

**GENOTYPE × ENVIRONMENT INTERACTION STUDIES ON THE
PERFORMANCE, ADAPTABILITY AND STABILITY OF PRE- RELEASE
FLUE-CURED TOBACCO HYBRID LINES IN ZIMBABWE**

By
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A research submitted in partial fulfilment of the requirements for the degree of Bachelor of
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DECLARATION

I hereby declare that the information herein presented is my own original work and a result of my effort. All additional information from secondary sources has been credited through acknowledgements and references.

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CERTIFICATION

I the undersigned confirm that Chikwature Nyasha Candidate for the Bachelor of Science Honours Degree in Crop Production and Horticulture has carried out a Research and presented on the project entitled:

GENOTYPE × ENVIRONMENT INTERACTION STUDIES ON THE PERFORMANCE, ADAPTABILITY AND STABILITY OF PRE- RELEASE FLUE-CURED TOBACCO HYBRID LINES IN ZIMBABWE

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ABSTRACT

The nature and magnitude of the genotype \times environment interactions is important to identify superior and stable genotypes under target environments. This will assist to maximize specific adaptation and to speed up the distribution of new cultivars to growers. Eleven pre-release flue cured tobacco hybrid lines were evaluated for yield and quality in four different tobacco growing regions thus, Kutsaga and Rusape representing the slow growing areas, Trelawney representing the medium growing areas and Tengwe representing the fast growing areas . The thrust of this study was to assess the stability and adaptability of these hybrid lines. KKK 26R was used as a positive check line because it is a cultivar that is adaptable and stable to a wide range of environments. The hybrid lines were raised with the new float tray system of seedling production and all cultural practises were done following the TRB handbook recommendations. Significant genotype \times environment variations were observed among the hybrid lines. For the total saleable yield, environmental high significant difference was observed ($P = 0.001$), the study revealed that Tengwe had the best yield of 2755kg/ha, while, Kutsaga had the least yield of 1934kg/ha. For the grade index genotypic highly significant difference was observed ($P = 0.001$) with G9 outperforming all other test genotypes including the check line with an index of 61.74, while, G1 least performed with an index of 51.0. For the top grades proportion Genotype \times Environment interaction was found and GGE biplot procedure was followed and results indentified the stability and adaptability of the performance of the hybrid lines. The GGE biplots indentified that G7, G8 and G11 as the high quality and stable genotypes. G1, G2 and G5 least performed and had low stability. It was observed that Kutsaga that is located in the slow growing areas as the ideal testing environment for these set of hybrids that were under test.

Key words: Genotype \times Environment interaction; Tobacco; Yield; Quality; Stability; Adaptability

DEDICATION

TO MY MOM AND DAD

An example of hard work, tenacity, strong will and great deal of faith that you have laid out for me, has made me to reach this far.

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Firstly, I am indebted to the Lord God Almighty who has granted me life, health, enthusiasm and opportunity, thus seeing me through the successful completion of this project. It is an immutable fact that this work could not have come to fruition was it not for the priceless and invaluable contributions of the following individuals and organisations. Mr Chaibva who supervised this work, provided technical insight and guidelines on the authorisation of this document. My profound gratitude goes to Dr Magama and Mr Shava who gave me an opportunity to carry out this study and assisted me in terms of the planning, logistics and overall execution of the project. The Plant Breeding technical team, who were always ready and willing to lend me a hand on matters concerning the project, The Tobacco Research Board (TRB), for awarding me a chance to conduct the research at Kutsaga Research Station and for availing all the necessary provisions for the successful completion of the project. Department of Horticulture at Midlands State University who have given me treasure that no one can take away. The list can never be fully exhausted, for there are so many whose noble participation is greatly appreciated. The gratitude is better felt than expressed.

