A Study of Heterotic Grouping, Gene Action and Genotype x Environment interactions of Mid-altitude and Highland Maize Inbred Lines in Rwanda

By

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General abstract

Maize (*Zea mays* L.), is one of the staple crops in Rwanda that contributes to national economic growth. Furthermore, the genetic plasticity of maize permits its adaptation to a wide spectrum of environments ranging from 900 m to over 2400 m above sea level (asl). However, grain yield is compromised by various limiting factors, among these, the lack of appropriate varieties, especially hybrids and scarcity of maize seed of varieties that can withstand various production constraints. Among other factors, productivity can be enhanced by developing a range of hybrids, which are higher yielding than open pollinated varieties. However, to lay a strong foundation for a viable hybrid-breeding programme, knowledge on genetic diversity, genetic effects governing yield and other traits in inbred lines and effective germplasm management requiring heterotic groups and patterns establishment is needed. The objectives of this study were, therefore; i) to determine the genetic distances and clusters among potential lines selected for the mid-altitudes and highlands of Rwanda; ii) to estimate the general and specific combining ability, heterosis and gene action for grain yield; iii) to determine heterotic groups and heterotic patterns among Rwandan newly developed lines and introduced lines based on line x tester mating scheme and diallel analysis, respectively; and iv) to investigate the magnitude of genotype–by-environment (G x E) interaction and stability of new hybrids for grain yield in the target environments.

To determine genetic diversity; 71 maize inbred lines selected for the mid-altitudes and highlands of Rwanda were genotyped with ninety two SNP markers. The unweighted pair group method with arithmetic mean (UPGMA) revealed a random allocation of the inbred lines into two major clusters regardless of their origin. Genetic clustering information acquired from the current study would be suitable information not only for maize hybrid programme establishment in Rwanda, but also for other collaborative tropical maize breeding programmes.

Estimation of the general and specific combining ability, heterosis and gene action for grain yield was done using forty-five single cross hybrids from a 10 x 10 half- diallel mating design. Among these parents, three of them were adopted as testers. The hybrids were evaluated in a 6 x 8 (forty-five crosses plus three checks) alpha-lattice design across twelve environments in Rwanda. General combining ability (GCA) and specific combining ability (SCA) effects were both highly significant ($P<0.001$-0.01) suggesting the presence of both additive and non-additive effects, but with higher magnitude of GCA for grain yield effects when all environments were combined.

The highest heterotic patterns were realized between groups S4 and S6/S7 (S4/S5) and within S4 group (S4/S8) and would be potentially useful for maize hybrid production in Rwanda.

Furthermore, nineteen maize inbred lines were crossed with four testers (20(T1), 21(T2), 22(T3), 23(T4)), following a line x tester mating scheme and generating 76 test crosses. These were evaluated together with two checks in 6 x 13 α-lattice design at four locations in 2015B and 2016A seasons, along with their 23 parental lines in adjacent trials. Generally, most of the lines exhibited positive heterosis with all testers. However, there was more inclination firstly towards tester T2 and then T3. The highest heterosis was displayed by line 8 with T3. Regardless of heterotic grouping method applied, the lines were discriminated in different heterotic groups different from the four heterotic groups of the testers. Two and nine heterotic groups were identified based on standard heterosis and SCA effects, respectively. Genetic distance was correlated to heterosis, SCA effects and test cross performance however, this was specific to some testers.

To investigate the magnitude of G x E interactions and stability of new hybrids for grain yield in the target environments; 126 experimental hybrids were evaluated in four environments representing the major agro-ecologies of Rwanda. One set of 78 hybrids was evaluated over two seasons (8 environments in total), while the other set of 48 hybrids was evaluated over three seasons (12 environments in total). Genotype and genotype by environment interaction (GGE) biplot method was applied for graphical display of the data. Hybrid 26 (ACR29 x 21) and 31(ECA1 x 22) from test crosses and diallel hybrid 3 (R10164 x ET4) and 25 (ET4 x ECA13) were identified as the best performers and then qualified as desirable hybrids. The GGE biplot revealed three mega-environments for test crosses and two mega-environments for diallel hybrids. Environments Rwerere first season (RWA), Rwerere second season (RWB) and Rubona second season (RBB) for test crosses and Rubona first season (RB1), Rubona second season (RB2) and Nyagatare second season (NY2) for the single crosses were the most powerful in discriminating genotypes.

Overall, the acquired information from genetic diversity and heterotic groupings is useful in designing the hybrid maize programme in Rwanda. This will guide the programme towards identifying suitable heterotic patterns as well as combining ability of the inbred lines selected from this study. Furthermore, the study revealed valuable maize inbred lines with desirable combining ability and new single cross hybrids. Consequently, the maize breeding programme will consider development of hybrids, such as single crosses and three way crosses using the inbred lines and F1 hybrids identified.

Declaration

I, **Alphonse Nyombayire**, declare that:

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

1. The research reported in this thesis, except where otherwise indicated, is my original research;
2. This thesis has not been submitted for any degree or examination at any other university;
3. This thesis does not contain other authors' data, graphs, pictures or other information unless specifically acknowledged as being sourced from other persons;
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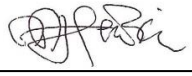
Signed 

Alphonse Nyombayire (Candidate)

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



Prof. John Derera (Supervisor)

Dr. Julia Sibiya (Co-supervisor)

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I am very grateful to many others who are not cited here but who have contributed a lot to this study. Last but not least, I am thankful to my family for their patience, prayers and encouragement during this study.

Dedication

I dedicate this thesis to my beloved wife, Goreth Umutesi, my daughter, Ketsia A. Gutabarwa and my sons Blessed A. Iratuzi, Heritier N. Shima and Victorieux H. Izerwe, My parents, brothers and sister.

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List of Acronyms and Abbreviations

ACCI:	African Centre for Crop Improvement
AD =	Anthesis Date
AEC:	Average Environment Coordination
AFLPS:	Amplified Fragment Length polymorphisms
AGRA:	Alliance for a Green Revolution in Africa
AMMI:	Additive Main Effects and Multiplicative Interaction
ANOVA:	Analysis of Variance
ASI:	Anthesis-Silking Interval
CIMMYT:	Centro Internacional del Mejoramiento de Maiz y Trigo
DNA:	Deoxyribonucleic acid
EA:	Ear Aspect
EDPRSII:	Second Economic Development and Poverty Reduction Strategy
EH:	ear height
EPP:	Number of Ears per Plant
ET:	Ear Texture
F1:	First generation
FW:	Field weight
GCA:	General Combining Ability
GD:	Genetic distance
GDP:	Gross Domestic Product

GEIs:	Genotypes by Environments Interactions
GGE:	Genotype and Genotype by Environment
GY:	Grain Yield
IPCA:	Interaction Principal Component Axes
HC:	Husk Cover
HPH:	High-parent Heterosis
Lat:	Latitude
Long:	Longitude
m asl:	metres above sea level
MC:	Moisture content
MPH:	Mid-parent Heterosis
MSV:	Maize Streak Virus
MT:	Mean of the testers
NCD:	North Carolina Design
NISR:	National Institute of Statistics of Rwanda
OPVs:	Open Pollinated varieties
PA:	Plant Aspect
PCA:	Principal Component Analysis
PIC:	Polymorphic Information Content
PN:	Number of plants at harvest
PH:	Plant height
PS:	Plant stand

RAB:	Rwanda Agriculture Board
RL:	Root Lodging
SCA:	Specific Combining Ability
SC:	Single Cross
SAS :	Statistical Analysis Software
SD:	Days to Silking
SH:	Standard Heterosis
SL:	Stalk or Stem Lodging
SNPs:	Single Nucleotide Polymorphisms
SSA:	Sub-Saharan Africa
SSRs:	Simple Sequence Repeats
T:	Tester
t:	Tonne
TLB:	Turcicum Leaf Blight
UPGMA:	Unweighted Pair Group Method with Arithmetic mean
USD:	United States Dollar
QTL:	Quantitative Trait Loci
%:	Percentage

Introduction

1. Importance of Maize

Worldwide, maize (*Zea mays* L.) is a major cereal crop in terms of production (FAO, 2012) with about 700 million tonnes produced annually. An annual average of 833.9 million tonnes of maize grain was produced in 2008-2010, making it the biggest crop grown worldwide (FAO, 2012; FAO, 2014). Of this, about 62.5 million tonnes were produced in sub-Saharan Africa, where eastern Africa alone produced 11.6 million tonnes. Globally, millions of people rely on maize as a staple food through economic necessity. In Africa, maize contributes at least one fifth of the total daily calories and accounts for 17 to 60% of the total daily protein supply of individuals in 12 countries as estimated by FAO food balance sheets (Krivanek et al., 2007; FAO, 2012; FAO, 2014).

In addition to its high demand as food in Africa, maize is also fast becoming a very important agricultural export crop within the region (Asea, 2005; Jayne et al., 2006; FARA, 2009; Smale et al., 2013). Maize exports estimated at 2.25 million tonnes have been reported between 2005 and 2007 (FARA, 2009). An even higher demand is projected with the region's rising population growth and expanding need for livestock feed. In developing countries, the demand for maize is expected to surpass the demand for both wheat and rice by the year 2020 (Pingali and Pandey, 2001; FARA, 2009). From 1995 to 2020, global and sub-Saharan Africa consumption is projected to increase by 50% and 93%, respectively (Pingali and Pandey, 2001). Therefore, it is crucial to overcome constraints threatening maize production as a critical crop to food security.

In Rwanda, maize is an important staple crop whose genetic plasticity has permitted its adaptation to a wide spectrum of environments in the country. It is grown in environments ranging from 900 m to over 2400 m above sea level (asl). The maturity cycle of maize varies with altitude and the variety type. Maize in Rwanda is consumed in various forms which include roasted or boiled green ears, boiled dry grain or mixed with legumes such as beans, or as *ugali* and *uji* (porridge) prepared from dry maize flour, or brewed into local beer. It is also increasingly becoming a major component of livestock and poultry feed in the country (Sallah et al., 2007)

Since 2006, in alignment with the broader millennium development goal aiming to eradicate extreme poverty and hunger, a crop intensification programme was initiated by the government of Rwanda, and maize was among the priority crops targeted. Consequently,

maize has become an important crop in production and ranks first among pulse and grain crops in Rwanda. From the 2005 cropping season, maize has experienced an unprecedented development more than any other crop (Figure 1). In 2011, about 525,679 tonnes of maize grain were produced on 223,414 ha (National Institute of Statistics of Rwanda (NISR), 2012). Recently, it was also reported that Rwandan agriculture contributes more than 30% of the GDP and employing over 70% of the population and thus a significant contributor to poverty reduction. Hence, in recognition to its potential in economic development, food security and poverty reduction, Rwanda has set a very ambitious agriculture agenda aiming at an annual average growth of 8.5% over the course of EDPRS II[1] (2013-2018) (NIS, 2015). The reasons for increased maize production are mainly due to: i) expansion in maize crop acreage from the highlands to other agro-ecologies of the country especially in semi-moist mid-altitudes and clearing of some new marshlands, ii) changes in maize cropping systems; that is from intercropping to mono-cropping and importation of improved seed, iii) availability of regional and internal markets, and iv) increased number of milling processors and industrial uses of maize.

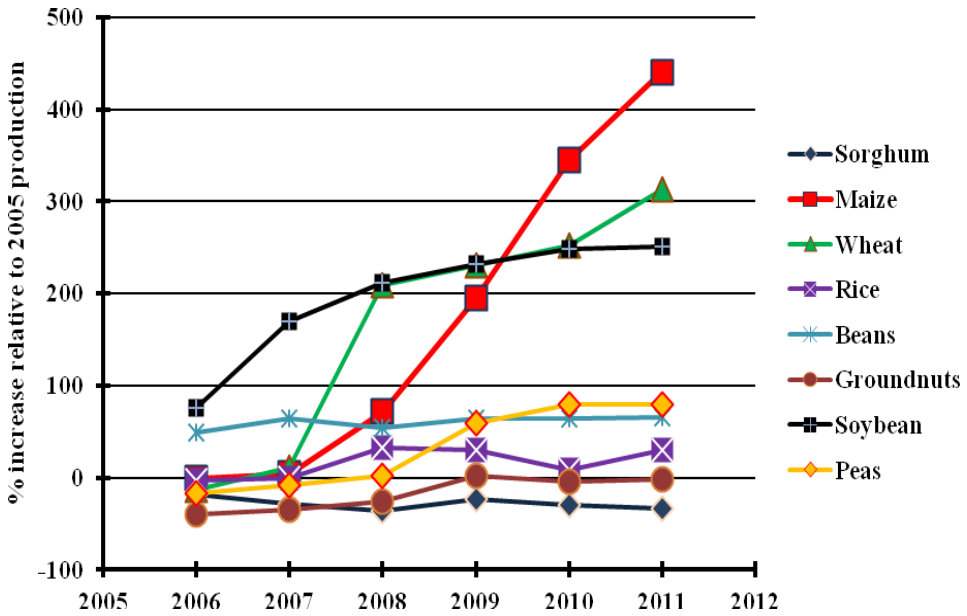

Figure 1.1: Increase (%) in crop production in Rwanda from 2006 relative to 2005. Source: NISR, 2012

[1] Second Economic Development and Poverty Reduction Strategy (EDPRS II) for 2013-2018. EDPRS II aims to implement Rwanda's Vision 2020, ensuring that the country achieves middle-income status by 2020 by accelerating economic growth to (11.5% average), reducing poverty to below 30%, and restructuring the economy towards services and industry.

2. Maize production constraints

Generally in sub-Saharan Africa and particularly in Rwanda, maize yields are much lower than in the temperate regions. Maize yields in Africa (1.7 tonnes/ha) account for 36% of global maize yields (4.9 tonnes/ha), even in regions with considerable rainfall and where farmers can invest in yield-increasing inputs (FARA, 2009). This is because of various production constraints which are primarily related to abiotic, biotic and socio-economic factors (Bänzinger et al., 2000; Ngaboyisonga et al., 2016).

Of the abiotic factors, maize production is mainly constrained by drought and low soil fertility (Bänzinger et al., 2000). Particularly in Rwanda, maize is produced under unpredictable climatic conditions on soils with low fertility, thus affecting maize production considerably. In most of the mid-altitude environments of Rwanda (67.5% of the national cultivated land area), in addition to growing maize under depleted soil nutrients, erratic and low rainfall amounts also significantly affect maize production (Ministry of Agriculture and Animal Resources (MINAGRI, 2009; Sallah et al., 2009; Kagabo et al., 2013;). This contributes greatly to the low yields observed in farmers' fields.

Additionally, maize productivity in SSA, including Rwanda, is affected by various biotic factors, with the most limiting factor being foliar diseases (Vivek et al., 2012; Sibiya et al., 2013). The conditions in Rwanda are favourable for the spread of these diseases; especially on maize grown in a monoculture system. The most economically important diseases in Rwanda are turcicum leaf blight (TLB), grey leaf spot (GLS) and maize streak virus (MSV), and most recently maize lethal necrosis disease (Adams et al., 2014). These diseases pose a serious threat to maize production in Rwanda (Sallah et al., 2007; REMA, 2011; Adams et al., 2014) as they have the potential to seriously affect grain yield especially when susceptible cultivars are grown.

Furthermore, maize production in Rwanda is also constrained by different socio-economic factors. These are mainly dominated by poor capital and poor infrastructure, lack of access to markets and the poor delivery of bulky inputs such as quality seed and fertilizer; and inappropriate production systems. This is also worsened because maize production in Rwanda is done by small scale farmers (MINAGRI, 2009; REMA, 2011; NISR, 2016) who have limited resources to purchase the required inputs.

Though efforts have been made to address these constraints in many different African countries including Rwanda, maize yields are still low. On average, yields on smallholder farmers' fields range from 0.8 t/ha to 1.1 t/ha against a potential of 12 t/ha from commercial

farmers. Most efforts focus on increased production levels based on existing land area. However, maize productivity based on cropping systems that use improved cultivars which can withstand various environmental stresses could considerably increase maize yields for small scale farmers (Hassan et al., 2001; Sallah et al., 2009). Therefore, developing maize cultivars that either escape or tolerate different harsh environmental conditions is one of the strategies for increasing maize yield.

Currently in Rwanda, with the policy of crop intensification, farmers often experience shortages of improved maize seed. The national maize research programme has evaluated and released some open pollinated maize varieties to farmers based on different attributes. However, these varieties are no longer performing well under the local biotic and abiotic stresses. To overcome this seed shortage problem, around 1,200 t of maize hybrid seed is annually being imported by the government from different countries in the region (Clement Urinzwenimana[2], *personal communication*, 2013). Nonetheless, this seed is still not enough to meet the farmers' needs, it is costly to the country and the practice is not sustainable. Therefore there is a need to look for sustainable alternatives. One of the strategies would be to develop maize hybrids locally, based on promising parental sources. However, the mode of gene action and heterotic groups prevailing in these parents as well as the type of adaptability in the resulting crosses needs to be identified

Information on genetic make-up and variability in the current maize germplasm in Rwanda as well as the interaction of this germplasm with local environmental conditions could be a key factor to be explored for yield improvement especially through development of high performing hybrids. It was revealed in some studies that genetic divergence of parents for a given cross could be very important in hybrid vigour expression of the cross (Hallauer et al., 2010; Semagn et al., 2012). However, the range of genetic distance could affect hybrid vigour or heterosis. Thus, genetic distances could be documented in order to define different heterotic groups among the Rwandan germplasm so that different heterotic groups existing in this germplasm could be exploited for high heterosis and selection of the best parental combinations for different traits. This could be complemented by genotype x environment interactions analysis in order to select hybrids with well-defined mode of adaptability**.**

[2] Rwanda Agriculture Board, Kigali-Rwanda

3. Problem statement

Scarcity of maize seed of varieties that can withstand various production constraints in major maize agro-ecologies of Rwanda is a major constraint to most small scale farmers and to the government. To fill this gap, the government of Rwanda has been spending around 6 billion USD (Patrick Karangwa[3], *personal communication*, 2016) every year since 2007 to import improved maize seed from different countries. However, these seeds are not sufficient and the imported varieties are also not well adapted to Rwandan agro-ecologies. This strategy is thus not reliable, hence the need for a sustainable solution to the problem.

There are some local open pollinated maize varieties (OPVs) that the farmers prefer previously selected and released in Rwanda based on their adaptability and other attributes that can be used in maize improvement. Suitable inbred lines can be extracted from these OPVs and used in hybrid production and investigations of heterosis and heterotic groups, combining ability as well as the interaction with environments of these genotypes for improved yield, disease resistance and other traits. However, to be beneficial, this depends on the parental lines involved in crosses. Therefore, investigating genetic divergence among the new locally developed maize inbred lines associated with field evaluations and finding out different heterotic groups as well as genotypes x environment interactions and mode of gene action could be a key factor for designing a sustainable maize hybrid programme in Rwanda.

4. Research objectives

Research aim

The overall aim of this study is to initiate a sustainable hybrid maize programme in Rwanda by developing heterotic groups and identifying heterotic patterns of the germplasm that is available. This could form the basis of the hybrid breeding strategy for Rwanda which would contribute to increased yield in both highland and mid-altitude environments in the country. Ultimately, this would impact positively on food security and income generation of small scale farmers in Rwanda. To achieve this, the following objectives were set out in the study:

[3] Rwanda Agriculture Board, Kigali-Rwanda

Specific objectives

The specific objectives of this study were:

1. To determine the genetic distances and clusters among potential maize inbred lines selected for the mid-altitudes and highlands of Rwanda;
2. To estimate the general and specific combining ability, heterosis and gene action for grain yield;
3. To determine heterotic groups and heterotic patterns among Rwandan newly developed inbred lines, and introduced lines, based on line x tester mating scheme and diallel analysis, respectively;
4. To investigate the magnitude of genotype x environment (G x E) interactions and stability of new hybrids for grain yield in the target environments.

5. Research hypotheses

The following hypotheses were tested under the current study:

1. There is useful genetic diversity among potentially selected maize inbred lines which could be sufficiently revealed through DNA fingerprinting;
2. The selected newly developed inbred lines could exhibit high combining ability which could be exploited in maize hybrid production;
3. Different heterotic groups and patterns could exist among the local and introduced maize inbred lines;
4. The hybrids resulting from the selected inbred lines could be stable across the target environments.

6. Thesis outline

The specific objectives and hypotheses of the thesis were tested and results reported in different chapters. The thesis comprises of six chapters in accordance with the number of activities associated with the specific objectives. Chapters 2-5 are written in form of discrete research papers, where each one follows the format of a stand-alone potentially publishable manuscript and as such overlapping of content and reference may be inevitable. The referencing system applied in different chapters is based on the journals of the American

Society for Agronomy, the Soil Science Society of America, and the Crop Science Society of America. Chapter 2 was published in Maydica Journal (vol. 61.2- M 17).

The chapters are outlined as follows:

1. Introduction to thesis.
2. Chapter 1: Literature review.
3. Chapter 2: Genetic diversity among maize inbred lines selected for the mid-altitudes and highlands of Rwanda.
4. Chapter 3: Combining ability and heterotic groups for grain yield and other agronomic traits among maize inbred lines selected for the mid-altitudes and highlands zones of Rwanda
5. Chapter 4: Heterotic groups, gene action and heterosis among maize inbred lines selected for the major agro-ecologies of Rwanda
6. Chapter 5: Genotype x Environment interaction and stability analysis of diallel and test cross maize hybrids across tropical medium and highland ecologies.
7. Chapter 6: General overview.

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1 Chapter One
Literature Review

1.1 Introduction

This chapter reviews relevant literature on major topics supporting the thesis research work and provides a theoretical basis for the study. This review starts by providing brief discussion on maize genetic diversity followed by a section describing tools applied for genetic diversity study. It presents essential key aspects related to the maize hybrid programme establishment, such as heterosis, determination of heterotic groups and patterns, combining ability, genotype stability and genotype x environment interaction (G x E). The last section highlights major mating designs applied in maize breeding.

1.2 Genetic diversity

Selecting suitable progenitors to use for generating crosses is a vital decision for plant breeders that could enable maximum exploitation of genetic variability and produce high performing recombinant genotypes. Therefore, diverse populations providing high performance, wide adaptability, and yield stability, have to be taken into account when selecting parental genotypes (Semagn et al., 2012; Mengesha, 2013; Wende et al., 2013). Additionally, genetic distance among individuals is a key factor to consider when predicting the genetic variability among parental combinations (Bertan et al., 2007; Semagn et al., 2012; Wende et al., 2013; Chanda et al., 2016). High yielding as well as genetically distant genotypes might represent inbred lines with different loci controlling the character and probably with high combining ability. Therefore, information on germplasm diversity and relationships existing among breeding materials is a key to crop improvement. Similarly, evaluation of genetic diversity, relationships, and structure in a given set of germplasm is valuable for selecting parental combinations aiming at developing progenies with high genetic variability (Semagn et al., 2012; Chanda et al., 2016).

Generally, phenotypic variation is positively associated with genetic diversity. Phenotypic variation depends on genotypes, environments and the interactions of the two factors. On the other hand, genetic diversity could be explained from various sources (Moose and Mumm, 2008); including breeding populations (which may occur either naturally or synthetically), segregating populations resulting from crosses of selected genotypes as parents, introductions not adapted to the specific environments, broad interspecific mating,

mutations, introduction of transgenic events, and the combinations of some of these sources. Nevertheless, not all of these sources are present at the same time for different breeding programmes to benefit from the genetic diversity.

In different research programmes, the use of exotic germplasm has been extremely successful for improving many beneficial traits in different crops, though difficulties might be encountered by introducing undesirable alleles. Hence the exploitation of genetic diversity must be balanced by elite performance, because choosing the best parents is an important key to maximize the probability for successful improvement (Moose and Mumm, 2008; Pan et al., 2012). Under this study, genetic diversity among maize inbred lines selected for the major agro-ecologies of Rwanda was explored.

1.3 Tools for studying genetic diversity

Currently, molecular tools have contributed to improved knowledge and the ability to explore genetic diversity in the germplasm pools from various crops. Especially in maize, this knowledge has permitted the investigation of plant evolution and genome exploration, thus contributing to the understanding of population structure, empirical measures of genetic responses to selection, and also in identifying and maintaining the reservoirs of genetic variability for future use (Slade et al., 2005; Pan et al., 2012). Knowledge of genetic relatedness among germplasm sources may guide the choice of source parents for production of hybrids or improved populations (Collard and Mackill, 2008; Hallauer et al., 2010; Pan et al., 2012; Wende et al., 2013; Chanda et al., 2016). Using single nucleotide polymorphism (SNPs) markers, Semagn et al. (2012) investigated the extent of genetic differentiation, population structure, and patterns of relationship among a diverse set of 450 CIMMYT maize inbreds and revealed the existence of three major groups. They reported high genetic distance and low kinship coefficients among most pairs of lines, implying the uniqueness of the majority of the inbred lines in breeding programmes for selecting promising parents in hybrid production.

Considering the weakness associated with morphological/phenotypic markers, that is influence from the environment, DNA markers have been shown to be useful tools for obtaining genetic information present in plant genomes and can be exploited in the estimation of genetic distances within and between plant species. Genetic distance can be assessed by using various types of molecular markers, comprising amplified fragment length polymorphisms (AFLPs), simple sequence repeats (SSRs), restriction fragment length polymorphisms (RFLPs), and single nucleotide polymorphisms (SNPs). However, current

advances in molecular technology have moved towards SNP markers (Bertan et al., 2007; Jones et al., 2007; Semagn et al., 2012; Chanda et al., 2016). This is because of their low cost, locus-specificity, codominance, high genomic abundance, potential for high throughput analysis, and lower genotyping error rates (Jones et al., 2007). The SNP markers have therefore become a powerful tool in different genetic applications: which include genetic diversity, linkage and quantitative trait loci mapping, and in marker assisted breeding (Hallauer et al., 2010; Semagn et al., 2012). Based on the above, SNP markers were therefore selected and applied in this study to determine the amount of genetic diversity among maize inbred lines selected for the mid-altitudes and highlands of Rwanda.

1.4 Heterosis in maize

Heterosis is hybrid vigour realized in performance of individual hybrids compared to their originating parents and is manifested in the progenies of parental lines with a high specific combining ability. Coined by Shull (1908), high heterosis was reported (Hallauer et al., 2010; Nepir et al., 2015) to result from unrelated parents than closely related ones. Frequently, crosses from maize parent lines of related origin consistently produce poor yield than crosses having one or no parent in common. However, this might not always be the case, hence the need for extensive evaluation trials to determine the best combinations among the parents (Hallauer et al., 2010; Fato et al., 2012).

In a maize hybrid development programme, identification of parental lines that result in superior crosses with high heterosis is the most time-consuming and costly operation. It is generally recommended that genotypes designated for crosses are well performing, adapted and stable especially for yield. Once these are fulfilled, it is possible to select for transgressive genotypes resulting from the occurrence of heterosis and the action of complementary dominant genes (Bertan et al., 2007). However, Hallauer and Miranda (1988) have reported that *per se* performance of given maize inbred lines does not predict the performance of the resulting maize hybrids for grain yield. On the other hand, Betrán et al. (2003) pointed out that the degree of heterosis depended on the performance of the parental lines and the resulting hybrids. They also added that environmental conditions could differentially affect the performance of the hybrids and the parents, thus altering the relationship between genetic distance and heterosis.

Nevertheless, heterosis has been widely exploited for maize hybrid development (Bidhendi et al., 2012; Fato et al., 2012; Abdel-Moneam et al., 2014), but its genetic basis is still unclear (Coors and Pandey, 1999). Though more research has been done in the past

several years, it is still debatable that heterosis has genetic basis or not (Hallauer et al., 2010). Many theories explained its causes based on the relationship between the level of dominance and the expression of heterosis without success. However, most of the present data agree with the dominance theory as the genetic basis of heterosis, which is due to the accumulation of favourable alleles showing incomplete to complete dominance. Most of these proposed and discussed theories can be fitted in allelic interaction or over dominance and the dominant favourable growth factors (Hallauer et al., 2010).

Regardless of these controversies, heterosis has highly boosted maize yield improvement worldwide (Hallauer et al., 2010; Iqbal et al., 2010). As long as the heterosis in hybrid production can be associated with genetic diversity of the parents, it can be the best option to exploit in order to increase yield without relying on more land under production as is the case in many developing countries, including Rwanda. However, to be profitable for farmers, heterosis should have significant benefits over other existing varieties for the traits under consideration.

1.5 Relationship between genetic diversity and heterosis

Investigation of genetic distance among maize population components and the relationship existing between genetic distances with heterosis could guide towards a suitable breeding strategy and predicting of hybrid performance. Genetically differing germplasm is a key factor for heterosis to occur and the best crosses have been reported to result from genetically unrelated maize parents (Hallauer and Miranda 1988; Reif et al., 2005; Semagn et al., 2012). Therefore, it might be necessary for genetic diversity studies among the populations to be conducted in hybrid programme as the level of genetic divergence could hinder the realization of heterosis. Thus, it is important to know which parents could manifest high heterosis in hybrid combinations (Hallauer et al., 2010).

