
SB20	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS) /4/…	CMSS0400421S-099Y-099ZTM-099Y-099M-3WGY-0B	CIMMYT- Mexico
SB21	PBW343*2/KUKUNA//PBW343*/TUKURU/3/PWB343	CGSS04B00047T-099Y-099ZTM-099Y-099M-8WGY-0B	CIMMYT- Mexico
SB22	PBW343*2/KUKUNA//PBW343*2/KUKUNA	CGSS04Y00099S-099Y-099M-099Y-099M-2WGY-0B	CIMMYT- Mexico
SB23	PBW343*2/KUKUNA//PBW343*2/KUKUNA	CGSS04Y00099S-099Y-099M-099Y-099M-21WGY-0B	CIMMYT- Mexico
SB24	CROC_1AE.SQUARROSA(205)//KAUZ/3/SASIA/4/TROST	CMSS04Y00467S-099Y-099ZTM-099Y-099M-4WGY-0B	CIMMYT- Mexico
SB25	MUNIA/CHTO/3/PFAU/BOW//VEE#9/4/CHEN/…	CMSS03M00096S-099ZTM-099Y-099M-5WGY-0B	CIMMYT- Mexico
SB26	INQALAB 91*2/KUKUNA//2*KRONSTAD F2004	CGSSO4B00056T-099Y-099ZTM-099Y-099M-9RDY-0B	CIMMYT- Mexico
SB27	PBW343*2/KUKUNA//PBW343*2/KUKUNA	CGSS04Y0099S-099Y-099M-099Y-099M-4WGY-0B	CIMMYT- Mexico
SB28	SERI.1B*2/3/KAUZ*2/BOW//KAUZ*2/5/CNO79//PF70354/MUS/3/…	CGSS04Y00058T-099M-099Y-099M-099Y-099M-2WGY-0B	CIMMYT- Mexico
SB29	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS) /4/…	CMSS04M01331S-0TOPY-099ZTM-099Y-099M-3WGY-0B	CIMMYT- Mexico
SB30	PBW343*2/KUKUNA//PBW343*2/KUKUNA	CGSS04Y00099S-099Y-099M-099Y-099M-10WGY-0B	CIMMYT- Mexico
SB31	SERI.1B*2/3/KAUZ*2/BOW//KAUZ*2/5/CNOP79//PF70354/MUS/3/…	CGSS04Y00058T-099M-099Y-099M-099Y-099M-11WGY-0B	CIMMYT- Mexico
SB32	WEAVER//VEE/PJN/3/MILAN/4/BL 1496/MILAN/3/CROC_1/…	CMSS04M00116S-0Y-099TZM-099Y-099M-5WGY-0B	CIMMYT- Mexico
SB33	SHA7/VEE#5/5/VEE#8//JUP/BJY/3/F3.71/TRM/42*WEAVER/6/…	CMSS04M01800S-0TOPY-099ZTM-099Y-099M-1WGY-0B	CIMMYT- Mexico
SB34	PBW343*2/KUKUNA//PBW343*2/KUKUNA	CGSS04Y00O99S-099Y-099M-099Y-099M-20WGY-0B	CIMMYT- Mexico
SB35	CROC_1AE.SQUARROSA(205)//KAUZ/3/ATTILA/4SW89.5193/…	CMSS04Y00455S-099Y-099ZTM-099Y-099M-1WGY-0B	CIMMYT- Mexico
SB36	PFAU/MILAN/4/CROC_1/AE.SQUARROSA(205)//KAUZ/3/…	CMSS04M00055S-0Y-099ZTM-099Y-099M-9WGY-0B	CIMMYT- Mexico
SB37	WAXWING//PBW343*2/KUKUNA	CGSS04Y00088S-099Y-099M-6WGY-0B	CIMMYT- Mexico
SB38	WAXWING*2/CIRCUS	CGSS04Y00021T-099M-099Y-099ZTM-099Y-099M-10WGY-0B	CIMMYT- Mexico
SB39	PBW343/HUTIES/4/YAR/AE.SQUARROSA (783)//MILAN/3/BAV92	CMSS04M00348S-0Y-099ZTM-099Y-099M-10WGY-0B	CIMMYT- Mexico
SB40	PBW343*2/KUKUNA//PBW343*2/KUKUNA	CGSS04Y00099S-099Y-099M-099Y-099M-7WGY-0B	CIMMYT- Mexico
SB41	PFAU/MILAN//TROST/3/PBW65/2*SERI.1B	CMSS04M1426S-0TOPY-99ZTM-099Y-099M-10WGY-0B	CIMMYT- Mexico
SB42	PBW343*2/KUKUNA//PBW343*2/KUKUNA/3/PBW343	CGSS04B00046T-099Y-099ZTM-099Y-099M-6RGY-0B	CIMMYT- Mexico

Appendix 4.1 continued

Variety	Name	Pedigree	Source
SB43	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS) /4/…	CMSS04Y00421S-099Y-99ZTM-099Y-099M-4WGY-0B	CIMMYT- Mexico
SB44	SERI.1B*2/3/KAUZ*2/BOW//KAUZ*2/5/CN079//PF70354/MUS/3/…	CGSS04Y00058T-099M-099M-099Y-099M-10WGY-0B	CIMMYT- Mexico
SB45	PASTOR/2*SITTA//PBW343*2/KUKUNA	CMSS04Y00333S-099Y-099ZTM-099Y-099M-3WGY-0B	CIMMYT- Mexico
SB46	ELVIRA//INGALAB91*2/KUKUNA	CMSS04Y00014S-099Y-099ZTM-099Y-099M-2GWY-0B	CIMMYT- Mexico
SB47	MESIA//PBW343*2/KUKUNA	CMSS04Y00262S-099Y-099ZTM-099Y-099M-5GWY-0B	CIMMYT- Mexico
SB48	WAXWING*2/KRONSTAD F2004	CGSS04Y00020T-099M-099Y-099ZTM-099Y-099M-3WGY-0B	CIMMYT- Mexico
SB49	SERI.1B*2/3/KAUZ*2/BOW//KAUZ*2/5/CNO79//PF70354/MUS/3/…	CGSS04Y00058T-099M-099Y-099M-099Y-099M-9WGY-0B	CIMMYT- Mexico
SB50	SONALIKA	II18427-4R-1M	CIMMYT- Mexico
20HRWT02	PBW343	CM85836-4Y-0M-0Y-8M-0Y-0IND	CIMMYT- Mexico
20HRWT03	D67.2PARANA 66.270//AE.SQUARROSA (320) /3/CUNNINGHAM/4/…	CMSA06M00431S-040ZTM-040ZTY-32ZTM-01Y-0B	CIMMYT- Mexico
20HRWT04	D67.2PARANA 66.270//AE.SQUARROSA (320) /3/CUNNINGHAM/4/…	CMSA06M00431S-040ZTM-040ZTY-34ZTM-01Y-0B	CIMMYT- Mexico
20HRWT05	WORRAKATTA/2*PASTOR//VORB	CMSA06M00468S-040ZTM-040ZTY-28ZTM-0Y-0B	CIMMYT- Mexico
20HRWT06	VORB/3/T.DICOCCON PI94625/AE.SQURROSA (372) //3*PASTOR	CMSA06M00667S-040ZTM-040ZTY-10ZTM-0Y-0B	CIMMYT- Mexico
20HRWT07	VORB/4/D67.2/PARANA 66.270//AE.SUQARROSA (320) /3/…	CMSA06Y00202S-040ZTP0Y-040ZTM-040SY-22ZTM-0Y-0B	CIMMYT- Mexico
20HRWT08	SOISSONS/KUKUNA//WBLL1*2/TURUKU	CMSA06Y00863T-036(WMC256+WMC032 SOI NEG)M-040ZTP0Y-040ZTM-040SY-4ZTM	CIMMYT- Mexico
20HRWT09	T.DICOCCON PI254156/3*KAUZ//2*STYLET	CMSA05M00628T-050Y-052ZTM-040ZTY-040ZTM-040SY-30ZTM-0Y-0B	CIMMYT- Mexico
20HRWT10	SERI*3//RL6010/4*YR/3/PASTOR/4/BAV92/5/VORB	CMSA06M0067S-040ZTM-040ZTY-6ZTM-0Y-0B	CIMMYT- Mexico
20HRWT11	C80.1/3*BATAVA//2*WBLL1/4/D67.2/PARANA 66.270//…	CMSA06M00135T-040-040ZTM-040ZTY-7ZTM-0Y-0B	CIMMYT- Mexico
20HRWT12	BABAX/LR39//BABAX/3/VORB/4/SUNCO/2*PASTOR	CMSA06Y00792T-040ZTM-040ZTP0Y-040ZTM-040SY-28ZTM-02Y-0B	CIMMYT- Mexico
20HRWT13	VORB/4/D67.2/PARANA 66.270//AE.SUQARROSA (320) /3/…	CMSA06Y00835T-040ZTM-040ZTP0Y-040ZTM-040P0Y-3ZTM-02Y-0B	CIMMYT- Mexico
20HRWT14	VORB/4/D67.2/PARANA 66.270//AE.SUQARROSA (320) /3/…	CMSA06Y00835T-040ZTM-040ZTP0Y-040ZTM-040P0Y-3ZTM-03Y-0B	CIMMYT- Mexico
20HRWT15	VORB/6/CPI8/GEDIZ/3/GOO//ALB/CRA/4/AE.SQUARROSA (208)	CMSA06Y00836T-040ZTM-040ZTO0Y-040ZTM-040P0Y-26ZTM-01Y-0B	CIMMYT- Mexico
20HRWT16	T.TAU.83.2.29/ATTILA//ATTILA/3/EXCALIBUR	CMSA06Y00221S-040ZTP0Y-040ZTM-040P0Y-5ZTM-0Y-0B	CIMMYT- Mexico
20HRWT17	KRICHAUFF/2*PASTOR/3/PFAU/WEAVER//KIRITATI/4/PFAU/…	CMSA06M00014T-040Y-040ZTM-0NJ-0NJ-9Y-3B-0Y-0B	CIMMYT- Mexico
20HRWT18	VORB*2/3/PFAU/WEAVER//KIRITATI	CMSA06M00111T-040Y-040ZTM-0NJ-0NJ-7Y-3B-0Y-0B	CIMMYT- Mexico
20HRWT19	VORB*2/3/PFAU/WEAVER//KIRITATI	CMSA06M00111T-040Y-040ZTM-0NJ-0NJ-15Y-3B-0Y-0B	CIMMYT- Mexico
20HRWT20	VORB*2/3/PFAU/WEAVER//KIRITATI	CMSA06M00111T-040Y-040ZTM-0NJ-0NJ-40Y-3B-0Y-0B	CIMMYT- Mexico

Appendix 4.1 continued

Variety	Name	Pedigree	Source
20HRWT21	VORB*2/3/PFAU/WEAVER//KIRITATI	CMSA06M00111T-040Y-040ZTM-0NJ-0NJ-44Y-2B-0Y-0B	CIMMYT- Mexico
20HRWT22	VORB*2/3/PFAU/WEAVER//KIRITATI	CMSA06M00111T-040Y-040ZTM-0NJ-0NJ-48Y-1B-0Y-0B	CIMMYT- Mexico
20HRWT23	MRUGA/KRONSTAD F2004	CMSA06Y00124S-0B-099Y-099ZTM-099Y-099M-9RGY-0B	CIMMYT- Mexico
20HRWT24	TACUPETO F2001/6/CNDO/R143//ENTE/MEXI_2/3/…	CMSS06Y00716T-099TOPM-099Y-099ZTM-099NJ-099NJ-11RGY-0B	CIMMYT- Mexico
20HRWT25	TACUPETO F2001/6/CNDO/R143//ENTE/MEXI_2/3/…	CMSS06Y00716T-099TOPM-099Y-099ZTM-099NJ-099NJ-2RGY-0B	CIMMYT- Mexico
20HRWT26	WBLL1*2/KUKUNA//KIRITATI/3/WBLL1*2/KUKUNA	CMSS06Y00742T-099TOPM-099Y-099ZTM-099NJ-099NJ-9RGY-0B	CIMMYT- Mexico
20HRWT27	NORM/WBLL1//WBLL1/3/TNMU/4/WBLL1*2/TUKURU	CMSS06Y00755T-099TOPM-099Y-099ZTM-099Y-099M-1RGY-0B	CIMMYT- Mexico
20HRWT28	PBW343*2//YANAC	CMSS06Y00849T-099TOPM-099Y-099ZTM-099Y-099M-5RGY-0B	CIMMYT- Mexico
20HRWT29	WBLL1*2/4/YACO/PBW65/3/KAUZ*2/TRAP//KAUZ*2/5/..	CMSS06Y01201T-099TOPM-099Y-099ZTM-099Y-099M-12RGY-0B	CIMMYT- Mexico
20HRWT30	FRANCOLIN #1/4/BABAX/LR42//BABAX*2/3/KURUKU	CMSS06B00010S0-0Y-099ZTM-099NJ-099NJ-9RGY-0B	CIMMYT- Mexico
20HRWT31	FRANCOLIN #1/4/BABAX/LR42//BABAX*2/3/KURUKU	CMSS06B00010S0-0Y-099ZTM-099NJ-099NJ-22RGY-0B	CIMMYT- Mexico
20HRWT32	PFAU/SERI.1B/ /AMAD/3/WAXWING/4/BABAX/LR42//BABX*2/3/…	CMSS06B00033S-0Y-099ZTM-099Y-099M-3RGY-0B	CIMMYT- Mexico
20HRWT33	WBLL1*2/KURUKU/4/BABAX/LR42//BABAX*2/3/KURUKU	CMSS06B0018S-0Y-099ZTM-099Y-099M-10RGY-0B	CIMMYT- Mexico
20HRWT34	WBLL1*2/KURUKU/4/BABAX/LR42//BABAX*2/3/KURUKU	CMSS06B0018S-0Y-099ZTM-099Y-099M-17RGY-0B	CIMMYT- Mexico
20HRWT35	WBLL1*2/BRAMBLING//JUCHI	CMSS06B00402S-0Y-099ZTM-099NJ-099NJ-15RGY-0B	CIMMYT- Mexico
20HRWT36	WBLL1*2/KKTS//KINGBIRD #1	CMSS06B00413S-0Y-099ZTM-099Y-099M-14RGY-0B	CIMMYT- Mexico
20HRWT37	WBLLI*2/KKTS//KINGBIRD #1	CMSS06B00413S-0Y-099ZTM-099Y-099M-15RGY-0B	CIMMYT- Mexico
20HRWT38	TACUPETO F2001//WBLL1*2/KKTS/3/WBLL1*2/BRAMBLING	CMS06B00699T-099TOPY-099ZTM-099Y-099M-1RGY-0B	CIMMYT- Mexico
20HRWT39	TACUPETO F2001/SAUAL/4/BABAX/LR42//BABAX*2/3/KURUKU	CMSS06B00700T-099TOPY-099ZTM-099NJ-099NJ-8RGY-0B	CIMMYT- Mexico
20HRWT40	WBLL1*2/KURUKU//KRONSTAD F2004/3/WBLL1*2/BRAMBLING	CMSS06B00720T-099TOPY-099ZTM-099Y-099M-3RGY-0B	CIMMYT- Mexico
20HRWT41	WBLL1*2/TURUKU*2//KRONSTAD F2004	CMSS06B00723T-099TOPY-099ZTM-099Y-099M-1RGY-0B	CIMMYT- Mexico
20HRWT42	YAV_3/SCO//JO69/CRA/3/YAV79/4/AE.SQUARROSA (498) /5/…	CMSS06B00762T-099TOPY-099ZTM-099Y-099M-18RGY-0B	CIMMYT- Mexico
20HRWT43	ATTILA*2/PBW65*2/5/REH/HARE//2*BCN/3/CROC_1/…	CMSS06B00786T-099TOPY-099ZTM-099Y-099M-7RGY-0B	CIMMYT- Mexico
20HRWT44	ATTILA*2/PBW65*2/5/REH/HARE//2*BCN/3/CROC_1/…	CMSS06B00786T-099TOPY-099ZTM-099Y-099M-11RGY-0B	CIMMYT- Mexico
20HRWT45	BABAX/LR42//BABAX/3/BABAX/LR42//BABAX/4/ATTILA/…	CMSS06B00795T-099TOPY-099ZTM-099NJ-099NJ-8RGY-0B	CIMMYT- Mexico
20HRWT46	BABAX/LR42//BABAX/3/BABAX/LR42//BABAX/4/ATTILA/…	CMSS06B00795T-099TOPY-099ZTM-099NJ-099NJ-9RGY-0B	CIMMYT- Mexico
20HRWT47	PRL/2*PASTOR*2//VORB	CMSS06BOO867T-099TOPY-099ZTM-099Y-099M-3RGY-0B	CIMMYT- Mexico