Contents

DECLARATION	ii
ABSTRACT	iii
DEDICATION.....	iv
ACKNOWLEDGMENTS	v
List of tables	x
List of appendices	xiii
ACCRONYMS AND ABBREVIATIONS.....	xiv
CHAPTER ONE.....	1
1.0 Introduction.....	1
1.1 Main objective.....	4
1.1.1 Specific objectives	4
1.1.2 Hypotheses	5
CHAPTER TWO	6
2.0 Literature Review	6
2.1 Account of tobacco domestication	6
2.2 Tobacco production and trends in Zimbabwe.....	6
2.3 Tobacco growing areas of Zimbabwe	7
2.4 Cooperative Cultivar Trials (CCT).....	7
2.5 Genotype × Environment (G×E) Interaction Effects	8
2.6 Gene and Environment	9
2.7 Classification of Genotype × Environment Interaction.....	9

2.7.1 No Interaction.....	10
2.7.2 Non Cross over GEI.....	11
2.7.3 Cross over interactions.....	12
2.8 Importance of Genotype × Environment interaction.....	13
2.9 Genotype × Environment interaction studies from other countries in different crops ..	14
2.10 Heritability and its estimations.....	14
2.11 Statistics tools that measure GEI.....	15
2.11.1 Conventional analysis of variance	16
2.11.2 Stability analysis approach.....	17
2.11.3 Crossover interaction and non-parametric techniques for stability analysis.....	20
2.11.4 Multiplicative analysis techniques.....	21
“CHAPTER THREE”	25
“3.0 Materials and Methods”	25
3.1 Research Sites	25
3.1.1 Harare (Kutsaga).....	25
3.1.2 Rusape.....	25
3.1.3 Trelawney.....	26
3.1.4 Tengwe.....	26
3.2 Experimental design	26
3.3 Description of plant material.....	26
3.4 Agronomic Practices	27

3.4.1 Seedling production	27
3.4.2 Land preparation.....	28
3.4.3 Planting	28
3.4.4 Fertilisation.....	28
3.4.5 Weed and pest control.....	28
3.4.6 Priming.....	29
3.4.7 Topping	29
3.4.8 De-suckering	29
3.4.9 Reaping	30
3.5 Data collection	30
3.5.1 Yield.....	30
3.5.2 Grade index	30
3.5.3 Top grades proportion.....	30
3.6 Data Analysis	30
CHAPTER FOUR.....	31
4.0 RESULTS.....	31
4.1. Total saleable yield of Genotypes in the test sites	31
4.1.1 Contribution of Genotypes to the total saleable yield	31
4.1.2 Contribution of different Environments to the total saleable yield	31
4.1.3 Interaction of Total saleable yield of genotypes in the test sites	32
4.2 Total Grade Index of genotypes in the test sites	32

4.2.1 Contribution of genotypes to the Grade Index	32
4.2.2 Contribution of different Environments to the Grade Index	33
4.2.3 Interaction of Grade Index of genotypes in the test sites	34
4.3 Top Grades proportion of 12 Genotypes under test	34
4.3.1 Contribution of the Genotype to the Top Grades Proportion.....	34
4.3.2 Contribution of Environment to the Top Grades Proportion	34
4.3.3 Interaction of Top Grade proportion of genotypes in the test sites	35
CHAPTER FIVE	46
5.0 DISCUSSION	46
5.1. Total saleable yield of genotypes in the test sites	46
5.2 Total Grade Index of genotypes in the test sites	48
5.3 Top grades proportion	49
5.3.1 Relations among Environments for the Top Grades Proportion	49
5.3.2 GGE biplot discriminating ability and representativeness of the test Environments for the Top Grades Proportion	50
5.3.3 Comparison of the test Environments for the Top Grades Proportion	51
5.3.4 Mean performance and stability of the test Genotypes for the Top Grades Proportion	51
5.3.5 Comparison of Genotypes to the ideal Genotype	52
5.3.6 Suitability of genotypes for particular environments using the “Which-Won- Where” function of a GGE biplot for the Top Grades Proportion.....	53
CHAPTER SIX.....	55