In the US Corn Belt, it was reported by Hallauer et al. (2010) that inbred lines of Reid origin when crossed with the ones originating from Lancaster exhibited high yield on average. A similar scenario was also realized in Europe and worldwide on crosses resulting from dent and flint maize germplasm. In all the cases, genetic diversity of the lines involved in the crosses was the most important factor to explain these variations in maize yield performance. However, Caixeta et al. (2013), in their study on relationship between heterosis and genetic divergence for phosphorus use efficiency and its components in

tropical maize, reported lack of relationship between genetic divergence and heterosis for this trait and its components in tropical maize.

In the study on association of parental genetic distance with heterosis and specific combining ability in quality protein maize, Wegary et al. (2013) found high significant positive correlations (though the values were not very high) between SSR marker-based genetic distance and hybrid performance for grain yield, signifying the possibility of molecular markers to predict the hybrid performance. However, they added that the correlations of SSR marker distance with heterosis were too low to be considered as predictive value except for the case of plant height. Similarly, Betran et al. (2003) reported a high and significant correlation ($r = 0.80$) between genetic distance and specific combining ability effects in tropical maize inbred lines grown under stress and non-stress environments. They suggested that the performance of hybrids and heterosis can better be predicted when genetic distance is smaller than a certain threshold, depending on the type of germplasm under study.

On the other hand, Dhliwayo et al. (2009) in their study on combining ability, genetic distances, and heterosis among elite International Maize and Wheat Improvement Center (CIMMYT) and International Institute of Tropical Agriculture (IITA) tropical maize inbred lines found no significant association of genetic distances with hybrid performance, specific combining ability effects, or mid-parent heterosis for grain yield. However genetic distances effectively grouped the inbred lines according to known pedigree relationships and approximately according to heterotic patterns used by CIMMYT and IITA. Similar trends were also revealed by Legesse et al. (2008), in their findings on relationship between hybrid performance and AFLP based genetic distance in highland maize inbred lines, where they found low correlation of AFLP measured genetic distance with hybrid performance. Later, Wegary et al. (2012) also mentioned that the degree of heterosis depends on the relative performance of parental lines and the corresponding hybrids. They also pointed out that the environment could have different effects on the performance of hybrids, and consequently varying the relationship between genetic distance and heterosis.

Different reasons related to low correlation which could exist between genetic distance with heterosis and the SCA have been given (Melchinger et al., 1990). This could result from the lack of linkage between genes controlling the traits to be measured, diversified effect of dominance, unequal genome coverage and random marker distribution (Melchinger et al., 1990).

1.6 Heterotic groups and heterotic patterns in maize

Initiating a maize hybrid breeding programme requires well documented germplasm that can be used. This therefore, requires the existence of parents to be involved in the crosses. However, parental development depends on good identification and utilization of heterotic groups and patterns (Melani and Carena, 2005; Hallauer et al., 2010; Wegary et al., 2013). In maize breeding programmes, the important factor for hybrid programme success is an appropriate choice of germplasm for inbred line development. Generally, broad populations, either from locally adapted or introductions have been used for breeding purposes. However, identification of promising heterotic patterns has also been reported to result from diversified maize gene pool (Melani and Carena, 2005; Hallauer et al., 2010; Semagn et al., 2012; Chanda et al., 2016). Considering the importance of genetic diversity in determining heterotic groups and heterotic patterns, open pollinated populations are valuable for the development of inbred lines given their diversity in the germplasm pool. This is because they have diversified backgrounds, origin, and level of heterozygosity within and among them as their basis for diversity.

Melchinger and Gumber (1998) defined a heterotic group as “a group of related or unrelated genotypes from the same or different populations, which display similar combining ability and heterotic response when crossed with genotypes from other genetically distinct germplasm groups”. By means of comparison, the same authors also defined the concept of heterotic pattern as referring to “a specific pair of two heterotic groups, exhibiting high heterosis and consequently high hybrid performance in their cross.” The latter concept requires partitioning the existing germplasm in a given hybrid breeding programme in at least two differing populations, and these are then improved with inter-population selection methods. It has a considerable impact in maize improvement as it predetermines to a large extent which type of germplasm to be used in a hybrid breeding programme over a long term period (Melchinger and Gumber, 1998; Reif et al., 2005).

This concept originates from breeding and selection principles outlined by Comstock et al. (1949) and designated as reciprocal recurrent selection where two populations with a given heterotic pattern, generally broad based, are improved by using progenies produced from within the same heterotic population. These are then crossed with a specific tester from differing heterotic population and then test crosses are assessed for their performance (Bernardo, 2001). Heterotic groups and patterns play an important role in breeding programmes for selecting parents of crosses for inbred line development as well as testers

for assessing combining ability of the new developed lines. They simplify and guide germplasm management for efficient utilisation (Reif et al., 2005; Nepir et al., 2015).

Based on a simulation study, Cress (1967) proposed that all available germplasm material designated for a long-term breeding programme of inter-population selection should be combined into one synthetic population and then create two populations to be used in heterotic grouping. However, his findings were reported based on a simple genetic model assuming; (i) a low number of QTLs, (ii) no linkage between the QTLs, (iii) two alleles per QTL, and (iv) absence of epistasis. Later, Melchinger and Gumber (1998) also suggested other criteria in order to find new heterotic patterns: (i) high mean performance and large genetic variance in the hybrid population; (ii) high *per se* performance and good adaptation of the parent populations to the target environment; and (iii) low inbreeding depression, when crosses are resulting from inbred lines. On the other hand, Reif et al. (2005) pointed out that the choice of heterotic patterns is primarily determined by the performance of the resulting crosses and added that field evaluation data considerably agree that crosses' performance increases following the divergence of the parent populations. Based on these different strategies in determining good heterotic patterns, Reif et al. (2005) mentioned that the wise decision on the best strategy should consider some factors such as: (i) the genetic basis of heterosis, (ii) the applied selection intensities for QTL, or (iii) the importance of favourable linkages.

Though genetic diversity has preponderant implication to determine heterotic groups for developing new inbred lines as potential seed stocks in hybrid production (Hallauer et al., 2010), it was reported that the only important component for breeding programmes is the recognition and utilisation of heterotic patterns (Sprague, 1984). However, considering the complexity existing in some traits and the lack of consistency between phenotypic and genotypic data, it has been suggested that in identifying promising heterotic patterns, extensive field evaluation data on the performance of crosses generated among heterotic groups should have more considerations (Melchinger, 1999; Barata and Carena, 2006).

1.7 Tools for establishment of heterotic groups and patterns

As maize parental lines development has been limited to their integration into particular heterotic groups, different tools have been tried to investigate good heterotic pattern for development and use of maize hybrids in efficient ways. This is because establishment of the best inbred combinations among heterotic groups is very crucial for the success of maize hybrids development (Barata and Carena, 2006; Fan et al., 2009; Chanda et al., 2016).

Generally, there are two major heterotic group-classification methods which have been used worldwide (Kauffman et al., 1982; Wu et al., 2007; Fan et al., 2009). The first method, also known as traditional, is based on field evaluation data. It uses specific combining ability (SCA) with some line-pedigree information and/or field hybrid-yield information in order to assign inbred lines into different heterotic groups (Kauffman et al., 1982; Wu et al., 2007; Fan et al., 2009; Fato et al., 2012). The second method assigns inbred lines into heterotic groups based on molecular tools, it uses molecular markers and then determines genetic distance (GD) or genetic similarity (GS) to assign maize lines to different heterotic groups (Barata and Carena, 2006; Wegary et al., 2013; Chanda et al., 2016). Though both methods are widely used in assigning maize inbred lines into different heterotic groups for heterosis exploitation; they have been criticized for providing different heterotic groupings (Menkir et al., 2004; Wu et al., 2007). Therefore, it was suggested (Menkir et al., 2004; Barata and Carena, 2006) that the heterotic grouping using molecular marker might only serve as a preliminary tool for designing and performing combining ability studies in the field evaluation in order to create clearly defined heterotic groups with a greater genetic similarity within groups.

However, heterotic grouping based on SCA has also some weaknesses. It has been reported (Fan et al., 2001; Wu et al., 2007) that SCA effects are considerably influenced by the interactions between the two inbred lines and by the interaction between hybrids and environments, and this could assign the same line in different heterotic groups. Hence, Fan et al. (2008 and 2009) proposed a third method: a heterotic group's specific and general combining ability (HSGCA), which includes both GCA and SCA effects. It is combining ability between a representative tester from a known heterotic group and another maize inbred line. Under the current study, combined methods comprising mid-parent heterosis, specific combining ability and molecular markers were applied for grouping different parental lines**.**

1.8 Combining ability

In any hybrid development programme, the main objective is to identify a new line that when combined with other lines, produces high performing hybrids. Consequently, recognition of the best combination of two (or more) parental genotypes to maximize variance within related breeding populations, and recognizing superior transgressive segregants in the segregating populations, are the most critical challenges to plant breeders. If resources were not limited, the best way would be to test immediately each new inbred line in combination with every other inbred with which it could be a parent in a hybrid cultivar. However,

considering a large number of progenies that would have to be tested, this is not feasible. Therefore, the breeder must identify a limited number of inbred lines having sufficient genetic potential prior to their evaluation in specific hybrid combinations (Felir, 1987; Nyombayire et al., 2011; Fasahat et al., 2016). Hence combining ability or productivity in crosses was introduced by Sprague and Tatum (1942).

Combining ability was defined as the potential of parents to produce superior progenies following hybridization. Later, Shattuck et al. (1993) defined it as the magnitude of additive and non-additive gene action. The concept of combining ability has become increasingly used in plant and animal breeding and is especially useful in connection with testing procedures, where it helps to study and compare the performance of a given parent in hybrid combination (Griffing, 1956). The performance can seldom be predicted only based on parental phenotype and hence it is measured by progeny testing. At the beginning, combining ability was a general concept used collectively for classifying an inbred line respective to its cross performance (Fasahat et al., 2016).

Two concepts of general combining ability (GCA) and specific combining ability (SCA) have been revealed and had important influence on inbred line evaluation and population development in crop breeding (Sprague and Tatum, 1942; Musila et al., 2010; Nyombayire et al., 2011; Wegary et al., 2014). General combining ability is used to designate the average performance of a line in hybrid combination, and specific combining ability is used to designate situations where certain crosses excel relatively better or do worse than expected based on the average performance of the lines involved. Parents exhibiting a high average combining ability in crosses are considered to have good GCA, while if their potential to combine well is restricted to a particular cross, they are considered to have good SCA. A parent with a GCA estimate of zero has an average combining ability and depending on the index used, parents with positive or negative GCA values perform above or below average. The SCA, on the other hand, expresses the performance of the progeny from a cross between two parents based on the average performance of the parents involved. The SCA estimates are either positive or negative. GCA is attributed to additive gene action; it is owing to the activity of genes which are largely additive in their effects as well as additive × additive interactions, while SCA is attributed to non-additive effects; that is regarded as an indication of loci with dominance variance (non-additive effects) and all the three types of epistatic interaction components if epistasis were present. They include additive × dominance and dominance × dominance interactions (Sprague and Tatum, 1942; Shattuck et al., 1993; Qu et al., 2012; Fasahat et al., 2016).

Furthermore, GCA and SCA can interact with the environment and cause changes in expected parental combining abilities over the environments (Pswarayi and Vivek, 2008; Nyombayire et al., 2011; Sibiya et al., 2012). Betran et al. (2003) evaluated seventeen maize inbred lines crossed in a diallel design under stress and non-stress environments and reported significant GCA and GCA x environment interaction effects for grain yield. Therefore, to obtain precise combining ability estimates, it may be necessary to evaluate parents in more than one environment.

The combining ability of lines for main characteristics is estimated by examining a set of designed progeny in well-designed trials accompanied by appropriate statistical analysis. Furthermore, parental selection for combining ability is conducted through growing and evaluating the progenies (Ai-zhi et al., 2012). General and specific combining ability effects and their implications in breeding are estimated using various mating designs that will be discussed in later sections. Combining ability has been applied in many crops ranging from cereals, roots to legumes, indicating that it is a crucial tool in plant breeding (Wegary et al., 2014; Fasahat et al., 2016). Similarly, in the current study, combining ability was estimated in the two sets of maize inbred lines being investigated.

1.9 Genotype x environment interaction and maize hybrid performance

Realisation of superior hybrid performance under different environmental conditions is the ultimate objective for most breeding programmes. Although traits of interest might vary among crop species and researchers over time, the ultimate goal remains the same (Hallauer, 2007). Generally, maize is produced under diverse environmental factors where interaction of the hybrid with the environment is more expressed, thus affecting the hybrid from showing its potential for grain yield and yield components (Bänzinger et al., 2000; Dhliwayo et al., 2009; Dehghanpour and Ehdaie, 2013).

These environmental factors are more pronounced on quantitative traits, especially yield compared to qualitative traits (Bernardo, 2002). Therefore, it has been reported that newly developed maize hybrids need to be evaluated in many locations and for several seasons before being recommended for release (Bernardo, 2002; Tonk et al., 2011; Dehghanpour and Ehdaie, 2013). This evaluation of genotypic performances across a number of environments offers useful information on adaptation and stability (Crossa, 1990; Meseka et al., 2008; Anley et al., 2013; Ngaboyisonga et al., 2016). Multi environment trials (METs) have been carried out to identify superior genotypes which can be recommended to farmers. Increasing the number of testing locations is a key factor for improving this effectiveness

(Sibiya et al., 2012). This is because in these METs, the genotypic contribution to total phenotypic variance is normally reduced, indicating that genotypic performance is not consistent across such environments. This is also sometimes accompanied by high error variances and sizeable G x E effects also limiting the effectiveness of selection. Presence of genotype by environment interaction (GEI) is a limiting factor in selecting varieties for wide adaptability. This is because significant GEI implies that genotypes selected from one environment may perform poorly in other environments, suggesting that breeding programmes should also focus on specific adaptation. Furthermore, cross-over interaction which results in changes in ranking order of genotypes constitutes another major breeding complication (Yan and Tinker, 2006; Oliveira et al., 2010; Ngaboyisonga et al., 2014). It tends to slow breeding progress when genotypes are selected in different environments. Generally, genotypes displaying minimum interaction with environments qualify as stable and are preferred by plant breeders as they express maximum genotypic potential (Khalil et al., 2011; Ngaboyisonga et al., 2014). Additionally, METs have been proven to guide selection of production environments suitable for specific genotypes (Kamut et al., 2013; Ngaboyisonga et al., 2014). Hence the necessity of carrying out such trials (METs) so that appropriate breeding objectives are set up.

Similarly in Rwanda, though maize is grown in all agro-ecologies of the country, it is important to identify varieties which are specific for the highlands and mid-altitude zones or those that are adapted across these two major agro-ecologies. This requires evaluating new maize varieties in some representative locations during several seasons in order to make relevant recommendations for each location and variety.

Studies have revealed that selection based on yield only may not always be adequate when genotype x environment (G x E) interaction is significant (Kang et al., 1991; Meseka et al., 2008). Importance of G x E interaction implies that the performance of the given genotype is then judged after averaging across all the testing locations. A hybrid can therefore be selected for one (specific adaptation) or many (wide adaptation) environments. Furthermore, a hybrid might be selected based on the expressed interaction with the environment compared to other genotypes in the same set under evaluation, hence the need for stability analysis to identify stable from unstable genotypes (Bernardo, 2002; Tonk et al., 2011). However, genotypes showing high performance in particular locations could be recommended as suitable in those locations.

Currently, some statistical models have been suggested for efficient analysis of genotype by environment interactions and the common ones being additive main effects and

multiplicative interaction (AMMI) and genotype (G) + genotype-by-environment (GE) interaction (GGE) (Bernardo, 2002; Chahal and Gosal, 2002). AMMI is a combination of analysis of variance and the principal component analysis (PCA). It uses the biplot to visualize relationships between eigenvalues for PCA and genotypic and environment means (Gauch, 2006). The main advantage of this model in comparison to regression linear models is that the interaction is allocated into many multiplicative parameters which are independent of each other.

On the other hand, GGE biplot analysis provides comprehensive visual information and was reported to be better, faster and easier to interpret than the results obtained from regression analysis (Yan and Kang, 2003; Meseka et al., 2008). The GGE biplot removes the large environmental effect (E) not necessary for genotype evaluation, and keeps only G and G x E that are more pertinent for making useful genotype evaluation and selection decisions (Yan et al., 2001; Yan and Kang, 2003; Dehghanpour and Ehdaie, 2013). Hence GGE becomes more efficient in G x E studies. In the current study, genotype (G) + genotype-by-environment (GE) interaction (GGE) was used.

1.10 Mating designs in maize breeding

Mating designs play a preponderant role in crop breeding. They are designated for producing progenies which are then assessed to estimate the magnitude and type of genetic variation present in a given population. Breeders could influence the outcome of a mating by the choice of parents, the control over the frequency with which each parent is involved in mating, and the number of offspring to be generated per mating, among other ways (Acquaah, 2012).

Though mating designs differ in their complexity; from a simple one factor design to the complex triallel or quadrallel designs, they are not routinely used in inbred and hybrid development programmes (Bernardo, 2002). A single mating design may not be efficient for all the goals; therefore, a complementary design with several simple designs may be used to achieve several objectives. Mating designs commonly used in maize breeding include; the Line x Tester, the diallel and North Carolina Designs (NCD) I, II and III (Griffing, 1956; Hallauer et al., 2010). The first two designs will be discussed in the following sections as they were used in this study.

1.10.1 Line x Tester (L x T) mating design

The line by tester (L x T) is considered as an extension of the top cross mating scheme when more than one testers are to be used. The design has been reported to be useful in hybrid breeding programmes (Kempthorne, 1957; Sharma, 2006) as it generates both full-sib and half-sib relatives.

In the crossing block, each tester is crossed to all lines involved and the resulting progenies are then evaluated in a replicated trial. Generally, in the crossing block, the test lines are used as female parents while the testers are used as males. However, when male sterile lines are used as testers, the test lines become the source of pollen (Singh and Chaudhary, 1977; Sharma, 2006). Testers confer a common genetic background, jointly as well as individually, against which the test lines are tested (Sharma, 2006). This design provides information on both GCA and SCA of parents and at the same time provides information on various types of gene effects. In addition, it can accommodate large numbers of genotypes and is suitable for testing early generation lines.

However, the usefulness of this design in hybrid programmes is influenced by the choice of testers which can be broad or narrow based. When a broad based population is used as tester, selection is suggested to be for GCA while narrow genetic based testers suggest selection for SCA (Kempthorne, 1957; Hallauer et al., 2010). Regardless of the different breeding objectives, the choice of the tester to be used was reported (Hallauer et al., 2010) to be the same as the ultimate aim is to find out a tester providing the best discrimination among inbred lines based on selection objectives.

In case of inbred lines evaluation, Matzinger (1953) defined a useful tester as the one combining the greatest simplicity in use with the maximum information on performance expected from tested lines when used in other combinations or grown in other environments. However, as reported by Hallauer et al. (2010), there is no tester that can fully meet these requirements. Therefore, according to Hallauer (1975), a suitable tester should be simple in use, provides information correctly classifying the relative merit of inbred lines and then maximises genetic gain.

Generally under L x T design, the choice of tester was reported to be related to heterotic groups and mainly to the hybrid product (Hallauer and Carena, 2009; Fato et al., 2012), thus the reason why the initial and even advanced evaluation on new inbred lines has to include testers representing elite germplasm in the breeding programme.

However, the choice of tester might also be governed by breeding objectives; if the priority is on development of new hybrids then inbred lines may be tested with different elite inbred line testers representing contrasting heterotic groups (Hallauer et al., 2010).

Some weaknesses related to L x T design were also pointed out by Kempthorne (1957); as the significance of σ^2_{GCA} and σ^2_{SCA} resulting from this design are not testable, these statistics are considered as exploratory nature only. Consequently the design might be recommended as a rapid method to screen genetic materials based on GCA/SCA effects rather than their variances. Therefore, more advanced designs are recommended later to develop more precise estimates of variance. Using L x T design in their study on a new maize heterotic pattern between temperate and tropical germplasms, Fan et al. (2008) identified one exotic line as a new heterotic group. Similarly, they reported also a new heterotic pattern in their findings. Other studies have also reported similar trends (Fan et al., 2009; Fato et al., 2012). In the current study, different maize inbred line testers with known heterotic groups were used to classify new inbred lines in different possible heterotic groups.

1.10.2 Diallel mating designs

Diallel mating designs involve a set of crosses generated by using inbred lines in all possible combinations. In comparison to the L x T, diallel mating design is appropriate when the number of parents is limited. Its analysis provides mainly information on the nature and amount of genetic parameters and GCA and SCA of parent and their crosses, respectively. It is widely used especially in maize hybrid breeding programmes to explore new heterotic patterns. Depending on the breeding objective, parental materials in a diallel could be populations (heterozygous) or inbreds. The two main approaches used for diallel analysis are Hayman's approach and Griffing's approach and four methods are used to generate progenies (Griffing, 1956; Singh and Chaudhary, 1977; Walter, 1987; Falconer and Mackay, 1996; Acquaah, 2012). The four methods vary either in the omission of the parents or the reciprocals. However, a diallel for a random model usually includes neither the parents nor the reciprocal crosses (Bernardo, 2002).

Method I is a full diallel including all the progenitors, F1 crosses and F1 reciprocal crosses, whereas method II includes the progenitors and the F1 crosses without reciprocals. Method III comprises F1 crosses and F1 reciprocal crosses while method IV only comprises F1 crosses. In addition, two important assumptions (fixed and random models) regarding parents involved in crosses are considered prior to producing crosses and their evaluation (Griffing, 1956). Therefore, the method and model selected can affect data interpretations.

Then, the accuracy of the analysis is improved by using the appropriate method and model. However, the programme objectives and parental sampling procedures could determine the most appropriate model to use (Shattuck et al., 1993).

If correctly analyzed, the diallel mating design was reported to be very powerful in determining alternative heterotic patterns (Shattuck et al., 1993; Hallauer et al., 2010). Similarly, using diallel method IV by classifying maize inbred lines into heterotic groups using diallel analysis, Bidhendi et al. (2012) were able to find best heterotic patterns useful in maize breeding programmes to obtain high-yielding hybrids. Also, in this study, ten maize inbred lines were crossed in the same diallel method to find out the mode of gene action and putative heterotic patterns for grain yield and other different traits for mid-altitude and highlands of Rwanda.

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2 Chapter two

Genetic diversity among maize inbred lines selected for the mid-altitudes and highlands of Rwanda[4]

Abstract

Understanding the genetic diversity and relationships among breeding materials is crucial in a hybrid-oriented programme. This study was carried out to apply specific single nucleotide polymorphism (SNP) markers to determine the amount of genetic diversity prevailing among maize inbred lines selected for the mid-altitudes and highlands of Rwanda and classify the inbred lines according to their genetic relationships. Seventy one maize inbred lines from different origins were genotyped with 92 SNP markers. The unweighted pair group method with arithmetic mean (UPGMA) revealed a random allocation of the inbred lines into different clusters. Lines were allocated to two major clusters regardless of their origin. Variation was observed among the SNPs for their efficacy. The highest (0.375) polymorphic information content (PIC) observed was exhibited by three markers; PZA00543_12, PZA00878_2, and PZA01735_1; while the lowest PIC value was revealed by the marker PZA01755_1 (0.1224). The PIC mean value of 0.30 revealed in this study indicates the potential of these SNP markers to discriminate inbred lines from diverse origins and their usefulness for diversity analysis of maize inbred lines. Genetic clustering information obtained from the current study would be used to organize the germplasm according to heterotic patterns and groups for the mid and highland maize in Rwanda and comparable tropical environments.

Keywords: Genetic diversity; Maize; Single nucleotide polymorphism.

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2.1 Introduction

Genetic diversity study is a valuable pillar in determining genetic relationships among maize inbred lines in a hybrid oriented programme. This would form the basis for designing appropriate hybrids. Genetic distance among breeding materials is a key factor to consider when predicting genetic variability among parental combinations (Mohammadi and Prasanna, 2003; Laborda et al., 2005; Bertan et al., 2007; Semagn et al., 2012; Wende et al., 2013). High yielding as well as genetically distant genotypes might represent parent inbred lines with different loci controlling the character and probably with high combining ability. Therefore, information on germplasm diversity and relationships existing among breeding materials is key to crop improvement. Evaluation of genetic diversity and relationships in a given set of germplasm is valuable for selecting parental combinations aimed at developing progenies with high genetic variability (Semagn et al., 2012).

Assessing genetic diversity and relatedness among breeding materials has a preponderant role in a breeding programme. Development of improved inbred lines and identification of suitable parental combinations to generate high performing hybrids is the leading task of maize breeders (Semagn et al., 2012). Information related to genetic diversity and relationships among diverse germplasm is valuable to plant breeders as this information leads the decision making during selection of parents for crossing and is useful for broadening the genetic basis of different breeding programmes (Laborda et al., 2005). Unfortunately, many maize breeding programmes depend on phenotypic evaluations. However, the presence of favorable alleles is difficult to detect among the germplasm mainly due to environment effects. This was earlier revealed by Leal et al. (2010) who reported that molecular markers have proved to have different advantages over other methods since they show genetic differences on a more detailed level without interferences from environmental factors and they involve techniques that provide fast results detailing genetic diversity. Therefore, for effective management of genetic diversity, there is need of well-characterized germplasm and genetic pools well classified into different clusters based on genetic diversity (Dhliwayo et al., 2009; Wende et al., 2013; Muhinyuza et al., 2015).

Genetic clustering of parental inbred lines will permit breeders to predict maize hybrid performance resulting from different intergroup crosses. However, the effectiveness of this will depend on genetic backgrounds of the germplasm being documented. Generally, high diversity is expected from inbred lines resulting from different clusters while, low diversity is expected between two inbred lines within the same cluster. Not only genetic diversity assessment is useful to identify parents for making crosses but also in predicting heterotic

groups. Increased allelic diversity will be responsible for the presence of discrete genetic groups among inbred lines, and this might result in high level of heterozygosity in the hybrid related to increased heterosis. However, confirming genetic grouping generated through molecular data is the most informative method and needs to be complemented with combining ability tests especially on yield and yield components (Adeyemo et al., 2012; Wende et al., 2013).

Various methods to identify the best progenitors for generating combinations and to cluster these progenitors to a given heterotic group have been reported (Bertan et al., 2007; Semagn et al., 2012) and include: (i) phenotypic performance for particular traits, (ii) pedigree relationships, (iii) adaptability and yield stability, (d) top crosses, (iv) diallel crosses, and (v) genetic distance assessed from morphological and molecular markers**.** Although each of these methods has its own advantages and disadvantages, using information resulting from them can contribute to identifying the best hybrid combinations (Dhliwayo et al., 2009; Wende et al., 2013).

DNA markers can assist in assessing the amount of genetic diversity available in breeding materials (Adeyemo et al., 2012; Muhinyuza et al., 2015). They have been reported to increase the efficiency of conventional breeding by shortening the time allocated to variety development (Semagn et al., 2012; Wende et al., 2013). Genetic distance assessed can be estimated using different types of molecular markers, comprising amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), simple sequence repeats (SSRs), and single nucleotide polymorphisms (SNPs) (Semagn et al., 2012). Of these markers, current advances in molecular technology have shown a shift heading towards SNPs (Jones et al., 2007; Semagn et al., 2012). This is because of their various attributes such as; locus-specificity, low cost per data point, codominance, high genomic abundance, potential for high throughput analysis, and lower genotyping error rates (Rafalski, 2002; Schlötterer, 2004; Chagné et al., 2007; Semagn et al., 2012). In their findings, Semagn et al. (2012) reported SNP markers as a powerful tool in genetic diversity studies and marker assisted breeding.

In the current study, SNP markers were used to assess the magnitude of genetic diversity and relationships among maize inbred lines selected for the mid-altitudes and highlands of Rwanda. This will be useful for establishment of a hybrid breeding programme in Rwanda. In other similar breeding programmes, it was realized that many undesirable crosses could be avoided by allocating inbred lines into well-differentiated clusters (Wende et al., 2013; Muhinyuza et al., 2015) and molecular markers have been reported to play a considerable

role in characterizing inbred lines and then generating diverse clusters of genotypes based on genetic diversity (Melchinger and Gumber, 1998; Reif et al., 2005; Wende et al., 2013). Earlier studies by other researchers, using molecular markers effectively allocated maize germplasm into different heterotic groups (Lee et al., 1989; Livini et al., 1992; Dubreuil et al., 1996; Wende et al., 2013).

Currently, the maize breeding programme in Rwanda performs selection and establishes genetic relationships of maize lines based on phenotypic characterization. However, this is known to be hindered by environmental effects. No study exists on genetic diversity assessment among maize inbreds in Rwanda based on molecular data. Earlier studies focused mostly on evaluation for adaptability of new introduced genotypes form different collaborators such as International Maize and Wheat Improvement Center (CIMMYT) and International Institute of Tropical Agriculture (IITA). Therefore, there is need to explore the genetic interrelationships existing among maize inbred lines selected for the major agro-ecologies of Rwanda and determining specific clusters and relationships in order to establish a sustainable maize hybrid programme. Consequently, the objective of the current study was to determine the genetic distances and clusters among potential maize inbred lines selected for the mid-altitudes and highlands of Rwanda using SNP markers.