Appendix 4.1 continued

Variety	Name	Pedigree	Source
20HRWT48	PFAU/WEAVER//KIRITATI/3/FRET2/TUKURU//FRET2/4/FRET2/…	CMSS06B00961T-099TOPY-099ZTM-099Y-099M-2RGY-0B	CIMMYT- Mexico
20HRWT49	KSW/5/2*ALTAR 84/AE.SQURROSA (221) //3*BORL95/3/URES/…	CMSS06B01003T-099TOPY-099ZTM-099Y-099M-9RGY-0B	CIMMYT- Mexico
20HRWT50	KFA/2*KACHU	CMSS06B01005T-099TOPY-099ZTM-099Y-099M-1RGY-0B	CIMMYT- Mexico
30SAWSN2	DHARWAR DRY	0IND	CIMMYT- Mexico
30SAWSN3	CHAM 6	CM40096-8M-7Y-0M-0AP-0LBN	CIMMYT- Mexico
30SAWSN4	PBW343	CM85836-4Y-0M-0Y-8M-0Y-0IND	CIMMYT- Mexico
30SAWSN5	VOROBEY	CMSS96Y02555S040Y-020M-050SY-020SY-27M-0Y	CIMMYT- Mexico
30SAWSN6	BERKUT	CMSS96M05638T-040Y-26M-010SY-010M-010SY-4M-0Y-011Y	CIMMYT- Mexico
30SAWSN7	W15.92/4/PASTOR//HXL7573/2*BAU/3/WBLL1	PTSS02B00102T-0TOPY-0B-0Y-11Y-0M-0SY	CIMMYT- Mexico
30SAWSN8	GK ARON/AG SECO 7846//2180/4/2*MILAN/KAUZ//…		CIMMYT- Mexico
30SAWSN9	BOW/VEE/5/ND/VG9144//KAL/BB/3/YACO/4/CHIL/6/..	CMSA04M01201T-050Y-040ZTP0M-040ZTY-040ZTM-040SY-…	CIMMYT- Mexico
30SAWSN10	BOW/VEE/5/ND/VG9144//KAL/BB/3/YACO/4/CHIL/6/..	CMSA04M01201T-050Y-040ZTP0M-040ZTY-040ZTM-040SY-…	CIMMYT- Mexico
30SAWSN11	D67.2/PARANA 66.270//AE.SQUARROSA (320)/3/…	CMSA06M00431S-040ZTM-040ZTY-31ZTM-02Y-0B	CIMMYT- Mexico
30SAWSN12	D67.2/PARANA 66.270//AE.SQUARROSA (320)/3/…	CMSA06M00431S-040ZTM-040ZTY-31ZTM-04Y-0B	CIMMYT- Mexico
30SAWSN13	H45/4/KRICHAUFF/FINSI/3/URES/PRL//BAV92	CMSA06M00501S-040ZTM-040ZTY-11ZTM-Y-0B	CIMMYT- Mexico
30SAWSN14	VORB/SOKOLL	CMSA06M00621S-040ZTM-040ZTY-16ZTM-01Y-0B	CIMMYT- Mexico
30SAWSN15	VORB/SOKOLL	CMSA06M00621S-040ZTM-040ZTY-16ZTM-03Y-0B	CIMMYT- Mexico
30SAWSN16	VORB/SOKOLL	CMSA06M00621S-040ZTM-040ZTY-16ZTM-04Y-0B	CIMMYT- Mexico
30SAWSN18	VORB/3/T.DICOCCON P194625/AE.SQUARROSA (372)…	CMSA06M00667S-40ZTM-040ZTY-50ZTM-2Y-0B	CIMMYT- Mexico
30SAWSN19	CNO79//PF70354/MUS/3/PASTOR/4/BAV92/5/MILAN/…	CMSA06Y00093S-40ZTP0Y-040ZTM-040SY-5ZTM-0Y-0B	CIMMYT- Mexico
30SAWSN21	EGA BONNIE ROCK/4/MILAN/KAUZ//PRINIA/3/BAV92	CMSA06Y00125S-040ZTPY-040ZTM-040SY-2ZTM-01Y-0B	CIMMYT- Mexico
19HRWSN2	KLEIN CHAMACO	KLB103.71-20Y-8M-1100YK-0ARG	CIMMYT- Mexico
19HRWSN3	HUAYUN INIA	0CHL	CIMMYT- Mexico
19HRWSN4	FUNDACEP30	OBRA	
19HRWSN6	KENYA HEROE	-0KEN	
19HRWSN7	PROINTA FEDERAL	CM33203-M-8M-8Y-1M-1Y-1M-0Y-1T-2T-0ARG	CIMMYT- Mexico
19HRWSN15	BRBT2/METSO	CMSA00M00142S-040P0M-040Y-030M-030ZTM-7ZTY-0M-0SY	CIMMYT- Mexico
19HRWSN16	BJY/COC//PRL/BOW/3/FRET2	CMSA00M00214S-040M-040Y-030M-030ZTM-8ZTY-0M-0SY	CIMMYT- Mexico
19HRWSN19	RABE/2*MO88/3/CAZO/KAUZ//KAUZ	CMSA00Y00199S-0P0Y-040M-010TSB-010ZTB-2ZTY-0M-0SY	CIMMYT- Mexico

Appendix 4.1 continued

Variety	Name	Pedigree	Source
19HRWSN21	VERDE/3/BCN//DOY1/AE.SQUARROSA (447)	CMSS00M0004S-030M-12Y-3SCM-1Y-0FGR-0Y	CIMMYT- Mexico
19HRWSN22	VERDE/3/BCN//DOY1/AE.SQUARROSA (447)	CMSS00M0004S-030M-12Y-6SCM-1Y-0FGR-0Y	CIMMYT- Mexico
19HRWSN23	VERDE/7/OPATA/6/68.111/RGB-U//WARD/3/FGO/4/…	CMSS00M00041S-030M-6Y-1SCM-1Y-0FGR-0Y	CIMMYT- Mexico
19HRWSN24	BCN/3/68112/WARD//AE.SQUARROSA (369)/4/…	CSS00GH00005S-0Y-5M-2Y-1FGR-1Y-0FGR-0BI-0Y	CIMMYT- Mexico
19HRWSN25	BCN/3/68112/WARD//AE.SQUARROSA (369)/4/…	CSS00GH00005S-0Y-5M-3Y-1FGR-2Y-0FGR-0BI-0Y	CIMMYT- Mexico
19HRWSN26	SHAAN 229/3/SHA/SERI//G.C.W 1/SERI	CMSW96WM00658S-15M-010Y-010M-010SY-3M-0Y-020SCM-0Y	CIMMYT- Mexico
19HRWSN27	BOW/GEN//DEN/3/TNMU	CMSS95M00672S-0100M-050Y-050M-1AL-14AL-2M-0Y-…	CIMMYT- Mexico
16HRWYT5	GUS/3/PRL/SARA//TSI/VEE#5/4/FRET2	CMSA00Y00819-040M-0P0Y-040M-040SY-030M-3ZTM-0ZTY…	CIMMYT- Mexico
16HRWYT7	PRL/SARA//TSI/VEE#5/3/FINSI	CMSA00M00066S-040P0M-040Y-030M-030ZTM-17ZTY-0M-0SY	CIMMYT- Mexico
16HRWYT9	BRBT2/METSO	CMSA00M00142S-040P0M-040Y-030M-030ZTM-7ZTY-0M-0SY	CIMMYT- Mexico
16HRWYT13	ATTILA/3*BCN/3/WUH1/VEE#5//CBRD	CMSS99Y01190S-040Y-040M-030Y-030M-23Y-1M-0Y	CIMMYT- Mexico
16HRWYT14	R37/GHL121//KAL/BB/3/JUP/MUS/4/2*YMI#6/5/…	CMSS99Y01443S-040Y-040M-030Y-030M-34Y-1M-0Y	CIMMYT- Mexico
16HRWYT18	CHEN/AE.SQ//2*WEAVER/3/BABAX/4/JARU	CMSS99Y03525T-040M-040Y-040M-040SY-040M-19Y-010M-…	CIMMYT- Mexico
16HRWYT19	JNRB.5/PIFED	CMSS99M00919S-0P0M-040SY-040M-040SY-10M-0ZTB-0SY-…	CIMMYT- Mexico
16HRWYT20	THELIN#2/TURUKU	CGSS02Y00118S-099M-099Y-099M-16Y-0B	CIMMYT- Mexico

HRWSN= high rainfall wheat screening nursery, HRWYT= high rainfall wheat yield trial, SAWSN= semi-arid wheat screening nursery and SB= 2nd CSISA spot blotch nursery.

Appendix 4.2

Mean disease reaction of 150 wheat germplasm screened against *Bipolaris sorokiniana* in 2013 and 2014 (0-9 scale)

Genotype number	Genotype	2013 mean score	Disease reaction	Genotype number	Genotype	2014 mean score	Disease reaction	Genotype number	Genotype	combined mean score	Disease reaction
1	16HRWYT13	5	MS	1	16HRWYT13	3	R	1	16HRWYT13	4	MR
2	16HRWYT14	4	MR	2	16HRWYT14	3	R	2	16HRWYT14	4	MR
3	16HRWYT18	5	MS	3	16HRWYT18	3	R	3	16HRWYT18	4	MR
4	16HRWYT19	5	MS	4	16HRWYT19	3	R	4	16HRWYT19	4	MR
5	16HRWYT20	5	MS	5	16HRWYT20	7	S	5	16HRWYT20	6	MS
6	16HRWYT5	5	MS	6	16HRWYT5	3	R	6	16HRWYT5	4	MR
7	16HRWYT7	4	MR	7	16HRWYT7	3	R	7	16HRWYT7	4	MR
8	16HRWYT9	4	MR	8	16HRWYT9	7	S	8	16HRWYT9	6	MS
9	19HRWSN15	4	MR	9	19HRWSN15	3	R	9	19HRWSN15	4	MR
10	19HRWSN16	4	MR	10	19HRWSN16	7	S	10	19HRWSN16	6	MS
11	19HRWSN19	4	MR	11	19HRWSN19	7	S	11	19HRWSN19	6	MS
12	19HRWSN2	5	MS	12	19HRWSN2	7	S	12	19HRWSN2	6	MS
13	19HRWSN20	5	MS	13	19HRWSN20	7	S	13	19HRWSN20	6	MS
14	19HRWSN21	5	MS	14	19HRWSN21	3	R	14	19HRWSN21	4	MR
15	19HRWSN22	5	MS	15	19HRWSN22	7	S	15	19HRWSN22	6	MS
16	19HRWSN23	5	MS	16	19HRWSN23	7	S	16	19HRWSN23	6	MS
17	19HRWSN24	5	MS	17	19HRWSN24	3	R	17	19HRWSN24	4	MR
18	19HRWSN25	5	MS	18	19HRWSN25	7	S	18	19HRWSN25	6	MS
19	19HRWSN26	5	MS	19	19HRWSN26	7	S	19	19HRWSN26	6	MS
20	19HRWSN27	4	MR	20	19HRWSN27	7	S	20	19HRWSN27	6	MS
21	19HRWSN3	5	MS	21	19HRWSN3	7	S	21	19HRWSN3	6	MS
22	19HRWSN6	3	R	22	19HRWSN6	3	R	22	19HRWSN6	3	R
23	19HRWSN7	2	R	23	19HRWSN7	3	R	23	19HRWSN7	3	R

Appendix 4.2 continued

Genotype number	Genotype	2013 mean score	Disease reaction	Genotype number	Genotype	2014 mean score	Disease reaction	Genotype number	Genotype	Combined mean score	Disease reaction
24	20HRWYT10	5	MS	24	20HRWYT10	4	MR	24	20HRWYT10	5	MS
25	20HRWYT11	4	MR	25	20HRWYT11	7	S	25	20HRWYT11	6	MS
26	20HRWYT12	4	MR	26	20HRWYT12	7	S	26	20HRWYT12	6	MS
27	20HRWYT13	4	MR	27	20HRWYT13	4	MR	27	20HRWYT13	4	MR
28	20HRWYT14	5	MS	28	20HRWYT14	4	MR	28	20HRWYT14	5	MS
29	20HRWYT15	4	MR	29	20HRWYT15	7	S	29	20HRWYT15	6	MS
30	20HRWYT16	5	MS	30	20HRWYT16	4	MR	30	20HRWYT16	5	MS
31	20HRWYT17	5	MS	31	20HRWYT17	4	MR	31	20HRWYT17	5	MS
32	20HRWYT18	5	MS	32	20HRWYT18	7	S	32	20HRWYT18	6	MS
33	20HRWYT2	4	MR	33	20HRWYT2	4	MR	33	20HRWYT2	4	MR
34	20HRWYT20	5	MS	34	20HRWYT20	7	S	34	20HRWYT20	6	MS
35	20HRWYT21	5	MS	35	20HRWYT21	7	S	35	20HRWYT21	6	MS
36	20HRWYT22	5	MS	36	20HRWYT22	4	MR	36	20HRWYT22	5	MS
37	20HRWYT23	3	R	37	20HRWYT23	4	MR	37	20HRWYT23	4	MR
38	20HRWYT24	5	MS	38	20HRWYT24	4	MR	38	20HRWYT24	5	MS
39	20HRWYT25	4	MR	39	20HRWYT25	7	S	39	20HRWYT25	6	MS
40	20HRWYT26	5	MS	40	20HRWYT26	4	MR	40	20HRWYT26	5	MS
41	20HRWYT27	4	MR	41	20HRWYT27	7	S	41	20HRWYT27	6	MS
42	20HRWYT28	4	MR	42	20HRWYT28	4	MR	42	20HRWYT28	4	MR
43	20HRWYT29	4	MR	43	20HRWYT29	7	S	43	20HRWYT29	6	MS
44	20HRWYT3	5	MS	44	20HRWYT3	7	S	44	20HRWYT3	6	MS
45	20HRWYT30	3	R	45	20HRWYT30	7	S	45	20HRWYT30	5	MS
46	20HRWYT31	4	MR	46	20HRWYT31	7	S	46	20HRWYT31	6	MS
47	20HRWYT32	4	MR	47	20HRWYT32	5	MS	47	20HRWYT32	5	MS
48	20HRWYT33	5	MS	48	20HRWYT33	7	S	48	20HRWYT33	6	MS

Appendix 4.2 continued

Genotype number	Genotype	2013 mean score	Disease reaction	Genotype number	Genotype	2014 mean score	Disease reaction	Genotype number	Genotype	Combined mean score	Disease reaction
49	20HRWYT34	4	MR	49	20HRWYT34	7	S	49	20HRWYT34	6	MS
50	20HRWYT35	4	MR	50	20HRWYT35	7	S	50	20HRWYT35	6	MS
51	20HRWYT36	3	R	51	20HRWYT36	5	MS	51	20HRWYT36	4	MR
52	20HRWYT37	4	MR	52	20HRWYT37	5	MS	52	20HRWYT37	5	MS
53	20HRWYT38	4	MR	53	20HRWYT38	7	S	53	20HRWYT38	6	MS
54	20HRWYT39	5	MS	54	20HRWYT39	7	S	54	20HRWYT39	6	MS
55	20HRWYT4	4	MR	55	20HRWYT4	7	S	55	20HRWYT4	6	MS
56	20HRWYT40	5	MS	56	20HRWYT40	5	MS	56	20HRWYT40	5	MS
57	20HRWYT41	4	MR	57	20HRWYT41	5	MS	57	20HRWYT41	5	MS
58	20HRWYT42	4	MR	58	20HRWYT42	7	S	58	20HRWYT42	6	MS
59	20HRWYT43	3	R	59	20HRWYT43	7	S	59	20HRWYT43	5	MS
60	20HRWYT44	4	MR	60	20HRWYT44	7	S	60	20HRWYT44	6	MS
61	20HRWYT45	4	MR	61	20HRWYT45	7	S	61	20HRWYT45	6	MS
62	20HRWYT46	4	MR	62	20HRWYT46	5	MS	62	20HRWYT46	5	MS
63	20HRWYT47	5	MS	63	20HRWYT47	7	S	63	20HRWYT47	6	MS
64	20HRWYT48	4	MR	64	20HRWYT48	7	S	64	20HRWYT48	6	MS
65	20HRWYT49	4	MR	65	20HRWYT49	7	S	65	20HRWYT49	6	MS
66	20HRWYT5	4	MR	66	20HRWYT5	5	MS	66	20HRWYT5	5	MS
67	20HRWYT50	4	MR	67	20HRWYT50	5	MS	67	20HRWYT50	5	MS
68	20HRWYT51	5	MS	68	20HRWYT51	5	MS	68	20HRWYT51	5	MS
69	20HRWYT6	4	MR	69	20HRWYT6	5	MS	69	20HRWYT6	5	MS
70	20HRWYT7	4	MR	70	20HRWYT7	5	MS	70	20HRWYT7	5	MS
71	20HRWYT8	4	MR	71	20HRWYT8	7	S	71	20HRWYT8	6	MS
72	20HRWYT9	5	MS	72	20HRWYT9	7	S	72	20HRWYT9	6	MS
73	30SAWSN10	4	MR	73	30SAWSN10	5	MS	73	30SAWSN10	5	MS
74	30SAWSN11	5	MS	74	30SAWSN11	7	S	74	30SAWSN11	6	MS
75	30SAWSN12	4	MR	75	30SAWSN12	7	S	75	30SAWSN12	6	MS

Appendix 4.2 continued

Genotype number	Genotype	2013 mean score	Disease reaction	Genotype number	Genotype	2014 mean score	Disease reaction	Genotype number	Genotype	Combined mean score	Disease reaction
76	30SAWSN13	3	R	76	30SAWSN13	5	MS	76	30SAWSN13	4	MR
77	30SAWSN14	4	MR	77	30SAWSN14	5	MS	77	30SAWSN14	5	MS
78	30SAWSN15	4	MR	78	30SAWSN15	7	S	78	30SAWSN15	6	MS
79	30SAWSN16	4	MR	79	30SAWSN16	5	MS	79	30SAWSN16	5	MS
80	30SAWSN18	5	MS	80	30SAWSN18	7	S	80	30SAWSN18	6	MS
81	30SAWSN19	5	MS	81	30SAWSN19	7	S	81	30SAWSN19	6	MS
82	30SAWSN2	5	MS	82	30SAWSN2	7	S	82	30SAWSN2	6	MS
83	30SAWSN21	5	MS	83	30SAWSN21	5	MS	83	30SAWSN21	5	MS
84	30SAWSN3	4	MR	84	30SAWSN3	7	S	84	30SAWSN3	6	MS
85	30SAWSN4	4	MR	85	30SAWSN4	7	S	85	30SAWSN4	6	MS
86	30SAWSN5	4	MR	86	30SAWSN5	7	S	86	30SAWSN5	6	MS
87	30SAWSN6	4	MR	87	30SAWSN6	5	MS	87	30SAWSN6	5	MS
88	30SAWSN7	7	S	88	30SAWSN7	7	S	88	30SAWSN7	7	S
89	30SAWSN8	7	S	89	30SAWSN8	6	MS	89	30SAWSN8	7	S
90	30SAWSN9	3	R	90	30SAWSN9	7	S	90	30SAWSN9	5	MS
91	Coucal	4	MR	91	Coucal	6	MS	91	Coucal	5	MS
92	Kwale	4	MR	92	Kwale	6	MS	92	Kwale	5	MS
93	Loerrie II	7	S	93	Loerrie II	7	S	93	Loerrie II	7	S
94	Mampolyo	4	MR	94	Mampolyo	7	S	94	Mampolyo	6	MS
95	Nkhanga	4	MR	95	Nkhanga	7	S	95	Nkhanga	6	MS
96	Nseba	4	MR	96	Nseba	6	MS	96	Nseba	5	MS
97	Pwele	8	S	97	Pwele	7	S	97	Pwele	8	S
98	Sahai	4	MR	98	Sahai	6	MS	98	Sahai	5	MS
99	SB1	3	R	99	SB1	7	S	99	SB1	5	MS
100	SB10	4	MR	100	SB10	6	MS	100	SB10	5	MS
101	SB11	3	R	101	SB11	6	MS	101	SB11	5	MS