6.0 CONCLUSIONS AND RECOMMENDATIONS	55
Reference	58
Appendices	62

List of tables

Table 3.1: Treatment table and description	27
Table 4.1: Mean performance of Total Saleable Yield for the hybrids at all sites	33
Table 4.3: Mean performance of the Top Grades Proportion for the hybrids at all sites	35
Table 4.4 Top Grades Proportion Index of 12 genotypes evaluated in four different growing environments	

List of figures

Figure 1.1 illustrations of the tobacco growing areas of Zimbabwe	2
Figure 2.1 illustrating no interaction effects of genotype \times environment for yield response	11
Figure 2.2 non-crossover interaction effects of genotype \times environment for yield response	12
Figure 2.3 cross over interaction effects of genotype \times environment for yield response	13
Figure 4.1 GGE-biplot based on genotype focused scaling for Top Grades Proportions	39
Figure 4.2 GGE-biplot view showing relationship among four testing environments	40
Figure 4.3 GGE-biplot showing a comparison of four testing environments with in ideal environment	
Figure 4.4 GGE ranking biplot shows the mean Top grades proportions and stability performance of the four tested genotypes	43
Figure 4.5 Comparison biplot views of Top grades proportions of genotypes with the ideal genotype	
Figure 4.6 which-won-where view of the GGE biplot of Top grades proportions	45

List of appendices

Appendix 1 Analysis of Variance for Total Sealable Yield.....	63
Appendix 2 analysis of variance for the Grade Index.....	63
Appendix 3 analysis of Variance for the Top Grades Proportion.....	63

ACCRONYMS AND ABBREVIATIONS

AMMI	Additive Main Effect and Multiplicative Interaction
GGE	Genotype Main Effects \times Environment Interaction Effects
ANOVA	Analysis of Variance
CV	Coefficient of Variance
FAO	Food and Agriculture Organisation
$G \times E$	Genotype \times Environment
GEI	Genotype \times Environment Interaction
Ha	Hectare
CCT	Cooperative Cultivar Trials
H^2	Broad Sense Heritability
h	Narrow sense heritability
cm	Centimetre
JLR	Linear Joint Regression
LSD	Least Significant Difference
PCA	Principal Coordinate Analysis

CHAPTER ONE

1.0 Introduction

Tobacco (*Nicotiana tabacum*.L) is a perennial crop grown as an annual in most of the countries in the world. According to (FAO, 2010) tobacco generates 20 billion dollars a year worldwide and about 3.9 million hectares of land are reserved for its production. Tobacco is the largest field crop income earner in Zimbabwe and its production is out-competing other cash crops such as cotton (TIMB, 2014). Approximately 700 000 families rely directly or indirectly on tobacco production in Zimbabwe, (Chivuraise, 2011) the majority of these families are rural based small scale farmers where tobacco is the primary source of income, thereby, directly contributes to rural economic development and livelihood (TRB, 2011).

There are well defined agro-ecological regions suited for tobacco production in Zimbabwe. These regions are termed fast, medium and slow growing areas and are classified to the growing regions based on the average cumulative rainfall and annual mean maximum temperature that prevails in the regions (TRB, 2011). The fast growing regions such as Guruve, Doma and Hurungwe, are characterised by low altitude, high rainfall and high temperatures. Medium growing regions such as Concession, Banket and Trelawney are characterised by medium altitude medium temperatures and slow growing areas such as Rusape and Headlands are characterised by high altitude high rainfall and warm-cool temperatures (TRB, 2011).