2.2 Materials and Methods

2.2.1 Plant materials

A total of 71 maize inbred lines; comprising 44 local inbred lines, 16 inbred lines from CIMMYT-Ethiopia and 11 lines from CIMMYT-Mexico were used in the study (Table 2.1). Most of inbred lines from CIMMYT were of tropical origin and they differed in their response to different foliar diseases and heterotic grouping. On the other hand, the local inbred lines were from nine maize open pollinated varieties (OPVs) and some of these populations have been grown by farmers for their different attributes. All these inbred lines were selected based on disease resistance, vigour, and adaptability to local environment.

Table 2.1: Description of maize inbred lines used in the study

No	**Code**	**Origin**	**No**	**code**	**Origin**
1	E1	CIMMYT-Ethiopia	37	M8144	Rwanda
2	E3	CIMMYT-Ethiopia	38	ACR3	Rwanda
3	E4	CIMMYT-Ethiopia	39	ACRO4	Rwanda
4	E5	CIMMYT-Ethiopia	40	ACR4	Rwanda
5	E8	CIMMYT-Ethiopia	41	ACRO29	Rwanda
6	E9	CIMMYT-Ethiopia	42	ACR29	Rwanda
7	E10	CIMMYT-Ethiopia	43	ECA1	Rwanda
8	E11	CIMMYT-Ethiopia	44	ECA13	Rwanda
9	E12	CIMMYT-Ethiopia	45	ECA18	Rwanda
10	E14	CIMMYT-Ethiopia	46	ECA1ECA2	Rwanda
11	E15	CIMMYT-Ethiopia	47	ECA1ECA1S5	Rwanda

No	**Code**	**Origin**	**No**	**code**	**Origin**
12	E17	CIMMYT-Ethiopia	48	ECA1ECA5	Rwanda
13	E18	CIMMYT-Ethiopia	49	ECA1ECA43	Rwanda
14	E19	CIMMYT-Ethiopia	50	ECAP3	Rwanda
15	E20	CIMMYT-Ethiopia	51	ECAP11	Rwanda
16	E21	CIMMYT-Ethiopia	52	ECAPO23	Rwanda
17	M351	CIMMYT-Mexico	53	ECAP23	Rwanda
18	M352	CIMMYT-Mexico	54	TQX7	Rwanda
19	M353	CIMMYT-Mexico	55	TQ7	Rwanda
20	M354	CIMMYT-Mexico	56	TQ8	Rwanda
21	M355	CIMMYT-Mexico	57	TQX31	Rwanda
22	M356	CIMMYT-Mexico	58	TQ31	Rwanda
23	M455	CIMMYT-Mexico	59	CM523	Rwanda
24	M456	CIMMYT-Mexico	60	CM506	Rwanda

No	**Code**	**Origin**	**No**	**code**	**Origin**
25	M457	CIMMYT-Mexico	61	MZ3	Rwanda
26	M459	CIMMYT-Mexico	62	MZ4	Rwanda
27	M464	CIMMYT-Mexico	63	MZ5	Rwanda
28	R10164	Rwanda	64	POL1	Rwanda
29	R10127	Rwanda	65	POL2	Rwanda
30	R10141	Rwanda	66	POL3	Rwanda
31	RM8147	Rwanda	67	POL4	Rwanda
32	RM8119	Rwanda	68	POL5	Rwanda
33	M8147	Rwanda	69	POL6	Rwanda
34	M8119	Rwanda	70	POL7	Rwanda
35	RM8144	Rwanda	71	POL8	Rwanda
36	RM8115	Rwanda			

2.2.2 Deoxyribonucleic acid (DNA) sampling and isolation

Deoxyribonucleic acid (DNA) was extracted from inbred lines planted in a nursery at Nyagatare research station in 2014B (March to July 2014) growing season. Using the punch method, at 4 weeks after planting, leaf sample tissue of each individual inbred line was harvested at the 3-4 leaf stage. Two leaf discs from each inbred line were then placed into 2 labelled 96-well blocks and each well representing an individual inbred line. Once the block

was completed, a sheet of air-pore tape was put on the top of the block for sealing and then placed inside plastic bags together with 50 g of silica gel for drying purpose. The samples were then sent to DNA Landmarks laboratory, Canada for genotyping. DNA was extracted and isolated following a proprietary Sarkosyl Nitrogen based method at the DNA Landmarks laboratory (Blin and Stafford, 1976).

2.2.3 Genotypic data analysis

Based on previous research studies on maize at CIMMYT, a total of 100 SNPs (Table 2.2) were used in the study. However, eight of them were not polymorphic with the genotypes involved in the study and therefore discarded from the analysis. For each SNP marker; number of alleles, allele frequency, number of genotypes, genotype frequency, observed heterozygosity, gene diversity, genetic distance, polymorphic information content (PIC), and cluster analysis based on similarity matrices obtained with Unweighted Pair Group Method with Arithmetic Average (UPGMA) to generate dendrograms were computed (Nei, 1991) using Power Marker version 3.25 (Liu and Muse, 2005).

2.3 Results

2.3.1 Characterisation of SNPs

Of the 100 SNPs genotyped, 92 with less than 10% missing data and considered to be high quality were used for subsequent analysis. These markers had high availability values (Table 2.2). The 92 SNPs revealed a total of 184 alleles (with an average of 2 alleles per marker). Genetic diversity varied from 0.014 to 0.500 with an average of 0.385. As a measure of allelic diversity at a locus, expected heterozygosity (He) values varied from 0.00 to 0.19 with a mean of 0.08, while the PIC estimates ranged from 0.014 to 0.375 with a mean of 0.303.The ten SNPs (Table 2.2) exhibiting the highest PIC and their potential to detect differences between the inbred lines were PZA00543_12 (0.3750); PZA00878_2 (0.3750); PZA01735_1 (0.3750); PZB00085_1 (0.3749); PZA00257_22 (0.3748); PZB01647_1 (0.3746); PZD00022_6 (0.3746); PZA02763_1 (0.3745); PZB02510_ (0.3742); PZD00022_6 (0.3742). Contrary to this, the following ten SNPs (Table 2) exhibited the lowest PIC; PZB01400_1 (0.1800); PZA02606_1 (0.1327); PZA01755_1 (0.1224); PZD00072_2 (0.1224); PZA02148_1 (0.1007); PZB00008_1 (0.1007); PZA00947_1 (0.0929); PZA02890_4 (0.0777); PZB00772_1 (0.0405); PZA03695_1 (0.0139).

Table 2.2: Characteristics of the 92 SNP markers used to genotype the 71 maize inbred lines

Marker	Av	He	PIC	Rank	Marker	Av	He	PIC	†Rank
PZA00543_12	0.9577	0.0882	0.3750	1	PZA00455_16	0.9859	0.0429	0.3466	47
PZA00878_2	0.9859	0.0571	0.3750	2	PZB02283_1	1.0000	0.0986	0.3448	48
PZA01735_1	1.0000	0.1127	0.3750	3	PZA03728_1	1.0000	0.1408	0.3421	49
PZA00257_22	0.9718	0.0580	0.3749	4	PZA03602_1	1.0000	0.0704	0.3392	50
PZB00085_1	1.0000	0.1268	0.3748	5	PZA03231_1	1.0000	0.1408	0.3362	51
PZB01647_1	1.0000	0.0563	0.3746	6	PZA03391_2	1.0000	0.1127	0.3362	52
PZD00022_6	1.0000	0.0282	0.3746	7	sh1_2	1.0000	0.0563	0.3362	53
PZA02763_1	0.9859	0.0714	0.3745	8	PZA01315_1	0.9718	0.0725	0.3304	54
PZA01142_4	1.0000	0.0704	0.3742	9	PZA00726_8	1.0000	0.0563	0.3228	55
PZB02033_2	1.0000	0.1268	0.3742	10	PZA02683_1	0.9859	0.0714	0.3212	56
PZB02510_5	1.0000	0.0986	0.3742	11	PZA03474_1	1.0000	0.0704	0.3111	57
PZA00948_1	0.9859	0.1143	0.3742	12	PZA03445_1	0.9859	0.0571	0.3091	58
PZB00109_2	1.0000	0.1408	0.3738	13	PZB02155_1	1.0000	0.0563	0.3069	59
PZA02676_2	0.9859	0.0429	0.3737	14	PZB01186_1	1.0000	0.0704	0.3025	60
PZA00223_2	0.9296	0.1061	0.3736	15	PZA01447_1	1.0000	0.0563	0.2979	61
PZA00827_1	1.0000	0.1127	0.3726	16	PZB01156_2	1.0000	0.0845	0.2979	62
PZA02068_1	1.0000	0.1127	0.3726	17	PZA02585_2	1.0000	0.0845	0.2882	63
PZA03404_1	1.0000	0.1127	0.3726	18	PZA02916_5	1.0000	0.0563	0.2882	64
PZA02564_2	0.9859	0.1286	0.3725	19	PZA01304_1	0.9859	0.0429	0.2854	65
umc128_2	1.0000	0.1268	0.3718	20	PZA02113_1	0.9859	0.0857	0.2800	66
PZA00266_7	0.9718	0.1304	0.3716	21	PZA03661_3	1.0000	0.0282	0.2777	67
PZA00352_23	0.9577	0.0882	0.3715	22	bt2_2	1.0000	0.0563	0.2665	68
PZA01396_1	0.9577	0.0588	0.3715	23	PZA02212_1	0.9859	0.0429	0.2629	69
PZA00343_31	0.9718	0.1159	0.3707	24	PZA03733_1	1.0000	0.0986	0.2606	70
PZA01292_1	1.0000	0.0986	0.3700	25	PZA00881_1	0.9577	0.1029	0.2550	71
PZA03507_1	1.0000	0.0423	0.3700	26	csu1171_2	1.0000	0.0563	0.2414	72
PZB02017_1	1.0000	0.1268	0.3700	27	PZA02367_1	0.9859	0.1000	0.2369	73
PZB01042_7	1.0000	0.1127	0.3689	28	PZB02480_1	1.0000	0.0423	0.2346	74
PZA02027_1	0.9718	0.1159	0.3686	29	PZB00175_6	1.0000	0.0423	0.2203	75
PZD00054_1	1.0000	0.0423	0.3678	30	PZB00232_1	1.0000	0.0704	0.2203	76
PZA00106_10	0.9577	0.0588	0.3671	31	PZA03644_1	1.0000	0.0704	0.2049	77
PZA01342_2	0.9718	0.1014	0.3645	32	PZB00068_1	1.0000	0.0704	0.2049	78
PZA02019_1	0.9859	0.1857	0.3633	33	PZB00869_4	1.0000	0.0282	0.1969	79
PZB01358_2	1.0000	0.0986	0.3620	34	PZA03395_3	0.9859	0.0143	0.1906	80
PZA00920_1	0.9718	0.1884	0.3612	35	PZA02386_2	1.0000	0.0141	0.1886	81
PZA02450_1	1.0000	0.0563	0.3603	36	PZA03470_1	1.0000	0.0704	0.1886	82
PZA00136_2	0.9296	0.1212	0.3599	37	PZB01400_1	1.0000	0.0282	0.1800	83
PZA03182_5	1.0000	0.0986	0.3584	38	PZA02606_1	1.0000	0.0423	0.1327	84
PZA00309_2	0.9718	0.1014	0.3574	39	PZA01755_1	1.0000	0.0563	0.1224	85
PZA02589_1	1.0000	0.0563	0.3522	40	PZD00072_2	1.0000	0.0563	0.1224	86
PZA03743_1	1.0000	0.1127	0.3522	41	PZA02148_1	1.0000	0.0000	0.1007	87
PZA01804_1	0.9859	0.0714	0.3515	42	PZB00008_1	1.0000	0.0563	0.1007	88

Marker	Av	He	PIC	Rank	Marker	Av	He	PIC	†Rank
PZA02957_5	1.0000	0.0704	0.3498	43	PZA00947_1	0.9577	0.0147	0.0929	89
PZA03116_2	1.0000	0.1549	0.3498	44	PZA02890_4	1.0000	0.0000	0.0777	90
PZD00027_2	1.0000	0.1127	0.3474	45	PZB00772_1	1.0000	0.0423	0.0405	91
ZHD1_1	1.0000	0.0563	0.3474	46	PZA03695_1	1.0000	0.0141	0.0139	92

Av, He and PIC, availability, means expected heterozygosity and polymorphic information content respectively; Av: number of observation over sample size. †Markers were ranked based on PIC values.

2.3.2 Genetic distance and relationships

The dendrogram generated using the UPGMA clustering algorithm based on SNPs data grouped all the 71 inbred lines into two major clusters (Fig. 2.1) with cluster one (I) having only two inbreds (MZ4 and MZ5) closely related in their pedigree information and originating from the same open pollinated variety. The remaining 69 inbred lines (97%) belonged to second cluster (II) also partitioned into many sub-clusters (from IIA-IIBc1a2) but also exhibiting distinct groupings within individual sub-clusters. There were two major sub-clusters within cluster II. The first one (IIA) consisted of four lines (ET17, ET18, ET12, and ET19) of the same origin (CIMMYT-Ethiopia) with inbred line ET 19 belonging to Ecuador heterotic group which is used as a tester (T2) in chapter four. The second sub-cluster (IIB) comprised all the rest (65) of the inbred lines. Of these 65 lines, 11 of them (IIB) fell in the same group and most of them (8) shared the same origin (CIMMYT-Ethiopia) where inbred line ET4 (referred to as S4 was used as tester in chapter three), and inbred lines ET21 and M464 (refers respectively as tester T3 and tester T4 in chapter four) were also placed in this group. The remaining 54 (76%) formed another group except for five (IIBa) (from ECA18 to RM8144) lines from Rwanda forming their own group. The remaining 49 (69%) inbred lines (IIBb-IIBc1a2) formed another major group having many small groups in it. However, some of the inbred lines within these groups were aligned based on their origin or their pedigrees. Inbred lines ET8 and ET9 (referred respectively as S6 and S7 in chapter three) and inbred ET5 (referred to as tester T1 in chapter four, fell also in this group (IIBb-IIBc1a2)).

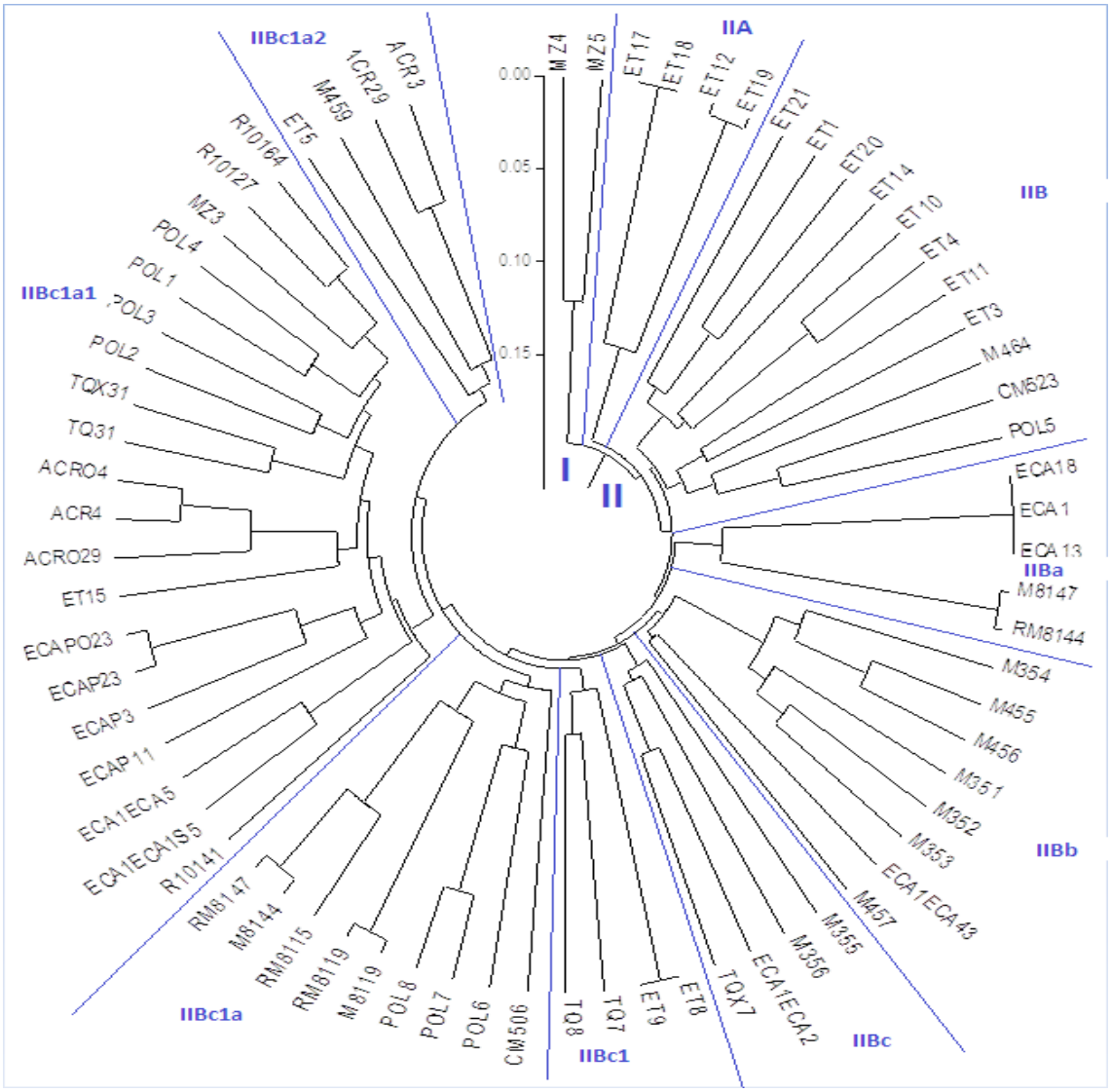

Figure 2.1: Radial dendrogram showing genetic relationships among 71 maize inbred lines tested using 92 SNP markers. The two clusters are denoted from I to II while sub-clusters are denoted from IIA to IIBc1a2

2.4 Discussion

2.4.1 Characterisation of markers

The 92 SNPs were effective in discriminating the 71 maize inbred line genotypes under study. As relative value of each marker with respect to the amount of polymorphism exhibited, the mean PIC value (0.303) observed in the current study was higher than the one reported in earlier findings (Lu et al., 2009; Hao et al., 2011). Using SNP markers for identification of functional genetic variations underlying drought tolerance in maize, Hao et al. (2011) pointed out an average PIC value of 0.239. A similar trend was also reported by

Lu et al. (2009) who reported a mean PIC value equivalent to 0.259 using 1034 SNPs to genotype 770 maize inbred lines. Therefore, the high PIC value revealed in this study might be a relevant indication confirming the potential for these SNP markers to discriminate maize inbred lines from diverse origins. This was also proven by the fact that the markers were able to separate closely related lines, indicating their usefulness for diversity analysis of maize inbred lines. On the contrary, when comparing SNPs and SSRs in assessment of genetic relatedness in maize, Yang et al. (2011) reported a higher PIC (0.340). A similar trend was also revealed by Wende et al. (2013) in their study on genetic interrelationships among medium to late maturing tropical maize inbred lines using selected SSR markers, where a PIC of 0.54 was reported. However, according to Srinivasan et al. (2004), the PIC values are dependent on the genetic diversity of the accessions chosen. Based on genetic diversity in combination with the revealed PIC, they would contribute in minimizing the use of closely related maize germplasm in maize breeding programmes which would otherwise lead to genetic depression and reduced genetic variation. Therefore, the current PIC demonstrates the usefulness of the SNPs and their potential to detect differences among the maize lines based on their genetic relationships.

2.4.2 Genetic distance and relationships

Generally, with some exceptions, there was a random allocation of the inbred lines into different clusters and / sub-clusters. Some of the inbred lines closely related were grouped in the same cluster or same sub-cluster (cluster I), confirming the presence of relationships between the pedigree and the SNPs marker groupings in this study. Though some of these inbred lines seemed to cluster according to their pedigree grouping (ECA18, ECA1 and ECA13), there were some inconsistencies; for instance: M355, M356, ECA1ECA2 and TQX7 clustered together despite being unrelated by pedigree. Similar findings were earlier reported (Dhliwayo et al., 2009; Yang et al., 2011; Semagn et al., 2012; Wende et al., 2013). There were some local lines (POL5, TQ7,TQ8, ACR3 and 1CR29) which clustered together with specific lines used as testers in chapter three (ET4, ET8 and ET9) and chapter four (ET5, ET19, ET21 and M464) indicating close relationships and similarities prevailing between these testers and these local inbred lines.

Discrepancies in classification of germplasm revealed when comparing molecular results with classification based on pedigree relatedness were earlier reported (Dhliwayo et al., 2009; Yang et al., 2011). They might have resulted in the fact that all the local inbred lines involved in the current study were developed from maize open pollinated varieties selected

from regional trials obtained from CMMYT-Kenya. There could have been exchanges of breeding materials among different CIMMYT breeding programmes, justifying the alignment of some inbred lines from different geographical locations in the same clusters or sub-clusters. Furthermore, these inconsistencies in inbred lines alignment may have resulted also from the effects of mutation, selection and genetic drift (Marsan et al., 1998; Senior et al., 1998; Wende et al., 2013).

Prasanna et al. (2004) mentioned that effective and reliable discrimination of inbred lines not only helps in the identification of genotypes, but also in promoting efficient utilization of genetic materials in breeding programmes. This was also earlier pointed out by Hallauer and Miranda (1988) who mentioned that the genetic divergence of parental varieties defines the manifestation of heterosis, and the heterotic pattern is determined by the genetic divergence of two parental lines. Therefore, crossing schemes comprising the more distant maize genotypes might allow for greater success in the production of genetic variability and thus might maximize the exploitation of heterosis and segregation (Molin et al., 2013). Consequently, the observed relationships in this study could be exploited accordingly in order to design a strong breeding maize hybrid programme in Rwanda.

2.5 Conclusion

Overall, the 92 SNP markers grouped the inbred lines into two major distinguishable clusters. In some case SNPs grouped the lines in contrast with the current pedigree records. However, for some of the sub-clusters, the SNP markers partitioned the inbred lines into distinguishable clusters in alignment with the pedigree records. Furthermore, in addition to high PIC exhibited by some individual markers and their mean, the PIC observed under this study confirmed how useful these SNP markers are for diversity investigation among the maize inbred lines under consideration. The acquired information regarding the amount of genetic diversity and relationships revealed in these lines together with combining ability and pedigree records would be explored to point out suitable heterotic patterns and group the inbred lines into specific heterotic groups.

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3 Chapter Three

Combining ability and heterotic groups for grain yield and other agronomic traits among maize inbred lines selected for the mid-altitudes and highlands zones of Rwanda

Abstract

Development and identification of maize parental lines that belong to different heterotic groups is a fundamental requirement for any hybrid production programme. The objective of this study was, therefore, to determine combining ability, heterosis and heterotic patterns for grain yield and other agronomic traits among ten selected local and exotic maize inbred lines and their progenies evaluated across the mid-altitude and highland zones of Rwanda. Forty-five single cross hybrids from a 10 x 10 half-diallel mating design plus three checks were tested in a 6 x 8 alpha-lattice design across twelve environments in Rwanda. General combining ability (GCA) and specific combining ability (SCA) effects were both highly significant ($P<0.001$-0.01), suggesting the presence of both additive and non-additive gene effects. The percentage mid-parent heterosis (MPH) for grain yield ranged from 36.4 (ET8/ET9) to 267.7% (ET4/ET8) with a mean of 164%, while high-parent heterosis (HPH) varied from 33.2% (ET8/TQX7) to 236% (ET4/TQX7) with a mean of 130.4%. Of the ten lines, using MPH, seven maize local inbred lines were discriminated and assigned into four different heterotic groups (S4, S7, S4/S6 and S6/S7). The highest heterotic patterns were realized between tester S4 and tester S6 (hybrid S4/S6) and between group S7 and tester S4 (hybrid S2/S4).These identified patterns would be potentially useful for maize hybrid production in Rwanda. Similarly, the resulting hybrids could be recommended in sub-Saharan African regions with similar ecosystems. Significance of both additive and non-additive genetic effects suggest that the breeding programme could apply both hybridization and recurrent selection as breeding strategies.

Keywords: Combining ability, grain yield, maize hybrids, heterosis, heterotic grouping and patterns.

3.1 Introduction

Worldwide and particularly in Rwanda, maize (*Zea mays* L) is the principal crop. It is a significant component of food security worldwide, providing food, feed and bioenergy (FAO, 2012). It is the most important staple food crop on which the livelihoods of more than 1.2 billion people in sub-Saharan Africa depend on (Krivanek et al., 2007; FAO, 2012). Likewise, it is important to Rwandan families who consume it in various forms which include roasted or boiled green ears, boiled dry grain or mixed with legumes such as beans, or as *ugali* and *uji* (porridge) prepared from dry maize flour, or brewed into local beer. Maize is also increasingly becoming a major component of livestock and poultry feed in the country (Sallah et al., 2007). As a vital component of food security across the world, maize improvement for yield potential is the most important for many genomics and breeding programmes (Hallauer and Miranda, 1988).

In developing countries, maize yields are much lower than in developed countries. Maize yields in Africa (less 1.7 tonnes/ha) account for 36% of global maize yields (4.9 tonnes/ha), even in regions with considerable rainfall and where farmers can invest in yield-increasing inputs (FARA, 2009; Shiferaw et al., 2011). In Rwanda, a similar trend of low maize yields (1.4 tonnes/ha) has also been reported (NIS, 2014). It is possible to obtain high yields with improved inputs and agronomic practices as well as use of genetically improved varieties. Therefore, appropriate breeding strategies are essential in order to develop maize varieties with increased yield and resisting various production constraints in the major maize agro-ecologies of Rwanda. The strategy could thus involve hybrid production as they are more productive than open pollinated varieties. However, such varieties are scarcely available (Sallah et al., 2007; Fato et al., 2012) in many developing countries including Rwanda. Production of hybrids implies selection of superior parents and precise identification of heterotic patterns (Hallauer and Miranda, 1988). Furthermore, the choice of selection methods for the improvement of traits in target germplasm will depend on the mode of gene action (Rovaris et al., 2014).

This phase of developing and identifying parents that form superior heterotic patterns, though fundamental to hybrid breeding, is the most costly and laborious in a maize hybrid programme. This is because *per se* performance of the parents does not predict the performance of maize hybrids for grain yield (Hallauer and Miranda, 1988; Dao et al., 2014). Heterosis will thus be an important predictor of the hybrid value in a given maize hybrid breeding programme. Consequently, laying a strong foundation for a viable hybrid maize

programme in Rwanda will require knowledge on combining ability and heterotic patterns among existing and introduced germplasm. Development of maize hybrids to exploit heterosis relies on genetically diverse and complementary elite inbred lines (Dao et al., 2014; Nyaligwa et al., 2015). This is because, as a key player for breeding progress, genetic diversity in any maize germplasm is a safeguard against vulnerability and is critical for increasing yields.

The concept of heterotic patterns is important in that it helps breeders in choosing parents of crosses for line development as well as testers to evaluate combining ability of newly developed inbred lines and therefore, simplifying germplasm management and organization (Reif et al., 2005; Nepir et al., 2015). For the Rwanda maize breeding programme, heterosis will be exploited through organised hybridization of desirable parents based on the heterotic patterns observed in the set of materials used in this study. The level of heterosis realised in F1 hybrids is highly associated with genetic diversity of the parental lines. Crosses between inbred lines from groups with differing genetic backgrounds are expected to exhibit high levels of heterosis than those among lines from the more genetically related groups (Hallauer and Miranda, 1988; Fato et al., 2012).

Similarly, combining ability analysis of maize (*Zea mays* L.) inbred lines and their hybrids are essential to develop new recombinants or hybrid varieties to exploit heterosis (Fato et al., 2012; Nyaligwa et al., 2015). The most relevant to a hybrid oriented breeding programme is the information on general combining ability (GCA) of the lines and specific combining ability (SCA) of their crosses, associated with the efficient exploitation of heterosis and heterotic patterns. Identification of inbred lines with good combining ability is a prerequisite for the success of any breeding programme aimed at hybrid development (Hallauer and Miranda, 1988; Dao et al., 2014; Nyaligwa et al., 2015). The information on both GCA and SCA effects, heterosis and heterotic patterns can be extracted based on many different mating schemes. Among these, diallel is one of the most widely used genetic designs in maize breeding programmes (Griffing, 1956; Hallauer et al., 2010) and was used in this study.

In Rwanda, scarcity of maize seed of improved varieties that can withstand various production constraints is a major challenge to small scale farmers and to the government. As a result there is a continued need to identify new sources of high performing maize hybrids using the available breeding genetic stocks and introduced germplasm to enhance maize productivity. Both, the heterotic effects and combining abilities of the newly developed and introduced germplasm has not been studied as yet in Rwanda. The objective of this study was, therefore, to determine combining ability and heterotic groups for grain yield and

associated traits among 10 maize inbred lines comprising seven locally developed and three introduced inbred lines and their progenies evaluated across the mid-altitude and highland zones of Rwanda.