Appendix 4.2 continued

Genotype number	Genotype	2013 mean score	Disease reaction	Genotype number	Genotype	2014 mean score	Disease reaction	Genotype number	Genotype	Combined mean score	Disease reaction
102	SB12	4	MR	102	SB12	7	S	102	SB12	6	MS
103	SB13	4	MR	103	SB13	7	S	103	SB13	6	MS
104	SB14	4	MR	104	SB14	7	S	104	SB14	6	MS
105	SB15	4	MR	105	SB15	6	MS	105	SB15	5	MS
106	SB16	4	MR	106	SB16	6	MS	106	SB16	5	MS
107	SB17	7	S	107	SB17	6	MS	107	SB17	7	S
108	SB18	3	R	108	SB18	7	S	108	SB18	5	MS
109	SB19	4	MR	109	SB19	6	MS	109	SB19	5	MS
110	SB2	4	MR	110	SB2	3	R	110	SB2	4	MR
111	SB20	7	S	111	SB20	7	S	111	SB20	7	S
112	SB21	4	MR	112	SB21	6	MS	112	SB21	5	MS
113	SB22	7	S	113	SB22	6	MS	113	SB22	7	S
114	SB23	4	MR	114	SB23	6	MS	114	SB23	5	MS
115	SB24	4	MR	115	SB24	6	MS	115	SB24	5	MS
116	SB25	4	MR	116	SB25	6	MS	116	SB25	5	MS
117	SB26	4	MR	117	SB26	6	MS	117	SB26	5	MS
118	SB27	4	MR	118	SB27	7	S	118	SB27	6	MS
119	SB28	4	MR	119	SB28	6	MS	119	SB28	5	MS
120	SB29	4	MR	120	SB29	7	S	120	SB29	6	MS
121	SB3	4	MR	121	SB3	6	MS	121	SB3	5	MS
122	SB30	4	MR	122	SB30	7	S	122	SB30	6	MS
123	SB31	4	MR	123	SB31	3	R	123	SB31	4	MR
124	SB32	3	R	124	SB32	6	MS	124	SB32	5	MS
125	SB33	3	R	125	SB33	8	S	125	SB33	6	MS
126	SB34	4	MR	126	SB34	6	MS	126	SB34	5	MS

Appendix 4.2 continued

Genotype number	Genotype	2013 mean score	Disease reaction	Genotype number	Genotype	2014 mean score	Disease reaction	Genotype number	Genotype	Combined mean score	Disease reaction
127	SB35	3	R	127	SB35	6	MS	127	SB35	5	MS
128	SB36	3	R	128	SB36	6	MS	128	SB36	5	MS
129	SB37	4	MR	129	SB37	6	MS	129	SB37	5	MS
130	SB38	3	R	130	SB38	8	S	130	SB38	6	MS
131	SB39	4	MR	131	SB39	6	MS	131	SB39	5	MS
132	SB4	3	R	132	SB4	8	S	132	SB4	6	MS
133	SB40	3	R	133	SB40	8	S	133	SB40	6	MS
134	SB41	4	MR	134	SB41	8	S	134	SB41	6	MS
135	SB42	4	MR	135	SB42	6	MS	135	SB42	5	MS
136	SB43	7	S	136	SB43	8	S	136	SB43	8	S
137	SB44	4	MR	137	SB44	8	S	137	SB44	6	MS
138	SB45	4	MR	138	SB45	6	MS	138	SB45	5	MS
139	SB46	3	R	139	SB46	6	MS	139	SB46	5	MS
140	SB47	3	R	140	SB47	6	MS	140	SB47	5	MS
141	SB48	4	MR	141	SB48	6	MS	141	SB48	5	MS
142	SB49	4	MR	142	SB49	6	MS	142	SB49	5	MS
143	SB5	2	R	143	SB5	6	MS	143	SB5	4	MR
144	SB50	8	S	144	SB50	8	S	144	SB50	8	S
145	SB6	4	MR	145	SB6	6	MS	145	SB6	5	MS
146	SB7	4	MR	146	SB7	6	MS	146	SB7	5	MS
147	SB8	4	MR	147	SB8	6	MS	147	SB8	5	MS
148	SB9	4	MR	148	SB9	6	MS	148	SB9	5	MS
149	UNZAWV1	4	MR	149	UNZAWV1	6	MS	149	UNZAWV1	5	MS
150	UNZAWV2	7	S	150	UNZAWV2	6	MS	150	UNZAWV2	7	S

HRWSN= high rainfall wheat screening nursery, HRWYT= high rainfall wheat yield trial, SAWSN= semi-arid wheat screening nursery and SB= 2nd CSISA spot blotch. Disease reaction: R= resistance, MR= moderate resistance, MS= moderate susceptible and S susceptible.

Chapter 5

Genetic analysis of resistance to spot blotch disease in rain-fed wheat (*Triticum aestivum* L.)

Abstract

Understanding the genetics of resistance to spot blotch disease is important in order to design the breeding strategy to improve the trait. The objective of this study was to determine the gene action and mode of inheritance of resistance to spot blotch in the rain-fed wheat lines. This was done by analyzing an 8 × 8 full diallel population and a six generation (P1, P2, F1, F2, BCP1 and BCP2) population derived from a cross between Loerrie II (susceptible) and 19HRWSN6 (resistant). Data was analysed using Hayman diallel analysis and generation mean analysis for the six generation population. The results obtained from the two biometrical methods matched well and suggested the importance of additive gene effects in controlling resistance to spot blotch. No epistasis, maternal or reciprocal effects were detected indicating that selection for resistance would be effective and the choice of the female parent was not critical in breeding for resistance to spot blotch disease. Both diallel and generation mean analyses revealed moderately narrow sense heritability of 56.0% and 55.5%, respectively. Resistance to the disease exhibited partial dominance. The Wr/Vr graph showed that the parental genotypes 30SAWSN10 (P1), 30SAWSN5 (P3) and Coucal (P4) possessed more dominant genes which makes them particularly suitable for inclusion in breeding for resistance to spot blotch. Furthermore, the resistant parent 19HRWSN6 could also be utilized as a donor parent in breeding. The overall results from this study indicated the possibility of improving resistance to spot blotch disease by utilizing these genotypes. Besides, exercising selection for resistance in the early segregating generation should be an effective approach due to predominance of additive gene effects.

5.1 Introduction

Wheat is one of the important cereal crops in Zambia. It is grown during the dry season under irrigation as well as in the summer rainy season. The production of wheat during the summer season is characterized by disease complexes which reduce wheat yields. Spot blotch disease is the major problem affecting summer rain-fed wheat production. The pathogen *Bipolaris sorokiniana* attacks all plant parts thus reducing the net photosynthetic area for the production of sugars for seed development resulting in reduced yield. Yield loss of more than 85% has been reported during summer season (Raemaekers, 1988). The disease also reduces wheat grain quality leading to poor acceptability by consumers. The cultural methods (uprooting disease plants, removing of diseased leaves and application of lime) used by most small-scale farmers who dominate summer wheat production to reduce the disease have not been effective. Use of resistant cultivars is thus the only viable, most economical and sustainable approach to reducing the disease. It is for this reason that spot blotch disease problem requires urgent attention from wheat breeders.

Thus, in order to address the spot blotch problem, the genetics of disease resistance need to be well understood. Understanding the genetics of resistance could greatly help in determining the breeding techniques to be implemented for the improvement of this trait (Ajith and Anju, 2005; Eshghi and Akhundova, 2009; Zaazaa et al., 2012). The gene effects for the inheritance of resistance to spot blotch disease have not been well established as there are conflicting reports regarding the type of gene action controlling resistance (Joshi et al., 2004b; Goel et al., 2010). Kuldeep et al. (2008) indicated that polygenes were important in the inheritance of resistance to spot blotch, while Neupane et al. (2007) reported that resistance was dominant and controlled by one major gene. Duveiller and Sharma (2009) reported that dominant and recessive genes controlled inheritance of resistance to the disease and in some instances epistasis has been reported. Sharma et al. (2006) found that partially dominant genes controlled the inheritance of resistance and that resistance was inherited quantitatively with moderate to high heritability estimates. Joshi et al. (2004a), on the other hand, indicated three additive genes controlled the inheritance of resistance. It would appear, therefore, that the inheritance of resistance for this disease depends on the genetic background of the material used. It is clear that more information about inheritance of resistance and the type of gene action controlling resistance to spot blotch is required for the successful breeding for resistance to the disease. Salama et al. (2006) and Ashghi and Akhundova (2009) indicated that

breeding procedures could only be efficient if the genetic effects controlling the expression of that trait are known.

In order to understand the genetics of resistance to spot blotch disease in the genotypes in this study, the Hayman`s diallel approach and generation mean analysis were used. Diallel mating design has been reported as the most powerful design in investigating combining abilities, gene action and heritability of different traits in plant species (Topal et al., 2004). Hallauer et al. (2010) reported that diallels were useful designs for self-pollinated crops. Additionally, Joshi et al. (2004a) indicated that the diallel cross was a good technique as it allowed crossing of genotypes in all possible combinations and thus delaying fixation of gene complexes. On the other hand, generation mean analysis technique allows the estimation of type and magnitude of gene action and determination of epistatic gene effects involved in the inheritance of a trait which cannot be detected using diallel analysis (Singh and Singh, 1992). Furthermore, they indicated a greater reliance on the results was obtained when generation mean analysis was used in combination with other designs. Genetic analysis for resistance to spot blotch disease in germplasm used for breeding in Zambia has not been done. The objectives of this study was thus to determine the gene action and mode of inheritance of resistance to spot blotch in rain-fed wheat.

5.2 Materials and methods

5.2.1 Diallel analysis

5.2.1.1 Experimental sites

The crossing block was established at Mt. Makulu Research Station in 2013 off season and 2013/14 summer season (Figure 5.1a). The evaluations of the plant material developed from the crosses were conducted during 2014/15 season at Seed-Co Research Farm in Mpongwe, Mt. Makulu research station in Chilanga and Golden Valley Agricultural Research Trust (GART) in Chisamba.

5.2.1.2 Plant materials and generation of segregating population

Eight parents (Table 5.1) with varied resistance to spot blotch disease were used in the study to generate F1 progenies using diallel mating design. Parents were crossed in all possible combinations (8 × 8 full diallel) at Mt. Makulu research station (Figure 5.1a). Seeds for each genotype were planted in a single 2 m row. Planting was staggered in time to ensure that all the selected genotypes would synchronize in their flowering. Split planting of parental genotypes was done three times at one week

intervals. Hand emasculation and pollination was used. Emasculation and pollination were done with the help of a pair of scissors, forceps and a dissecting needle. Emasculation of the individual floret (female plant) was done in the morning between 8 am and 12 noon (Figure 5.1b). The emasculated head was covered with a well labelled glassine bag to avoid contamination. Pollination of the emasculated head was done in the morning between 8 am and 12 noon, two to three days after emasculation. Hand collection and transfer of pollen was employed (Figure 5.1c). The generated F1 seed was selfed to generate 56 F2 progeny in 2014 dry season (May-Sepetmber).

a b c

Figure 5.1: a) the crossing block during 2013/14 season b) hand emasculation c) hand pollination

Table 5.1: Genotypes used in an 8 × 8 full diallel cross

Parent	Name	Pedigree	Source	Reaction to spot blotch
P1	$30^{th}10$	CMSA04M01201T-050Y-040ZTP0M-040ZTY-040ZTM-040SY-…	CIMMYT- Mexico	Resistance
P2	$30^{th}18$	CMSA06M00667S-40ZTM-040ZTY-50ZTM-2Y-0B	CIMMYT- Mexico	Moderately resistant
P3	$30^{th}5$	CMSS96Y02555S040Y-020M-050SY-020SY-27M-0Y	CIMMYT- Mexico	Resistance
P4	Coucal	CM70377-3MB-0MM-1MB0MM-2MM-0MM	Mt. Makulu- Zambia	Moderately susceptible
P5	Loerrie II	CM33027-F-15M-500Y-0M-87B-0Y	Mt. Makulu- Zambia	Susceptible
P6	Kwale		Mt. Makulu- Zambia	Moderately susceptible
P7	SB50	II18427-4R-1M	CIMMYT- Mexico	Susceptible
P8	$19^{th}15$	CMSA00M00142S-040P0M-040Y-030M-030ZTM-7ZTY-0M-0SY	CIMMYT- Mexico	Moderately resistant

$19^{th}15$ =19HRWSN15, $30^{th}18$= 30SAWSN18, $30^{th}5$=30SAWSN5, $30^{th}10$=30SAWSN10, SB50=Sonalika. HRWSN=High rainfall wheat screening nursery and SAWSN=Semi-arid wheat screening nursery

5.2.1.3 Experimental layout for the evaluation of trials

Sixty-four progenies which included the parents and F2 populations were planted in a simple 8 × 8 lattice design with two replications. Rows were 3 m long with 20 cm spacing between rows and 40 cm between plots. The parental genotypes were planted in two rows and the F2 populations were planted in four rows. Each row was seeded with 60 plants which was later thinned out to have 30 plants per row. To create enough disease pressure on to the plants, one row of susceptible parent SB50 (Sonalika) was planted in the alleyways and borders (Joshi et al., 2004a). Standard agronomic practices were followed for good crop management.

5.2.1.4 Data collection

Disease presence was evaluated based on foliar symptoms. All plants were scored for disease severity on appearance of symptoms, five times at seven day intervals. The disease severity score was based on Saari and Prescott`s scale for assessing foliar disease (Eyal et al., 1987). The scores ranged from 0 – 9. In a 0- 9 scale, 0 was scored on leaves with no symptoms, 1 was scored on leaves having one or two necrotic spots to score of 9 on leaves having many extensive necrotic spots with pronounced chlorosis as indicated in Table 5.2. Disease severity of each plot was found by averaging the severity ratings of all plants. Genotypes falling in the 1-3 category were recorded as resistant, 4 as moderately resistant, 5-6 as moderately susceptible and 7-9 as susceptible (Chaurasia et al., 1999).

5.2.2 Generation of mean analysis

Two parental genotypes; spot blotch susceptible line Loerrie II (P1) and 19HRWSN6 ($19^{th}6$) a resistant lines (P2), were crossed to generate F1 progenies. Loerrie II was obtained from Mt. Makulu Research while 19HRWSN6 was an introduced genotype from CIMMYT- Mexico. Crossing was done in the 2013 off-season under irrigation at Mt. Makulu Research Station (May-September). Seeds for each genotype were planted in a single 2 m row. Planting was staggered in time to ensure that all the selected genotypes were at about the same stage of growth for crossing. Hand emasculation and pollination was used for crossing the wheat lines. To generate F2 and backcross populations (BCP1 and BCP2), F1 was selfed to produce F2 and also backcrossed to both resistant parent (P1) and susceptible parent (P2) to produce BCP1 and BCP2, respectively, in the summer rainy season of 2013/14 (November-April). The six generations developed (P1, P2, F1, F2, BCP1 and BCP2) were evaluated in 2014/2015 summer rainy season (November-April) at Mt. Makulu Research Station and Seed-Co Research farm in Mpongwe.

Table 5.2: Rating scale of diseased plants

Score	Rating scale %	Symptom description	Disease reaction
0	0	No symptoms	Immune
1	< 1%	One or two small necrotic spots without chlorosis	Resistant
2	1-3	Few small necrotic spots without chlorosis	Resistant
3	4-6	Few small necrotic spots with chlorosis	Resistant
4	7-12	Medium size necrotic spots with distinct but restricted chlorotic margin	Moderate resistant
5	13-24	Medium to large size necrotic spots with distinct but restricted chlorotic margin	Moderate susceptible
6	25-48	Large abundant necrotic spots with distinct chlorotic margin	Moderate Susceptible
7	49-60	Large necrotic spots linked together with pronounced chlorosis	Susceptible
8	61-75	Extensive necrotic spots fully merge expanding longitudinally with pronounced chlorosis	Susceptible
9	76-100	Extensive necrotic spots almost covering the entire leaf area expanding longitudinally with pronounced chlorosis	Susceptible

Source: Adapted from Fetch Jr and Steffenson (1999)

5.2.2.1 Experimental layout and scoring of diseases

The generations P1, P2, F1, F2 and BCP1 and BCP2 were planted in a randomised complete block design with two replications with the parents and F1 consisting of 1 row, F2 population 6 rows and BCP1 and BCP2 families 4 rows per plot of 3 m long and 20 cm between plots. Each row was seeded with 60 plants which were later thinned out to 30 plants per row. Plant to plant distance was 10 cm. Early planting was done to enhance disease spread during flowering. To create enough disease pressure on to the plants, one row of SB50, a susceptible genotype, was planted in the alleyways and borders. Data on spot blotch was collected on 20 plants for F1 and parental genotypes, 120 plants for F2 and BCP1 and BCP2 generations. Standard agronomic practices were followed for good crop management. Weeding was done using hand hoes. All plants were scored for disease severity using a 0-9 scale based on Saari and Prescott's scale for assessing foliar disease (Eyal et al., 1987).