Tobacco Growing Areas of Zimbabwe

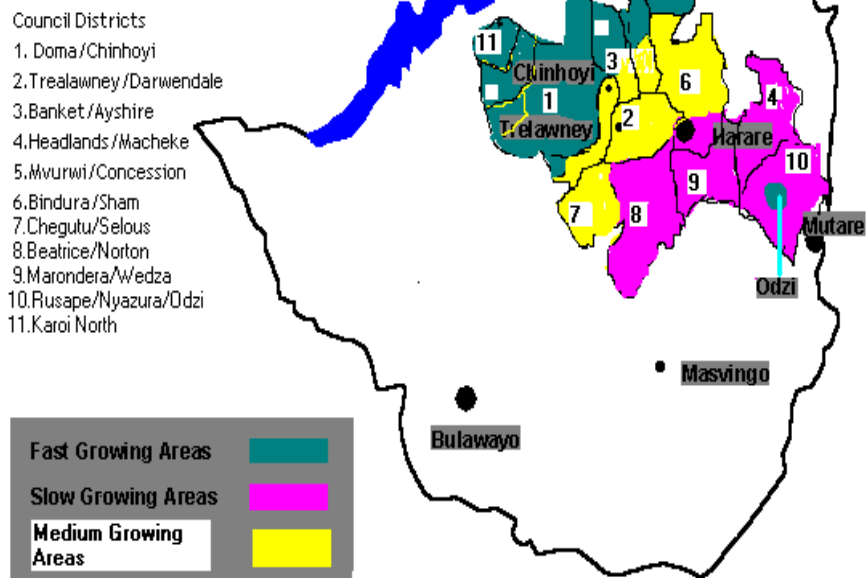


Figure 1.1 illustrations of the tobacco growing areas of Zimbabwe (TRB, 2011)

Although there are well known traditional growing areas, one of the major constrain of tobacco production in Zimbabwe is the farmer’s inability to select appropriate tobacco varieties for their environment and management practises. Recent trends have revealed that yield and quality of tobacco presented for marketing can be significantly improved if farmers adopted the correct varieties for their respective regions (TIMB, 2014). Two common mismatches observed in small scale tobacco farming are:

- Selecting fast maturing and ripening varieties for fast growing regions, resulting in excessive pressure during reaping, and poor biomass accumulation, which ultimately result in poor leaf quality and reduction in yield.
- Selecting slow maturing varieties for slow growing regions that commonly results in delayed reaping and consequently, increased reduction in yield and poor curing qualities (TRB, 2011).

Variety adaptation is largely based on the growth habit, in relation to the rate at which an environment promotes growth and ripening (*Fan et al., 2007*). To a lesser extent, variety adaptation is also based on the inherent varietal resistance to naturally prevailing biotic and abiotic stresses in selected area (*Yan et al., 2010*). Thus, it is very important to identify genotypes that perform well in specific environment for plant improvement so as to recommend best ideal genotypes for specific locations. Genotype \times environment interaction is when dissimilar genotypes react in a different way to environmental fluctuations and this concept is very essential in plant breeding as it influences the selection of superior genotypes in trials (*Yan and Rajan, 2002*). G \times E Interaction complicates the selection process because of the difference in performance of different genotypes in the same environments and has been reported in different crops and plants and the occurrence of GEI causes genotypes to perform differently from one environment to other (*Van de Merwe, 2012*). Thus, it is important to have information of the influence of GEI on variety evaluation to aid decisions before cultivar recommendations.

Leaf quality, plant stature, adaptability of crop genomes as well as the general performance of different cultivars is the concern of farmers striving to achieve high productivity and quality tobacco. Although there are well defined tobacco growing areas in Zimbabwe (slow, fast and medium), proper study has not been done in trying to understand the concept of the genotype \times environment interaction (*TRB, 2011*). Currently, varieties are prescribed to their growing areas based only on their ripening rates, there is lack of information about variety performance in particular areas in terms of the influence of the genotype \times environment interaction.

National yield of tobacco in Zimbabwe averages to 912 kg/ha against the varieties potential of 4000-4500 kg/ha, while quality of the tobacco leaf is very poor (TIMB, 2014). Poor variety selection in the tobacco growing regions is among the major factors contributing to such low yields and poor quality. Tobacco growers are not accessing the best information on how best they can grow their varieties in their locations (Factsheet, 2008). Cultivar trials like the Cooperative Cultivar Trials enable the recommendation of the best varieties for specific areas, however, these trials tends to be confounded by seasonal growing conditions. It is therefore imperative that formal testing of varieties with sophisticated analytical tools to decipher the G×E studies should be conducted. Varieties that are found to be stable in each environment are the ones that should be grown by farmers and this information can only be found if the trials like the genotype × environment Interaction are conducted (Mazarura, 2010).