3.2 Materials and methods

3.2.1 Germplasm

Forty-five single cross hybrids were derived from a half-diallel cross of ten inbred lines (Table 3.1) composed of seven inbred lines originating from seven populations adapted to the mid-altitude of Rwanda and three (S4,S6 and S7) highland inbred lines from CIMMYT-Ethiopia with different genetic backgrounds and these were adopted as testers. The S4 inbred line is in Kitale heterotic group, S6 is in Ecuador heterotic group, while S7 is from pool 9A. Furthermore, the three highland inbred lines were selected based on their adaptability to the Rwandan environmental conditions. They were included in the diallel study to determine heterotic divergence and guide in the discrimination of the seven maize local inbred lines into different heterotic groups.

Table 3.1: Germplasm involved in the study

No	Name	Pedigree	Heterotic Group	Origin
S1	R10164	RM101 5-6 (64)	-	Rwanda
S2	RM8147	RMO81 9-2 (47)	-	Rwanda
S3	ACRO29	ACROSS8762 4-5 (29)II	-	Rwanda
S4	ET4	SRSYN95[KIT//N3/TUX]F1-##(GLS=2)-22-2-2-2-2-#-#-#-#-#-#	Kitale	CIMMYT
S5	ECA13	ECA16-STR 4-7 (13)	-	Rwanda
S6	ET8	[ECU/SNSYN[SC/ETO]]c1F1-##(GLS=2.5)-31-1-1-1-1-1-#-#-#-#-#	Ecuador	CIMMYT
S7	ET9	[POOL9Ac7-SR(BC2)]FS89-1-2-4-2-1-2-2-###-#-#-#	Pool 9A	CIMMYT
S8	TQX7	[TUXSEQ]C1 5-8 (7)I	-	Rwanda
S9	MZ5	ZM607-80-4-1-B*4(5)	-	Rwanda
S10	POL6	POOL32-6-1-1-B-B(6)	-	Rwanda

3.2.2 Site descriptions

The study was carried out in four research sites representative of major Rwandan maize growing agro-ecologies (Table 3.2). Bugarama site is located in the semi-arid mid-altitude, ranging from 900-1200 metres above sea level (m.a.s.l). Nyagatare and Rubona are located in the moist mid-altitude ranging from 1200-1700 m.a.s.l, while Rwerere is located in the highlands which are above 1700 m.a.s.l. Supplementary information on the experimental sites is given in Table 3.2 and Figure 3.1.

Table 3.2: Description of testing environments

Agro-ecology	Site	Season	Rainfall(mm)†	Description
Mid-altitude	Nyagatare	15A	379.3	Lat. 1° 20' S, Long. 30° 20' E, 1450 masl
		15B	-	
		16A	-	
Mid-altitude	Rubona	15A	562.90	Lat. 2° 29′ S, Long. 29° 46′E, 1650 mas
		15B	344.1	
		16A	817.1	
Mid-altitude	Bugarama	15A	599.50	Lat 2°28S,Long 29°00E, 900 masl
		15B	446.71	
		16A	868.90	
Highland	Rwerere	15A	715.80	Lat. 1° 29' S, Long. 29° 52' E;2,100 m asl
		15B	-	
		16A	-	

† Rainfall amount received from planting to harvesting

Season A =from September to February, Season B=from March to July

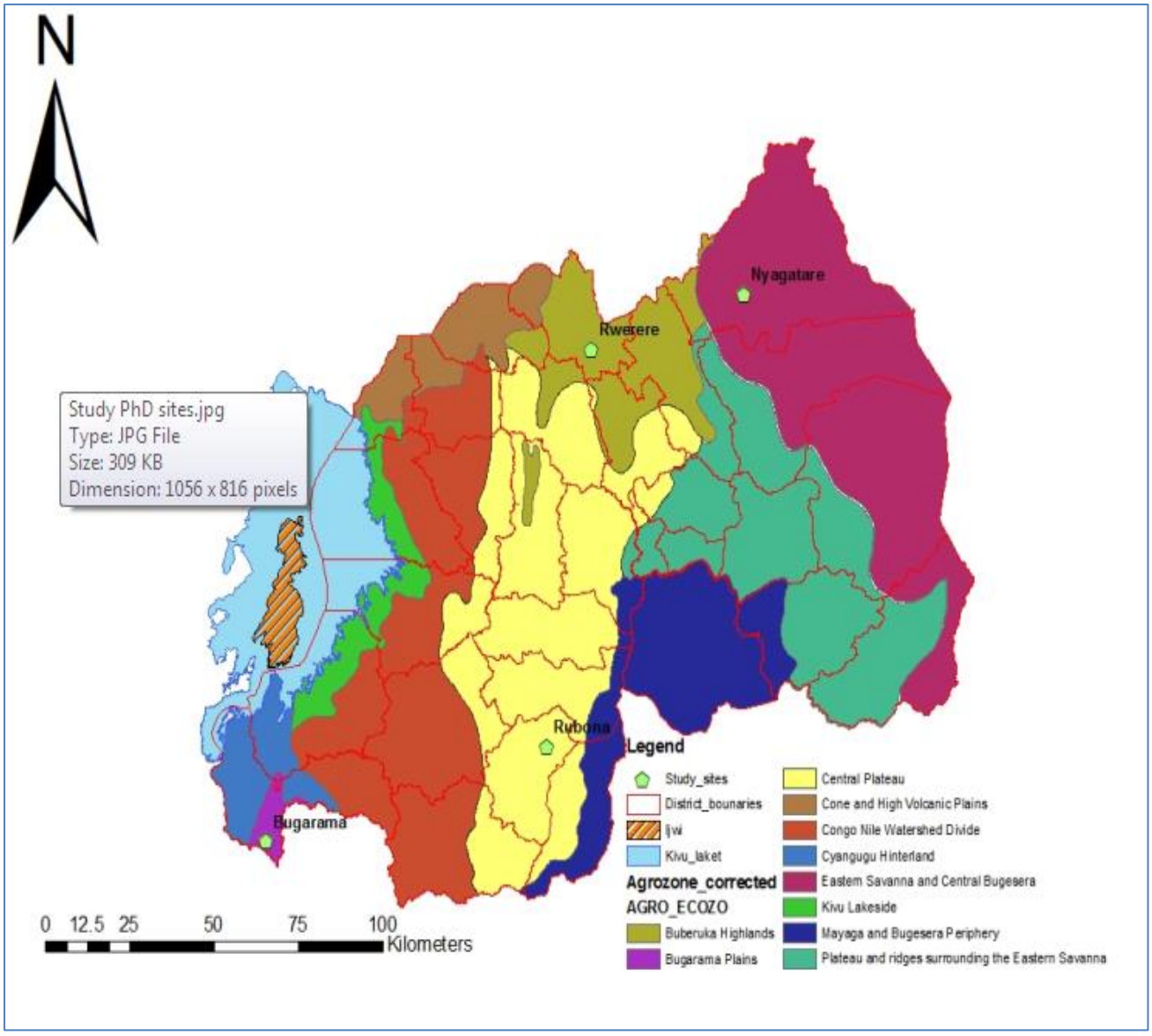

Figure 3.1: Evaluation site locations

3.2.3 Experimental design and trial management

The 45 F1 progenies together with three checks and 10 inbred parents were evaluated at Bugarama, Nyagatare, Rubona and Rwerere during three consecutive seasons; 2015A, 2015B, and 2016A growing seasons representing twelve testing environments. The F1 progenies plus checks and their parents were evaluated in the same sites but in adjacent experiments to avoid competition. A 6 x 8 alpha-lattice design for the progenies and a randomized complete block design for the parental experiment with two replications for each experiment were used. Each plot comprised one row of 5.0 m in length (except Nyagatare site in 16A season and Rwerere site in 16A season where row length was 4.0 m) and 0.75 m

between rows in all experiments, while the intra-row spacing was 0.25 m. The maize seedlings were thinned to one plant per hill giving a stand of approximately 53,333 plants ha^{1}. At all sites, 200 kg/ha of N-P-K (17-17-17) were applied at two weeks after planting. At six weeks after planting, 50 kg N/ha were applied as top dressing using urea (46-0-0). Hand-weeding was done using the hoe when necessary to keep the plots free of weeds. In each agro-ecology, maize genotypes of similar vigour were used as borders.

3.2.4 Data collection

Data were recorded on a plot basis and comprised different variables following standard procedures used at CIMMYT (CIMMYT, 1985). Grain yield (t/ha), as grain mass per plot adjusted to 12.5 % moisture content**.** Field weight (FW) (weight of the harvested ears) per plot was multiplied by 0.80 shelling percentage to obtain grain yield (t/ha), adjusted to 12.5% grain moisture. Grain yield was computed based on the formula:

Grain yield (t/ha) = field weight (kg)/ [(plot size) x (100-grain moisture content) / (100-12.5) x10 x 0.8].

Moisture content (MC) was measured as percentage grain moisture content using a moisture meter at harvest. Days to anthesis (AD), as number of days from planting to 50% of plants shedding pollen and days to silking (SD) as number of days from planting to 50% of plants showing silk emergence while anthesis-silking interval (ASI) was computed as difference between SD and AD. Plant stand (PS) was counted as the number of plants per plot determined at three weeks after planting. Number of plants at harvest (PN) was counted as the number of plants in each plot at harvest, regardless whether plants had one ear, two, or were barren. Ears per plant (EPP) were determined as the number of ears with at least one fully developed grain, expressed as a fraction of the number of plants at harvest. Plant height (cm) (PH) was measured as distance from the base of a plant to the auricle of the flag leaf, while ear height (cm) (EH) was the distance between the ground level and the base of the primary ear. Stalk or stem lodging (SL) was computed as percentage of plants per plot that had their stems broken below the ear and root lodging (RL) was determined as the percentage of plant per plot which had their stems inclined by at least 45^{o}. Plant aspect (PA), ear aspect (EA) and ear texture (ET) were rated using a scale of 1 – 5, where 1 was very good and 5 bad.

Similarly, husk cover (HC) was assessed using a visual scale of 1-5; where 1 designated very short husks and 5 very long as the best husk cover of cob.

Disease scores were mainly focused on major foliar diseases: turcicum leaf blight (*Exserohilum turcicum,*), grey leaf spot (*Cercospora zeina*), phaeosphaeria leaf spot (*Phaeosphaeria maydis*), maize streak virus (MSV), and common rust (*Puccinia sorghi)*. The rating score for all these diseases was based on a 1 to 9 disease scale where 1 denotes clean plants, no disease symptom and 9 indicates high disease severity. The rating scales were as follows; 1 = 0%, 2 = <1%, 3 = 1-3%, 4 = 4-6%, 5 = 7-12%, 6 = 13-25%, 7 = 26-50%, 8 = 51-75%, and 9 = 75-100% leaf surface showing symptoms of the disease. In addition to MSV severity, its incidence was also computed as % of plants with symptoms within a plot.

3.2.5 Data analysis

Data on the measured traits were analyzed using GLM procedures of SAS statistical package version 9.3 (SAS Institute, 2002) complemented by Genstat 17th edition computer software (Payne et al., 2014). Accordingly, significance tests were performed in each and across locations using the analysis of variance (ANOVA). Bartlet homogeneity of variances was performed prior to the combined analysis of variance. A mixed model was used for data analysis. In this regard, genotypes were used as fixed factor while locations, replications and incomplete blocks within replications were considered as random factors.

3.2.6 Estimates of combining ability effects and heterosis

Both the GCA and SCA effects were estimated from inbred parents and crosses, respectively. Standard checks were not used for this analysis. The GCA effects of lines, the SCA effect of crosses, their interactions with the environment as well as their mean squares in each environment and across environments were estimated following Griffing's model 1 (fixed parental effects), method 4 (crosses only) (Griffing, 1956). The following statistical model (Griffing, 1956; Hallauer et al., 2010) for the combined diallel analysis across environments was applied;

$$Y_{ijkl} = \mu + E_e + k(re)_k + g_i + g_j + s_{ij} + gE_{ie} + sE_{eij} + \varepsilon_{ijkl}$$

where Y_{ijkl} is the measurement observed for the ijth cross in the lth environment; μ is the grand mean; E_e is environment effect; $k(re)_k$ is the estimate of the kth incomplete block within replications nested in the environment; $g_i + g_j$ are GCA effects; s_{ij} is the SCA effect; gE_{ie} is the interaction effect between GCA and the environment; sE_{eij} is the interaction effect between

SCA and the environment; while ε_{ijkl} is the error term associated with the ijth cross evaluated in the kth replication and E_e environment.

The restrictions $\Sigma\ gi$=0 and $\Sigma\ sij$=0 were imposed on the combining ability effects. The significance of GCA and SCA effects was verified using a t-test. As the combining ability mean squares were calculated based on cross means of each genotype from each location, the error mean square was used for GCA and SCA significance. The standard errors of the GCA and SCA effects were estimated as the square root of the GCA and SCA variances (Griffing, 1956).

To determine gene action model for different traits, the relative importance of additive and non-additive effects (GCA and SCA, respectively) were estimated according to GCA and SCA mean squares ratios (Baker, 1978). Ratio close to the theoretical maximum of one (unity) indicated the importance of additive genetic effects while ratio much lower than unity implied the importance of dominance genetic effects for a given trait. The formula was as follows:$\frac{2MS_{GCA}}{2MS_{GCA}+MS_{SCA}}$; where MS_{GCA} and MS_{SCA} were the mean squares for GCA and SCA, respectively.

Mid-parent heterosis (MPH) as the performance of the hybrid compared to the average parental performance was calculated as follows (Hallauer et al., 2010);

$$MPH = \frac{\mathrm{F1-MP}}{MP} X100.$$

Further, high-parent heterosis (HPH) as the performance of the hybrid compared to its best parent performance was calculated as follows:

$$HPH = \frac{\mathrm{F1-HP}}{HP} X100;$$

Where F1 is the mean performance of the cross and MP is mean of the two inbred parents and HP is the mean value of the highest performing parent.

Standard heterosis (SH) in addition to mid-parent heterosis (MPH) was computed as:

SH = ((F1- MT)/ MT) *100, where MT = Mean of the testers, best hybrid or the trial mean, F1 = F1 hybrid mean performance.

Heterotic groups were defined using MPH.

3.3 Results

3.3.1 Analysis of variance

The study was effective in discriminating the hybrids for yield and secondary traits. The analysis of variance for individual location displayed significant differences (P<0.001-0.01) among crosses for grain yield and most of the other agronomic traits (data not displayed) in all the twelve testing environments except Rwerere 15B site. Within sites across the three seasons, highly significant differences were observed among crosses for grain yield and most of the other measured traits in all the sites (data not displayed) and effects of season, season x crosses were also significant. Similarly, when all environments were combined (Table 3.3) a highly significant difference was observed among the genotypes for all traits measured. The trend was similar for GCA and SCA, except for the SCA for PH. In addition, the environment variance, GCA x E, SCA x E, genotype x environment interaction were significant for all the traits. However, the magnitude of these interactions were lower compared to the main effects. The proportions of GCA effects for all traits were larger than SCA effects for the combined environments.

Table 3.3: A 10 x 10 diallel cross analysis for grain yield and associated traits over twelve environments in Rwanda

Source	DF	Yld‡	EPP	AD	SD	PH	EH	TLB	MSV
Environments (E)	11	443.350***	1.254***	23030.5***	24666.1***	128362.841***	41826.970***	80.959***	126.27***
E.REP	12	9.954	0.044	34.0	37.1	1054.794	462.366	4.0481	0.6509
Genotypes (G)	44	33.375***	0.104***	166.9***	180.0***	6273.414***	2328.225***	4.6875***	5.4005***
GXE	484	3.879***	0.024***	10.9***	11.2***	294.821***	132.512***	0.8768***	1.5612***
GCA	9	107.121***	0.325***	750.6***	819.1***	25142.361***	9590.9561***	16.666	18.462**
SCA	35	14.411***	0.047*	16.8ns	15.6***	1421.399ns	460.6661 ns	1.6074***	2.042***
GCA x E	99	8.196**	0.037***	17.5***	17.5***	577.400***	229.440***	1.7352*	4.4113**
SCA x E	385	2.769***	0.021***	9.2***	9.6***	222.160*	107.590 ns	0.6561***	0.8283***
Error	528	1.615	0.014	6.0	6.6	189.818	98.839	0.5481	0.4786
Mean		7.376	1.052	76.6	77.8	200.069	97.305	3.3611	1.7676
CV (%)		17.23	11.06	3.20	3.30	6.89	10.22	22.03	39.148

*, **, *** indicates significance at 0.05, 0.01, and 0.001 probability levels, respectively

‡ AD, anthesis days; SD, silking days; EPP, ears per plant; PH & EH, plant & ear height; MSV, maize streak virus; TLB, turcicum leaf blight; yld, grain yield.

DF, Degree of freedom; CV, Coefficient of variation.

3.3.2 Combining ability effects and heterosis

3.3.2.1 General combining ability effects

There were significant differences among maize inbred lines for GCA effects. The GCA effects for grain yield for the combined environments ranged from -0.936 t/ha to 1.184 t/ha (Table 3.4). Sixty percent of the maize inbred lines displayed negative GCA effects for grain yield, with inbred line S5 showing the highest positive (1.184) GCA effects while inbred line S2 had the lowest (-0.936) GCA effects. Similarly for this trait, 50% of the inbred lines exhibited significant variations (P< 0.01-0.0001) for GCA effects with inbred lines S4, S5, S2 displaying the highest or lowest GCA effects. Furthermore, in relation to other traits studied, GCA effects among the inbred lines showed different trends depending on the inbred line and considered trait.

Figure 3.2 shows the GCA effects for grain yield across the three seasons (15A, 15B and 16A) in different locations. The inbred lines S4 and S5 displayed consistently positive GCA effects in all environments with the highest values in NYT location. Contrary to this, S2 and S8 performed poorly in all environments and showed the worst performance in NYT and RBN locations. The other lines exhibited different behavior in various locations.

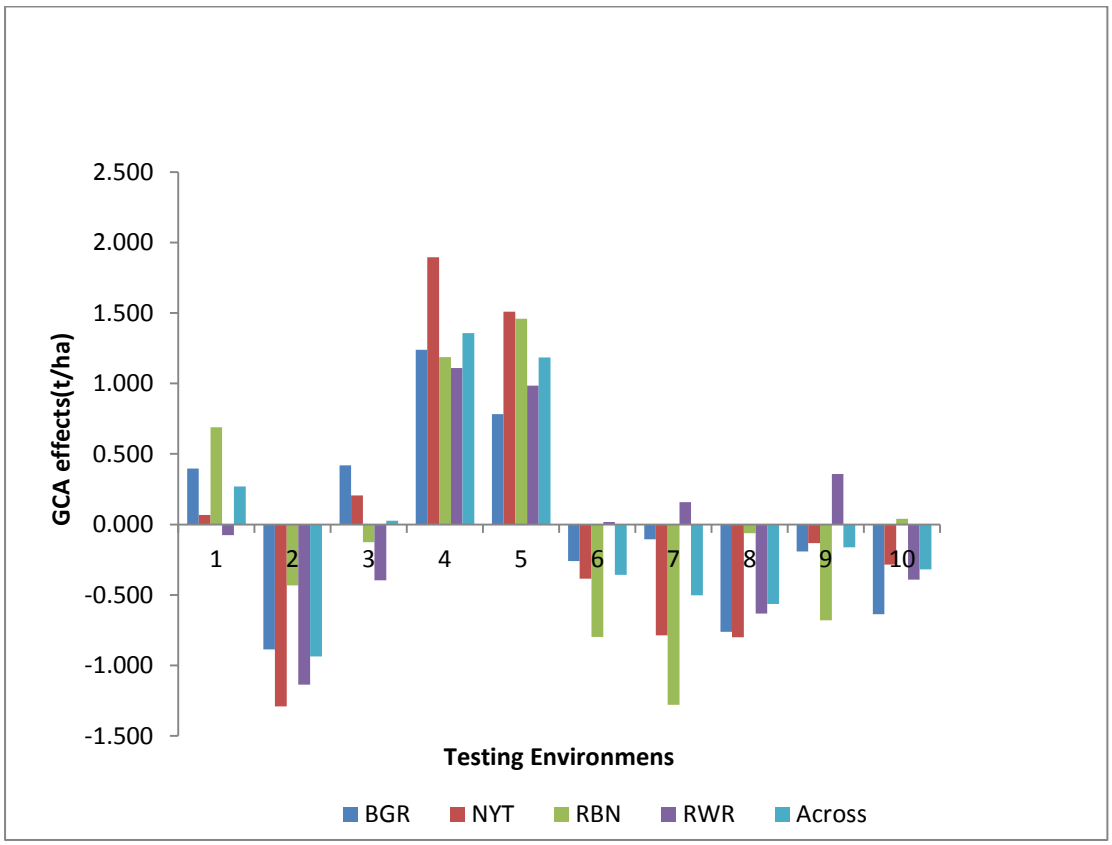

Figure 3.2: GCA estimates for grain yield across 3 seasons in different environments

BGR= Bugarama, NYT = Ntagatare; RBN = Rubona; RWR = Rwerere; across = combined environments. 1= S1; 2=S2; 3= S3; 4= S4; 5= S5; 6= S6; 7= S7; 8= S8; 9= S9; 10= S10.

Table 3.4: Estimates of GCA effects and means for grain yield and other agronomic traits of ten parental inbred lines across 12 environments

Parent	†Yld		EPP		AD		SD		PH		EH		TLB		MSV	
	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA	Mean	GCA
S1	3.42	0.270	1.06	0.038*	80	-1.446*	81	-1.674	126.11	-9.061**	56.88	-3.405	3.2	0.073	1.5	0.053
S2	1.67	-0.936***	0.86	-0.066^{+}	75	-3.066**	76	-3.346**	89.87	-20.066***	44.20	-9.338***	4.1	0.656***	1.3	0.001
S3	3.10	0.026	1.02	0.029*	84	0.752	86	0.883	131.55	0.317	58.79	-1.917	3.3	-0.104	1.9	0.053
S4	2.68	1.358***	1.03	0.068^{+}	87	1.700	86	1.524	149.03	14.958***	82.84	15.992***	3.6	0.021	1.8	0.261*
S5	3.93	1.184***	1.02	0.011*	85	2.075^{+}	87	1.982^{+}	167.46	18.222***	70.84	4.762**	3.5	-0.234**	3.0	0.480***
S6	2.23	-0.356	1.02	-0.041*	86	1.169	88	1.347	132.60	-2.037	63.17	1.233	3.3	-0.260**	1.1	-0.494
S7	2.34	-0.504**	1.03	-0.037*	87	2.075^{+}	89	2.451*	137.10	-0.785	66.68	0.723	3.3	-0.385***	1.1	-0.551***
S8	1.78	-0.564**	0.94	0.017*	86	0.799*	86	0.680	143.26	2.950	55.25	-4.612**	4.9	0.125	1.5	0.053***
S9	3.48	-0.161	0.96	0.002**	81	-1.196**	83	-1.007	163.53	5.311^{+}	77.71	2.724^{+}	3.6	-0.089	1.5	0.058
S10	3.94	-0.318	1.03	-0.023*	77	-2.863**	78	-2.841*	133.05	-9.809***	65.32	-6.161***	3.7	0.198*	2.0	0.084

$^{+}$, *, **, and ***, indicate significance of GCA effects at 0.1, 0.05, 0.01 and 0.001 probability, respectively

† AD, anthesis days; SD, silking days; EPP, ears per plant; Yld, grain yield (t/ha); PH, plant height; EH, ear height; TLB, Turcicum leaf blight; MSV, maize streak virus.

3.3.2.2 Estimates of specific combining ability effects

Hybrids were significantly different for specific combining ability effects. Specific combining ability estimates of the 45 hybrids averaged across the 12 testing environments for major traits are presented in Table 3.5. The SCA effects for grain yield ranged from -3.399 (hybrid S6/S7) to 0.883 (hybrid S7/S8). When arranged by descending order, hybrids such as S7/S8 (0.883), S2/S5 (0.821), S6/S10 (0.781) and S6/S9 (0.749) displayed the highest positive SCA effects. Conversely, by descending order hybrids S6/S7 (-3.399), S1/S10 (-0.954), S1/S2 (-0.813) and S4/S8 (-0.761) displayed the lowest SCA effects for grain yield.

Overall, around 56% of the hybrids had positive SCA effects for grain yield, but only a few of them showed significant SCA effects (Table 3.5).

Table 3.5: Estimates of the SCA effects for 45 single cross hybrids for yield and other agronomic traits evaluated across twelve environments

No	Cross	†Yld	EPP	AD	SD	PH	EH	TLB	MSV
1	S1/S2	-0.813	-0.048	0.041	0.157	-13.243	-6.531	0.493*	-0.155
2	S1/S3	0.140	0.009	-0.777	-0.447	6.133	1.072	-0.163	-0.082
3	S1/S4	0.451	0.008	-0.350	0.079	-2.071	-1.986	-0.372	0.043
4	S1/S5	-0.120	0.026	-0.600	-0.921	5.858	2.693	-0.116	0.074
5	S1/S6	0.282	0.025	0.515	0.547	1.483	1.308	-0.007	-0.160
6	S1/S7	0.534	0.004	0.150	0.235	9.622	7.896	-0.049	-0.186
7	S1/S8	0.679	0.038	-0.948	-1.078	2.478	2.671	-0.101	-0.041
8	S1/S9	-0.200	-0.003	0.796	0.235	-4.610	-0.814	0.155	0.413
9	S1/S10	-0.95	-0.058	1.171	1.193	-5.651	-6.307	0.160	0.095
10	S2/S3	-0.221	-0.028	0.635	0.016	-3.105	4.267	0.337	-0.072
11	S2/S4	-0.074	-0.058	0.604	0.417	1.395	2.179	-0.080	0.220
12	S2/S5	0.821	0.038	-0.063	0.042	-2.412	-0.650	-0.366	0.126
13	S2/S6	0.287	0.023	-1.115	-0.906	-3.830	-5.273	-0.340	-0.025
14	S2/S7	0.219	0.009	-0.438	-0.843	2.582	-0.798	-0.215	0.032
15	S2/S8	-0.345	0.029	0.880	0.803	2.957	2.074	0.149	-0.072
16	S2/S9	0.308	0.001	-0.709	-0.343	9.843	3.181	-0.095	0.006
17	S2/S10	-0.182	0.033	0.166	0.657	5.813	1.551	0.118	-0.061
18	S3/S4	-0.086	0.054	-0.006	0.021	-7.573	-7.199	0.139	-0.082
19	S3/S5	-0.755	-0.024	0.244	0.563	-4.009	-3.346	0.102	0.199
20	S3/S6	0.170	-0.001	0.442	0.115	2.428	2.486	-0.372	-0.285
21	S3/S7	0.346	0.003	-1.589	-1.531	6.226	1.091	-0.247	-0.103
22	S3/S8	-0.177	0.011	0.187	0.573	-6.094	-3.885	0.201	0.293
23	S3/S9	0.015	-0.018	0.890	0.553	2.478	3.290	0.040	0.121
24	S3/S10	0.568	-0.006	-0.027	0.136	3.515	2.225	-0.038	0.012
25	S4/S5	-0.219	-0.009	0.630	0.464	0.838	1.185	0.227	0.491

No	Cross	†Yld	EPP	AD	SD	PH	EH	TLB	MSV
26	S4/S6	0.655	0.004	-0.256	-0.359	7.877	5.114	0.045	0.006
27	S4/S7	0.030	0.059	-0.370	-0.296	0.509	-2.315	-0.080	-0.270
28	S4/S8	-0.761	-0.118	0.239	0.433	-1.020	-0.080	0.243	-0.082
29	S4/S9	0.022	0.035***	-0.641	-0.671	-2.007	-2.009	-0.085	-0.296
30	S4/S10	-0.018	0.025	0.150	-0.088	2.052	5.112	-0.038	-0.030
31	S5/S6	0.172	-0.001	-0.672	-0.484	3.103	1.024	-0.033	-0.379
32	S5/S7	0.622	-0.001	-0.745	-0.796	6.518	1.338	0.259	-0.405
33	S5/S8	-0.064	-0.033	0.197	0.433	3.172	1.301	-0.043	-0.343
34	S5/S9	-0.070	-0.006	1.525	1.579	-4.769	-1.800	-0.205	0.236
35	S5/S10	-0.388	0.010	-0.516	-0.880	-8.299	-1.746	0.175	0.001
36	S6/S7	-3.399***	-0.140	1.703	1.297	-29.256	-11.034	0.826	1.111
37	S6/S8	0.302	0.064**	0.437	0.318	0.508**	-0.185**	-0.101***	-0.160***
38	S6/S9	0.749	0.008*	-0.777	-0.369	8.240	6.334	0.113	-0.124
39	S6/S10	0.782	0.016	-0.277	-0.161	9.447	0.227	-0.132	0.017
40	S7/S8	0.884	0.036	-0.553	-0.244	1.934	-0.076	-0.267	0.105
41	S7/S9	0.231	0.022	0.859	1.068	2.124	3.538	-0.054	-0.108
42	S7/S10	0.533	0.008	0.984	1.110	-0.260	0.361	-0.174	-0.176
43	S8/S9	-0.617	-0.019	-0.365	-0.661	-4.309	-6.058	0.061	-0.046
44	S8/S10	0.099	-0.008	-0.073	-0.578	0.373	4.239	-0.142	0.345
45	S9/S10	-0.439	-0.019	-1.579	-1.390	-6.990	-5.662	0.071	-0.202

+, *, **, and ***, indicate significance of SCA effects at 0.1, 0.05, 0.01 and 0.001 probability, respectively

† AD, anthesis days; SD, silking days; EPP, ears per plant; Yld, grain yield (t/ha); PH, plant height; EH, ear height; TLB, Turcicum leaf blight; MSV, maize streak virus.