5.3 Data analysis

5.3.1 Diallel analysis

The data collected were subjected to analysis of variance to determine whether there were significant differences. Analysis of variance was conducted using the general linear model procedure (PROC GLM) in SAS version 9.3 (SAS Institute, 2011). Genetic analysis tests were conducted following significant analysis of variance results. The model used was,

$Yijk = \mu + Gi + Ej + G \times E + rk(E) + eijk$

Where Yijk= spot blotch disease score of i^{th} genotype in j^{th} environment of k^{th} replication, μ= overall mean, gi= ^{ith} genotype, Ej= j^{th} environment, G × E= genotype × environment interaction, rk (E) = k^{th} replication within E environment and eijk= residual factor.

Genetic analysis was performed by diallel cross analysis method by Hayman (1954b) using GenStat software version 14 (Payne et al., 2011). Both numerical (analysis of variance) and graphical approach were used to determine the gene effects.

The analysis of the diallel table was used to estimate the additive and non-additive genetic effects among the genotypes and also to determine whether maternal effects contributed to the inheritance of resistance to spot blotch in wheat or not. The following linear model was used for the Hayman analysis;

Y = μ + site + a + b + c + d + a×site + b×site+ c×site + d×site, where

- μ = grand mean
- sites= site effects
- a = additive effects
- b = dominance effects

b = is partitioned into:

- b_1, indicates direction of dominance (unidirectional if significant; equiv. to parent vs. crosses contrast)
- b_2, tests asymmetry of alleles
- b_3, shows that some dominance is peculiar to some crosses

- b × site partitioned into:
    - site × b_1, site × b_2 and site*b_3
- c = maternal effects
- d = reciprocal effects other than maternal effects

5.3.1 The adequacy of additive–dominance model

The adequacy of the additive–dominance model was tested through Wr- Vr analysis of variance, regression analysis and t^2 test (uniformity of Wr, Vr) as described by Singh and Chaudhary (1995), and Dabholkar (1999). The model was considered adequate when the analysis of Wr-Vr was found not to be significant indicating the absence of epistasis. If epistasis was present Wr-Vr varied between arrays. Additionally, the model was considered adequate when the regression coefficient significantly deviated from zero and not from unity. Furthermore, significant value of the t^2-test indicated the inadequacy of the model. If dominance is present the Wr +Vr values must change from array to array.

5.3.2 Estimation of genetic variance components

The genetic components of variation (Table 5.3) were estimated following the procedure given by Hayman (1954a, b).

5.3.3 Graphical analysis

The graphic representation of the covariance (Wr) of all the offspring's in each parental arrays with the non-recurring parents and the variance (Vr) of all components of the rth array was done. By plotting Wr/Vr (covariance/variance) graph, the information about the presence of dominant and recessive genes in the parental genotypes was evaluated. The distribution of dominant and recessive genes among parents was determined by order of array points along the regression line. Parents with preponderance of dominant genes were located near the origin while those with array points located very far away from the origin possessed more recessive genes. Parents with array point located at the middle of the regression line had equal frequencies of dominant and recessive genes. For the average level of dominance, over-dominance was indicated when the intercept was negative, complete dominance when regression line passed through the origin and partial dominance when the slope of regression line intercepted Wr-axis above the origin. In the absence of epistasis, Wr is linked to Vr by regression line of a unit slope (Singh and Chaudhary, 1995).

Table 5.3: Components of genetic variation and genetic parameters

Serial	Components
1	D= additive
2	H_1= variation due to dominance effects of genes
3	H_2= variation due to dominant effect of genes correlated for gene distribution
4	F= Relative frequency of dominant and recessive alleles- it determines the relative frequency of dominant and recessive genes in the parental population. F is positive when dominant genes are more than recessive genes
5	h^2 = overall dominance effect of heterozygous loci
6	E= environmental variance
7	$\sqrt{H_1}/D$ = Average degree of dominance
8	$H_2/4H_1$ = proportion of genes with positive and negative effects in the parents. If the ratio is equal to 0.25 it indicates symmetrical distribution of the positive and negative genes.
9	$\sqrt{4DH_1} + F) / (\sqrt{4DH_1}$- F= proportion of dominant and recessive genes in the parents. If the ratio is 1, then the dominant and recessive genes in parents are equal in proportion. The ratio > 1 indicates more dominant genes and when the ratio < 1 it indicates more recessive genes.
10	Heritability (narrow- sense) was estimated based on Verhalen and Murray (1969) formula as cited by (Singh and Chaudhary, 1995). F2 heritability= $1/4D/(1/4D+1/16H_1-1/8F+E)$
11	Correlation coefficient between the parental order of dominance (Wr + Vr) for each array and mean of common parent of array (Yr).
12	r^2 = prediction of measurements of completely dominant and recessive parents

5.3.2 Generation mean analysis

The data collected was subjected to combined analysis of variance using the general linear model procedure (PROC GLM) in SAS version 9.3 (SAS Institute, 2011) to determine whether there were significant differences. The model used was,

$Yijk = \mu + Gi + Ej + G \times E + rk (E) + eijk$

Where Yijk= spot blotch disease score of i^{th} generation in j^{th} environment of k^{th} replication, μ= overall mean, gi= generation mean, Ej= j^{th} environment, G × E=

generation × environment interaction, rk= k^{th} replication within E environment and eijk= residual factor.

Mean separation between generations was done in SAS version 9.3 (SAS Institute, 2011) using least significance difference (LSD) procedure for pair wise comparison (P≤ 0.05) as suggested by Kang (1994).

Data was submitted to generation mean analysis (GMA) using the methodology proposed by Mather and Jinks (1971) following the significant analysis of variance. The GMA was performed using PROC GLM and PROC REG procedures in accordance with SAS macros described by Kang (1994). The genetic model used was;

$$\mathbf{Y = m + \alpha a + \beta d + \alpha^2 aa + 2\alpha\beta ad + \beta^2 dd}$$

Where;

- α and β are the coefficients for a and d, respectively
- Y = generation mean
- m = mean of the F2 generation as the base population and intercept value
- a = additive effects
- d = dominance effects
- aa = additive x additive gene interaction effects
- ad = additive x dominance gene interaction effects
- dd = dominance x dominance gene interaction effects

A stepwise linear regression was used to estimate the additive and dominant parameters. The regression analysis was carried out using PRO REG macros in SAS developed by Kang (1994). The regression analysis was weighted based on the inverse of the variance of means and matrix parameter (Checa et al., 2006). To establish the parameters that were acceptable within the model, R^2 and F-test (goodness of- fit) were used (Ceballos et al., 1998). The F-test was calculated using the formula below (Checa et al., 2006):

$$Fc = \frac{\text{(SSq general model) - (SSq reduced model) /difference in df}}{\text{SSq residual from the general model / df residual from the general model}}$$

Where SSq = sums of squares, df = degrees of freedom, Fc = F calculated

To determine the importance of additive, dominance and epistatic effects, the model's parameters were tested sequentially one at a time starting with additive effects and then

in combination with other parameters of the model (Ceballos et al., 1998). The importance of the gene effect estimates was based on the ratio between the sums of squares and the total sums of square after entering the different elements in the model. Significance of the genetic estimates was also determined by dividing the estimated parameter values with their standard errors, if the value exceeded 1.96 then it was considered significant (Singh and Chaudhary, 1995).

Variance components (additive, dominance and environment) were estimated as described by Mather and Jinks (1982) using the equation below,

- $A = (2\sigma^2F2) – \sigma^2BCP1 + \sigma^2BCP2$
- $D = \sigma^2G\ (F2) - \sigma^2A\ (F2)$
- $E = 1/4\ (\sigma^2P1 + \sigma^2P2 + (2\sigma^2F1)$

Where: A = Additive genetic variance, D = Dominance variance and E = Environmental component of variance

Where: σ^2P1 = variance of parent 1; σ^2P2 = variance of parent 2; σ^2F1 = variance of F1; σ^2F2 = variance of F2 generation; σ^2BCP1 = variance of backcross to parent 1; σ^2BCP2 = variance of backcross to parent 2.

Narrow sense heritability (h^2) was estimated as follows; (Warner, 1952).

$h^2 = [\sigma^2F2 – (\sigma^2BCP1 + \sigma^2BCP2)\ /2] / \sigma^2F2.$

Where, σ^2F2 = variance of F2 generation, σ^2BCP1 = variance of backcross to parent 1; σ^2BCP2 = variance of backcross to parent 2.

The coefficient of dominance (F) was calculated by the formula: (Mather and Jinks, 1982),

$F = \sigma^2BCP_2 – \sigma^2BCP1$

5.3 Results

5.4.1 Analysis of variance (diallel)

Highly significant differences were observed among the genotypes for resistance to spot blotch disease and also the environments ($P < 0.001$) (Table 5.4). This indicated the influence of the genotypes and the environment in the expression of the disease. The genotype by environment interaction was not significant. Disease pressure was high at Golden Valley Agricultural Research Trust (GART) (mean disease score of 6.0) and Seed-Co research farm in Mpongwe (5.7). At Mt. Makulu research station the mean

disease score was 5.1. At all the sites none of the genotypes (parents and crosses) were symptomless.

Table 5.4: Combined analysis of variance for spot blotch disease resistance in wheat across three sites

Source	Degrees of freedom	Mean Square
Environment (Env)	2	27.27***
Replication (Env)	3	1.38
Genotype (G)	63	1.04***
G × Env	126	0.41^{ns}
Error	189	0.34
Corrected total	383	
CV (%)	10.47	
Mean	5.60	
R^2	73.03	

*** Highly significant P<0.001; ns=non- significant

5.4.2 Performance of parents and crosses at different sites and across sites

At GART, out of 56 F2 populations one was found to be moderately resistant, forty-nine moderately susceptible and six susceptible (Figure 5.2). The moderately resistant cross was between Coucal and $30^{th}5$ (score of 4.5). In Mpongwe 55 of 56 (98.2%) crosses were moderately susceptible while 1.8% were susceptible. The parents Coucal, $30^{th}5$ and $30^{th}10$ performed better (score of 5.2) than other parents. The cross between $30^{th}18$ and Coucal performed better (score of 5) than both parents. At Mt. Makulu, 23.2% of the crosses were moderately resistant, 73.2% moderately susceptible and 3.6% susceptible. The crosses with lowest disease reaction were between Kwale × Coucal and $30^{th}18$ × $30^{th}10$. Among the parents, $30^{th}10$ was resistant with a mean score of 3.0. Across sites, 5.4% of the crosses were moderately resistant, 87.5% moderately susceptible and 7.1% susceptible. The moderately resistant crosses across sites were between $30^{th}18$ × Coucal, Kwale × Coucal and Coucal × $30^{th}5$ (Appendix 5.1).

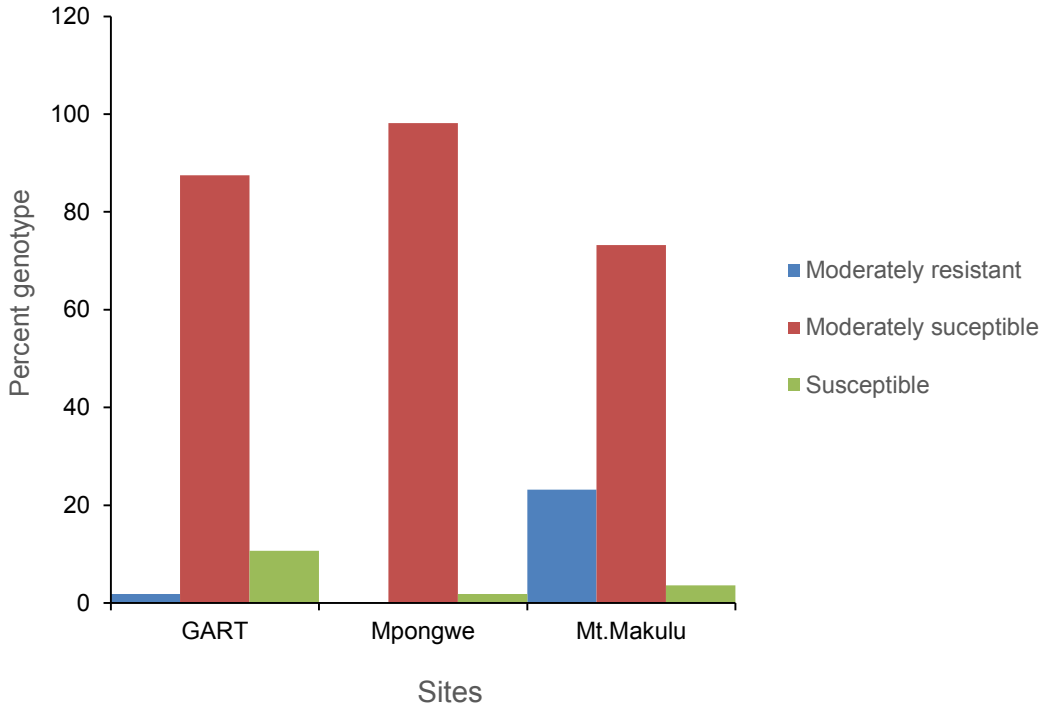

Figure 5.2: Performance of crosses to spot blotch disease across sites

5.4.3 Genetic analysis - Hayman Approach

5.4.3.1 Analysis of a diallel table

The diallel analysis (Table 5.5) showed that the 'a' item was significant ($P < 0.05$) indicating the presence of additive gene effects. Non- significant b_1 ($P > 0.05$) component indicated the absence of directional dominance of the genes for resistance. The items b_2 and b_3 were also not significant ($P > 0.05$). The non-significance of item b_2 represented the non-asymmetrical gene distribution in the parents and b_3 indicated the absence of dominance effects specific to individual crosses. The 'b' component was not significant showing the absence of general dominance effects. The 'c' and 'd' components were also non-significant indicating the absence of maternal and non-maternal reciprocal effects respectively. The non-significance of 'c' and 'd' items suggested that there was no need to retest the 'a' component.

Table 5.5: Hayman diallel analysis for spot blotch disease resistance in an 8 × 8 full diallel cross in wheat

Item	Sums of squares	Degree of freedom	Mean square.	F-test
a	18.56	7	2.65	7.39*
b_1	2.20	1	2.19	8.87^{ns}
b_2	2.20	7	0.31	0.98^{ns}
b_3	3.99	20	0.20	1.26^{ns}
b	8.39	28	0.30	1.48^{ns}
c	1.29	7	0.18	1.29^{ns}
d	4.03	21	0.19	0.98^{ns}
total	32.26	63	0.51	
Block (B)	26.81	2	13.40	
B × a	5.02	14	0.36	
B × b_1	0.49	2	0.25	
B × b_2	4.48	14	0.32	
B × b_3	6.33	40	0.16	
B × b	11.31	56	0.20	
B × c	1.99	14	0.14	
B × d	8.20	42	0.19	
B × total	26.52	126	0.21	

*Significant at 5 %, ns= non-significant. a= additive effects, b= dominance effects

5.4.3.2 The additive – dominance model

The analysis of variance of Wr - Vr and Wr + Vr (Table 5.6) to test the adequacy of the additive–dominance model showed that Wr-Vr and Wr + Vr were non-significant for spot blotch disease resistance. The results for t^2 test and the regression analysis are as presented in Table 5.7. The t^2 test was non-significant. The regression analysis for spot blotch disease resistance showed that regression coefficient 'b' differed significantly from zero but it was not significantly different from unity. From these analyses, the additive-dominance model was adequate for the data set.

Table 5.6: Analysis of variance of Wr - Vr and Wr + Vr for spot blotch disease resistance estimates in an 8 × 8 diallel

	Source of Variation	df	Sus of square	Mean square
Wr - Vr	Rep	2	0.29	0.12
	Wr - Vr	7	0.06	0.01^{ns}
	Residual	14	0.09	0.01
	Total	23	0.39	
Wr + Vr	Rep	2	0.43	0.22
	Wr + Vr	7	0.23	0.03^{ns}
	Residual	14	0.87	0.06
	Total	23	1.53	

*Significant at 5 %, ns= non-significant; Wr-Vr; differences over the arrays; Wr+Vr, parental order of dominance

Table 5.7: Regression analysis and t^2 test for resistance to spot blotch

Trait	a	b	t^2 test	S.E(b)	T value of b b=0	T value of b b=1
Spot blotch disease	0.005	1.108	ns	0.24	*	ns

*Significant at 5 %, ns= non-significant

5.4.3.3 Genetic components of variation

The estimates of the components of variance (Table 5.8) showed that additive variance (D) and environmental variance (E) were highly significant ($P < 0.001$) for the control of spot blotch disease resistance. The components H_1, H_2, F and h^2 were negative and non-significant for spot blotch disease resistance. The degree of dominance ratio ($\sqrt{H_1}/D$) was less than unity. The parameter $H_2/4H_1$ was not equal to 0.25. Furthermore, the results showed that the ratio of the proportion of dominant and recessive genes in the parents ($\sqrt{4DH_1} + F$) / ($\sqrt{4DH_1} - F$) was not greater than unity. The correlation coefficient (r) between the parental order of dominance (Wr + Vr) for each array and mean of common parent of array (Yr) was positive (0.67) and not significant. The r^2 estimate for spot blotch disease resistance was less than unity. Narrow-sense heritability was 56%.

5.4.3.4 Graphical representation.

The graphical representation of Wr/Vr (Figure 5.3) showed that regression line intercepted the W-axis just above the point of origin with the intercept (a) value of 0.005. Figure 5.3 also displayed scattered array points along the regression line on the graph signifying the genetic diversity among parents for resistance to spot blotch disease. From the array points position on the regression line, parents P3, P4 and P1 were near to the origin, parents P6 and P5 were in-between the regression line while P2, P8 and P7 were far away from the origin.