Therefore, this research seeks to evaluate the influence of the GEI on the newly bred tobacco hybrids lines on how best famers can choose cultivars that best perform in their areas in terms of yield and quality.

1.1 Main objective

To determine the genotype × environment Interaction and establish the adaptability and stability of 11 pre-release flue-cured tobacco hybrids in four different tobacco growing areas in comparison to KRK 26R (Check)

1.1.1 Specific objectives

- To measure the total saleable yield of 11 new pre-release flue cured tobacco hybrids in four different tobacco growing areas to the popular variety KRK26R as the check
- To compare the grade index of 11 new pre-release flue cured tobacco hybrids in four different growing areas to the check KRK26R)

- To assess the top grades proportion of 11 new pre-release flue cured tobacco hybrids in four different growing areas to the check KRK26R)

1.1.2 Hypotheses

- There is significant difference in the total saleable yield (tonnes/ha) of 11 new pre-release flue cured tobacco hybrids in four different tobacco growing areas to the popular variety KRK26R as the check
- There is significant difference in the grade index of 11 new pre-release flue cured tobacco hybrids in four different growing areas to the check KRK26R)
- There is significant difference in the top grades proportion of 11 new pre-release flue cured tobacco hybrids in four different growing areas to the check KRK26R)

CHAPTER TWO

2.0 Literature Review

2.1 Account of tobacco domestication

Tobacco originated in South and North America and first known by the European explorers in the 15th and 16th century where it was used as medicine, psychoactive substance, narcotic, painkiller, and pesticides and later on used for rituals and ceremonies. Due to the demand of the plant, distributions outside its origins were done through trade in the 300 BC.

Tobacco smoking in Zimbabwe is thought to have been through primitive landraces such as the Nyoka landraces and wild species of the *Nicotiana rustica* (TRB, 2011). Successful cultivation of the flue-cured tobacco in Zimbabwe was made by Jesuit priest at Chishawasha in the 1890s (TRB, 2011). The establishment of tobacco in Zimbabwe came with changes in culture, economy and different stages in political and trading importance. Through breeding over 1000 different varieties of *N. tabacum* are the most economic in the world today. One hundred and twenty five countries now cultivate the crop with over 4 million hectares of land (TIMB, 2014).

2.2 Tobacco production and trends in Zimbabwe

The land reform program of the early 2000 saw an increase in tobacco production from 22000 to about 64775 farmers by 2013 with over 77910 hectares under use. The rise contributed to the country's Gross Domestic Product (GDP) from 2009- 2015 with about USD\$174,457,761 to USD\$586,400,000 respectively (TIMB, 2016), the fast land reform program brought about change and thus expanding the tobacco industry.

2.3 Tobacco growing areas of Zimbabwe

The Zimbabwean natural environment consists of two major climatic divisions with the semi-dry regions covering the tobacco growing belt. The region is then divided into three agro-ecological sub-areas namely fast, slow and medium growing areas. The sub-areas vary in the climatic attributes thus giving differential growth patterns for tobacco which lead to different growth habits, variations in yield and quality of the leaf (TRB, 2011).

However, it should be noted that although there is a rise in tobacco production, the industry is confounded by various challenges, one of which is of the planting material being used which does not suit areas considered marginal for tobacco production. Thus current varieties are not bred for these areas so there is a gap that needs research in addressing this expansion in the tobacco industry (TRB, 2011).

2.4 Cooperative Cultivar Trials (CCT)

To ensure proper variety recommendations plant breeders perform Cooperative Cultivar Trials (*Crossa et al., 2002*). These are trials meant to agronomically test new hybrids in the various tobacco growing areas under farmer management practices and in different seasons. These trials are done three to five years and new hybrids showing better performance under these trials are then recommended to be grown in these growing areas and specific districts. Interestingly, by growing genotypes in diverse locations, the utmost yielding or genotypes that outperform other genotypes in all the growing areas can be identified (*Yan et al., 2007*). There are differences in the performance of genotypes tested in different environments due to the variations in the factors such as soil. Cultivars tested in diverse locations or years often have significant fluctuations in yield, and or quality due to the response of genotypes to environmental factors such as soil fertility or presence of diseases (*Kamara et al., 2006*).