Regarding foliar diseases among the 45 hybrids evaluated across the 12 environments (Table 3.5), 58% of the hybrids exhibited negative SCA effects for TLB disease reaction, however, none of these effects was significant. Overall, only 4.5% of the hybrids showed significant SCA effects for TLB. With regards to the other traits under study, 40-56% of the hybrids displayed negative SCA effects depending on the trait.

When averaged across seasons within sites (data not shown), SCA effects for grain yield did not exhibit any clear consistent pattern. However, most of the SCA effects were not significant in all sites. Hybrid S2/S5 displayed the highest positive (1.933) and no significant SCA effects in Rubona station, while hybrid S7/S8 displayed the consistent highest and no significant SCA effects in the three sites (Nyagatare, Rubona and Rwerere stations).

3.3.3 Heterosis and Heterotic patterns and alignment for grain yield and other agronomic traits

There was significant variation for levels of heterosis between the lines. Mean performance of the hybrids and heterosis for grain yield and TLB across the four testing locations in three seasons (15A, 15B and 16A) are summarized in Table 3.6. The percentage mid-parent heterosis (MPH) for grain yield ranged from 36.4% (S6/S7) to 267.7% (S4/S6) with a mean of 164%, while high-parent heterosis (HPH) varied from 33.2% (S6/S8) to 236% (S4/S8) with a mean of 130.4%.

In general, 91% of the crosses exhibited MPH≥100%, whereas 78% of the crosses displayed HPH≥100%. In the top 10 crosses showing high MPH, around 50% of them comprised parent 4 (S4) and 5 (S5), the same parents were involved in the highest yielding cross S4/S5 (9.70 t/ha).

There were also differences for standard heterosis (SH) for yield which was calculated based on relative trial mean (%), relative best check hybrid mean (%), and relative mean of the testers (%) (Table 3.7). Most of the crosses displayed positive SH except for heterosis relative to the highest performing check (best check) where all the crosses exhibited negative SH.

Table 3.6: Means, mid-parent and high parent heterosis for grain yield and TLB across environments

			†YLD			TLB	
			Heterosis			Heterosis	
No	**Cross**	**Mean**	**MPH (%)**	**HPH (%)**	**Mean**	**MPH (%)**	**HPH (%)**
1	S1/S2	5.90	131.5	72.4	4.583	25.7	44.7
2	S1/S3	7.81	139.6	128.4	3.167	-2.6	0.0
3	S1/S4	9.46	209.7	176.5	3.083	-9.2	-2.6
4	S1/S5	8.71	137.0	121.8	3.083	-8.1	-2.6
5	S1/S6	7.57	167.8	121.4	3.167	-1.9	0.0
6	S1/S7	7.68	166.4	124.5	3.000	-7.7	-5.3
7	S1/S8	7.76	198.1	127.0	3.458	-14.4	9.2
8	S1/S9	7.29	110.9	109.1	3.500	3.7	10.5
9	S1/S10	6.37	73.1	86.4	3.792	11.0	19.7
10	S2/S3	6.25	161.9	101.6	4.250	14.0	27.5
11	S2/S4	7.72	254.8	188.0	3.958	2.2	9.2
12	S2/S5	8.45	201.7	115.1	3.417	-10.9	-3.5
13	S2/S6	6.37	226.5	185.5	3.417	-7.9	3.8
14	S2/S7	6.16	207.0	163.2	3.417	-8.4	2.5
15	S2/S8	5.53	220.3	210.3	4.292	-5.1	4.0
16	S2/S9	6.59	155.5	89.0	3.833	-0.5	7.0
17	S2/S10	5.94	111.6	73.7	4.333	11.2	18.2
18	S3/S4	8.67	200.2	180.0	3.417	-1.8	2.5
19	S3/S5	7.83	123.0	99.4	3.125	-9.1	-6.2
20	S3/S6	7.22	170.8	133.0	2.625	-20.8	-21.2
21	S3/S7	7.24	166.5	133.9	2.625	-21.2	-21.2
22	S3/S8	6.66	173.0	115.1	3.583	-13.1	7.5
23	S3/S9	7.26	120.5	108.2	3.208	-7.2	-3.8
24	S3/S10	7.65	117.4	123.7	3.417	-2.4	2.5

			†YLD		TLB		
			Heterosis			Heterosis	
No	**Cross**	**Mean**	**MPH (%)**	**HPH (%)**	**Mean**	**MPH (%)**	**HPH (%)**
25	S4/S5	9.70	193.5	147.0	3.375	-5.8	-4.7
26	S4/S6	9.03	267.7	236.8	3.167	-8.4	-3.8
27	S4/S7	8.26	229.0	208.0	2.917	-16.2	-12.5
28	S4/S8	7.41	231.9	176.3	3.750	-12.2	3.4
29	S4/S9	8.59	178.7	146.6	3.208	-11.0	-11.5
30	S4/S10	8.40	153.6	145.6	3.542	-2.9	-2.3
31	S5/S6	8.38	172.0	113.3	2.833	-17.1	-13.9
32	S5/S7	8.68	177.0	121.0	3.000	-12.7	-10.0
33	S5/S8	7.93	177.9	102.0	3.208	-24.1	-9.4
34	S5/S9	8.33	124.7	112.1	2.833	-20.5	-20.0
35	S5/S10	7.86	99.6	129.7	3.500	-2.9	-1.2
36	S6/S7	3.12	36.4	33.2	3.542	6.9	7.6
37	S6/S8	6.76	236.7	202.9	3.125	-23.9	-5.1
38	S6/S9	7.61	166.2	118.3	3.125	-9.1	-5.1
39	S6/S10	7.48	142.4	118.8	3.167	-9.0	-3.8
40	S7/S8	7.19	249.0	207.5	2.833	-31.3	-13.9
41	S7/S9	6.94	138.4	99.2	2.833	-18.1	-13.9
42	S7/S10	7.09	125.7	107.2	3.000	-14.3	-8.9
43	S8/S9	6.03	129.1	73.2	3.458333	-18.6	-3.5
44	S8/S10	6.59	130.3	92.8	3.541667	-17.5	-3.4
45	S9/S10	6.46	73.9	88.8	3.541667	-2.3	-1.2

† YLD, grain yield (t/ha); TLB, Turcicum leaf blight; MPH, mid-parent heterosis; HP, high parent heterosis.

With regards to major foliar diseases, the most important disease realized was TLB. Heterosis for TLB ranged from -31.7% (S7/S8) to 25.7% (S1/S2) with a mean of -7.9% for MPH and from -21.2% (S3/S6 and S3/S7) to 44.7% (S1/S2) with a mean of -0.80 % for HPH. The hybrid (S1/S2) exhibited the maximum heterosis for both MPH and HPH. In general, most of the hybrids displayed negative heterosis, 60% and 58% respectively for HPH and MPH.

Table 3.7: Top 15 maize hybrid yield across twelve environments with standard heterosis higher than 6% of trial mean

		Relative yield to				
No	**Cross**	**Trial mean (%)**	**Best check (%)†**	**Tester 1(S4) mean (%)**	**Tester 2(S6) mean (%)**	**Tester 3(S7) mean (%)**
1	S4/S5	31.4	-39.2	119.9	164.3	152.1
2	S1/S4	28.1	-19.5	191.3	250.1	234.0
3	S4/S6	22.4	-2.6	252.6	323.7	304.2
4	S1/S5	18.0	-10.2	224.8	290.4	272.4
5	S5/S7	17.6	-22.0	182.4	239.3	223.7
6	S3/S4	17.5	-20.9	186.2	244.0	228.2
7	S4/S9	16.5	-20.0	189.4	247.8	231.8
8	S2/S5	14.4	-24.9	171.7	226.5	211.5
9	S4/S10	13.8	-34.3	137.7	185.6	172.5
10	S5/S6	13.5	-35.6	132.9	179.9	167.0
11	S5/S9	12.9	-20.4	188.0	246.1	230.2
12	S4/S7	11.9	-13.0	214.9	278.5	261.1
13	S5/S8	7.5	-34.3	137.6	185.5	172.4
14	S5/S10	6.4	-36.6	129.5	175.9	163.2
15	S3/S5	6.1	-43.0	106.3	147.9	136.5

† The highest performing check

3.3.4 Heterotic patterns and grouping

New heterotic patterns among inbred lines and their alignment with programme testers were observed. Heterotic patterns are shown in Table 3.6. Among the top 10 hybrids, 60% had S4 as progenitor, 30% were between lines from S4 with the others from the 2 groups (S6 and S7). In addition, the cross S4/S6 was ranked third among the top 10 hybrids, while the cross S4/S7 appeared among the top 12.

Since most of the SCA effects were not significant for grain yield, heterotic alignment was performed based on mid-parent heterosis (Table 3.8). Three varieties (Table 3.1) with known heterotic groups were considered as testers (S4, S6 and S7) and were included in the diallel study to determine heterotic divergence and guide in the discrimination of the seven maize local inbred lines into different heterotic groups.

Table 3.8: Heterotic grouping of the inbred lines using mid-parent heterosis (%)

		Heterosis with Testers (%)			
Line	**Pedigree**	S4	S6	S7	Alignment with testers†
S1	R10164	209.70	167.78	166.38	S6/ S7
S2	RM8147	254.84	226.48	206.97	S7
S3	ACRO29	200.17	170.83	166.53	S6/ S7
S5	ECA13	193.53	267.69	229.03	S4
S8	TQX7	231.90	236.71	248.99	S4/S6
S9	MZ5	178.74	166.17	138.41	S7
S10	POL6	153.58	142.44	125.67	S7

† S4, S6, and S7 heterotic grouping.

All the lines displayed positive heterosis with all the three testers; however, most of the inbreds were inclined towards tester S7 or displayed similar levels of heterosis with both S6 and S7 testers (Table 3.8), while the remainder aligned with either S4 or S4/S6. The highest (267.69%) mid-parent heterosis was realized in the cross: S5/S6. On the contrary, the lowest (125.67%) mid-parent heterosis was observed in the cross: S7/S10.

3.4 Discussion

3.4.1 Combining ability effects and gene action

Significant combining ability effects and its interaction with environments has implications for breeding strategy for the maize programme in Rwanda. Analyzed across seasons in four environments, GCA effects were significant and their mean squares were higher than SCA mean squares for all traits analyzed, suggesting that additive gene action was more important than non-additive in controlling these traits. These findings are consistent with previous studies (Musila et al., 2010; Rovaris et al., 2014; Nepir et al., 2015). This implies that selection processes such as recurrent selection for GCA could be applied in the base populations from which the inbred lines were derived to obtain lines with traits in consideration. However, SCA effects were also significant except for AD, PH and EH implying non-additive effects also played a role in controlling some of the traits and suggests the breeding programme could also benefit from hybridization. The significance of GCA x E and SCA x E for all traits, except SCA x E for EH, indicated that effects associated with these traits for genotypes varied with the environment in the current study.

This is comparable to previous studies (Ali et al., 2012; Rovaris et al., 2014; Nepir et al., 2015). The relative performance of hybrids in this study depended on specific testing environments. Additionally, the highly significant differences observed among genotypes for all traits implied that there were large differences among the performance of the genotypes under this study, while the higher magnitude of mean squares for G and GCA than G x E and GCA x E justifies that environment effects had less influence on the genotypes and additive gene action. A similar trend was reported by other researchers for various crops (Musila et al., 2010; Rovaris et al., 2014; Wegary et al., 2014; Nepir et al., 2015).

For rapid advance of maize inbred lines and hybrids in a breeding programme, GCA and SCA effects should be taken into account as major criteria. Under the current study, lines S4 and S5 displayed significant, consistent positive GCA effects for yield which are desirable implying a positive attribute as good combiners in contributing to increased grain yield in their crosses. High positive GCA values indicate that the parent in question is greatly superior to the other parents in relation to mean progeny performance. Hallauer and Miranda (1988) stated that inbred lines which have superior GCA effects should be retained for further use in a breeding programme. This, therefore confirms, suitability of S4 and S5 inbred lines for inclusion in the Rwanda maize breeding programme and can be used directly for hybrid production. This is in agreement with other earlier studies where positive and

significant GCA effects were also reported for lines useful for use in hybrid production (Rovaris et al., 2014; Nepir et al., 2015). In addition to this, line S5 had desirable significant negative GCA effects for TLB which was the most important disease observed. This line would thus contribute to reduced TLB disease when combined in a hybrid. On the contrary, the consistent negative GCA effects for yield exhibited by line S2, demonstrates a negative contribution by the line in grain yield. In regards to individual locations across seasons, except for S4, S5 and S2, the GCA effects for yield in other lines varied implying their usefulness in specific environments. However, consistent poor performance observed in S2 and S8 indicated their weakness across all the testing environments.

The SCA effects across environments for grain yield were positive and significant for crosses S7/S8 and S2/S5. However, lines S2, S7 and S8 had negative GCA effects for the same trait. This indicated that high yielding hybrids could be gained not only by relying on crossing good x good GCA lines but also by crossing bad x good GCA lines. It was earlier stated (Nepir et al., 2015) that high SCA values indicate the significance of non-additive gene action and thus it is manifested between crosses of two genetically divergent parental lines, mainly due to the preponderance of dominance gene effects. Significantly variable SCA effects observed under the current study among the crosses implied that a breeding strategy based on SCA effects like hybridization could be used to select good hybrids.

3.4.2 Heterosis

Mid-parent heterosis analysis of grain yield in the present study revealed that all hybrids were superior to their parents, suggesting the potential of these inbred lines in hybrid development to exploit hybrid vigor and suggests the positive role of non-additive gene effects. A similar trend was also realized for HPH, highlighting that the newly bred hybrids can perform better than their high parent in grain yield which could be recommended for hybrid production. Consequently, hybrids selected based on both MPH and HPH can be selected for release and/or for further breeding in the maize programme in Rwanda. The level of mean based on mid parent (164%) and high parent (130.4%) heterosis shown for grain yield in the current study was however lower than that previously reported by Nepir et al. (2015) when studying heterosis and combining ability of highland quality protein maize inbred lines. This difference in levels of heterosis might have resulted in dissimilarities of germplasm involved in the two studies.

Furthermore, standard heterosis for yield taken into account based on relative trial mean (%), relative best check hybrid mean (%), and relative mean of the testers (%) revealed that most of the crosses displayed positive SH except heterosis relative to the best check where all the crosses exhibited negative SH.

Therefore, standard heterosis (SH) observed in the crosses for grain yield, indicated that the hybrids had added advantage of being superior to the trial, testers mean and some other checks, except the best check hybrid mean due to negative HS (however, this is not applied when specific individual environments are considered). This implies that selection should also be done based on other advantages when comparing the hybrids of the current study and the checks. In addition to this, not only is grain yield a polygenic trait, it depends also on a large number of other related traits and environments. Hence selection on the basis of grain yield alone is usually not effective. Therefore, selection along with its component characters and specific environments could be more effective and reliable (Fasahat et al., 2016).

In regard to TLB, contrary to positive heterosis preferred for yield, negative heterosis is the desirable effect for TLB due to the rating scale used, where 1 denotes resistance and 9 susceptibility. Therefore, hybrids such as S3/S6 and S3/S7 that exhibited the maximum negative heterosis for both HPH and other hybrids that displayed negative heterosis are the more preferred. However, hybrids combining both positive heterosis for gran yield and negative heterosis for TLB are the most suitable because they have potential to reduce damage caused by the disease resulting in increased yield.

3.4.3 Heterotic patterns and grouping

Heterotic groups A and B at CIMMYT have been aligned similar to some of the well-known heterotic patterns across the globe. These include Tuxpeño vs. ETO Blanco of Mexico, Reid Yellow Dent vs Lancaster of the USA, Kitale vs. Ecuador of the east African highlands and N3 vs SC of southern Africa among other genetic backgrounds. It was cited by previous researchers (Pswarayi and Vivek, 2008) that group A is expected to exhibit heterosis similar to Kitale, Tuxpeño, N3, and Reid, while group B would exhibit heterosis similar to Ecuador, ETO, SC, Blanco, and Lancaster.

Similarly, for the seven local lines and three basic testers of different background, it was possible to demonstrate some heterotic patterns. The seven lines were assigned to four major heterotic groups based on mid-parent heterosis magnitude when crossed to the

testers. Hence, a cross between a line and a tester revealing low mid-parent heterosis level had the line assigned to the same heterotic group as the tester. Although, theoretically no heterotic patterns are expected from crosses of inbred lines from the same group some heterotic patterns have been realized within groups (Fato et al., 2012; Nepir et al., 2015; Richard et al., 2016). It was earlier reported that sufficient MPH could exist between parents of high GCA within the same heterotic groups. This is because in general, tropical maize germplasm is known to have an intra-group diversity that is sufficient to exploit heterosis contributed by additive genetic effects (Pswarayi and Vivek, 2008).

On the other hand, lines exhibiting high magnitude of mid-parent heterosis were aligned to different heterotic groups, implying that good heterotic patterns are expected from crosses of lines identified in different groups (Pswarayi and Vivek, 2008) as realized in cross S5/S6. Therefore, the maize breeding programme in Rwanda can exploit heterosis by crossing the lines from different heterotic groups. As this programme is geared towards development of three-way hybrids, this could be a better opportunity where hybrids could be developed using the two heterotic groups (e.g. A x A' crossed to a line from the group B). Single crosses with higher yield can be developed from higher-yielding as well as good combining inbred lines that belongs to the same heterotic group by largely exploiting additive variance, while retaining the dominance effects to be fully exploited in the final cross of a three-way cross hybrids (Fato et al., 2012; Nepir et al., 2015). In other words, fifty percent of the heterosis will be obtained from the male parent from the group that is opposite to constituent parents of the single cross in a three-way cross hybrid.

Nevertheless, as heterotic patterns are specific to the group of parents being tested, changes might be expected in the heterotic behavior observed in the current study. It was earlier stated (Rawlings and Thompson, 1962) that lines belonging to the same heterotic group may not have absolutely identical heterotic patterns because of small differences in the alleles they may be carrying. Similarly, in this study, lines that were derived from the same genetic background were not necessarily assigned to the same heterotic group. On the other hand, lines derived from different genetic background may have absolutely identical heterotic patterns (Dao et al., 2014). This indicates that genetic diversity of constituent parents of a hybrid is not necessarily correlated with hybrid performance.

3.5 Conclusions

The results of this study revealed the presence of high variability among hybrids for grain yield and other traits. Therefore it would be possible to select maize hybrids that are suitable

for the mid and high altitudes of Rwanda. Maize inbred lines S4 and S5 displayed consistently positive GCA effects in all environments with line S4 qualifying as the best combiner. Among the top10 crosses showing high heterosis, 50% of them comprised parent 4 (S4), the same parent was also involved in the highest yielding cross S4/S5 (9.70 t/ha). This hybrid and others would be used directly as single cross hybrids or as potential single-cross testers for development of three-way hybrids in the maize programme for the mid and highland ecologies of Rwanda. Similarly, in regards to the important disease (TLB), the cross S3/S6 and S3/S7 exhibited the highest desirable heterosis (-31.7%) and could be used in developing three-way maize hybrids resistant/tolerant to TLB. Three maize inbred lines (S4, S6 and S7) that were considered as testers discriminated the seven local lines into three heterotic groups that could form the basis of the maize hybrid programme in Rwanda.

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4 Chapter Four

Heterotic groups, gene action and heterosis among maize inbred lines selected for the major agro-ecologies of Rwanda

Abstract

Maize breeding programmes exploit inbred lines with superior combining ability for grain yield and other agronomic traits to create competitive hybrids. Therefore, the objectives of this study were to determine heterotic groups of locally developed maize inbred lines, their heterotic relationships, genetic distances with exotic testers as well as the gene action controlling the different traits. Nineteen maize inbred lines were crossed to four testers, following a line x tester mating scheme resulting in 76 test crosses. These crosses were evaluated together with four checks in 10 x 8 α-lattice design across four locations in 2015B and 2016A seasons. Both additive and non-additive gene action were important for grain yield with preponderance of additive gene action. The most desirable GCA effects for grain yield were realized in inbred line 8 while the highest desirable SCA effects were displayed by the test cross 18x20. Generally, most of the lines exhibited positive heterosis with all testers. However, there was more aligning firstly towards tester 2 and then to 3. The highest heterosis was displayed by the combination of line 8 with 3. Regardless of the heterotic grouping method applied, the lines were discriminated into different heterotic groups; two and nine heterotic groups were identified based on standard heterosis and SCA effects; respectively. Genetic distance was correlated to heterosis, SCA effects and test cross performance, although this was more oriented to specific testers. The information would be useful in optimizing the maize hybrid breeding programme in Rwanda

Keywords: Gene action, genetic distance, heterosis, heterotic group.

4.1 Introduction

Worldwide and particularly in sub-Saharan Africa, maize (*Zea mays* L.) is a major staple cereal crop serving as human main diet especially for small income families and considerable production area is allocated to this crop (Dao et al., 2014; Ranum et al., 2014).

In Rwanda, maize has become a leading crop in agricultural production and ranks first among pulse and grain crop production in Rwanda. It has seen an unprecedented development and radical changes in the past seven years resulting in increased national production from 96,662 t in 2006 to 525,679 t in 2011 (NISR, 2012). This increased maize production was mainly due to a shift in using only open pollinated varieties (OPVs) towards maize hybrids. However, these hybrids are imported from outside hence the need for development of local maize hybrids with high yield potential. After realizing this problem, the maize programme in Rwanda Agriculture Board (RAB) focused on hybrid development using germplasm from various sources. Therefore, a large number of inbred lines were developed from different adapted and adopted OPVs.

Since knowledge of heterotic groups is important in any hybrid breeding programme (Dao et al., 2014), this study aimed at addressing this aspect with a special focus on the lines developed by the Rwandan maize breeding programme. Heterotic groups enable the exploitation of heterosis in an efficient as well as in a consistent manner through identification of complementary lines that can be used in the crosses. In addition, the heterotic groups can be used to reduce the number of germplasm in a breeding programme while preserving diversity within that germplasm. Heterotic grouping results in maximizing combining ability (Barata and Carena, 2006) while helping the breeder to make documented decisions on suitable hybrid combinations (Fato et al., 2012), thus minimizing the possibility of assessing a high number of undesirable crosses. This concept was also reported (Prasanna, 2012) to be important for the development of climate-change resilient maize cultivars. Thus, breeders have been identifying multiple heterotic groups and patterns to improve maize hybrid breeding or monitor changes in heterotic patterns after prolonged breeding (Fato et al., 2012, Wegary et al., 2013, Richard et al., 2016).

Initiating a maize hybrid breeding programme requires well documented germplasm (parental lines) that can be used, and thus good identification and utilization of heterotic groups and patterns for these lines (Melani and Carena, 2005, Hallauer et al., 2010, Wegary et al., 2013). Generally, broad populations, either from locally adapted or introductions have been used for breeding purposes. However, identification of promising heterotic groups has

also been reported to result from diverse maize gene pools (Melani and Carena, 2005, Hallauer et al., 2010, Semagn et al., 2012). Hence the knowledge regarding germplasm diversity and genetic relationships among breeding materials is invaluable in crop improvement strategies (Mohammadi and Prasanna, 2003)**.**

Furthermore, it has been reported that the best heterotic responses are obtainable when crosses are made between parents originating from genetically diverse populations (Dhliwayo et al., 2009, Fato et al., 2012). Therefore, in any maize breeding programme, it is essential to establish the probable heterotic groups to ensure maximum exploitation of heterotic patterns as this will guide the choice of parents and breeding strategies for the success of maize hybrid production (Bidhendi et al., 2012) hence its implementation to the maize hybrid programme in Rwanda. Molecular markers are useful tools in evaluation of genetic diversity and relationships and in heterotic groups' identification (Semagn et al., 2012). Similarly, Information on heterotic groups could be availed through different mating schemes. However, with established testers for a hybrid-breeding programme, the line x tester mating scheme, was earlier reported by Kempthorne (1957) and Akula et al. (2015) to be simpler and effective in revealing the information. The design offers the possibility of crossing given germplasm to two or more genetically different testers. Consequently, this scheme was applied in the current study. Thus, this study was undertaken to determine heterotic groups prevailing in locally developed maize inbred lines, their heterotic relationships, and genetic distances with exotic testers and mode of gene action governing the traits evaluated.

4.2 Materials and Methods

4.2.1 Germplasm

Nineteen maize inbred lines and four testers (Table 4.1) were involved. The inbred lines were derived from seven populations adapted to the mid-altitudes of Rwanda introduced from the International Maize and Wheat Improvement Center (CIMMYT).The four testers resulted from different genetic backgrounds and were introduced from CIMMYT (Ethiopia and Mexico). These testers were selected among many others based on their adaptability to local conditions and their genetic background. The lines were crossed with the testers, following a line x tester mating scheme and generated 76 test crosses.

Table 4.1: Maize inbred lines and testers involved in the study

No	Line	Pedigree	Heterotic Group	Origin
1	R10164	ISARM101 5-6 (64)	Not Assigned (N/A)	Rwanda
2	R10127	ISARM101 2-3 (27)	N/A	Rwanda
3	ACR3	ACROSS8762 4-5 (3)	N/A	Rwanda
4	ACRO4	ACROSS8762 6-5 (4)	N/A	Rwanda
5	ACR25	ACROSS8762 8-4 (25)	N/A	Rwanda
6	ACRO29	ACROSS8762 4-5 (29)	N/A	Rwanda
7	ACR29	ACROSS8762 4-9 (29)	N/A	Rwanda
8	ECA1	ECAVEL16-STR 9-4 (1)	N/A	Rwanda
9	ECA1ECA5	ECAVEL1/ECAVEL16-STR 3-10 (5)	N/A	Rwanda
10	TQ7	[TUXSEQ]C1 5-8 (7)II	N/A	Rwanda
11	TQX31	[TUXSEQ]C1 3-1 (31)I	N/A	Rwanda
12	MZ1	ZM607-38-4-1-B*4(1)	N/A	Rwanda
13	MZ2	ZM607-79-1-1-B*4(2)	N/A	Rwanda
14	MZ3	ZM607-38-1-1-B*4(3)	N/A	Rwanda
15	POL1	POOL32-70-2-1-B*4(1)	N/A	Rwanda
16	POL2	POOL32-76-1-1-B*4(2)	N/A	Rwanda
17	POL4	POOL32-17-1-1-B*4(4)	N/A	Rwanda
18	POL6	POOL32-6-1-1-B-B(6)	N/A	Rwanda
19	POL7	POOL32-6-3-1-B*4(7)	N/A	Rwanda
	Testers		N/A	
20	1	[POOL9Ac7-SR(BC2)]FS59-4-1-2-1-1-2-1-2-#-#-#-#-#-#	Pool9A	CIMMYT-Ethiopia
21	2	SRSYN95[ECU//SC/ETO]F1-##(GLS=3.5)-20-2-1-1-#-#-#-#-#	Ecuador	CIMMYT-Ethiopia
22	3	[KIT/SNSYN[N3/TUX]]c1F1-##(GLS=2)-23-3-3-1-1-#	Kitale	CIMMYT-Ethiopia
23	4	Pool9AC6HM3-1-3-1-1-2P-2P-1-1-2-1-B-B	AB	CIMMYT-Mexico

4.2.2 Field evaluation and measurements

The resulting 76 test crosses plus four checks were evaluated in an 8 x 10 alpha-lattice design. On the other hand, the 19 parental lines were evaluated in a randomized complete

block design. Both experiments had two replications each with plots consisting of one row of 5.0 m (except Nyagatare site which was 4.0 m length in 16A season) in length with 0.75 m and an intra-row spacing of 0.25 m. They were planted on the same day and managed in the same way. At all sites, 200 kg/ha of N-P-K (17-17-17) were applied at two weeks after planting. At six weeks after planting, 50 kg N/ha was applied as top dressing using urea (46-0-0). Hand-weeding was done using the hoe when necessary to keep the plots free of weeds. In each agro-ecology, maize genotypes of similar vigour were used as borders.

The study was carried out in four research sites representative of major Rwandan maize growing agro-ecologies (Table 3.2). Bugarama site is located in the semi-arid mid-altitude, ranging from 900-1200 metres above sea level (m.a.s.l). Nyagatare and Rubona are located in the moist mid-altitude ranging from 1200-1700 m.a.s.l, while Rwerere is located in the highlands which are above 1700 m.a.s.l. The four sites were used in two consecutive seasons (2015B and 1206A) resulting in eight testing environments. Supplementary information on the experimental sites is given in Table 3.2 and Figure 3.1. Field measurements were performed as described in section 3.3.4 of Chapter 3.

4.2.3 Data analysis

Analysis of variance within and across environments was performed using GLM SAS software programme (SAS Institute, 2002) to test significant differences among the genotypes including checks. This was followed by the line x tester analysis following the general model:

Y_{ijk} = n + r(ek) + ek + li + tj + (l x t) ij + (l x e)ik + (t x e)jk + (l x t x e)eijk + εijk

Where: Y_{ijk} is the measured trait on genotype of ith line crossed with jth tester evaluated in r replications across k environments; n is the overall mean; r (ek) = effect of replication nested within ek environments; ek is the environmental main effects; l and t are average effects of lines and testers; respectively which is equivalent to GCA effects of lines and testers, respectively; l x t is line x tester interaction effects corresponding to the SCA effects of the crosses; l x e, t x e and l x t x e are the interactions of the lines, testers and the lines x testers with the environments, and eijk = a random experimental error.