Table 5.8: Estimates of components of variation (± SE) for spot blotch disease of 8 × 8 diallel cross

Components	Estimates
D= additive	0.12 ± 0.02***
H_1= variation due to dominance effects of genes	-3.51 ± 0.05 ^{ns}
H_2= variation due to dominant effect of genes correlated for gene distribution	-0.24 ± 0.04 ^{ns}
F= relative frequency of dominant and recessive alleles	-0.13 ± 0.05 ^{ns}
h^2 = overall dominance effect of heterozygous loci	-0.09 ± 0.03 ^{ns}
E= environmental variance	0.219 ± 0.007***
$\sqrt{H_1/D}$ = average degree of dominance	-2.67
$H_2/4H_1$ = proportion of genes with positive and negative effects in the parents	0.02
$(\sqrt{4DH_1} + F) / (\sqrt{4DH_1} - F)$= proportion of dominant and recessive genes in the parents	0.64
Heritability (narrow- sense)	0.56
correlation coefficient between the parental order of dominance (Wr + Vr) for each array and mean of common parent of array (Yr)	0.67
r^2 = prediction of measurements of completely dominant and recessive parents	0.45

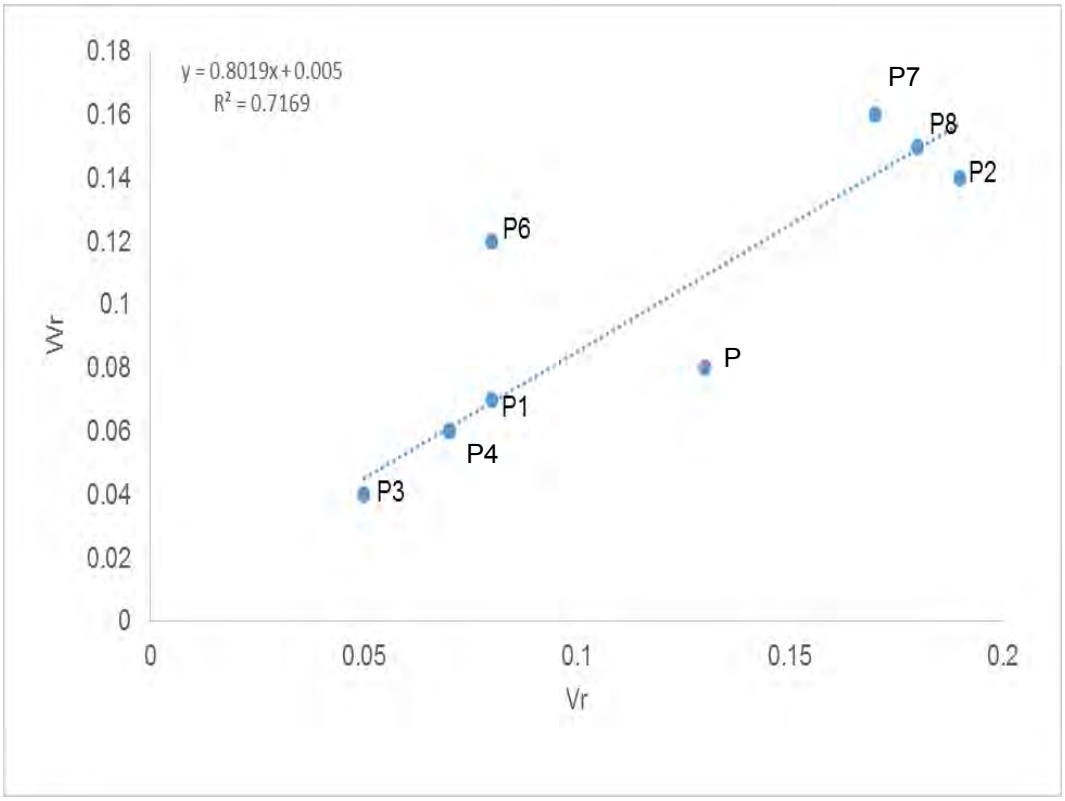

Figure 5.3: Linear regression of Wr on Vr for spot blotch disease showing distribution of parental lines in the 8 × 8 diallel cross. P3=30SAWSN5, P4=Coucal, P1=30SAWSN, P6=Kwale, P5=Loerrie II, P2=30SAWSN18, P8=19HRWSN15 and P7=SB50 (Sonalika)

5.4.4 Generation mean analysis

The combined analysis of variance for two environments (Table 5.9) revealed highly significant differences among generations for resistance to spot blotch disease ($P<0.001$). Environments and generation × environment interaction were not significantly different ($P> 0.05$) for spot blotch scores.

Table 5.9: Generation mean squares of spot blotch disease severity scores

Source	Mean Square
Environment (E)	0.25992228^{ns}
Replication(E)	13.74049192
Generation (G)	2.89831068***
G×E	0.17709515^{ns}
Error	2.3282
Mean	5.94
R^2	94.87
Coefficient of variation	8.1

*** Significant at p<0.001, ns= not significant

The mean score (Table 5.10) showed that F1 population had less disease compared to P1, F2, BCP1 and BCP2 but not with P2 (resistant parent). The F2 generations had a mean severity score less than the mean of the susceptible parent (P1). Nonetheless, no significant differences were observed between the means of the susceptible parent P1, BCP1, BCP2 and F2 generations (Table 5.10). The means of the F2 generations were not significantly different from the means of F1. Table 5.10 also shows that the F1 was significantly different from P1 and BCP1. The resistant parent P2 was significantly different from F1 and P1, F2, BCP1 and BCP2 generations. The two parents were contrasting in terms of disease resistance.

Table 5.10: Mean comparison between various generations for spot blotch disease resistance

Generation	Mean
P1	6.68 A
BCP1	6.54 A
F2	6.34 AB
BCP2	5.99 AB
F1	5.73 B
P2	4.36 C

P1= Loerrie II (susceptible parent), P2=19HRWSN6 (resistant parent). Means followed by the same letter for each cross are not significantly different at P<0.05.

5.4.4.1 Genetic effects

The analysis of variance for generation means for spot blotch disease reaction (Table 5.11) revealed highly significant differences for additive effects (P < 0.001). The additive × additive, additive × dominance and dominance × dominance effects were not significant (P > 0.05). The additive gene effects accounted for 94.79% of the total genetic variation, dominance effects explained 1.19% of the total variation (Table 5.11). Additive × dominance gene interaction contributed most of the total genetic variation (5.34%) amongst other epistasis gene interaction.

Table 5.11: Mean squares of gene effects for spot blotch mean scores and relative contribution of gene effects to the model total sums of square

Source	Mean Square	Relative contribution of gene effects (%)
Replication	26.83**	
Additive (a)	10.81***	94.79
Dominance (d)	0.14^{ns}	1.19
Additive × additive (aa)	0.01^{ns}	0.12
Additive × dominance (ad)	0.61^{ns}	5.34
Dominance × dominance (dd)	0.38^{ns}	3.34

*** highly significant p <0.001,** significant p< 0.01 ns= not significant

5.4.4.2 Gene effects estimates

The results in Table 5.11 did not give any evidence for epistasis, hence, additive-dominance model of a three parameters, mean (m), additive (a) and dominance (d), was used. The gene effects estimates of (m), (a) and (d) are as presented in Table 5.12. The

mean effect was highly significant (P <0.001) while the additive effect was significant at P <0.01. The dominance effect was not significant. It was also observed that the additive effects were higher in magnitude than the dominance.

Table 5.12: Estimates genetic effects (± SE) for spot blotch resistance

Model	Gene effects estimates
Mean (m)	5.60 ± 0.43***
Additive (a)	1.26 ± 0.43**
Dominance (d)	1.13 ± 0.99^{ns}
R^2	0.32

***, ** indicate significance at P <0.001 and P <0.01, respectively. ns= not significant

5.4.4.3 Heritability estimates and degree of dominance

The narrow sense heritability estimate and coefficient of degree of dominance are presented in Table 5.13. The narrow sense heritability estimate was moderately high (55.45%). The coefficient of degree of dominance was found to be negative and less than unity.

Table 5.13: Narrow sense heritability and coefficient of degree of dominance

Parameter	Estimates
Narrow-sense heritability	55.45
Coefficient of degree of dominance (F)	-0.07

5.5 Discussion

5.5.1 Diallel analysis

The significant differences in the reaction to spot blotch disease among the genotypes, indicated the presence of considerable amount of genetic variation in the parents and their respective crosses. This is an indication that there is a greater possibility of selecting resistant genotypes in the material under study. No parental genotypes or crosses were immune to spot blotch disease across sites. Parents such as P1 (30th10, Resistant (R)), P3 (30th5 (R)) and P4 (Coucal, (MS)) showed lower disease severity (moderately susceptible) across sites than the other parental genotypes. Loerrie II (P5)

and SB50 (Sonalika) (P7) showed high disease severity across sites. Parent P4 had better disease resistance compared to P5 and P6 which are the other adapted parents. The parents P1, P3, and P4, had low disease severity, and could be good sources of resistance to spot blotch disease. Three crosses were moderately resistant (score of 4) to the disease across sites. The moderately resistant crosses were, $30^{th}18$ × Coucal (MR × MS), Kwale × Coucal (MS × MS), and Coucal × $30^{th}5$ (MS × R). These crosses could be incorporated in breeding for resistance to spot blotch disease. Furthermore, crosses between susceptible parents P5 (Loerrie II) and P7 (SB50) were also susceptible (disease score of 7-9). From these results, it is suggested that to obtain adequate resistance in the progenies one or both of the parents should have some level of resistance. This information is very useful for successful breeding for spot blotch resistance.

The analysis of variance of the diallel table showed that additive gene action controlled inheritance of resistance of spot blotch disease. This was shown by the significant item 'a'. The presence of additive gene effects in the inheritance of resistance to spot blotch disease was validated by the significant 'D' variance component and the non-significance of H_1 and H_2 dominance components (Table 5.8). The results showed that additive gene effects were predominant in controlling inheritance of resistance to spot blotch disease. These results are in line with those of Khani et al. (2010) and De et al. (2014) who reported additive gene action in the inheritance of resistance to spot blotch disease. Yang et al. (2002) and Singh et al. (2007) reported that if the additive gene effects were greater than non-additive gene effects, then selection could be done in the early segregating generations. In this case, selection for resistance to spot blotch disease could be highly effective in the early segregating generations. A recurrent selection could be suggested as it would effect in accumulation of desirable genes for resistance. This can be done by intermating outstanding F3 plants (Wiersma et al., 2001). Jiang et al. (1993) reported an increase in the frequency of number of plants resistant to scab disease caused by *Gibberella zeae* in wheat after three cycles of recurrent selection.

The component 'b' was not significant indicating the non-involvement of dominant gene effects in the inheritance of resistance to spot blotch disease. Additionally, the non-significant b_1, b_2 and b_3 items indicated the absence of, directional dominance effects, due to parents having different number of dominant genes and dominance effects specific to individual crosses, respectively. The negative value of h^2 provided further evidence of the absence of dominant genes in the inheritance of resistance to spot blotch disease. This is in line with the finding by Sharma et al. (2006). However, the findings of Neupane et al. (2007) were not in line with the results of the present study as they reported the importance of the dominant gene effect in the inheritance of resistance

to the disease. Duveiller and Sharma (2009) indicated the importance of both dominant and recessive genes in controlling resistance to spot blotch disease.

The H_1 value was greater than H_2 indicating unequal distribution of positive and negative gene frequency in the parents (Hayman, 1954a). This was supported by the value of the proportion of genes with positive and negative effects in the parents ($H_2/4H_1$) which deviated from 0.25, its expected value, indicating asymmetrical distribution of the positive and negative genes for resistance. The negative and non-significant F value suggest that the recessive genes are more frequent than the dominant genes. This was held by the less than unity value of the ratio of proportion of dominant and recessive genes in the parents ($\sqrt{}$ $4DH_1$ + F) / ($\sqrt{}4DH_1$- F). The correlation coefficient (0.67) was found to be positive and nearing unity indicating that recessive genes were mostly positive (Hayman 1954a). The non-significant correlation coefficient revealed that the dominant genes in the parental genotypes were equally positive and negative (Dabholkar, 1999). Furthermore, the estimate of the r^2 which was less than unity revealed the impossibilities of predicting the degree of complete dominance and recessive in parents. This study found that the recessive genes, with their decreasing effect in resistance, were more prevalent in the parental genotypes than the dominant genes.

The scaling test of additive–dominance model revealed the absence of non-allelic gene interactions in the inheritance of resistance to spot blotch disease. This was established by the non-significance of Wr – Vr analysis. Further confirmation of the absence of epistasis in the inheritance of resistance to spot blotch disease was presented by the non-significance of t^2 test. The regression coefficient significantly deviated from zero but not unity further indicating the absence of epistasis in the control of inheritance of resistance to spot blotch disease. However, studies done by Duveiller and Sharma (2009) reported presence of epistasis. This study, showed the complete absence of epistasis in the control of inheritance of resistance to spot blotch disease. The current study also established absence of maternal and non-maternal reciprocal effects in the inheritance of resistance to spot blotch disease. This implies that the choice of the female parent was not important in breeding for resistance and that the reciprocal crosses do not have to be evaluated separately. The mean degree dominance ratio $\sqrt{}$H/D was less than unity indicating the presence of partial dominance and was supported by the slope of regression line which intercepted Wr-axis just above the origin (Figure 5.4). The present results are in conformity with those of Sharma et al. (2006). The absence of epistasis coupled with the presence of partial dominance suggest that selection for resistance to spot blotch disease can be done in early segregating generations (Khan et al., 2000; Chowdhry et al., 2002).

The graphical analysis showed that parents P2, P7 and P8 contained more recessive genes as revealed by their position on the regression line, farthest from the origin. Parents P7 and P8 were rated susceptible to spot blotch while P2 as moderately susceptible (MS). The parents P5 and P6, situated at the middle of the regression line, possessed equal proportion of dominant and recessive genes and these were rated MS. Parents P1, P3 and P4 appeared to possess most of dominant genes for resistance as revealed by the position of their array points on the regression line, closer to the origin, while they were rated moderate susceptible in this study. Some crosses from these parents (Appendix 5.1) were rated as moderately resistant (MR) confirming that indeed they exhibited dominance of resistance. Thus, these parental genotypes (P1, P3 and P4) could be utilized in the development of resistance to spot blotch disease to help improve on the effect of disease in rain-fed wheat. On the other hand, the scattered distribution of array points on the regression line showed that genetic variation among parents existed for resistance to spot blotch disease which could be further be exploited in the breeding program.

The significant environmental variance indicated the influence of environment in the expression of the disease. A similar finding was reported by De et al. (2014). However, the moderately high (0.56) narrow sense heritability estimates (h^2) that was found suggested that the trait could be improved through simple selection in segregating generations despite the effects of the environment (Verhalen and Murray, 1969; De et al., 2014). Additionally, the moderately high narrow-sense heritability implied that 56% variation was heritable in nature and governed by the additive gene effects. Dubin and Rajaram (1996) reported heritability estimates of between 0.60-0.89 while Sharma et al. (1997a) reported heritability estimates ranging between moderate to high (0.47- 0.67).

5.5.2 Generation mean analysis

The significant differences established from the analysis of variance (P1, P2, F1, F2, BCP1 and BCP2) revealed different responses of the generations to spot blotch disease, indicating the contrast between P1 and P2 which is a requirement for GMA. The mean disease severity score of the susceptible and resistant parent were very different from the means of F1 and F2. Additionally, the mean comparisons revealed significant differences of the parental genotypes. Furthermore, the means of BCP1 and BCP2 tended to be closer to the respective recurrent parent indicating the divergence of the parents which satisfied the basic prerequisite for generation mean analysis (Mather and Jinks, 1949).

The resistant parent P2 showed resistant reaction to spot blotch disease due to the low disease severity score observed. For this reason, P2 could be identified as a good

source of resistance. The mean disease severity of F1 was in between the parental genotypes, suggesting partial dominance (Checa et al., 2006). The coefficient of dominance F was negative and nearly zero implying that the dominant genes were low in 19HRWSN6, the resistant parent (Mohammed, 2014). According to the mean comparison, the mean of BCP1 and BCP2 were not significantly different. Even though this was so, it was observed that backcross breeding method could provide a useful means of improving resistance to spot blotch disease, because BCP2 had disease severity scores lower than the susceptible parent indicating a reduction in the disease in each backcross.

The additive gene effects were significant and positive showing that they were important in controlling resistance to spot blotch disease (Table 5.13). Besides, the higher magnitude of contribution of additive effects (94.79%) to the total variation of generation further indicated a much larger role of additive effects in the inheritance of resistance to spot blotch disease compared to dominance effects. Sharma et al. (2004) reported similar findings. This suggests that the resistance levels in wheat genotypes could be improved through simple mass selection in early segregating generations (Mumtaz et al., 2015). The narrow sense heritability were moderately high (55.45%) revealing a large contribution of additive effects in controlling resistance to spot blotch disease. Moderate narrow sense heritability estimates for spot blotch disease agreed well with several earlier reports by Sharma et al. (1997a, b). In this study non-allelic interactions were found not important in controlling resistance to spot blotch disease.

5.6 Conclusion

Additive gene effects were important in the inheritance of resistance to spot blotch disease as established from both the diallel and generation mean analyses. This was further confirmed by moderate high narrow sense heritability estimates which suggests a great involvement of additive gene effects in the expression of resistance to the disease. There were no maternal effects involved in the inheritance of resistance to spot blotch disease signifying that the choice of female parent in hybridization of resistance to spot blotch disease was not important. Additionally, as estimated by both diallel and generation mean analyses, epistasis had no effect in the control of resistance to spot blotch disease which is very important in breeding for resistance to spot blotch disease. This implies that selection would be effective in early generations. Resistance to spot blotch disease exhibited partial dominance. The absence of epistasis coupled with the presence of partial dominance, high additive effects and moderately high narrow sense heritability effects showed that selection for resistance to spot blotch disease might be

highly effective in early segregating generations through simple selection. The parents 30SAWSN10 (P1), 30SAWSN5 (P3) and Coucal (P4) were found to contain more dominant resistant genes than recessive ones as revealed by the relative position on the regression line. The generation mean analysis genotype found 19HRWSN6 to be resistant. Therefore, 30SAWSN10 (P1), 30SAWSN5 (P3), Coucal (P4) and 19HRWSN6 could further be incorporated in breeding programme to improve resistance to spot blotch disease. Furthermore the identified moderately resistant crosses ($30^{th}18$ × Coucal, Kwale × Coucal and Coucal ×30th5) could be advanced in the breeding programme.