The linear mixed model was adopted for data analysis. In the analysis, entries were regarded as fixed factors while sites, replications and incomplete blocks within a replication were considered as random factors. Test crosses variation was partitioned into tester and lines main effects then generating two independent estimates of GCA effects (GCA for

testers and for lines), while the interaction of tester and line (tester × line) estimated the SCA effects (Hallauer and Miranda, 1988; Kearsey and Harpal, 1996). Furthermore, GCA effects for individual parents were computed as follows: GCAl = Xl – μ and GCAt = Xt – μ, Where: GCAl and GCAt = GCA of female (line) and male (tester) parents, respectively; Xl and Xt= mean of the female and male parents, respectively; while μ = overall mean of all test crosses. The standard error (SE) for male and female GCA effects were also computed as follows: MSEm = √MSE/rm where MSE= mean square error r = reps; m = number of males; MSEf= √MSE/rf where MSE= mean square error, r = reps; f = number of females.

The effects of SCA were calculated as follows: $SCA_X = X_X - E(X_X) = X_X – [GCAl + GCAt+ μ]$, Where: SCA_X = SCA effects of the two parents in the cross; X_X = observed mean value of the cross; $E(X_X)$ = expected value of the cross based on the GCA effects of the two parents involved; GCAf and GCAm = GCA of line and tester parents, respectively. The standard error (SE) for the SCA effects was also performed as follows: SE = √(MSE/r), Where: MSE = error mean square; r = number of replications.

The significance of GCA and SCA effects were tested by dividing the corresponding GCA and SCA values by their respective standard error and then comparing the obtained t with tabular t-value at error degrees of freedom.

Standard heterosis (SH) was computed as SH = ((F1- MT)/ MT) *100, where MT = Mean of the testers, best hybrid or the trial mean, F1 = F1 hybrid mean performance. Heterotic grouping was defined using SCA and heterosis. When a cross between an inbred line and a tester exhibited high SCA estimates, then that inbred line was assigned to a different heterotic group with that tester and the opposite applied when the cross exhibited a low SCA effect. Similarly, using standard heterosis, an inbred line was classified in the same heterotic group with a tester when there was low standard heterosis with regard to that inbred line relative to the tester and the opposite applied when a high standard heterosis was observed.

4.2.4 Molecular analysis and correlations

Genetic distance (GD) was estimated using data for inbreds and testers which was sent to DNA landmarks for genotyping. However, lines 5, 13 and 14 were excluded from analysis because of missing genetic data. Hence data used in regard to genetic distance in the current chapter involved a total of 16 lines and 4 testers. Detailed information regarding these molecular markers is provided in section 2.2.3. Pearson’s correlations between genetic

distance, grain yield, heterosis and specific combining ability effects were computed using Genstat version 17 computer software (Payne et al., 2014).

4.3 Results

4.3.1 Test crosses variation and gene action

Mean squares for test crosses showed significant differences (P=0.05-0.001) for all traits measured (Table 4.2). However, their interactions with testing environments were also significant except for EPP. Lines mean squares considered as GCA females representing additive gene action were also significant for grain yield and other traits and similar results were observed for lines interaction with environments except for EPP and PH. However, lines mean squares magnitude were more important than the interaction. With regards to mean squares of testers considered as male GCA effects representing additive gene action, significant differences were revealed for all traits and a similar trend was also realized for environment x testers except for EPP. Nonetheless, testers' mean squares magnitude were more important than the interaction.

Considered as SCA effects representing non-additive gene action, line x tester mean squares exhibited significant differences for all traits and a similar trend was observed in their interaction with environments except for EPP, PH and EH. However, the main effects were larger than the interactions.

Table 4.2: Mean squares for yield and other traits across eight environments

Source	DF	†GY	EPP	AD	SD	PH	EH	TLB
Site	7	639.82***	1.02***	48841.73***	49158.91***	179603.71***	62585.70***	128.01***
Test crosses	75	16.37***	0.14*	61.49***	101.63***	4941.00***	980.90***	1.60**
Lines	18	39.41***	0.15	161.95***	202.46***	7533.91***	1730.21***	1.97***
Testers	3	36.84**	0.46**	221.14***	837.57***	6611.59	5019.42***	9.62***
Lines*Testers	54	7.55***	0.12	19.33***	27.67***	3984.49	502.63***	1.03***
Site*Test crosses	525	4.01***	0.10	10.15***	11.60***	3472.00	226.40*	0.94**
Site*Lines	126	4.95***	0.11	11.69***	13.81***	3604.33	251.39***	0.91***
Site*Testers	21	9.32***	0.12	26.49***	38.45***	6354.33**	524.40***	2.87***
Site*Lines*Testers	378	3.40***	0.10	8.61***	9.27*	3267.18	201.25	0.85***
Error	600	2.01	0.10	5.77	7.73	3281.60	173.28	0.49

*, **, and ***, indicate significance at 0.05, 0.01 and 0.001 probability, respectively

† AD, anthesis days; SD, silking days; EPP, ears per plant; GY, grain yield (t/ha); PH, plant height; EH, ear height; TLB, Turcicum leaf blight (score).

4.3.2 General and specific combining ability estimates

4.3.2.1 Estimates of general combining ability effects

Estimates of GCA effects for grain yield as presented in Table 4.3 revealed that 10 inbred lines out of 19 exhibited significant differences. Among these, 4 of them displayed positive GCA effects, with inbred line (8) showing the highest (1.85 t/ha) significant positive GCA effects. This line also exhibited significantly different GCA effects in other traits under the current study except for EPP, however, with GCA effects of various signs. In the negative and significant GCA effects, inbred line (10) displayed the highest negative (-1.38 t/ha) value. With regards to testers involved in this study, none of them were significant for grain yield and this trend was also realized in other traits except testers 22 (3) and 23 (4) which showed significantly different GCA effects for SD, however with different signs.

Table 4.3: Estimates of general combining ability effects for grain yield and other traits across eight environments

Lines	† GY	EPP	AD	SD	PH	EH	TLB
1	-0.31	0.01	-1.38***	-1.73***	-7.95	-1.11	-0.04
2	0.25	-0.02	1.70***	1.63***	-6.40	-5.40*	0.22
3	0.38	0.03	2.29***	2.33***	5.20	7.29***	-0.19
4	0.59*	-0.02	1.04**	1.77***	-8.85	-1.17	-0.23*
5	1.10***	-0.03	2.08***	2.72***	4.25	2.42	0.06
6	-0.03	-0.02	0.29	0.88^{+}	-1.15	-1.63	-0.03
7	0.79***	0.04	1.73***	1.56**	11.96	8.72***	-0.28*
8	1.85***	0.04	2.11***	1.52**	18.99*	9.63***	-0.22
9	0.32	0.07	-1.60***	-1.98***	6.86	0.86	0.21
10	-1.38***	0.05	-0.11	0.05	24.24**	-4.09	0.00
11	0.33	0.14**	1.33***	1.63***	1.26	6.03**	0.03
12	-0.29	-0.02	-0.63	-0.53	-6.72	-2.46	0.00
13	-0.91***	-0.06	-1.38***	-1.25**	2.02	4.63*	0.02
14	-0.66**	-0.08	-0.55	-0.39	-8.30	-5.35*	0.10

Lines	† GY	EPP	AD	SD	PH	EH	TLB
15	-0.62**	-0.04	-0.27	-0.56	2.30	0.17	-0.04
16	-0.91***	-0.06	-2.00***	-2.27***	-5.37	-4.41*	0.42***
17	0.31	0.01	-0.66	-0.55	2.11	-0.33	-0.01
18	-0.19	0.00	-0.75	-0.94*	-17.47	-8.61	0.22
19	-0.61**	-0.03	-3.25***	-3.83***	-16.97	-4.73*	-0.06
‡SEL	0.23	0.05	0.39	0.45	9.29	2.14	0.11
Testers							
20 (T1)	-0.05	-0.02	-0.50	0.17	3.21	4.37	-0.13
21 (T2)	0.21	-0.05	0.02	0.36	3.70	2.32	0.25
22 (T3)	0.31	0.05	-0.71	-2.26*	-0.52	-4.59	0.05
23 (T4)	-0.47	0.02	1.19	1.74*	-6.40	-2.00	-0.13
SET	0.50	0.11	0.85	0.98	20.25	4.65	0.25

*, **, and ***, indicate significance at 0.05, 0.01 and 0.001 probability, respectively, † AD, anthesis days; SD, silking days; EPP, ears per plant; GY, grain yield (t/ha); PH, plant height; EH, ear height; TLB, Turcicum leaf blight (score). ‡ SEL and SET; Standard error for lines and testers, respectively

For the other agronomic traits, in general lines showed different trends (Table 4.3); some lines had significant GCA effects with favorable and unfavorable signs depending on the trait and the corresponding lines. However, none of the lines exhibited significant GCA effects for EPP except for line 11.

4.3.2.2 Estimates of specific combining ability effects

With respect to estimates of SCA effects, most of the test crosses were not significant for grain yield and other traits (Table 4.4). The highest proportion (7%) of significant test crosses was realized for grain yield while it was not significant in test crosses for some traits such as EPP and TLB. Regarding grain yield, both positive and negative significant SCA effects were observed. The highest and desirable significant positive (3.81 t/ha) SCA effect was displayed by the test cross 18x20, while the lowest and undesirable significant negative (-2.94 t/ha) SCA effects was displayed by 12x22 test cross. Lines 12 and 18 were involved in most of the test crosses displaying significant SCA effects.

Table 4.4: Estimates of specific combining ability effects for grain yield and other traits across eight environments

Test Crosses	†GY	EPP	AD	SD	PH	EH	TLB
1X20	-0.20	-0.03	0.51	1.36	2.21	0.56	0.18
1X21	0.20	0.06	0.06	0.84	5.79	-0.26	-0.51
1X22	-0.15	0.00	-0.58	-2.30	-6.75	-0.53	0.25
1X23	0.15	-0.03	0.01	0.10	-1.25	0.13	0.05
2X20	0.78	-0.02	-0.63	-0.07	11.58	6.63	-0.21
2x21	0.06	0.02	-0.08	0.48	-0.61	0.29	0.04
2X22	-0.32	-0.03	0.90	-0.29	-9.42	-4.31	0.05
2X23	-0.52	0.02	-0.19	-0.13	-1.56	-2.72	0.09
3x20	0.06	-0.02	-0.22	0.36	-2.78	-0.99	-0.06
3x21	0.34	-0.03	-0.55	0.09	7.01	4.77	-0.18
3x22	-0.17	-0.03	1.18	-0.43	-6.53	-2.45	0.08
3x23	-0.23	0.09	-0.41	-0.02	2.30	-1.42	0.13
4x20	-0.64	-0.10	-0.53	0.67	-6.40	-6.05	-0.07
4x21	0.57	0.07	1.08	1.03	8.64	4.92	-0.20
4x22	-0.11	0.01	-0.75	-2.99	-5.01	-2.37	0.25
4x23	0.18	0.02	0.21	1.29	2.77	3.39	-0.01
5x20	-0.38	0.00	-0.44	0.72	-4.41	-5.13	0.07
5x21	-0.09	-0.01	0.73	1.01	1.92	-2.39	0.07
5x22	1.06	-0.02	-0.04	-2.82	-2.24	3.27	-0.11
5x23	-0.59	0.02	-0.26	1.09	4.74	4.14	-0.06
6x20	-0.31	-0.05	0.09	0.81	1.28	-1.35	-0.03
6x21	0.19	0.01	0.01	0.05	4.39	1.94	-0.09
6x22	-0.29	0.00	-0.07	-1.22	-6.14	-1.61	0.23
6x23	0.42	0.03	-0.04	0.37	0.47	0.92	-0.16
7x20	-0.19	0.02	0.03	1.12	-10.61	-3.46	-0.03
7x21	0.05	-0.03	-0.11	0.61	2.07	-2.57	0.29

Test Crosses	†GY	EPP	AD	SD	PH	EH	TLB
7x22	-0.08	0.07	-0.88	-3.47	2.14	3.12	-0.08
7x23	0.21	-0.06	0.96	1.74	6.40	2.81	-0.22
8x20	0.22	0.07	-1.22	-0.52	5.50	4.59	0.04
8x21	-0.61	0.00	-0.86	-0.09	1.14	-2.90	0.10
8x22	1.19	-0.06	1.06	-1.36	7.95	3.78	-0.33
8x23	-0.79	-0.01	1.02	1.98	-14.60	-5.57	0.16
9x20	0.01	0.03	0.54	0.79	1.87	-0.14	-0.14
9x21	0.61	-0.01	-0.16	-0.28	9.77	6.10	0.11
9x22	-0.92	-0.03	-0.68	-1.49	-7.58	-1.34	0.31
9x23	0.30	0.01	0.29	0.98	-4.07	-4.72	-0.33
10x20	-0.05	0.09	-0.19	0.01	-29.73	-6.72	0.00
10x21	0.11	-0.01	0.55	0.44	-28.74	2.05	0.32
10x22	0.40	-0.07	-1.04	-2.46	85.99*	6.43	-0.36
10x23	-0.46	-0.01	0.68	2.01	-27.52	-1.86	0.00
11x20	0.03	-0.18	-0.32	0.75	1.63	2.42	0.29
11x21	0.06	-0.16	0.42	1.11	-0.46	-1.48	-0.09
11x22	-0.48	0.49	-1.79	-3.97*	-7.55	-3.87	-0.27
11x23	0.39	-0.15	1.68	2.12	6.38	2.84	0.03
12x20	-2.71**	-0.02	-0.49	-0.41	1.15	0.13	0.13
12x21	-1.25	0.03	1.00	1.08	3.89	0.08	-0.12
12x22	-2.94**	-0.05	-0.65	-1.57	-11.34	-3.17	-0.24
12x23	-1.89	0.04	0.13	0.90	6.30	2.87	0.19
13x20	0.35	0.05	0.76	1.31	5.92	3.19	0.11
13x21	-1.31	0.05	-3.44*	-3.14	-11.36	-10.42	0.05
13x22	1.09	-0.09	1.79	0.78	7.14	8.27	0.06
13x23	-0.13	-0.01	0.88	1.06	-1.70	-1.14	-0.26
14x20	-0.05	0.01	0.87	1.39	-0.40	3.77	-0.03
14x21	-2.00*	-0.11	-2.20	-2.25	-16.83	-17.32	0.22

Test Crosses	†GY	EPP	AD	SD	PH	EH	TLB
14x22	0.62	0.03	2.03	0.73	6.01	5.88	-0.14
14x23	1.43	0.07	-0.69	0.13	11.21	7.57	-0.09
15x20	0.29	0.03	0.84	1.18	-1.06	-4.70	-0.01
15x21	0.62	0.01	0.26	0.42	10.79	9.03	-0.01
15x22	-0.90	-0.04	-0.50	-1.54	-9.10	-1.47	-0.13
15x23	-0.01	0.00	-0.60	-0.07	-0.63	-2.95	0.11
16x20	-0.22	0.04	-0.11	0.01	3.41	-3.12	-0.42
16x21	0.14	0.03	1.87	2.75	-1.42	4.77	0.14
16x22	0.30	-0.05	0.48	-0.02	0.62	3.28	0.15
16x23	-0.21	-0.02	-2.24	-2.74	-2.62	-5.03	0.08
17x20	-0.43	-0.01	0.11	0.79	-1.41	-5.01	-0.17
17x21	-0.15	0.03	0.66	1.16	0.33	1.44	-0.11
17x22	0.84	-0.03	-0.80	-2.43	-11.24	-3.13	0.34
17x23	-0.27	0.01	0.04	0.48	12.33	6.60	-0.11
18x20	3.81***	0.07	-0.43	0.43	12.87	5.52	-0.03
18x21	2.18*	-0.01	0.19	0.55	0.39	-0.65	-0.46
18x22	1.84	-0.05	1.04	-0.35	-20.52	-7.61	0.48
18x23	2.57*	-0.01	-0.80	-0.63	7.26	2.64	-0.03
19x20	0.04	0.01	0.82	1.89	9.37	9.39	0.19
19x21	0.68	0.05	0.56	0.62	3.29	2.11	0.25
19x22	-0.57	-0.05	-0.71	-2.15	-6.42	-2.63	-0.74
19x23	-0.14	-0.01	-0.68	-0.37	-6.23	-8.97	0.25
Error	1.00	0.22	1.70	1.97	40.51	9.31	0.50

*, **, and ***, indicate significance at 0.05, 0.01 and 0.001 probability, respectively, † AD, anthesis days; SD, silking days; EPP, ears per plant; GY, grain yield (t/ha); PH, plant height; EH, ear height; TLB, Turcicum leaf blight (score).

4.3.3 Heterosis and heterotic groups

Standard heterosis for 76 test crosses computed relative to testers (T1-T4), trial mean, best check and mean of checks are presented in Table 4.5. It was revealed that all the test crosses exhibited positive standard heterosis with all testers, with higher heterosis realized in test crosses with testers T1 and T2. With regards to the trial mean, 51% of the test crosses displayed positive standard heterosis with the highest value observed in the test crosses 8/22 (48.9%) and 5/22 (36.03%).

Relative to the best check, only 4% of the test crosses exhibited positive standard heterosis, while 51.3% displayed positive standard heterosis relative to the mean yield for the checks.

Table 4.5: Standard heterosis for grain yield across eight environments for 76 test crosses

No	Test Cross	Relative to †T1 mean (%)	Relative to T2 mean (%)	Relative to T3 mean (%)	Relative to T4 mean (%)	Relative to trial mean (%)	Relative to best check mean (%)	Relative to mean of checks (%)
1	1X20	180.64	75.41	79.10	171.49	-8.08	-24.55	-8.08
2	1X21	209.86	93.68	97.75	199.77	1.49	-16.69	1.49
3	1X22	198.74	86.73	90.66	189.01	-2.15	-19.68	-2.15
4	1X23	177.22	73.28	76.92	168.19	-9.20	-25.47	-9.20
5	2X20	249.42	118.41	123.00	238.04	14.45	-6.05	14.45
6	2x21	229.08	105.69	110.02	218.36	7.79	-11.52	7.79
7	2X22	216.30	97.70	101.86	205.99	3.60	-14.96	3.60
8	2X23	172.69	70.45	74.03	163.81	-10.68	-26.68	-10.68
9	3x20	223.04	101.92	106.17	212.52	5.81	-13.15	5.81
10	3x21	247.36	117.12	121.68	236.04	13.77	-6.61	13.77
11	3x22	228.83	105.54	109.86	218.12	7.70	-11.59	7.70
12	3x23	191.53	82.22	86.06	182.04	-4.51	-21.62	-4.51
13	4x20	201.18	88.25	92.21	191.37	-1.35	-19.02	-1.35
14	4x21	266.78	129.26	134.08	254.83	20.13	-1.39	20.13
15	4x22	240.62	112.91	117.38	229.52	11.57	-8.42	11.57
16	4x23	219.07	99.43	103.63	208.67	4.51	-14.22	4.51

No	Test Cross	Relative to †T1 mean (%)	Relative to T2 mean (%)	Relative to T3 mean (%)	Relative to T4 mean (%)	Relative to trial mean (%)	Relative to best check mean (%)	Relative to mean of checks (%)
17	5x20	235.32	109.59	114.00	224.40	9.83	-9.85	9.83
18	5x21	259.91	124.97	129.70	248.19	17.89	-3.23	17.89
19	5x22	315.31	159.59	165.05	301.78	36.03	11.66	36.03
20	5x23	207.04	91.92	95.95	197.04	0.57	-17.45	0.57
21	6x20	187.97	79.99	83.78	178.58	-5.68	-22.58	-5.68
22	6x21	221.73	101.10	105.33	211.25	5.38	-13.50	5.38
23	6x22	204.89	90.57	94.58	194.96	-0.14	-18.03	-0.14
24	6x23	201.56	88.49	92.45	191.73	-1.23	-18.92	-1.23
25	7x20	230.25	106.42	110.76	219.49	8.17	-11.21	8.17
26	7x21	252.46	120.31	124.94	240.98	15.44	-5.24	15.44
27	7x22	251.21	119.53	124.14	239.77	15.04	-5.57	15.04
28	7x23	229.43	105.91	110.24	218.70	7.90	-11.43	7.90
29	8x20	295.37	147.13	152.33	282.49	29.50	6.30	29.50
30	8x21	270.01	131.27	136.14	257.95	21.19	-0.52	21.19
31	8x22	354.62	184.16	190.14	339.81	48.90	22.23	48.90
32	8x23	231.69	107.33	111.69	220.89	8.64	-10.82	8.64
33	9x20	218.00	98.77	102.95	207.64	4.16	-14.50	4.16
34	9x21	255.93	122.47	127.15	244.33	16.58	-4.31	16.58
35	9x22	192.56	82.87	86.71	183.03	-4.18	-21.34	-4.18
36	9x23	211.91	94.96	99.06	201.75	2.16	-16.14	2.16
37	10x20	139.60	49.76	52.91	131.79	-21.52	-35.58	-21.52
38	10x21	158.09	61.32	64.71	149.68	-15.47	-30.61	-15.47
39	10x22	175.81	72.39	76.02	166.82	-9.66	-25.85	-9.66
40	10x23	102.35	26.48	29.14	95.76	-33.72	-45.60	-33.72
41	11x20	219.43	99.66	103.86	209.02	4.62	-14.12	4.62
42	11x21	232.18	107.63	112.00	221.36	8.80	-10.69	8.80

No	Test Cross	Relative to †T1 mean (%)	Relative to T2 mean (%)	Relative to T3 mean (%)	Relative to T4 mean (%)	Relative to trial mean (%)	Relative to best check mean (%)	Relative to mean of checks (%)
43	11x22	212.91	95.58	99.70	202.71	2.49	-15.87	2.49
44	11x23	216.62	97.90	102.07	206.30	3.70	-14.87	3.70
45	12x20	167.67	67.31	70.83	158.95	-12.33	-28.03	-12.33
46	12x21	243.90	114.95	119.48	232.69	12.64	-7.54	12.64
47	12x22	173.02	70.65	74.24	164.12	-10.58	-26.60	-10.58
48	12x23	185.08	78.19	81.94	175.79	-6.63	-23.35	-6.63
49	13x20	178.27	73.94	77.59	169.21	-8.85	-25.18	-8.85
50	13x21	116.07	35.06	37.90	109.03	-29.23	-41.91	-29.23
51	13x22	227.34	104.60	108.91	216.67	7.22	-11.99	7.22
52	13x23	138.08	48.81	51.94	130.33	-22.02	-35.99	-22.02
53	14x20	171.85	69.92	73.49	162.99	-10.96	-26.91	-10.96
54	14x21	96.34	22.72	25.30	89.94	-35.69	-47.21	-35.69
55	14x22	217.67	98.56	102.74	207.32	4.05	-14.59	4.05
56	14x23	218.92	99.34	103.53	208.53	4.46	-14.26	4.46
57	15x20	188.55	80.36	84.15	179.15	-5.49	-22.42	-5.49
58	15x21	214.68	96.69	100.83	204.42	3.07	-15.40	3.07
59	15x22	151.30	57.08	60.38	143.11	-17.69	-32.44	-17.69
60	15x23	156.55	60.36	63.73	148.20	-15.97	-31.02	-15.97
61	16x20	153.16	58.24	61.57	144.91	-17.08	-31.93	-17.08
62	16x21	180.58	75.38	79.07	171.44	-8.10	-24.56	-8.10
63	16x22	192.14	82.60	86.44	182.62	-4.31	-21.46	-4.31
64	16x23	134.58	46.62	49.71	126.94	-23.17	-36.93	-23.17
65	17x20	198.07	86.31	90.23	188.36	-2.37	-19.86	-2.37
66	17x21	222.03	101.28	105.52	211.54	5.48	-13.42	5.48
67	17x22	270.73	131.72	136.60	258.65	21.43	-0.33	21.43
68	17x23	186.54	79.10	82.87	177.20	-6.15	-22.96	-6.15

No	Test Cross	Relative to †T1 mean (%)	Relative to T2 mean (%)	Relative to T3 mean (%)	Relative to T4 mean (%)	Relative to trial mean (%)	Relative to best check mean (%)	Relative to mean of checks (%)
69	18x20	248.95	118.11	122.70	237.58	14.29	-6.18	14.29
70	18x21	187.65	79.80	83.58	178.28	-5.78	-22.66	-5.78
71	18x22	176.86	73.05	76.69	167.84	-9.32	-25.56	-9.32
72	18x23	174.92	71.84	75.46	165.97	-9.95	-26.08	-9.95
73	19x20	177.97	73.75	77.40	168.92	-8.95	-25.26	-8.95
74	19x21	217.99	98.76	102.94	207.63	4.15	-14.51	4.15
75	19x22	167.00	66.89	70.40	158.30	-12.55	-28.22	-12.55
76	19x23	151.18	57.00	60.30	143.00	-17.73	-32.47	-17.73
Means		2.24	3.59	3.52	2.32	6.85	8.35	6.97

†T1; T2; T3 and T4 testers T1=20, T2=21, T3=22, and T4=23

To document the inbred lines for their heterotic groups and orientations regarding grain yield, various tools were applied. Based on standard heterosis relative to the respective testers, inbred lines were aligned in two different groups: T1/T3/T4 and T1/T2/T3/T4 (Table 4.6). However, some of them displayed some common patterns somehow (Table 4.6).The group T1/T3/T4 comprised only inbred line 14, while the remaining 18 lines aligned to T1/T2/T3/T4 group. Generally, most of the lines exhibited positive heterosis with all testers. However, there was more inclination firstly to tester T2 and then to T3 which had the highest heterosis with the maximum (354.62%) displayed by line 8 with T3. On the contrary, line 14 showed the lowest (96.34%) heterosis with T2.

Table 4.6: Inbred lines heterotic grouping based on standard heterosis relative to testers

	Standard heterosis				Heterotic grouping	
Line	†T1	T2	T3	T4	T1/T3/T4	T1/T2/T3/T4
1	180.64	209.86	198.74	177.22		+
2	249.42	229.08	216.30	172.69		+
3	223.04	247.36	228.83	191.53		+
4	201.18	266.78	240.62	219.07		+
5	235.32	259.91	315.31	207.04		+
6	187.97	221.73	204.89	201.56		+
7	230.25	252.46	251.21	229.43		+
8	295.37	270.01	354.62	231.69		+
9	218.00	255.93	192.56	211.91		+
10	139.60	158.09	175.81	102.35		+
11	219.43	232.18	212.91	216.62		+
12	167.67	243.90	173.02	185.08		+
13	178.27	116.07	227.34	138.08		+
14	171.85	96.34	217.67	218.92	+	
15	188.55	214.68	151.30	156.55		+
16	153.16	180.58	192.14	134.58		+
17	198.07	222.03	270.73	186.54		+
18	248.95	187.65	176.86	174.92		+
19	177.97	217.99	167.00	151.18		

†T1; T2; T3 and T4 testers: T1=tester 1, T2=tester 2, T3=tester 3, and T4=tester 4

By using SCA estimates for grain yield (Table 4.7), inbred lines were discriminated based on the four testers. The lines were assigned into different groups of the testers depending on the direction of the SCA estimate. Except inbred line 11 which aligned with T3 by showing negative SCA estimates, most of the other lines exhibited negative SCA estimates with more than one tester. It was realized that inbred line 12 had a negative sign for SCA estimates with all the testers. On the contrary, inbred line 18, showed consistent positive SCA estimates with all the testers.

Table 4.7: Heterotic alignment of 19 inbred lines based on SCA estimates for grain yield

Line	SCA effects				Grouping of Lines			
	T1	T2	T3	T4	†T1	T2	T3	T4
1	-0.20	0.20	-0.15	0.15	+		+	
2	0.78	0.06	-0.32	-0.52			+	+
3	0.06	0.34	-0.17	-0.23			+	+
4	-0.64	0.57	-0.11	0.18	+		+	
5	-0.38	-0.09	1.06	-0.59	+	+		+
6	-0.31	0.19	-0.29	0.42	+		+	
7	-0.19	0.05	-0.08	0.21	+		+	
8	0.22	-0.61	1.19	-0.79		+		+
9	0.01	0.61	-0.92	0.30	+		+	
10	-0.05	0.11	0.40	-0.46	+			+
11	0.03	0.06	-0.48	0.39			+	
12	-2.71	-1.25	-2.94	-1.89	+	+	+	+
13	0.35	-1.31	1.09	-0.13		+		+
14	-0.05	-2.00	0.62	1.43	+	+		
15	0.29	0.62	-0.90	-0.01			+	+
16	-0.22	0.14	0.30	-0.21	+			+
17	-0.43	-0.15	0.84	-0.27	+	+		+
18	3.81	2.18	1.84	2.57				
19	0.04	0.68	-0.57	-0.14			+	+

†T1; T2; T3 and T4 testers: T1=tester 1, T2=tester 2, T3=tester 3, and T4=tester 4

4.3.4 Genetic distance and relationships for grain yield

Genetic distances (GD) between inbred lines and testers as well as within testers are presented in Table 4.8. High mean (0.383) GD was observed among lines with tester T3. The maximum GD (0.468) was realized between the tester 3 with line 3. A similar trend (Table 4.6) was realized in this tester where the highest heterosis (354.62%) was realized with line 8, whereas, minimum GD (0.254) was displayed by L2xT2.

Table 4.8: Genetic distance between 19 inbred lines and 4 testers for grain yield

	Genetic distance			
Lines	**T1**	**T2**	**T3**	**T4**
1	0.274	0.332	0.332	0.354
2	0.273	0.254	0.305	0.309
3	0.340	0.409	0.468	0.429
4	0.319	0.311	0.326	0.391
6	0.329	0.339	0.390	0.395
7	0.268	0.412	0.395	0.465
8	0.427	0.354	0.413	0.407
9	0.402	0.460	0.427	0.410
10	0.370	0.401	0.384	0.432
11	0.342	0.328	0.338	0.343
14	0.319	0.293	0.373	0.338
15	0.313	0.337	0.406	0.303
16	0.276	0.371	0.360	0.360
17	0.341	0.403	0.386	0.320
18	0.272	0.387	0.429	0.407
19	0.301	0.428	0.401	0.383
Min	**0.268**	**0.254**	**0.305**	**0.303**
Mean	**0.323**	**0.364**	**0.383**	**0.378**
Max	**0.427**	**0.460**	**0.468**	**0.465**
Testers				

T1	0.000	0.418	0.361	0.432
T2	0.418	0.000	0.397	0.358
T3	0.361	0.397	0.000	0.424
T4	0.438	0.358	0.424	0.000

Regarding correlation (Table 4.9), genetic distance GD was significantly and positively correlated with grain yield related to tester T4. This also applied for GD with SCAT1 and HT4, while significant and negative correlation was observed between GD and SCAT4.However, in relation to individual testers (T1-T4) various trends were observed (Table 4.9).

Table 4.9: Pearson's correlations of Genetic distance, Standard heterosis, SCA effects and test crosses performance for grain yield across eight environments

	†GD	GDT1	GDT2	GDT3	GDT4	HT	HT1	HT2	HT3	HT4	SCAT1	SCAT2	SCAT3	SCAT4	Yld	YldT1	YldT2	YldT3	YldT4
GD	-																		
GDT1	0.61	-																	
GDT2	0.86	0.29	-																
GDT3	0.84	0.38	0.72	-															
GDT4	0.76	0.21	0.60	0.51	-														
HT	0.21	0.37	-0.03*	0.10	0.23	-													
HT1	0.15	0.23	-0.08^{+}	0.19	0.15	0.86	-												
HT2	0.27	0.25	0.21	0.10	0.26	0.76	0.60	-											
HT3	0.17	0.44	-0.09^{+}	0.02*	0.18	0.84	0.62	0.43	-										
HT4	0.07^{+}	0.23	-0.15	0.03*	0.14	0.80	0.61	0.43	0.63	-									
SCAT1	0.03*	-0.26	0.02*	0.27	0.07^{+}	-0.05^{+}	0.40	-0.16	-0.23	-0.13	-								
SCAT2	0.20	-0.26	0.37	0.25	0.23	-0.07^{+}	0.15	0.36	-0.43	-0.30	0.59	-							
SCAT3	0.11	0.02*	-0.01	0.17	0.16	0.13	0.27	-0.31	0.41	0.02*	0.54	0.00**	-						
SCAT4	-0.06^{+}	-0.34	-0.04*	0.16	0.06^{+}	-0.20	0.04*	-0.43	-0.33	0.19	0.68	0.28^{+}	0.40	-					
Yld	0.21	0.37	-0.03*	0.10	0.23	1.00	0.86	0.76	0.84	0.80	-0.05^{+}	-0.07^{+}	0.13	-0.20	-				
YldT1	0.15	0.23	-0.08^{+}	0.19	0.15	0.86	1.00	0.60	0.62	0.61	0.40	0.15	0.27	0.04*	0.86	-			

	†GD	GDT1	GDT2	GDT3	GDT4	HT	HT1	HT2	HT3	HT4	SCAT1	SCAT2	SCAT3	SCAT4	Yld	YldT1	YldT2	YldT3	YldT4
YldT2	0.27	0.25	0.21	0.10	0.26	0.76	0.60	1.00	0.43	0.43	-0.16	0.36	-0.31	-0.43	0.76	0.60	-		
YldT3	0.17	0.44	-0.09^{+}	0.02*	0.18	0.84	0.62	0.43	1.00	0.63	-0.23	-0.43	0.41	-0.33	0.84	0.62	0.43	-	
YldT4	0.07^{+}	0.23	-0.15	0.03*	0.14	0.80	0.61	0.43	0.63	1.00	-0.13	-0.30	0.02*	0.19	0.80	0.61	0.43	0.63	-

†GD, genetic distance, GDT1; GDT2; GDT3 and GDT4, genetic distance with tester 1,2,3 and tester 4 respectively; HT, mean heterosis for all testers,HT1 ,HT2, HT3 and HT4, heterosis relative to tester 1, 2, 3, and tester 4 respectively; SCA, specific combining ability, SCAT1, SCAT2, SCT3 and SCAT4, specific combining ability relative to tester 1, 2, 3, and tester 4 respectively; Yld, grain yield, YldT1, YldT2, YldT3 and YldT4, grain yield for tester 1, 2, 3 and tester 4 respectively;

4.4 Discussion

4.4.1 Gene action and test crosses variation

Significant differences among test crosses realized for all traits showed that the test crosses were adequately different from each other for these traits and thus implying a possibility of selecting most desirable test crosses for these traits. Similar findings were previously reported (Fato et al., 2012; Akula et al., 2015).

Mean squares of lines and testers for grain yield and other traits representing GCA females and males, respectively were significant and greater than lines x testers mean squares suggesting preponderance of additive gene action. Therefore, selection procedures such as recurrent selection for GCA in the base populations could be applied for improvement of these traits. Furthermore, line x tester mean squares representing SCA effects were significant for grain yield and other traits, thus denoting the importance of non-additive gene action as well, indicating that these traits could be improved through development of hybrids between the complementary inbred lines and testers. The main effects showed interactions with environments, indicating different performances under different environments. However, the main effects mean squares were much higher such that they masked the effect of these interactions. Diverse ecologies and more replications for testing would be recommended for precise results. Similar findings were reported by other researchers working on different maize genotypes (Musila et al., 2010; Abrha et al., 2013, Wegary et al., 2013, Abdel-Moneam et al., 2014, Rovaris et al., 2014).

4.4.2 Combining ability effects

Estimates of GCA for individual lines revealed some favorable general combiners for grain yield. Among these, inbred line 8 showed the highest value (1.85 t/ha) and could thus contribute favorable alleles for the development of new varieties for increased yield. This line and others having similar GCA estimate patterns exhibited their value as testers in selection for high yield. These lines identified as good combiners could, therefore, be utilized in maize improvement programmes for improvement of the traits of interest as they have high potential of transferring desirable traits to their cross progenies in mid-altitudes and highlands. They can be used directly for hybrid production such as in three-way hybrids where they can be used as males and the single cross hybrids with high levels of heterosis

as females. These results are in line with reports by Rovaris et al. (2014). On the contrary, other lines such as inbred line 10 showed negative significant GCA effects (-1.38t/ha) and were observed to be poor combiners contributing to reduced grain yield. The GCA estimate was reported by Rovaris et al. (2014) as an important tool for the breeder to select better parents. This is because a low estimate, whether positive or negative, indicates that the GCA value of the parent, obtained based on its hybrid combinations, does not differ greatly from the general mean of the other populations assessed. On the other hand, high positive or negative GCA values indicate that the parent in question is greatly superior or inferior to the other parents in relation to mean progeny performance.

With regards to estimates of SCA effects, though most of the test crosses were not significant for grain yield, the highest positive and significant SCA estimates realized in test crosses such as 18x20 implies the presence of good specific combiners in the germplasm under the current study. However, the opposite applies for some test crosses such as 12x22 that showed the highest undesirable SCA effects for grain yield. Significant positive SCA effects for the test crosses indicated a significant deviation from what would have been predicted based on performance of the parents. Therefore, these test crosses with highly positive and significant estimates of SCA effect could be selected based on their specific combining ability and used in maize improvement programme.

With regards to testers involved in this study, none of them were significant for GCA effects for grain yield and this trend was also realized in other traits except testers 3 and 4 which showed significant GCA effects for SD although with different signs. Possibly, testing the current inbred lines using more testers could provide different trends with regard to GCA effects significance.

4.4.3 Heterosis and Heterotic groupings

Genetic variation and heterosis are the basic reasons that many breeding programmes always prefer hybrid maize rather than open pollinated varieties or synthetic varieties (Ali et al., 2012). Similarly, the positive standard heterosis values realized in the current study demonstrated the potential available in some of the test crosses. High heterosis exhibited with testers 1 and 4 implies that heterosis in the current germplasm could be maximized by crossing specific lines with these two testers. This was also emphasized in some specific test crosses that exhibited higher heterosis than the best checks showing their usefulness in the maize breeding programme than the currents checks. This is in agreement with previous reports on the maize crop (Ali et al., 2012; Wegary et al., 2013; Rovaris et al., 2014)

With regards to heterotic groups, though it was possible to reveal some patterns, heterotic grouping based on standard heterosis classified the lines into two groups (T1/T3/T4 and T1/T2/T3/T4) and each group comprising more than one tester. This implies that high heterosis could be expected from crosses of same inbred lines aligning with many testers. Therefore, breeding management of these inbred lines should take into account the two groups. Possibly, classifying these lines based on more/or other testers would have availed more clusters and specific heterotic groups not formed by one or two testers. Similar findings were earlier reported on different maize germplasm (Fato et al., 2012; Rovaris et al., 2014).

However, using the magnitude of SCA estimates for grain yield, inbred lines were classified into nine groups. Inbred lines in crosses showing low magnitude of SCA effects were aligned to the same heterotic group, while those displaying high magnitude of SCA effects belonged to different heterotic groups (Fato et al., 2012; Wegary et al., 2013). Only two lines (9 and11) were aligned to a heterotic group composed by one tester (T3) whereas the remaining lines were aligned to heterotic groups formed by more than one tester. Therefore, discriminating the current lines based on more testers would have enhanced the probabilities of identifying test crosses with larger specific combining ability effects and heterotic groups composed by one or two testers.

Discrepancies in number of heterotic groupings provided by SCA and heterosis grouping in maize were earlier reported by other researchers (Menkir et al., 2003; Wegary et al., 2013; Richard et al., 2016) who pointed out that heterotic grouping can be influenced by the method used in assigning lines to the groups. Some lines 4,6,15 and 19 were aligned based on their origin and were consistent with their pedigree alignment and specific combing ability heterotic grouping. This was also in agreement with reports by Wegary et al. (2013), though the germplasm used is different.

4.4.4 Genetic distance and correlations with specific combining ability and heterosis

The analysis showed high means for GD among lines and tester T3. Generally, a high genetic distance between pairs of lines indicates unrelatedness between the lines and high heterosis could be expected from a cross between them. Hence high heterosis (354.62%) exhibited by test cross 8xT3 tallied with the high genetic distance (0.413) shown by this test cross. High genetic distances mostly realized between lines with testers T3 and T4 corresponded with the positive and significant correlations observed between GD withT3 andT4, with heterosis (HT3 and HT4), grain yield (YldT3, YldT4) and with specific combing

ability effects (SCAT1 and SCAT4) which is in agreement with prior findings by de MC. Pinto et al. (2003) and Wegary et al. (2013).

Test crosses performance for grain yield in relation to some testers (T1 and T4) was also shown to be correlated positively and significantly with the SCA effects (SCAT3 and SCAT4), showing that SCA effects with these testers were effective in predicting hybrid performance compared to *per se* performance of their parents. This might be supported by favorable SCA effects realized in test crosses such as 18 x T1, 18xT2 and 18xT4 which were among the high yielding entries. A similar trend was reported by Drinic et al. (2002) where a high correlation between genetic distance and SCA (0.63) was observed. However, it was also noted (Reif et al., 2003; Wegary et al., 2013) that a significant genotype x environment interaction for grain yield affected the correlation between SSR markers and yield or yield heterosis, which might have been the case in the current study where yield was averaged across 8 environments.

4.5 Conclusions

In general, the results of this study revealed the importance of both additive and non-additive gene action in controlling grain yield and some other traits. Therefore, not only selection would be effective for yield improvement in the current germplasm but also in developing and identifying superior hybrids. The promising test crosses could be exploited for future breeding work as well as for direct release. The magnitude of standard heterosis observed in the current test crosses guarantees the development of commercial hybrids, as some of the test crosses out-yielded the best check. Heterotic grouping based on different methods classified lines differently. However, regardless of the method used, the four testers discriminated the current lines in different heterotic groups allowing their rational breeding management and initiating hybrid breeding programme in Rwanda. Genetic distance was correlated to heterosis, SCA effects and test cross performance but this was related to specific testers. Information generated from the current findings might be useful for laying a foundation for hybrid maize programme in Rwanda and for other researchers for high yielding maize variety development.

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5 Chapter Five:

Genotype x Environment interaction and stability analysis of diallel and test cross maize hybrids across tropical medium and highland ecologies

Abstract

Genotype x environment (G x E) interaction is the differential performance of genotypes across environments, especially in the tropics where seasonal and spatial variability is large. This results in serious challenges of product selection across environments. The objectives of this study were to determine G x E interaction and yield stability of new diallel cross and test cross hybrid and to identify suitable genotypes for the medium and highland ecologies in Rwanda. One set of 76 test cross hybrids plus two commercial control hybrids, and another set of 45 diallel cross hybrids and three commercial control hybrids were evaluated in four locations representing the major agro-ecologies of Rwanda over seasons. The test crosses (line x tester) were evaluated over two seasons; while diallel crosses were evaluated over three seasons. Therefore, environments were defined by site and season combination. The data were subjected to genotype and genotype by environment interaction (GGE) biplot analysis, using Genstat statistical package. The analysis revealed three mega-environments for test crosses and two mega-environments for diallel crosses, which discriminated the hybrids. Two test cross hybrids ACR29 x 21 and ECA1 x 22 and two diallel crosses R10164 x ET4 and ET4 x ECA13 displayed specific adaptation qualifying them as candidates for further testing in respective mega-environments. Test crosses 19(ACR25 x ET21), 29(ECA x ET5) and 69(POL6 x ET5) and two diallel crosses R10164 x ET4 and ET4 x ET9 demonstrated high yield and stability. Overall, the study revealed crossover interaction and need to breed for both broad and specific adaptation in these medium and high altitude environments.

Key Words: Genotype by environment interaction, GGE biplot, maize grain yield, Stability.

5.1 Introduction

Maize is an important staple crop for Sub-Saharan Africa, including Rwanda. It grows in a wide range of environmental conditions from sea level to highlands in the region (Nzuve et al., 2013; Ngaboyisonga et al., 2014; Shi et al., 2015; Ngaboyisonga et al., 2016). Therefore, grain yield of maize is highly influenced by genotype x environment interactions (GxE). It is prudent to characterize the behavior of new experimental hybrids such as test crosses and the diallel cross hybrids in medium (800 - 1600 m above sea level) and highland (>1600 m) environments in East Africa. Similar maize production environments are found in Southern Africa and elsewhere.

There are also other factors that call for GxE analysis of experimental hybrids in the region. Currently maize is exposed to changing environmental conditions. These include biotic and abiotic stresses due to global climatic changes that influence behaviour of hybrids in space and time. Maize growing areas are changing because of its displacement from its traditional production belts by higher-value crops such as vegetables. It is increasingly being grown in more difficult and marginal production environments, which are characterised by declining soil organic matter, reduced soil fertility, and soils with low water-holding capacity among other challenges in tropical areas and developing countries. These dynamic environmental conditions are particularly evident in sub-Saharan Africa including Rwanda where limited resources do not allow additional inputs and irrigation to be supplied (Bänziger and Cooper, 2001; Nyombayire et al., 2011). Production of maize grain is dominated by smallholder farmers (less than 3 ha) who lack the means to condition the environment. This calls for development of high yielding stable hybrids.

The consequences of environment and genotype interaction in the selection and release of improved genotypes cannot be ignored. For this reason, plant breeders have been striving to develop genotypes with superior and stable grain yield, quality and other desirable characteristics over a wide range of environmental conditions. Genotype x environment (G x E) interaction is one of the main complications in the selection of broadly adapted varieties in many breeding programmes. Various studies (Fan et al., 2007; Bisawas et al., 2014) have shown that a proper understanding of the environmental and genetic factors causing the interaction as well as an assessment of their importance in the relevant G x E system could have a large impact on plant breeding. In many countries including Rwanda, research programmes are regularly testing many varieties in various locations and for several years before giving recommendations to farmers of which varieties to grow where (Fan et al., 2007; Sallah et al., 2009; Bisawas et al., 2014). In this regard, newly developed maize

hybrids, from diallel cross and line x tester mating were evaluated in multi environment trials across agro-ecologies in Rwanda. The objectives were to determine G x E interaction and yield stability and identify suitable hybrids for medium and highland ecologies.

5.2 Materials and Methods

5.2.1 Germplasm

Two sets of germplasm were evaluated under this study; one trial comprising 45 F1 diallel crosses from a 10 x 10 diallel cross and three commercial hybrid checks: RH104, PAN4m-19 and SC637. The second trial comprised 76 test crosses from the 19 x 4 Line x Tester mating, respectively, and two commercial hybrid checks: PAN4m-19 and H629. The commercial hybrid checks used are registered and widely grown by farmers in Rwanda. The details related to the test germplasm are provided in the materials and methods in section 3.3.1 of Chapter 3 and section 4.2.1 of Chapter 4.

5.2.2 Sites descriptions

Details to this section are provided in the materials and methods in section 3.3.2 of Chapter 3.

5.2.3 Field evaluation and measurements

The four evaluation sites described in section 3.2.2 of Chapter 3 were used to evaluate this germplasm. The environments were defined by site and season combination. The 45 diallel crosses and their respective checks were evaluated in three consecutive seasons (2015 season A (season A=from September to February), 2015 season B (season B=from March to July) and 2016 season A) making a total of twelve testing environments, while the test crosses and their respective checks were evaluated under the same four sites in two consecutive seasons (2015 season B and 2016 season A) making a total of eight testing environments.

Field measurements were performed on a plot basis and followed standard procedures used at CIMMYT (CIMMYT, 1985). The following variables were measured: Grain yield (t/ha), as grain mass per plot adjusted to 12.5 % moisture content**.** Field weight (FW) (weight of the

harvested ears) per plot was multiplied by 0.80 shelling percentage to obtain grain yield (t/ha), adjusted to 12.5% grain moisture. Grain yield was computed based on the formula:

Grain yield (t/ha) = field weight (kg)/ [(plot size) x (100-grain moisture content) / (100-12.5) x10 x 0.8].

Moisture content (MC) was measured as percentage grain moisture content using a moisture meter at harvest. Days to anthesis (AD), as number of days from planting to 50% of plants shedding pollen and days to silking (SD) as number of days from planting to 50% of plants showing silk emergence while anthesis-silking interval (ASI) was computed as difference between SD and AD. Plant stand (PS) was counted as the number of plants per plot determined at three weeks after planting. Number of plants at harvest (PN) was counted as the number of plants in each plot at harvest, regardless whether plants had one ear, two, or were barren. Ears per plant (EPP) were determined as the number of ears with at least one fully developed grain, expressed as a fraction of the number of plants at harvest. Plant height (cm) (PH) was measured as distance from the base of a plant to the auricle of the flag leaf, while ear height (cm) (EH) was the distance between the ground level and the base of the primary ear. Stalk or stem lodging (SL) was computed as percentage of plants per plot that had their stems broken below the ear and root lodging (RL) was determined as the percentage of plant per plot which had their stems inclined by at least 45^{o}. Plant aspect (PA), ear aspect (EA) and ear texture (ET) were rated using a scale of 1 – 5, where 1 was very good and 5 bad.

Similarly, husk cover (HC) was assessed using a visual scale of 1-5; where 1 designated very short husks and 5 very long as the best husk cover of cob.

Disease ratings mainly focused on major foliar diseases: turcicum leaf blight (*Exserohilum turcicum*), grey leaf spot (*Cercospora zeina*), phaeosphaeria leaf spot (*Phaeosphaeria maydis*), maize streak virus (MSV), and common rust (*Puccinia sorghi)*. The rating score for all these diseases was based on a 1 to 9 disease scale where 1 denotes clean plants, no disease symptom and 9 indicates high disease severity. The rating scales were as follows; 1 = 0%, 2 = <1%, 3 = 1-3%, 4 = 4-6%, 5 = 7-12%, 6 = 13-25%, 7 = 26-50%, 8 = 51-75%, and 9 = 75-100% leaf surface showing symptoms of the disease. In addition to MSV severity, its incidence was also computed as % of plants with symptoms within a plot.

5.2.4 Data analysis

Prior detailed analyses, to determine the existence of G x E interaction on grain yield, data from individual sites was first submitted to ANOVA using Genstat 17th edition computer software (Payne et al., 2014). Genotypes were also treated as fixed effects and environments (both temporal and spatial), replications within environments and blocks within replications were considered as random effects. This analysis was complemented by the use of genotype main effect (G) and genotype-by-environment (G x E) interaction (GGE) biplot analysis (Yan et al., 2007). The GGE biplot model was applied based on singular value decomposition (SVD) of principal components as follows:

$$\bar{Y}_{ij} - \mu_i - \beta_j = \sum_{k=1}^{t} \lambda_k \alpha_{ik} \gamma_{jk} + \varepsilon_{ij}$$

Where: $\bar{Y}_{ij}$ =the performance of genotype i in the environment j, μ = the grand mean, β_j = the main effect of environment j, *k* is the number of principal components (PC); λ_k is singular value of the kth PC; and α_{ik} and γ_{jk} are the scores of ith genotype and jth environment, respectively for PC_k; while ε_{ij} is the residual associated with genotype i in environment j.

The analysis was interpreted based on studies by Yan and Yan et al. (2000, 2006, 2007, and 2011). To assess visual relationships among genotypes and their testing environments, the GGE biplot based on the PCA of environment-centred data was applied (Meseka et al., 2008; Yan et al., 2000). Ideal genotypes were the ones showing high PC1 values (related to high mean grain yield) and PC2 values close to zero. On the other hand, the best testing environments were those providing better discrimination of the genotypes (show a high PC1 value) and PC2 values close to zero (Yan et al., 2000; Yan et al., 2011).

5.3 Results

5.3.1 Polygon view of the GGE biplot analysis for test cross hybrids

Based on the GGE biplot (Figure 5.1), the first two PCs explained 62.84% (PC1=47.90 and PC2=14.94%) of the total GGE variation for grain yield. The polygon view is useful in visualizing the “which won-where” pattern of the multi-environment trials. It provides a good visualization of crossover G x E interactions (Yan and Tinker, 2006). It was drawn such that

environments fall into different sectors of the polygon with the sectors divided by perpendicular lines drawn to each side of the polygon starting from the biplot origin. High yielding genotypes for each sector appeared on the vertices of the polygon (Yan et al., 2007).

The plot in Figure 5.1 was divided into six sectors based on the rays of the biplot where the eight environments were grouped into two major sectors. There was one big sector comprising six environments (NYA, RBA, BGA, BGB, RWA and RWB), while the other small sector comprised two environments (NYB, RBB). Therefore, the analysis revealed the presence of two mega-environments. Additionally, the 76 test cross hybrids and the two checks were distributed in the six sectors where genotypes 26 (7 x 21) was a winner in the small mega-environment, genotype 31 (8 x 22) won in the bigger mega-environment, while genotypes 40 (10 x 23) and 54 (14 x 21) were winners but in low yielding environments

None of the checks displayed clear pattern of winning in the two mega-environments.

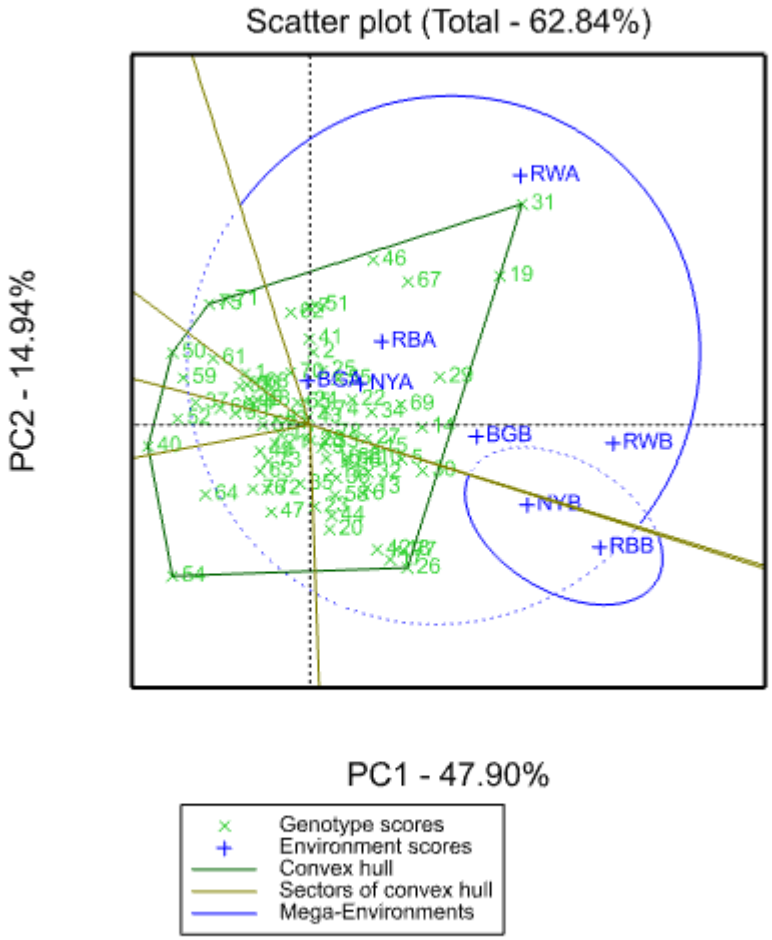

Figure 5.1: Polygon view of the GGE biplot based on grain yield for 76 test crosses (G1-G76) across eight environments (location x season).Environments are: NYA=Nyagatare first season, NYB= Nyagatare second season; RBA=Rubona first season; RBB=Rubona second season; BGA=Bugarama first season; BGB=Bugarama second season; RWA=Rwerere first season and RWB=Rwerere second season.

5.3.2 Polygon view of the GGE biplot analysis of diallel cross maize hybrids

Figure 5.2 presents the schematic view of mega-environment classification and the winning genotypes. Based on the GGE biplot (Figure 5.2), the first two PCs explained 66.12% (PC1=52.58 and PC2=13.55%) of the total GGE variation for grain yield. Consequently, eight sectors were drawn from the polygon, where environments fell into three sectors

representing mega-environments. The environments were grouped as follows: six environments BG1, BG2, BG2, RW1, RW2 and NY2 in one sector, five environments RB1, RBB, RB2, NY1 and NYB in another sector and one environment RWB appeared in its own sector. The vertex genotype for the mega-environments composed by the 6 environments was genotype 25(S4/S5), whereas vertex genotype for the mega-environment of five environments was genotype 3(S1/S4) and the sector with one environment was genotype 34(S5/S9).Though vertex single crosses 15(S2/S8), and 36(S6/S7) were observed, none of these genotypes fitted in any of the mega-environments as they were displayed out of all the mega-environments. Genotype 39 (S6/S10) and others were also located very close to the origin while others were located far from the origin. Both checks (genotype 47 and 48) were located in the same mega-environment with genotype 47 closer to the origin.