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Appendix 5.1

Performance of the wheat parental and F2 genotypes for response to spot blotch disease, 2014/15 season

Genotypes		Seed-Co	Mt.Makulu	GART	Across sites	Disease Reaction
19th15		6	7	6	7.3	S
30th10		5	3	6	5.1	MS
30th18		6	6	7	6.1	MS
30th5		5	5	6	5.4	MS
Coucal		5	5	5	5.1	MS
30th10 ×	30th18	5	5	6	5.3	MS
Kwale ×	30th10	6	4	6	5.4	MS
30th10 ×	SB 50	6	5	7	5.8	MS
SB 50 ×	30th10	6	6	6	6.0	MS
30th10 ×	19th15	6	6	6	5.8	MS
19th15 ×	30th10	5	6	5	5.4	MS
30th18 ×	30th 5	6	5	6	5.5	MS
30th18 ×	30th10	6	3	6	5.1	MS
30th 5 ×	30th18	6	4	6	5.3	MS
30th18 ×	Coucal	5	4	5	4.8	MR
Coucal ×	30th18	6	4	6	5.1	MS
30th18 ×	Loerrie II	5	4	6	5.2	MS
Loerrie II x	30th18	6	5	6	5.6	MS
30th 18 ×	Kwale	6	4	6	5.2	MS
Kwale ×	30th18	5	5	6	5.7	MS
30th18 ×	SB 50	6	6	6	5.7	MS
SB 50 ×	30th18	7	5	7	7.3	S
30th10 ×	30th5	5	6	6	5.8	MS
30th18 ×	19th15	6	5	6	5.5	MS
19th15 ×	30th18	6	4	6	5.3	MS
30th 5 ×	Coucal	5	5	6	5.4	MS
Coucal ×	30th 5	5	5	4	4.9	MR
30th 5 ×	Loerrie II	5	5	6	5.4	MS
Loerrie II x	30th 5	6	5	6	5.4	MS
30th 5 ×	Kwale	5	5	6	5.5	MS
30th 5 ×	30th10	6	6	6	5.8	MS
Kwale ×	30th 5	5	4	5	5.0	MS
30th 5 ×	SB 50	6	5	6	5.7	MS
SB 50 ×	30th 5	6	5	7	6.1	MS
30th 5 ×	19th15	5	5	6	5.5	MS
19th15 ×	30th 5	5	4	6	5.2	MS
Coucal ×	Loerrie II	6	6	6	5.8	MS
30th10 ×	Coucal	6	5	5	5.3	MS

Appendix 5.1 continued

Genotypes		Seed-Co	Mt.Makulu	GART	Across sites	Disease Reaction
Loerrie II x	Coucal	6	5	6	5.6	MS
Coucal ×	Kwale	6	5	6	5.5	MS
Kwale ×	Coucal	5	3	5	4.8	MR
Coucal ×	SB 50	6	5	6	5.7	MS
SB 50 ×	Coucal	6	5	6	5.4	MS
Coucal ×	19th15	5	4	6	5.2	MS
19th15 ×	Coucal	5	5	5	5.2	MS
Coucal ×	30th10	5	5	6	5.3	MS
Loerrie II x	Kwale	6	6	7	6.0	MS
Kwale ×	Loerrie II	5	5	5	5.1	MS
Loerrie II x	SB 50	6	7	6	7.1	S
SB 50 ×	Loerrie II	6	6	7	7.0	S
Loerrie II x	19th15	6	6	6	5.8	MS
19th15 ×	Loerrie II	6	5	6	5.7	MS
30th10 ×	Loerrie II	6	7	6	5.9	MS
Kwale ×	SB 50	6	5	6	5.9	MS
SB 50 ×	Kwale	6	5	6	5.5	MS
Kwale ×	19th15	5	6	6	5.7	MS
19th15×	Kwale	6	6	6	5.8	MS
Loerrie II x	30th10	6	6	6	5.8	MS
SB 50 ×	19th15	6	5	7	6.0	MS
19th15 ×	SB 50	6	6	6	7.0	MS
30th10 ×	Kwale	5	4	6	5.3	MS
Kwale		7	7	6	7.0	S
Loerrie II		7	7	6	7.0	S
SB 50		7	7	7	7.4	S

MR=Moderate resistant, MS=Moderate susceptible, S=Susceptible19th15=19HRWSN15, 30^{th}18= 30SAWSN18, 30^{th}5=30SAWSN5, 30^{th}10=30SAWSN10, SB50=Sonalika. HRWSN=High rainfall wheat screening nursery and SAWSN=Semi-arid wheat screening nursery

Chapter 6

Validation of microsatellite molecular markers linked with resistance to *Bipolaris sorokiniana* in wheat (*Triticum aestivum* L.)

Abstract

Spot blotch disease caused by *Bipolaris sorokiniana* (Sacc.) Shoem causes yield losses and reduces grain quality in wheat. Molecular markers reported to be linked with resistance to *Bipolaris sorokiniana* could accelerate the identification of resistant genotypes as they are independent of environmental effects. However, before they can be utilized for marker assisted selection, validation in an independent population is required. The objective of this study was to validate three simple sequence repeat (SSR) molecular markers (*Xwgm570*, *Xgwm544* and *Xgwm437*) previously reported to be linked with resistance to *Bipolaris sorokiniana.* The markers were validated using 66 wheat genotypes comprising eleven parental genotypes and fifty-five F2 progenies. The eleven parental genotypes included three locally adapted genotypes from Zambia (Coucal, Kwale and Loerrie II) and the rest were from the International Maize and Wheat Improvement Centre (CIMMYT) in Mexico. The fifty- five F2 progenies were derived from diallel crosses inovolving 8 parents; Coucal, Kwale, loerrie II, 30th5, 30th10, 30th8, SB50 and 19th15. SB50 was used as a susceptible check while 19th6 and 30th5 were used as resistant checks. All the markers differentiated between resistance and susceptible genotypes and the results corresponded with the field screening results. In addition, significant associations with resistance to the pathogen (*Xgwm570*, P=0.003, *Xwgm544* and *Xgwm437*, P=0.03) were observed. The high adjusted R^2 further indicated the markers' association with resistance to *Bipolaris sorokiniana.* Marker *Xgwm570* (adjusted R^2=11.0%) had the strongest association with resistance to *Bipolaris sorokiniana* followed by *Xgwm544* (adjusted R^2=10.0%) and then *Xgwm437* (adjusted R^2=7.0%). Therefore, these markers could be useful in increasing the efficiency of selection for resistant genotypes in wheat breeding in Zambia. Since R^2 values are low, a combination of two or three markers can be employed during marker assisted selection.

6.1 Introduction

Production of summer rain-fed wheat in Zambia is challenged by the prevalence of spot blotch disease (Figure 6.1a) caused by *Bipolaris sorokiniana* (Figure 6.1b) which results in significant yield losses (85%) (Raemaekers, 1988) and grain quality deterioration. The rain-fed wheat cultivars grown in Zambia are susceptible to spot blotch and there is need for urgent control measures. Utilization of resistance genotypes to *Bipolaris sorokiniana* is the best approach to manage the disease because it is economical and environmentally friendly. To date, selection of resistant genotypes has been through screening wheat genotypes in several environments through use of disease phenotyping to identify resistance sources in order to develop new genotypes with improved resistance.

Screening for resistance has been done in one season only (summer) due to the presence of high disease epiphytotic conditions. Phenotypic markers such as leaf angle (Joshi and Chand, 2002), leaf tip necrosis (Joshi et al., 2004) and stay green (Joshi et al., 2007), that have been associated with resistance to spot blotch disease, have also been used for selection purposes. The problem associated with phenotypic evaluation of genotypes is that it requires large sample sizes for screening; it is time consuming, greatly relies on repeated ratings in diverse environments, laborious, confounded by environmental factors and incurs high experimental errors (Fernando et al., 2015). Nonetheless, molecular markers linked to *Bipolaris sorokiniana* resistance genes can provide an alternative approach to overcome these drawbacks and accelerate identification and development of resistant genotypes (Concibido et al., 2004; Islam et al., 2011). Duveiller and Sharma (2013), and Sharma et al. (2007) reported that the use of molecular markers linked to *Bipolaris sorokiniana* resistance genes in combination with field selection could increase the efficiency and speed of improving resistance in wheat genotypes. This is because use of molecular markers saves time, reduces experimental errors and is more reliable and accurate as the markers are not confounded by environmental factors (Concibido et al., 2004).

Quantitative trait loci (QTL) and simple sequence repeat markers (SSR) linked with resistance to *Bipolaris sorokiniana* have been identified in wheat. The identification of QTL and SSR markers associated with resistance to *Bipolaris sorokiniana* presents new opportunities for improving resistance in wheat. With molecular markers, screening and identification of resistant genotypes in parental and segregating generations could be done in the absence of disease epiphytotic conditions (Mondal et al., 2007). Kumar et al. (2009) identified four QTLs linked to *Bipolaris sorokiniana* resistance genes on chromosomes 2AL, 2BS, 5BL and 6BL. However, only two QTLs located on the short

arm of 2B were found to be consistent in their three year trial. Awasthi and Lal (2014) indicated that a QTL or molecular marker that is present in numerous genetic backgrounds is important for marker assisted breeding. Kumar et al. (2005) reported two SSR markers on chromosome 7D (*Xgwm437*) and on chromosome 5B (*Xgwm544*) linked with resistance to spot blotch. Das et al. (2002) identified 18 random amplified polymorphic DNA (RAPD) that discriminated resistance from susceptible genotypes. Ragiba and Prabhu (2009) also identified four RAPD markers associated with resistance to spot blotch in wheat genotype 'Chirya 3'. Additionally, three SSR markers linked to spot blotch resistance were identified in wheat genotype 'G162' on chromosome 5B (*Xgwm67*), 6A (*Xgwm570*) and on 6D (*Xgwm469*) by Sharma et al. (2007). Adhikari et al. (2012), on the other hand, using Diversity Arrays Technology (DArT) markers identified four genomic regions (chromosome 1A, 3B, 7B and 7D) associated with resistance to *Bipolaris sorokiniana* at seedling stage.

Validation of molecular markers using another population is required before utilizing them for marker assisted breeding to determine their effectiveness (Anitha et al., 2013). No previous studies have been conducted to validate the SSR markers previously reported to be linked to resistance genes of *Bipolaris sorokiniana.* Validation establishes the value of a molecular marker reported to be linked to a particular trait in an independent population with varying genetic background (Sharp et al., 2001; Islam et al., 2011). Marker-trait association has been done through various analysis approaches such as single marker analysis (SMA), simple interval mapping (SIM), composite interval mapping (CIM) and also multiple interval mapping (MIM) (David et al., 2011). David et al. (2011) indicated that single marker analysis could be conducted using several statistical analyses such as regression analysis, analysis of variance (ANOVA), t-tests, log likelihood ratios, and maximum likelihood estimations. The present study was carried out to validate three SSR markers *Xgwm544* and *Xgwm437* (Kumar et al., 2005) and *Xgwm570* (Sharma et al., 2007), reported to be associated with *Bipolaris sorokiniana* resistance to determine their usefulness in breeding for resistance in Zambian wheat genotypes. In this study single marker analysis using regression analysis in SAS version 9.3 (SAS Institute, 2011) was used to determine the relationship between the corresponding marker scores and the phenotypic trait observed.

a b

Figure 6.1: a) symptoms of spot blotch disease on leaves b) Conidia of *Bipolaris sorokiniana* observed at Mt. Makulu Laboratory, 2013 (magnification: x1000)

6.2 Materials and methods

6.2.1 Plant material

A total of 66 wheat genotypes comprising eleven parental genotypes and fifty- five F2 progenies were used for screening for resistance to *Bipolaris sorokiniana*. The wheat materials used in the study are presented in Appendix 6.1. The eleven parental genotypes included three locally adapted genotypes from Zambia (Coucal, Kwale and Loerrie II) and the rest were introduced from International Maize and Wheat Improvement Centre (CIMMYT)-Mexico). The 55 F2 progenies were derived from diallel crosses involving 8 parents; Coucal, Kwale, loerrie II, 30th5, 30th10, 30th8, SB50 and 19th15. Sonalika (SB50) was used as a susceptible check, while 19th6 and 30th5 were used as resistant checks.

6.2.2 Phenotypic evaluation

Screening for resistance of the 66 wheat genotypes was done under natural epidemics in three sites Mpongwe Seed-Co Research Farm, Golden Valley Agricultural Research Trust (GART) and Mt Makulu Research Station during 2013/14 season. The experimental field was laid out in a 6 × 11 alpha lattice design with two replications. Rows were 3 m long with 20 cm spacing between rows and 40 cm between plots. Each row was seeded with 60 plants which was later thinned out to have 30 plants per row. Standard agronomic practices were followed for good crop management. All plants were scored for disease severity on symptom appearance, at seven day intervals. The

severity score on the last day of scoring was used for analysis. The disease severity score was based on Saari and Prescott`s scale for assessing foliar disease (Eyal et al., 1987). The severity score ranged from 0 – 9. Zero was scored on leaves with no symptoms, 1 was scored on leaves having one or two necrotic spots, 2 on leaves with few small necrotic spots without chlorosis, 3 on leaves with few small necrotic spots with chlorosis, 4 on leaves with medium size necrotic spots with distinct but restricted chlorotic margin, 5 on leaves with medium to large necrotic spots with distinct but restricted chlorotic margin, 6 on leaves having large abundant necrotic spots with distinct chlorotic margin, 7 on leaves with necrotic spots linked together with pronounced chlorosis, 8 on leaves with extensive necrotic spots fully merge expanding longitudinally with pronounced chlorosis and 9 on leaves having many extensive necrotic spots with pronounced chlorosis (Figure 6.2). Disease severity of each plot was found by averaging the severity ratings of the plants. The genotypes were classified using the resistance criterion proposed by Chaurasia et al. (1999). Genotypes falling in the 1-3 category were recorded as resistant, 4 as moderately resistant, 5-6 moderately susceptible and 7-9 as susceptible.

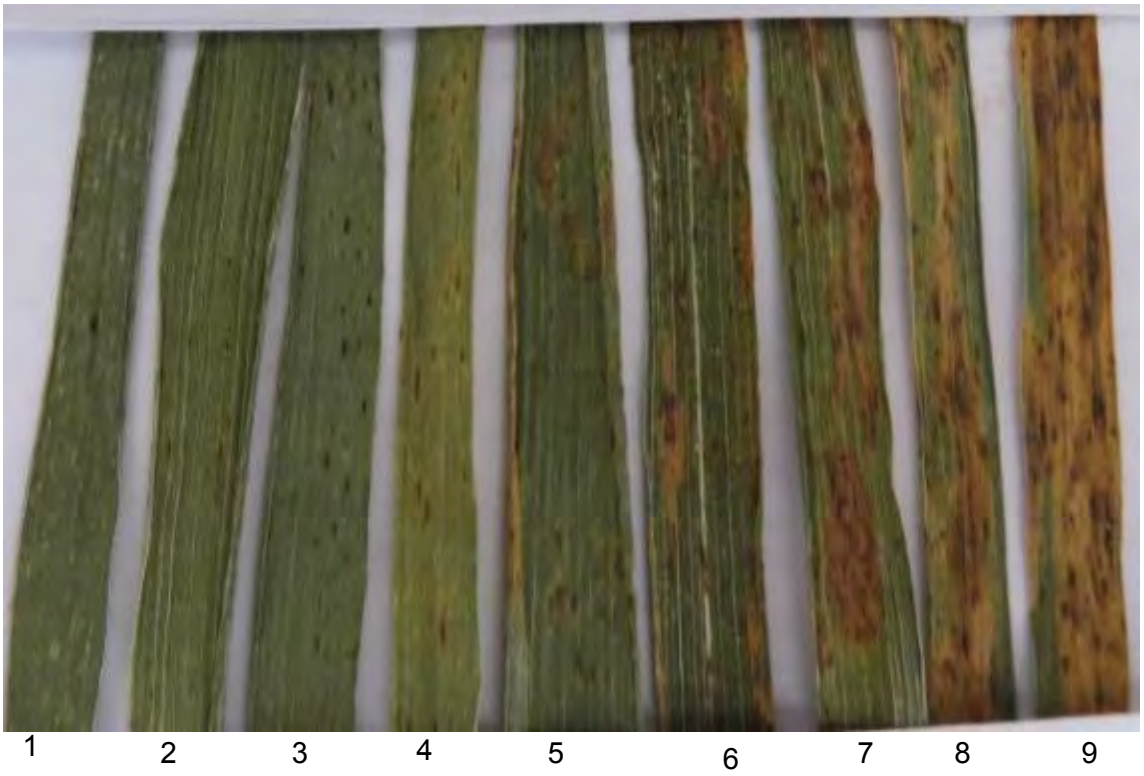

Figure 6.2: Visual rating scale for assessment of the severity of spot blotch disease on wheat.

(Photo: B. Tembo)

6.2.3 Genotypic evaluation

6.2.3.1 Genomic DNA extraction

DNA for each genotype was extracted according to International Maize and Wheat Improvement Centre (CIMMYT) (2005) protocol. Twenty seeds for each genotype were ground separately using a mortar and pestle into a fine powder. About 70 mg of seed meal was transferred to 1.5 ml centrifuge tube and labelled. The extraction buffer of 700 µl (1% SDS, 700 mM NaCl, 100 mM Tris and 50 mM EDTA) was added to the meal and incubated for 10 minutes at 65^{o}C. After incubation, 200 µl of 5M potassium acetate was added to the mixture. The mixture was then vortex and placed on ice for 10 minutes, and then centrifuged at 12,000 rpm for 10 minutes at 4^{o}C. Then 400 µl of the supernatant was placed in a new 1.5 ml tube in which 400 µl of iso-propyl alcohol was added. The mixture was mixed gently by inverting the tube 3 times and then centrifuged at 800 rpm for 3 minutes at 4^{o}C. The supernatant was discarded and the DNA pellet washed twice by adding 500 µl ethanol and centrifuging at 8000 rpm for 3 minutes each wash. The pellet was dried at room temperature and re-suspended in 50-100 µl of deionised water.