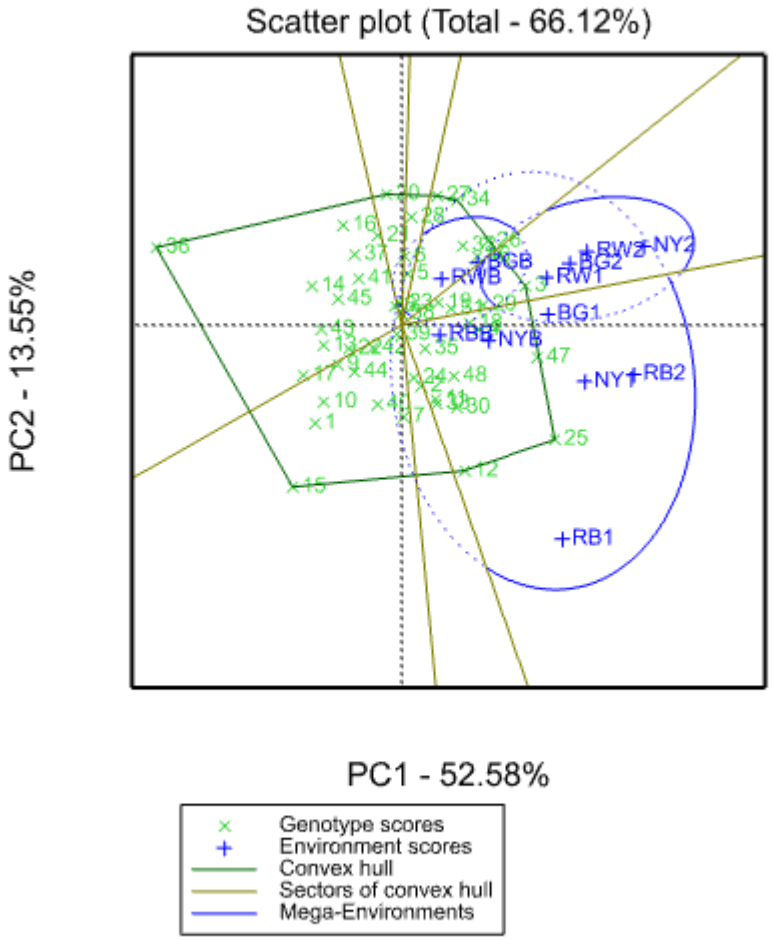

Figure 5.2: Polygon view of the GGE biplot based on grain yield for 45 diallel cross hybrids (G1-G45) across twelve environments (location x season).Environments are: NY1=Nyagatare first season; NYB=Nyagatare second season; NY2=Nyagatare third season; RB1=Rubona first season; RBB=Rubona second season; RB2=Rubona third season; BG1=Bugarama first season; BGB=Bugarama second season; BG2=Bugarama third season; RW1=Rwerere first season; RWB Rwerere second season and RW2=Rwerere third season

5.3.3 Ranking of test cross hybrids based on mean performance and stability

Figure 5.3 displays the average environment coordination (AEC) view of the GGE biplot showing stability and mean performance ranking of genotypes.

It was possible to reveal the best means as well as the stability of the tested genotypes. This was achieved by drawing an average environment coordinate (AEC) on the genotype-focused biplot. The arrow headed line points to higher mean yield across environments while the crossing lines point to greater variability (poor stability) in either direction. Consequently, in descending order, the highest yielding groups of genotypes were: 31,19,29,67 and 69. On the other hand, the worst yielding genotypes in ascending order were: 40, 54, 52, 50 and 59. With regards to stability represented by short crossing line from AEC, genotypes 29, 69, 34, and 64 demonstrated stability, whereas genotypes 26, 31, 46, 67, 71 and 75 exhibited lack of stability represented by longer crossing line from AEC. Regarding the checks, hybrid 77 exhibited yield above mean with some level of variability. No clear pattern was displayed by the other check.

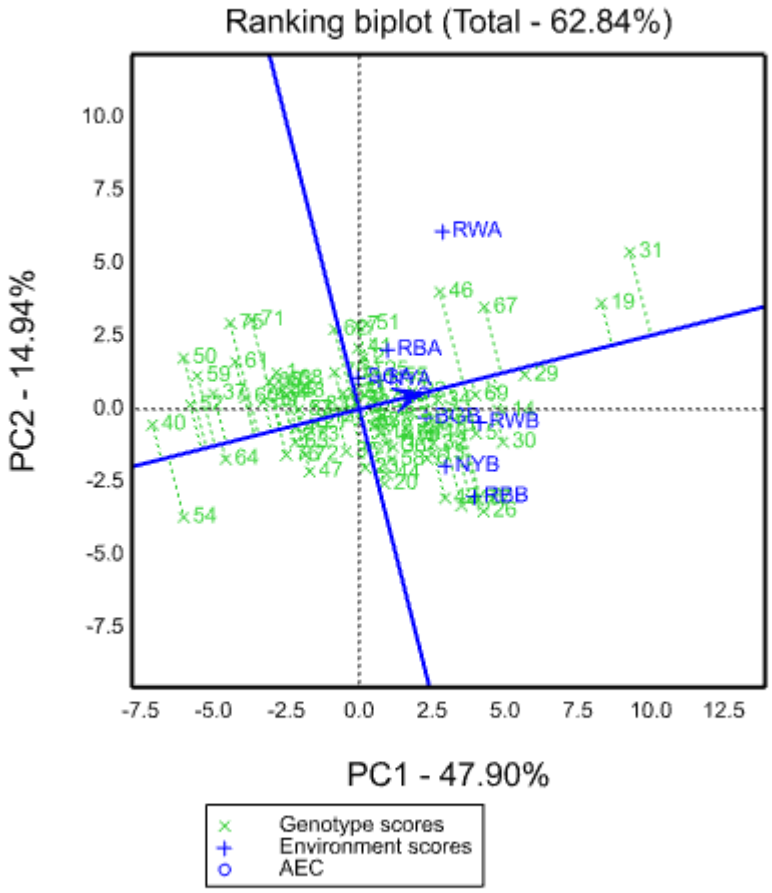

Figure 5.3: Biplot of the average environment coordination (AEC) view showing mean performance and stability of all test cross genotypes (G1-G78) across eight environments (location x season).Environments are: NYA=Nyagatare first season, NYB= Nyagatare second season; RBA=Rubona first season; RBB=Rubona second season; BGA=Bugarama first season; BGB=Bugarama second season; RWA=Rwerere first season and RWB=Rwerere second season.

5.3.4 Ranking of diallel cross hybrids based on mean performance and stability

Figure 5.4 displays the average environment coordination (AEC) view of the GGE biplot showing stability and performance ranking of diallel cross maze hybrids across twelve environments. High performance as well as the stability of the tested genotypes was revealed. This was achieved by drawing an average environment coordinate (AEC) on the genotype-focused biplot. The arrow headed line points to higher performing genotypes across environments while the crossing lines point to greater variability (poor stability) in either direction. It was revealed that high yielding hybrids were 3, 25, and 29. On the contrary, hybrids 1, 15, 17 and 36 exhibited the poorest yields. Regarding hybrids stability across the testing environments, hybrids 3, 13, 29, and 42 demonstrated high stability. On the other hand, the worst stable hybrids were 12, 15, 25, 27 and 34. Both high yield as well as high stability were displayed by hybrids 3, and 29. This trend was also exhibited by one of the checks (47) while the other check did not display a clear pattern.

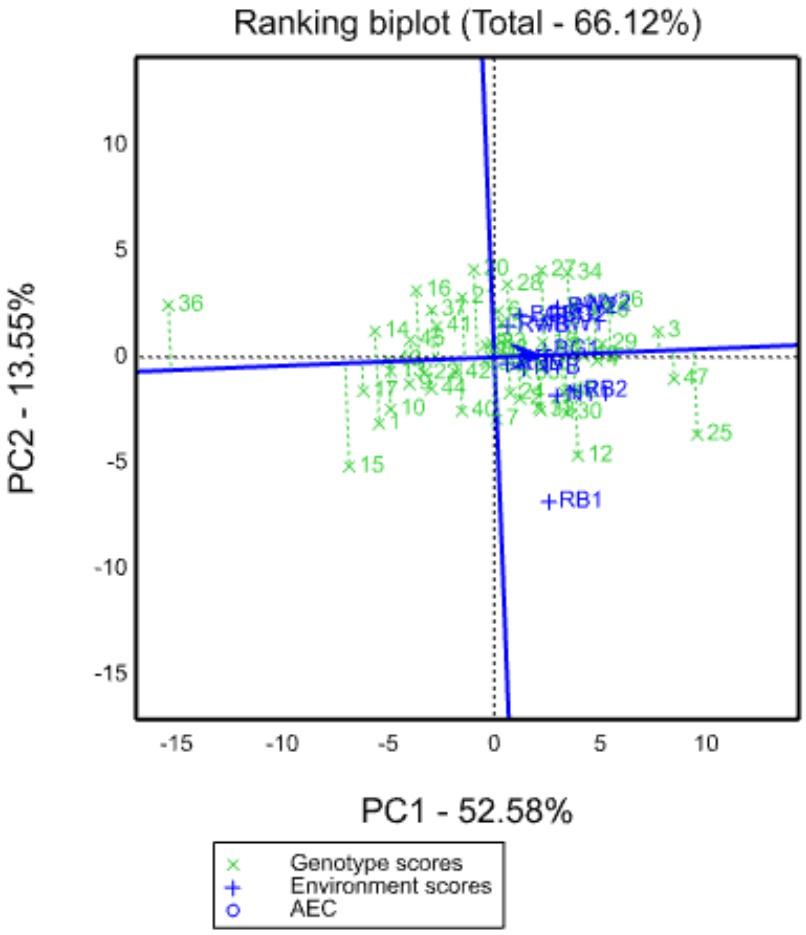

Figure 5.4: Biplot of the average environment coordination (AEC) view showing mean performance and stability of diallel cross hybrids (G1-G48) across twelve environments (location x season).Environments are: NY1=Nyagatare first season; NYB=Nyagatare second season; NY2=Nyagatare third season; RB1=Rubona first season; RBB=Rubona second season; RB2=Rubona third season; BG1=Bugarama first season; BGB=Bugarama second season; BG2=Bugarama third season; RW1=Rwerere first season; RWB Rwerere second season and RW2=Rwerere third season

5.3.5 GGE biplot showing the discriminating power and representativeness for test cross hybrids

To reveal environment patterns (Figure 5.5), environmental vectors were drawn from the biplot origin to join the environments for genotypes evaluation based on environment focused scaling.

Based on the length of vectors from the biplot origin, the eight environments clustered into three groups. Environments RWA, RWB and RBB formed their own group displaying the longest vectors from the biplot origin, followed by the group of environments composed by RBA, BGB and NYB and lastly the group of shortest vectors formed by two environments: BGA and NYA.

With regards to angles among environments as approximated based on the cosine of the angle between the vectors of two environments, all the eight environments showed an acute angle (less than 90).The widest angle was observed between environments RWA and RBB while the smallest angle was realized between environments BGB and RWB. It was also observed that all the sites in season A were grouped together based on the angle among them.

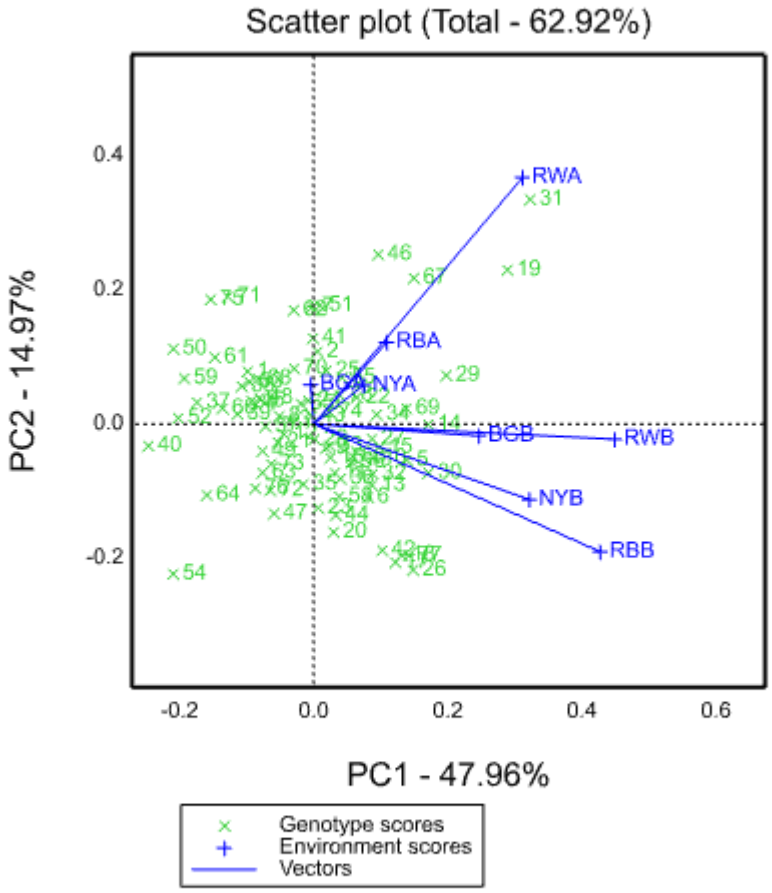

Figure 5.5: GGE biplot based on grain yield for eight environments (location x season) showing the relationship among the environments for test crosses (G1-G78). Environments are: NYA=Nyagatare first season, NYB= Nyagatare second season; RBA=Rubona first season; RBB=Rubona second season; BGA=Bugarama first season; BGB=Bugarama second season; RWA=Rwerere first season and RWB=Rwerere second season.

5.3.6 GGE biplot showing the discriminating power and representativeness of the test environments for diallel cross hybrids

Environment patterns to display different behavior of diallel cross hybrids were revealed in Figure 5.6. Environmental vectors were drawn from the biplot origin to join the environments for genotypes evaluation based on environment focused scaling.

Except environment RB1 displaying obtuse angles (greater than 90) with environments BGB and RWB, the rest of the eleven environments exhibited among them an acute angle (less than 90), however, with variable angle size among environments. The smallest acute angle was observed in three groups of environments; group: NY2, RW1, RW2 and BG2, followed by group RBB, NYB and RB2, also followed by group: BGB and RWB.

Regarding the length of vectors from the biplot origin to discriminate the genotypes. The twelve testing environments aligned into three groups. Environments RB1, RB2,and NY2 clustered in their own group with the longest vectors from the biplot origin followed by the cluster of NY1, BG1, RW1, RW2, and BG2 and finally the cluster of RWB, BGB, RBB and NYB.

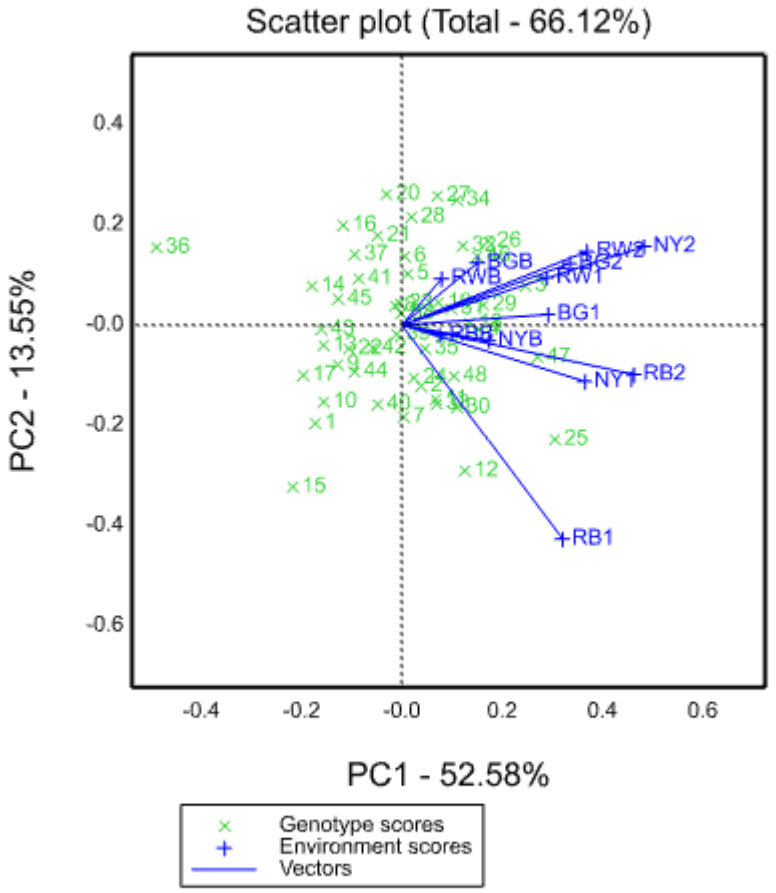

Figure 5.6: GGE biplot based on grain yield for twelve environments (location x season) showing the relationship among the environments for diallel cross hybrids (G1-G48). Environments are: NY1=Nyagatare first season; NYB=Nyagatare second season; NY2=Nyagatare third season; RB1=Rubona first season; RBB=Rubona second season; RB2=Rubona third season; BG1=Bugarama first season; BGB=Bugarama second season; BG2=Bugarama third season; RW1=Rwerere first season; RWB Rwerere second season and RW2=Rwerere third season

5.4 Discussion

5.4.1 Polygon view of GGE biplot analysis of test cross and diallel cross maize hybrids

Polygon view of GGE biplot analysis was required to present the schematic view of mega-environment classification and point out genotypes possibly suitable to specific mega-environments (Yan et al., 2007; Nzuve et al., 2013). It was earlier reported that a mega-environment denotes a group of fairly homogeneous environments steadily sharing the best genotypes (Yan et al., 2007; Meseka et al., 2008). With regards to test cross hybrids, variation explained by the two PCs was high (62.84%). This revealed that the GGE biplot was efficient in representing variation due to G and G x E. A similar trend was also revealed in single cross hybrids where the two PCs explained 66.12% of the variation.

Regarding hybrids response in different environments, various mega-environments were identified (two and three mega-environments respectively for test cross and single cross hybrids). These mega-environments displayed different high yielding genotypes thus indicating presence of cross-over G x E interaction and inconsistent performance for these genotypes across environments. It was reported that dividing the target environments into different mega-environments and deploying different hybrids in these mega-environments is helpful to make use of GEI (Gauch and Zobel, 1997).

Different test cross hybrids (G26, G31 and others) and single cross hybrids (G3, G25 and others) were located on the vertices of the polygon and then identified as winning genotypes in different mega-environments. These winning hybrids are environment specific and can be recommended for production in their respective mega-environments as more responsive to environments, while the remaining hybrids were less responsive to environments. It was earlier (Yan and Kang, 2003) pointed out that cultivar evaluation within a mega-environment should be based on both mean performance and stability to avoid the random GEI rather than trying to exploit it.

Although the hybrids were specific to certain mega-environments, they were more fitting in environments where they were closer within these mega-environments.

Some hybrids were located on the vertices of the polygon however not fitting in any of the mega-environments, suggesting that such hybrids were among the superior hybrids but in lower yielding environments.

5.4.2 Hybrids rank based on mean yield and stability

According to Yan and Tinker (2006), genotypes exhibiting both high mean performance and high stability across environments are qualified as ideal genotypes. Consequently, under this study, test cross hybrids 19, 29 and 69, diallel cross hybrids 3 and 29 and check 47 in diallel crosses displayed both high mean yield and high stability. High yield was defined using the single-arrowed line which is the AEC abscissa pointing to higher mean yield across environments. On the other hand, high stability was defined using the perpendicular lines in either direction, the shorter the line for a genotype from the AEC line, the higher he stability of that genotype. It implies that these stable hybrids were broadly adapted and were consistently ranked the same across environments under the current study. On the other hand, test crosses 31, 46 and 67 and diallel crosses 12, 25 and 26 exhibited high mean yield but were unstable, suggesting their adaptation to specific environments.

5.4.3 GGE biplot showing the discriminating power and representativeness of the test environments for test cross hybrids

Environment patterns were revealed based on the length of vectors drawn from the biplot origin based on environment focused scaling and the cosine of the angle between the vectors of two environments (Yan, 2002; Sibiya et al., 2012; Nzuve et al., 2013). Consequently, all and eleven testing environments respectively for test cross and single cross hybrids were positively correlated. They had an acute angle between them (less than 90). However, the strength of correlation among them varied following the size of their acute angle among them. Hence environments RWA, RBA and BGA for test crosses and NY2, RW2, BG2 and RW1 for diallel crosses were revealed as redundant testing environments and the similar applied to environments BGB and RWB. These environments displayed very small angles showing strong positive associations among them across the two seasons of evaluation. Therefore, the presence of close associations among test environments suggests that a single environment could have sufficed to obtain information on the hybrid genotypes to reduce the cost and increase breeding efficiency

If two test environments are closely correlated consistently across years, one of them can be dropped without loss of much information about the genotypes (Yan and Kang, 2003; Yan and Tinker, 2006).

With regards to discriminating the test crosses and diallel cross hybrids, all environments clustered into three groups based on the length of vectors from the biplot origin. Hence environments RWA, RWB and RBB for test crosses and RB1, RB2 and NY2 for the diallel crosses were identified as the most discriminating. This is because they had longer vectors than other environments for the genotypes. The vector length of an environment measures the discriminating power of its ability to differentiate the cultivars (Yan and Tinker, 2006; Kamut et al., 2013), signifying that these three environments were the best for genetic differentiation of the genotypes. On the contrary, environments BGA and NYA for test crosses and RWB, BGB, RBB and NYB for the diallel crosses appeared the least discriminating. This was justified by their very short vectors and qualified as non-discriminating test environments hence considered as less useful because they provided little discriminating information about the genotypes. According to Yan and Tinker (2006), test environments that are consistently non-discriminating (non-informative) provide little information on the genotypes and, therefore, should not be used as test environments.

5.5 Conclusions

Applying GGE biplot analysis under the current study enabled the visual comparison and identification of superior genotypes and environments for breeding purposes in variety selection and making assured recommendation in different environments of Rwanda. Different mega-environments were revealed among the genotypes studied justifying presence of variation in Rwandan environments regarding genotype separation. Hybrids such as 26 and 31 among the test crosses and 3, 25 and 34 among the diallel crosses were identified as winning genotypes in mega-environments and could be recommended for production in their respective mega-environments. The test cross hybrids 19, 29 and 69, diallel crosses 3, 29 and check 47 were qualified as high yielding and highly stable genotypes and can be used as a reference genotype for evaluation and used for broad selection. Some of the testing environments displayed strong positive association among each other suggesting that a single testing environment could have been recommended to obtain sufficient information on the genotypes for rational resource management.

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6 Chapter Six

Overview of research findings

6.1 Introduction

In Rwanda, agriculture contributes to more than 30% of the GDP and employing over 70% of the population and thus a significant contributor to poverty reduction. Hence, in recognition to its potential in economic development, food security and poverty reduction, Rwanda has set a very ambitious agriculture agenda aiming at an annual average growth of 8.5% over the course of EDPRS II[5] (2013-2018) (NIS, 2015). Maize (*Zea mays* L.), is one of the staple crops in Rwanda that contributes to national economic growth. However, scarcity of maize seed of varieties withstanding production constraints is a major problem. The current chapter highlights the study objectives with subsequent summary on major findings for each objective, and their implications toward a sustainable hybrid maize programme in Rwanda.

This study was formulated to address the following objectives:

1. To determine the genetic distances and clusters among potential maize inbred lines selected for the mid-altitudes and highlands of Rwanda;

2. To estimate the general and specific combining ability, heterosis and gene action for grain yield;

3. To determine heterotic groups and heterotic patterns among Rwandan newly developed and introduced maize inbred lines from a line x tester mating scheme, and diallel crosses respectively; and

4. To investigate the magnitude of G x E interactions and stability of new hybrids for grain yield in the target environments.

[5] Second Economic Development and Poverty Reduction Strategy (EDPRS II) for 2013-2018. EDPRS II aims to implement Rwanda's Vision 2020, ensuring that the country achieves middle-income status by 2020 by accelerating economic growth to (11.5% average), reducing poverty to below 30%, and restructuring the economy towards services and industry.

6.2 Major Findings

6.2.1 Genetic diversity among maize inbred lines selected for the mid-altitudes and highlands of Rwanda

Seventy one maize inbred lines from different sources were genotyped with ninety two SNP markers. It was revealed that;

- There was a random allocation of the inbred lines into different clusters and lines were allocated into two major clusters regardless their origin.
- The highest (0.375) polymorphic information content (PIC) observed was exhibited by three markers; PZA00543_12, PZA00878_2, and PZA01735_1.
- The acquired information from genetic diversity will be a useful key for designing hybrid maize program in Rwanda. It will guide towards suitable heterotic patterns as well as the combining ability of the inbred lines selected from this study.

6.2.2 Combining ability and heterotic patterns for grain yield and other agronomic traits among maize inbred lines selected for the mid-altitudes and highlands zones of Rwanda

Forty-five diallel cross hybrids from a 10 x 10 half-diallel mating design plus three checks were tested in a 6 x 8 alpha-lattice design across twelve environments in Rwanda. It was realized that;

- General combining ability and specific combining ability effects were both highly significant (P<0.001-0.01) but with high magnitude of GCA for grain yield when all environments were combined, suggesting the presence of both additive and non-additive effects.
- Inbred line S5 showed the highest positive (1.184) GCA effects for grain yield.
- Hybrids such as S2/S5 and S4/S6 exhibiting grain yield>8.5 t/ha and high specific combining ability, could be used for direct production or serve as testers for three way hybrids.
- Hybrids S1/S4, S3/S4, S4/S6 and S4/S7 displayed high grain yield and high heterosis.

- All the lines displayed positive heterosis with all the three testers. However, most of the inbreds displayed similar levels of heterosis with both S6 and S7 testers.

6.2.3 Heterotic orientations, gene action and heterosis among maize inbred lines selected for the major agro-ecologies of Rwanda

Nineteen maize inbred lines were crossed with four testers, following a line x tester mating scheme and generated seventy six test crosses, which were evaluated together with two checks in 6 x 13 α-lattice design at four locations in 2015B and 2016A seasons. It was revealed that;

- Both additive and non-additive gene action were important for grain yield with preponderance of additive gene action over non-additive gene action.
- The most desirable GCA effects for gran yield were realized in inbred lines 5, 7 and 8
- Test crosses such as 17/T2 and18/20 exhibited grain yield >7.3 t/ha and high specific combining ability and could be used for direct production or serve as testers for three way hybrid crosses.
- Based on standard heterosis relative to the respective testers, inbred lines were aligned in two different groups (T1/T3/T4 and T1/T2/T3/T4). However, some of them somehow displayed some common patterns.
- Genetic distance was correlated to heterosis, SCA effect and test crosses performance however, though this was related to specific testers.

6.2.4 Genotype x Environment interaction and stability analysis of diallel and test cross maize hybrids across tropical medium and highland ecologies

Seventy-six test crosses plus two checks and 45 diallel cross hybrids plus three checks were evaluated in four environments. Genotype and genotype by environment interaction biplot method was applied for graphical display of the data. It was revealed that;

- Hybrid 26 (ACR29 x 21) and 31(ECA1 x 22) from test crosses and 3 (R10164 x ET4), 25 (ET4 x ECA13) and 34 (ECA13 x ZM5) from single crosses were identified as the best performers and then qualified as desirable hybrids in specific environments.
- Test crosses 19 (5 x 22), 29 (8 x 20) and 69 (18 x 20) and diallel crosses 3 (R10164 x ET4), 29 (ET x ZM5) and 47(Check) were revealed as high yielding and highly stable
- The GGE biplot revealed three mega-environments for test crosses and two mega-environments for diallel crosses.

- Environments RWA, RWB and RBB for test crosses and RB1, RB2 and NY2 for the single crosses were the most discriminating the genotypes.
- Overall, the study revealed stability and presence of crossover interactions signifying the need to breed for both broad and specific adaptation.

6.3 Implications of the research findings and way forward

The PIC values revealed in this study confirmed the utility of the SNP markers to discriminate between inbred lines from diverse origins. This was proven more in their discrimination of closely related lines, indicating their usefulness for diversity analysis of maize inbred lines under the current study. High genetic distances realized among some pairs of inbred lines is an indication of distinctiveness of these lines and could be considered for hybrid development. Genetic clustering information acquired from the current study will be suitable information for maize hybrid program establishment in Rwanda and for other collaborative tropical maize breeding programs. This will also guide towards suitable heterotic patterns and groups as well as the combining ability of the inbred lines involved in this study. It would be worthy to mention that caution should be taken when using these findings due to lack of consistency in inbred line groupings based on these SNP markers and different testers.

Results on mode of gene action revealed the preponderance of additive gene action, suggesting that hybrid performance prediction for grain yield will be mainly based on parental lines with high (S4, S5, L5, L7, and L8) general combining ability effects. This will be accomplished by accumulating favorable alleles from these parents based on different breeding methods like recurrent selection and backcross breeding. Hybrids such as 18/T1, 18/T2 and 18/T4 displayed favourable SCA estimates for grain yield and they could be used directly as hybrids or potential single cross testers for development of three-way hybrids. Minor presence of non-additive effects observed in controlling grain yield should also be taken into account when using the current findings. This suggests that further breeding gain can be achieved through developing hybrids based on crosses with both high mean and specific combining ability effects.

Overall, the current findings suggests the need of considering both the average performance of inbred lines in hybrid combinations and the specific hybrid combinations in the course of variety development.

The few hybrids that exhibited significant SCA effects for grain yield, demonstrated also relatively high mid-parent heterosis and good hybrid *per se* performances.
This implies the potential of these varieties in hybrids production. The revealed heterotic groups, high heterosis and genetic distance among identified genotypes, suggests the usefulness of combining different breeding methods. In a special way, findings based on genetic distances could be used to guide in selecting which parents to combine to minimize unwanted crosses.

For appropriate choice of test environments, high yield and stability of the new hybrids developed under the current study, genotype x environment interaction revealed cross-over interactions. This suggests that selection for specific adaptation of genotypes would be the best method to increase genetic gains. On the other hand, both high yield and high stability displayed by some test cross hybrids (19, 29 and 69) and diallel cross hybrids (3, 29, 47) suggest presence of promising hybrids which could be exploited across all agro-ecologies of Rwanda and in other maize programmes having similar agro-ecologies. Strong correlation observed among some of testing environments revealed redundancy in some of the testing environments. Therefore, to minimize evaluation cost, few testing environments could be suffice to obtain information on the genotypes.

Screening the germplasm for tolerance to abiotic stresses identified as constraints in the current study could improve the stability of performance of the varieties when grown in diverse agro-ecologies.