6.2.3.2 Genotyping with SSR markers

The markers *Xgwm570* (Sharma et al., 2007), and *Xgwm437*and *Xgwm544* (Kumar et al., 2005) previously reported to be linked to genes for resistance to *Bipolaris sorokiniana* causing spot blotch disease in wheat were used in this study. Primer sequences (Table 6.1) were obtained from http://wheat.pw.usda.gov/cgi-bin/graingenes.

To amplify regions of genomic DNA, polymerase chain reaction (PCR) was performed in a 12µl volume reaction mixture containing 2 µl of template DNA (10 ng/µl), 1.2 µl dNTPs (25 Units/ µl), 0.72 µl MgCl2 (50 mM), 1.2 µl buffer (10X), 0.12 µl *Taq* polymerase (5U/ µl), 6.16 µl PCR grade water, 0.24 µl Dye (10 µM), 0.06 µl forward primer and 0.3 reverse primer. The amplification reactions were performed in a heated lid thermal cycle programmed at 93^{o}C for 1 minute for 1 cycle, followed by 30 cycles of denaturing at 93^{o}C for 30 seconds, primer annealing at 60^{o}C for 30 seconds and an extension of 1 cycle of 72^{o}C for 5 minutes followed by a final extension at 72^{o}C for 10 minutes. The PCR products were fluorescently labelled and separated by capillary electrophoresis on an ABI 3130 automatic sequencer (Applied Biosystems, Johannesburg, SA) amplification products were scored using GeneMapper 4.1 (CIMMYT, 2005).

6.2.3.3 Data analysis

The phenotypic data collected were subjected to analysis of variance to determine whether there were significant differences among genotypes. Analysis of variance was conducted using the general linear model procedure (PROC GLM) in SAS version 9.3 (SAS Institute, 2011).

Single marker analysis was done to determine the association between field spot blotch resistance value and the marker genotype data. The band amplified from each marker data was scored as either 0 to indicate absence of the marker and 1 to indicate presence of the marker (David et al., 2011). Single marker analysis was conducted in SAS version 9.3 (SAS Institute, 2011) using simple linear regression analysis (PROC REG) method. Significance of the regression coefficient suggests that there is a relationship between the marker and the trait (Anandhan et al., 2010). The molecular marker with high adjusted R^2 implies that is has strongest relationship with resistance (Anandhan et al., 2010). The analysis was conducted following the linear model below,

$Yi = a + bXi + error$

Where, Y= trait value, a = constant, $X_i = i^{th}$ marker

A multiple linear regression analysis was conducted in Genstat version 14 (Payne et al., 2011) to investigate impact of the three markes on marker assisted breeding. The following linear model was used,

$Yi = a + b_1X_1 + b_2X_2 + b_3X_3 + error$

Where, Y= trait value, a = constant, X_1= marker *Xgwm570*, X_2= marker *Xgwm544* and X_3= marker *Xgwm437*.

Table 6.1: SSR primer sequence used for validation of resistance to *Bipolaris sorokiniana* in wheat

SSR Primer	Forward primer sequence	Reverse primer sequence
Xgwm570	5' TCGCCTTTTACAGTCGGC 3'	5' ATGGGTAGCTGAGAGCCAAA 3'
Xgwm544	5' TAGAATTCTTTATGGGGTCTGC 3'	5' AGGATTCCAATCCTTCAAAATT 3'
Xgwm437	5' GATCAAGACTTTTGTATCTCTC 3'	5' GATGTCCAACAGTTAGCTTA 3'

6.3 Results

6.3.1 Phenotypic evaluation

Siginificant differences (P < 0.001) were observed among genotypes to the reaction spot blotch disease (Table 6.2). Among the eleven parental genotypes, three (19^{th}6, 30^{th}5, 30^{th}10) were recorded as moderately resistant, four as moderately susceptible (Coucal, SB1, 19^{th}19, 30^{th}18,) and four as susceptible (SB50, 19^{th}15, Kwale and Loerrie II) (Appendix 6.1). Among the Zambian genotypes one (Coucal) was moderately susceptible while the other two (Loerrie II and Kwale) were susceptible. Of the fifty-five F2 progenies, three were recorded as moderately resistant (score of 4.8), forty-eight as moderately susceptible (score of 5-6) and four as susceptible (score of 7-9). No genotype was immune to the disease.

Table 6.2: Combined analysis of variance of spot blotch disease reaction evaluated in three locations

Source	Degree of freedom	Mean square
Environment (Env)	2	27.61***
Replication (Env)	3	1.38
Entry	65	1.18***
Entry × Env	130	0.41^{ns}
Error	195	0.36
Corrected total	395	
CV	10.81	
R^2	72.88	
Mean	5.57	

*** Highly significant P<0.001; ns=non- significant

6.3.2 Molecular marker analysis

Marker *Xgwm570* amplified PCR product of 155 base pair (bp) in the parental genotypes 30th5, 19th19 and 30th18 (Figure 6.3a), whereas 19th6 (moderately resistant) showed a 164 bp in addition to 155 bp. The 155 bp was also present in 25 moderately resistant F2 progenies from these parental genotypes which was absent in the susceptible genotypes. A susceptible parental genotype SB50 (Sonalika) (Figure 6.3b) and some F2 progenies with SB50 segments such as F2plot53, F2plot54, F2plot63 and F2plot64 showed a 172 bp fragment upon amplification with marker *Xgwm570*. These genotypes had 7.0 as a mean disease score. The 172 bp amplicon was present in all susceptible genotypes and not in the resistant and moderately resistant genotypes.

There was no amplification with marker *Xgwm544* for moderately resistant parental genotypes $19^{th}6$ and $30^{th}5$ (Figure 6.4a). However, 22 F2 progenies derived from these parental genotypes, $30^{th}5$ and $19^{th}6$, showed band sizes of 196 bp, 198 bp and 200 bp (Figure 6.4b). Parents $30^{th}10$ and $30^{th}18$ showed a similar band to that observed in the F2 progenies. The susceptible parent, SB50 upon screening with marker *Xgwm544*, a 192 bp fragment was observed (Figure 6.4c). This amplicon only appeared in all the susceptible parents which included Kwale and Loerrie II and some F2 progenies derived from these parents.

Marker *Xgwm437* amplified a 121 bp fragment in the susceptible parent SB50 and F2 progenies with fragments of SB50 (Figure 6.5a). Marker *Xgwm437* amplified an identical fragment size of 129 bp and 138 bp in the resistant parental genotype $30^{th}5$ (Figure 6.5b) and $19^{th}6$, and 11 F2 progenies derived from them.

Single marker analysis showed highly significant association (P=0.003) between phenotype trait and associated genotyped results from marker *Xgwm570* (Table 6.3). Marker *Xgwm570* accounted for 14.0% phenotypic variation (R^2), while the adjusted R^2 was 11.0%. Significant association was also observed between genotyped results of molecular marker *Xgwm544* (P= 0.03) and the associated phenotypic trait. The R^2 and the adjusted R^2 for marker Xgwm544, were 13.0% and 10.0%, respectively. The association between genotyped results of molecular marker *Xgwm437* and the phenotype was significant (P=0.03). The phenotypic variance explained (R^2) by *Xgwm437* was 9.5%, while the adjusted R^2 was found to be 7.0%.

The multiple regression model with all the three markers showed a significant association (P<0.01) with the phenotypic trait (Table 6.4). The proportion of phenotypic variation (R^2) explained by all the three marker is 18.0%. However, marker *Xgwm570* contributed significantly (P<0.001) to the multiple regression model (Table 6.2) than molecular marker *Xgwm544* and *Xgwm437*.

Table 6.3: Single marker and multiple regression accumulated analysis for resistance to *Bipolaris sorokiniana* in wheat

	Single marker analysis			Multiple regression accumulated analysis
Marker	Probability value	R^2 (%)	Adjusted R^2 (%)	Probability value
Xgwm570	0.003	14.0	11.0	<0.001
Xgwm437	0.03	9.5	7.0	0.71
Xgwm544	0.03	13.0	10.0	0.10

Table 6.4: Multiple regression analysis of resistance to *Bipolaris sorokiniana* in wheat using three molecular markers, *Xgwm570*, *Xgwm544* and *Xgwm437*

Source	Degree of freedom	Mean square
Regression	3	2.63**
Residual	63	23.94
Total	66	0.58

** indicate significance at P <0.01

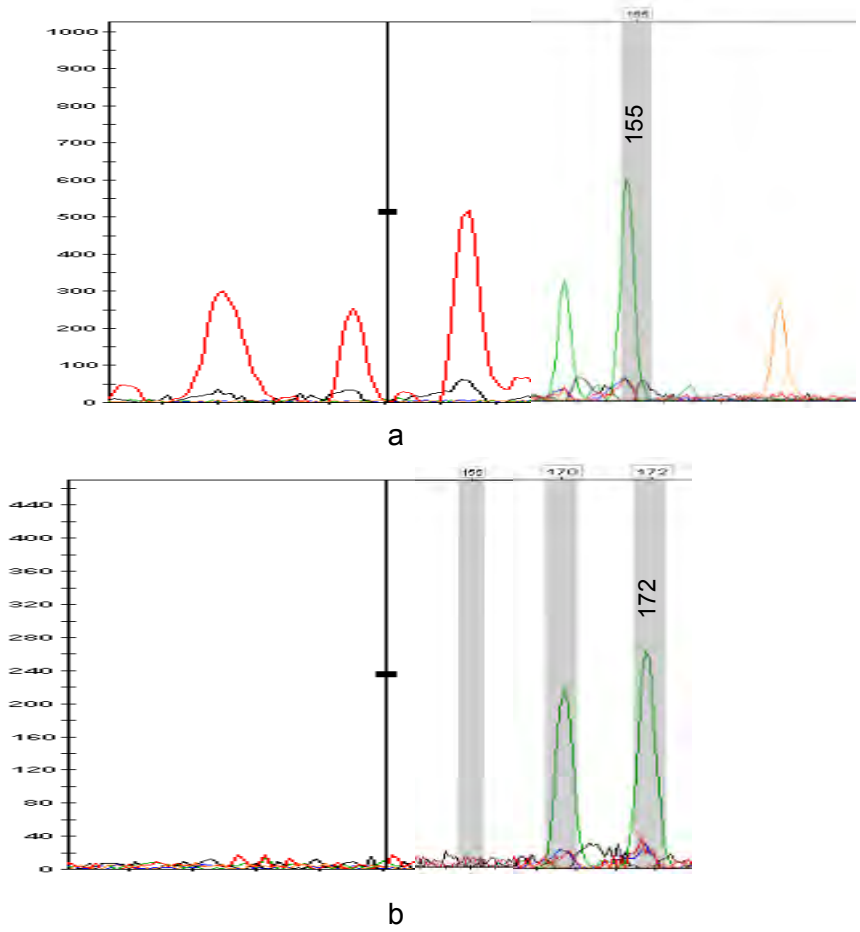

Figure 6.3: Electropherogram of the ampilication products of marker *Xgwm570* in a) 30th5 a resistant genotype, b) SB50 a susceptible genotype

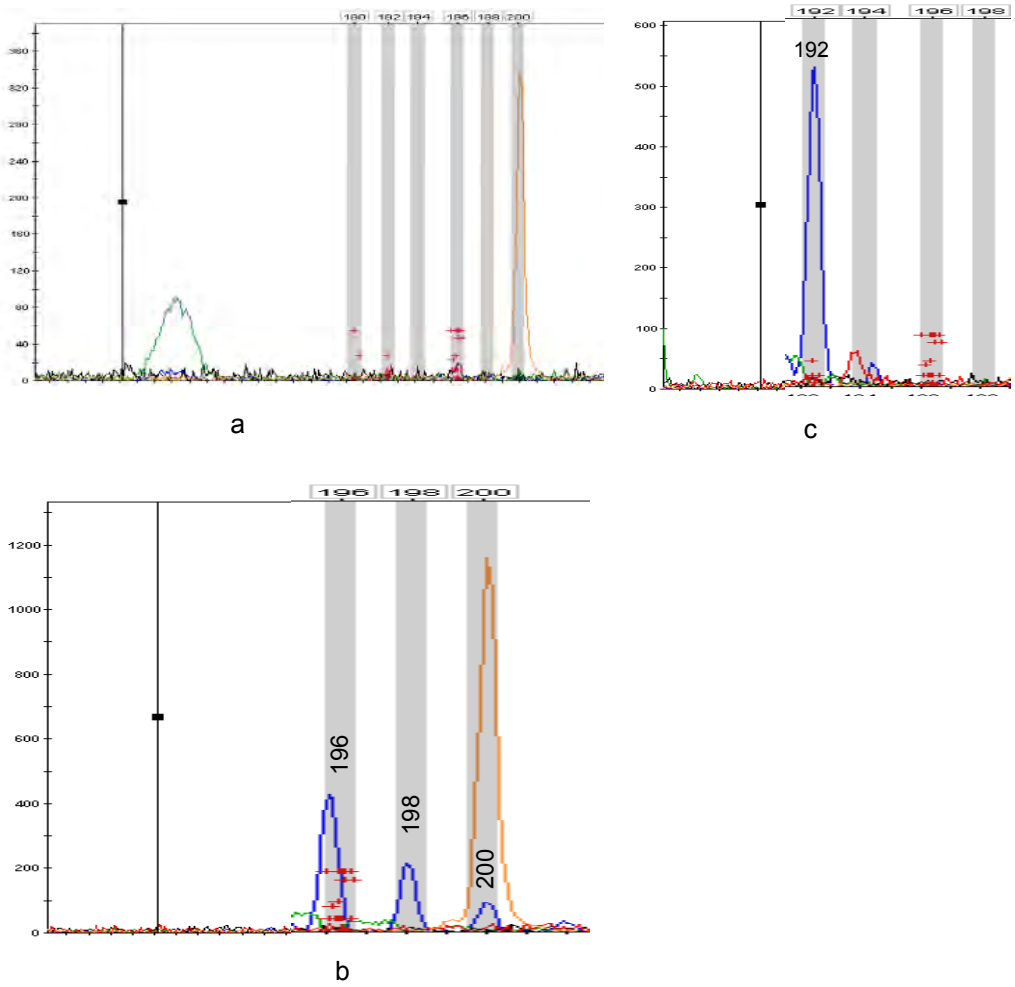

Figure 6.4: Electropherogram of the amplification products of marker *Xgwm544* in a) 30th5 a resistant, b) F2 progeny 20 (30th5 × 30th18, c) SB50 susceptible genotype

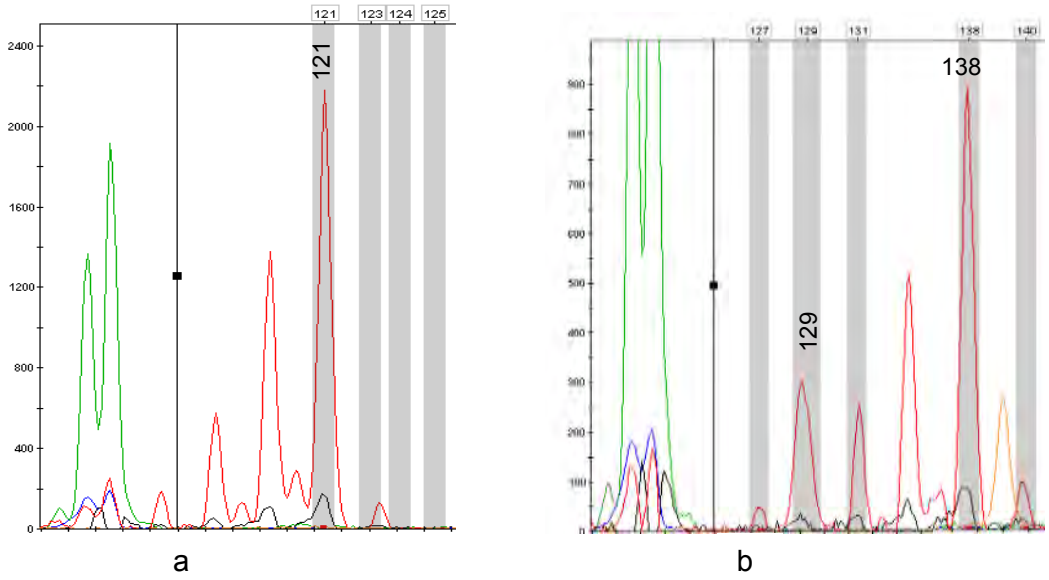

Figure 6.5: Electropherogram of the amplification products of marker *Xgwm437* in a) SB50 a susceptible genotype b) 30th5 a resistant genotype

6.4 Discussion

Variations in the phenotypic data implies that the genotypes differed at genotypic level hence, a population was ideal for validation.

The results indicated that all the three SSR markers *Xgwm570, Xgwm544* and *Xgwm437* showed an association with resistance to *Bipolaris sorokiniana* which causes spot blotch disease. However, marker *Xgwm570* had the highest adjusted R^2 (11.0%) implying that it had the strongest association with resistance to *Bipolaris sorokiniana,* followed by *Xgwm544* (10.0%) and then *Xgwm437* (7.0%). Anandhan et al. (2010) indicated that the relationship between the marker and the trait could be judged from the adjusted R^2. The marker with high adjusted R^2 has the relative strongest relationship with the trait.

Marker *Xgwm570* differentiated between resistant and susceptible genotypes by the amplicon size of 155 bp that amplified only in the resistant and moderately resistant parental genotypes and their derived 25 F2 progenies but not in the susceptible genotypes. The similarity observed in the amplified fragments in the resistant parental and the F2 progenies, resistant and moderately resistant ones, is one of the indications that the marker was associated with resistance (Reena and Jaiwal, 2014; Sehrawat et al., 2015). However, all the susceptible parental genotypes (SB50, Kwale and Loerrie II) and their derived F2 population showed a 172 bp amplification product confirming the

absence of gene for *Bipolaris sorokiniana* resistance in them. Similar to this study, Aggarwal et al. (2011) using SCAR marker ($SCAR_{600}$) amplified a 600 bp band in all leaves infected with *Bipolaris sorokiniana* but not in healthy leaves. The significance of the regression coefficient (P=0.003) showed that marker *Xgwm570* was highly associated with resistance to *Bipolaris sorokiniana.* Additionally, the relatively high adjusted R^2 (11.0%) observed with this marker implies the association with resistance to *Bipolaris sorokiniana* was strong (Anitha et al., 2013). Marker *Xgwm570* explained 14% phenotypic variance (R^2). This shows that marker *Xgwm570* has a high potential for use in marker assisted selection for resistance to *Bipolaris sorokiniana.* These results confirm the earlier findings by Sharma et al. (2007) who suggested that the *Xgwm570* was linked to resistance genes of *Bipolaris sorokiniana* that cause spot blotch disease.

Marker *Xgwm544* also discriminated susceptible and resistant genotypes effectively and the results corresponded with the disease reaction. Marker *Xgwm544* amplified a 192 bp fragment in susceptible parental genotypes and their derived F2 progenies but not in the resistant genotypes, an indication of the absence of gene for resistance to *Bipolaris sorokiniana* in them. Marker *Xgwm544* could not produce any amplicons in the moderately resistant parental genotypes 30th5 and 19th6. This could be due to the absence of tested linked SSR polymorphism in these genotypes (Mondal et al., 2007; 2012) or due to crossing over between the marker and the allele for resistance in these parental genotypes (Mondal et al., 2007). Additionally, it could be due to the marker not being tightly linked to the resistant allele in the corresponding genotypes (Gajjar et al., 2014) and/or other genetic factors conditioning resistance (Young and Kelly, 1997).

On the other hand, the F2 progenies from the resistant parental genotypes (30^{th} 5 and 19^{th}6) and, 30^{th}10 and 30^{th} 18 upon screening with *Xgwm544* produced 196 bp,198 bp and 200 bp fragments that were highly associated with resistance (P=0.03) to *Bipolaris sorokiniana.* This implies that the F2 progenies may have resistance genes in common. Additionally, some F2 progenies that were phenotypically identified as moderately susceptible also showed 196 bp, 198 bp and 200 bp upon amplification with *Xgwm544* suggesting that they had some resistant genes. Marker *Xgwm544* showed a strong relationship with resistance to *Bipolaris sorokiniana* as shown by the high adjusted R^2 (10.0%) and also the significance regression coefficient observed from the regression analysis (P=0.03). *Xgwm544* explained 13% of the observed total phenotypic variation for resistance. This finding confirmed the earlier study by Kumar et al. (2005) that *Xgwm544* was linked to *Bipolaris sorokiniana* resistant genes indicating its usefulness as a tool for identifying resistant genotypes in early breeding generations.

In this study, marker *Xgwm437* discriminated resistant from susceptible genotypes in parents and their F2 derived progenies. This was observed from the products of amplification that were identical in the parental and the F2 progenies. That is, marker *Xgwm437* amplified a 121 bp fragment that was only present in all susceptible genotypes confirming the field screening results, thus, suggesting the absence of resistance genes to *Bipolaris sorokiniana*. Aggarwal et al. (2010) using universal rice primer (URP)-2F observed three bands of 600 bp, 800 bp and 900 bp only in wheat leaves infected with *Bipolaris sorokiniana* and not in the healthy leaves. Marker *Xgwm437* amplified identical fragment of 129 bp and 138 in resistant parental genotypes (30^{th}5 and 19th6) and F2 moderately resistant progenies confirming the presence of resistance genes to *Bipolaris sorokiniana*. Marker *Xgwm437* also displayed a relationship with resistance to *Bipolaris sorokiniana* considering the significance of the regression coefficient (P=0.03) (Anandhan et al., 2010). Furthermore, the adjusted R^2 of 7.0% reveals presence of association between the marker genotype and the phenotype, and the marker explained 9.5% of the observed variation. Thus, marker *Xgwm437* previously reported by Kumar et al. (2005) to be linked with resistance to *Bipolaris sorokiniana* was also confirmed in this study. These results mean that marker *Xgwm437* will be useful in screening for the resistant genotypes as it would accelerate the identification of resistant genotypes during early generations (Bernando et al., 2013). Multiple regression analysis showed that the addition of markers explained more of the phenotypic variation as observed from the R^2 value. A significant association between the markers and the phenotypic trait implies that there was a positive interaction effect between the markers (Haley and Knott, 1992). Hence, therefore, a combination of the markers can employed during marker assisted to accelerate identification of resistant genotypes.

6.5 Conclusion

The SSR markers *Xgwm570*, *Xgwm544* and *Xgwm437* previously reported to be linked with resistance to *Bipolaris sorokiniana,* which causes spot blotch disease in wheat, were validated in this study. The markers discriminated between resistant and susceptible genotypes in the populations used in the study. The markers amplified identical fragments in resistant parental genotypes and resistant and moderately resistant F2 progenies that were not present in the susceptible genotypes. Likewise, the fragments that were observed in the susceptible genotypes were absent in the resistant ones. The significance of the analysis coupled with adjusted R^2 value observed from the markers, *Xgwm570* (11.0%), *Xgwm544* (10.0%) and *Xgwm437* (7.0%), further showed that there was association between the marker genotype and the phenotype. Therefore, these markers could be useful in Zambia as they would increase the efficiency for identification

of resistant genotypes in the seedling stage and even in the absence of the disease epiphytotic conditions. This would allow screening for resistance to spot blotch in both summer and winter season. Since R^2 values are low, a combination of two or three markers can be employed during marker assisted selection.

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Appendix 6.1

Wheat genotypes used for validation of SSR molecular markers (*Xgwm570*, *Xgwm544* and *Xgwm437*) associated with resistance to spot blotch disease

Genotype	Cross		Field screening	*Xgwm570*	*Xgwm544*	*Xgwm437*
19th15	Parent		MS	-	-	-
30th10	Parent		MR	0	+	+
30th18	Parent		MS	+	+	+
30th5	Parent		MR	+	0	+
Coucal	Parent		MS	-	-	-
SB1	Parent		MS	-	-	-
19th6	Parent		MR	+	0	-
19 th19	Parent		MS	+	-	-
F2plot1	30th10 x	30th18	MS	+	+	+
F2plot10	Kwale x	30th10	MS	-	-	-
F2plot11	30th10 x	SB50	MS	-	-	-
F2plot12	SB50 x	30th10	MS	-	-	-
F2plot15	30th10 x	19th15	MS	-	-	-
F2plot16	19th15 x	30th10	MS	-	-	-
F2plot19	30th18 x	30th5	MS	+	+	+
F2plot2	30th18 x	30th10	MS	+	+	+
F2plot20	30th5 x	30th18	MS	+	+	-
F2plot21	30th18 x	Coucal	MR	+	+	-
F2plot22	Coucal x	30th18	MS	-	-	-
F2plot23	30th18 x	Loerrie II	MS	+	+	+
F2plot24	Loerrie II x	30th18	MS	+	+	+
F2plot25	30th 18 x	Kwale	MS	+	0	-
F2plot27	30th18 x	SB50	MS	+	0	-
F2plot28	SB50 x	30th18	S	+	+	+
F2plot3	30th10 x	30th5	MS	+	+	+
F2plot31	30th18 x	19th15	MS	+	+	-
F2plot32	19th15 x	30th18	MS	0	-	-
F2plot35	30th 5 x	Coucal	MS	+	+	+
F2plot36	Coucal x	30th5	MR	+	+	+
F2plot37	30th 5 x	Loerrie II	MS	+	-	+
F2plot38	Loerrie II x	30th5	MS	+	-	-
F2plot39	30th5 x	Kwale	MS	+	+	+
F2plot4	30th5 x	30th10	MS	+	+	-
F2plot40	Kwale x	30th5	MS	-	-	-
F2plot41	30th5 x	SB50	MS	-	-	-
F2plot41	SB50 x	$30^{th}5$	MS	-	-	-
F2plot45	30th5 x	19th15	MS	+	+	-
F2plot46	19th15 x	30th5	MS	+	+	-

Appendix 6.1 continued

Genotype	Cross			*Xgwm570*	*Xgwm544*	*Xgwm437*
F2plot49	Coucal x	Loerrie II	MS	-	-	-
F2plot5	30th10 x	Coucal	MS	+	+	-
F2plot50	Loerrie II x	Coucal	MS	+	-	-
F2plot51	Coucal x	Kwale	MS	-	-	-
F2plot52	Kwale x	Coucal	MR	0	0	0
F2plot53	Coucal x	SB50	MS	-	+	-
F2plot54	SB50 x	Coucal	MS	-	+	-
F2plot57	Coucal x	19th15	MS	-	-	-
F2plot58	19th15 x	Coucal	MS	-	-	-
F2plot6	Coucal x	30th10	MS	-	+	-
F2plot61	Loerrie II x	Kwale	MS	+	-	-
F2plot62	Kwale x	Loerrie II	MS	-	-	-
F2plot63	Loerrie II x	SB50	S	+	-	-
F2plot64	SB50 x	Loerrie II	S	-	-	-
F2plot67	Loerrie II x	19th15	MS	-	-	-
F2plot68	19th15 x	Loerrie II	MS	-	-	-
F2plot7	30th10 x	Loerrie II	MS	-	-	-
F2plot71	Kwale x	SB50	MS	-	+	-
F2plot72	SB50 x	Kwale	MS	-	+	-
F2plot75	Kwale x	19th15	MS	-	-	-
F2plot76	19th15 x	Kwale	MS	-	-	-
F2plot8	Loerrie II x	30th10	MS	0	-	-
F2plot81	SB50 x	19th15	MS	-	-	-
F2plot82	19th15 x	SB50	S	+	-	-
F2plot9	30th10 x	Kwale	MS	-	-	-
Kwale	Parent		S	-	-	-
Loerrie II	Parent		S	-	0	+
SB50	Parent		S	-	-	-

'+' indicate the presence and '–' absence of genes for resistance. '0' indicate that no amplification, R- Resistant, MR- moderately resistant, MS-moderately susceptible, S-susceptible, 19th- HRWSN, 30th- SAWSN (HRWSN- high rainfall wheat screening nursery, SAWSN- semi-arid wheat screening nursery)

Chapter 7

Overview of research findings

7.1 Introduction

This chapter provides a summary of the main findings, their implications and suggestions for future research on breeding for resistance to spot blotch disease in wheat.

The specific objectives of the study were:

1 To determine farmers` preferences for rain-fed wheat cultivars and identify production constraints.
2 To assess genetic diversity in wheat germplasm adapted to summer rain-fed conditions in Zambia.
3 To screen germplasm from Zambia and CIMMYT-Mexico for resistance to spot blotch.
4 To determine gene action controlling the inheritance of resistance to spot blotch disease caused by *Bipolaris sorokiniana*.
5 To validate three simple sequence repeat (SSR) (*Xgwm544*, *Xgwm570* and *Xgwm437*) markers previously reported linked with resistance to spot blotch disease.

7.2 Summary of research findings and implications

7.2.1 Production constraints and farmers' preferences of summer rain-fed wheat

The participatory rural appraisal was conducted in Mpika district of Muchinga Province of Zambia mainly in Mufubushi and Mpika–Main areas to establish wheat production constraints and farmers' preferred traits for rain-fed wheat cultivars. The study established that:

- Coucal (amber colour) was the only cultivar grown since 1980s when it was introduced. This shows that there has been no active breeding of rain-fed wheat cultivars for the past years.
- Bird and termite damage, disease complexes among them spot blotch disease, drought, weeds, lack of good seed source and lack of readily available market

were the most important constraints identified by farmers. These contributed to the abandonment of summer wheat production by most farmers.

- Farmers desired to have a wide range of cultivars to boost summer wheat production. They preferred the cultivars to be high yielding with white coloured grain, resistant to diseases, resistant to bird and termite damage and also drought resistant.

The results provide important information to the wheat research team at Zambia Agricultural Research Institute (ZARI) that to enhance wheat production amongst small-scale farmers, it is essential to develop more rain-fed cultivars while taking into account the important biotic and abiotic stresses and their preferred traits.

7.2.2 Genetic diversity using agro-morphological traits and the association between traits

On evaluation of locally adapted genotypes and introductions from CIMMYT-Mexico, the study revealed:

- The existence of significant amount of variation among genotypes for the agro-morphological traits under study.
- Principal component analysis identified plant height, tillers/m^2, peduncle length, days to heading, days to maturity and grain yield as the main traits that described the variability among the genotypes implying that they were useful traits for classifying genotypes.
- Clustering based on Ward's method and squared Euclidean distance, grouped 150 genotypes into five clusters suggesting that sampling and utilizing genotypes from appropriate contrasting groups could be good for genetic improvement.
- Hectolitre weight, tiller/plant, thousand grain weight (TGW), grains/spike, peduncle length, and tillers/m^2 could be effective selection criteria for high yield as they exhibited positive direct effects on yield and also significant and positive association with yield.

7.2.3 Genetic variability among wheat (*Triticum aestivum* L.) germplasm for resistance to spot blotch disease in Zambia

One hundred and fifty genotypes from Zambia and the International Maize and Wheat Improvement Centre (CIMMYT) Mexico were screened for spot blotch resistance, the findings were as follows:

- The 150 wheat genotypes showed great variation in their reaction to spot blotch disease.

- The genotypes were classified into resistant, moderately resistant, moderately susceptible and susceptible groups.
- Genotypes 19HRWSN6, 19RWSN7 and 19HRWSN15 were among the resistant cultivars across environments.
- Among the locally adapted genotypes, Coucal was moderately susceptible while Kwale and Loerrie II were susceptible. This underlines the need for improving resistance in the locally adapted genotypes and also developing new genotypes with high levels of resistance.
- The identified resistant and moderately resistant genotypes could be used to enhance resistance in the locally adapted genotypes.

7.2.4 Genetic analysis of resistance to spot blotch disease

The genetic analysis for resistance to spot blotch disease was done using an 8 x 8 full diallel Hayman approach and generation mean (GMA) analysis from a cross between 19HRWSN6 a resistant genotype and Loerrie II a susceptible genotype. The results from the two biometrical methods were similar. The following were the findings:

- Additive gene effects were important in controlling resistance to spot blotch disease an indication that spot blotch resistance could be improved through selection.
- Maternal and non-maternal reciprocal effects were not important in inheritance of resistance to the disease implying that the choice of female parent in breeding for resistance to spot blotch is not critical.
- Epistatic gene effects were absent an indication that selection for resistance could be done in early segregating generations.
- Hayman diallel analysis showed that resistance to spot blotch disease exhibited partial dominance. Narrow-sense heritability was moderately high (56.0%).
- The Wr/Vr graph showed that the parents 30SAWSN10 (P1), 30SAWSN5 (P3) and Coucal (P4) displayed the maximum number of dominant genes while parents SB50 (P7), 19HRWSN15 (P8) and 30SAWSN18 (P2) had the highest frequency of recessive genes for resistance.
- Parental genotypes which displayed maximum number of dominant genes can be used in breeding for resistance to spot blotch.
- From GMA resistant parent P2 (19HRWSN6) was identified as a good source of resistance due to the low disease severity observed.

7.2.5 Validation of molecular markers linked with resistance to spot blotch disease caused by *Bipolaris sorokiniana*

Sixty-six wheat genotypes comprising 11 parental genotypes and 55 F2 progenies were screened with the SSR markers *Xgwm570*, *Xgwm544* and *Xgwm437* to confirm their reported association with resistance. The findings were:

- All the markers showed a significant relationship with resistance
- Marker *Xgwm570* amplified a 155 bp fragment in all resistant and moderately resistant parental and F2 progenies which was not present in susceptible genotypes.
- The marker explained 14% of the phenotypic variance and gave a high adjusted R^2 of 11.0% indicating a strong association with resistance.
- Marker *Xgwm544* amplified PCR fragments 196 bp, 198 bp and 200 bp which were only present in all the moderately resistant F2 progenies.
- The adjusted R^2 (10.0%) observed with this maker showed that there was an association with resistance.
- Marker *Xgwm437* amplified 129 and 138 bp fragments in resistant genotypes which was absent in the susceptible ones.
- The adjusted R^2 of 7.0% showed that there was a relationship with resistance to *Bipolaris sorokiniana.*

The study confirmed the association of SSR markers *Xgwm570, Xgwm544* and *Xgwm437* previously reported to be linked with resistance to spot blotch disease. Therefore, the markers can be used in the identification of genotypes resistant to *Bipolaris sorokiniana* in marker assisted selection. This information is important to wheat breeders in Zambia as it would accelerate identification of resistance genotypes early in the developmental stage of the plant without waiting for high disease epiphytotic conditions during rainy season. It would also allow off-season screening of the germplasm.

7.3 Recommendations and way forward

The study established that genetic diversity exists among the wheat genotypes in Zambia which is the building block for any breeding program. Furthermore, the opportunity exists of improving resistance to spot blotch disease in the adapted susceptible and moderately susceptible genotypes by utilizing the identified resistant and moderately resistant genotypes. Use of molecular markers linked with resistance to spot blotch could accelerate the process of identifying resistant genotypes. The choice of a female parent is not important in breeding for resistance to spot blotch disease due to

the absence of maternal effect. Exercising selection for resistance in the early segregating generation should be an effective approach in breeding for resistance due to predominance additive gene effects. Identification of farmers preferred traits implies that incorporating these traits in new improved varieties would facilitate adoption of the varieties.

It is thus recommended that the progenies identified as moderately susceptible and moderately resistant be evaluated further to identify and select superior lines. Government intervention in marketing of wheat among small-scale farmers is important. This would help to encourage summer production which will in turn compliment winter production to help attain self-sufficiency in wheat. Wheat is a crop that is widely consumed in most households in Zambia. It is also recommended for the wheat research team at ZARI to focus on developing more rain-fed wheat genotypes that incorporate farmers' preferred traits established in this study. This would give farmers a wide range of varieties to choose from. Additionally, breeding for resistance to spot blotch disease should be among the top priorities. Small-scale wheat farmers should also be encouraged to form cooperatives, as through cooperatives it will be easy to market their produce.