These fluctuations are referred to as $G \times E$ interaction and are common and have been studied in trying to improve crop productivity.

2.5 Genotype \times environment ($G \times E$) interaction effects

Successful cultivars should have outstanding yield, quality and other essential agronomic characters. Performance of cultivars should be dependable and consistency from environment to environment. Occurrence of Genotype \times Environment interaction is one of the major causes of the difference in the performance of genotypes in different environments. GEI is the failure of cultivars to attain the similar comparative performance in different environments. More so, it should be noted that the causes of Genotype \times Environment interaction have become a combination of both genetic and non-genetic effects on the developments of crops (*Fan et al., 2007*).

Phenotype of living organisms is mostly is controlled by genotypic and the environmental variations and these variations are rarely independent to each other (Bernardo, 2002). The way a phenotype respond to variations in environments vary from genotype to genotype. Thus, plant breeders are embarking on programs to match the genotype and environment in a way that genotypes are designed to grow in environments they do best. In this exercise, breeders come across situations where the comparative ranking of cultivars changes from one environment to the other and this affects the continuation of breeding on cultivar evaluation in terms of how genotypes perform in respective environments (Yan and Hunt, 2001).

Furthermore, there are problems rising in the genotype \times environment interaction and these problems influence several stages in plant breeding programs by reducing correlation between genotype and by complicating the evaluation of selection. Genotype \times environment interaction exist where there are differences among genotypes that are not consistent from

environment to environment. Kang (2005) is of the view that genotype \times environment interaction is relevant only when cultivars are tested in various locations, thus in various locations, years as well as varying growing seasons. Experiments conducted in single environments do not allow breeders to draw general conclusion regarding performance of test genotypes and a general conclusion relative to performance in a wide range of environment is what the breeder should know. Conducting experiments in several environments is necessary to analyze and identify stable adaptable and high yielding genotypes.

2.6 Gene and environment interaction

Survival of living organisms is determined either by their genes or by the environment and these are consequences of the gene and environment interaction. A gene is genetic constituents of an individual that determines characteristics (phenotype) inherited, while the environment may be divided into predictable and unpredictable categories (*Kaya et al., 2006*). The predictable category includes variables that occur under human control such as the agronomic practices and the unpredictable category includes the unpredictable variables such as rainfall and temperature. The environment has become a state of change and the pace of the evolution has become vital to the plant breeders. The ability or inability of plants to adapt to the changes in the environment to the speed necessary determines the continuation, extinction or the evolution of species.

2.7 Classification of genotype \times environment interaction

GEI can influence the nature of cultivars that breeders may want to release for production in different growing areas thereby affecting the breeding progress. Statistics in genotype \times environment arise only when there are differences in the performance of genotypes cause lack consistency over different areas (*Magagane, 2012*). The performance of genotypes in environments determines how vital interaction is in G \times E studies, thus when the performance

of a genotype remain constant from one environment to the other, it is eluded that there is absence of G×E.

Statistics in genotype × environment arise only when there are differences in the performance of genotypes cause lack consistency over different areas (Magagane, 2012) :

$$A_1 - B_1 \neq A_2 - B_2 \text{ or } A_1 - B_1 - (A_2 - B_2) \neq 0$$

Considerable interaction rise as :

$$A_1 - B_1 - A_2 + B_2 \neq 0$$

Where $A_1, A_2, B_1,$ and B_2 characterize different cultivars

There are different types of genotype × environment interaction and these are:

2.7.1 No Interaction

No interaction GEI happens when one genotype, genotype A (Figure 2.1) performs superior than genotype (B) with the similar magnitude across all locations integrated in test locations (Magagane, 2012)

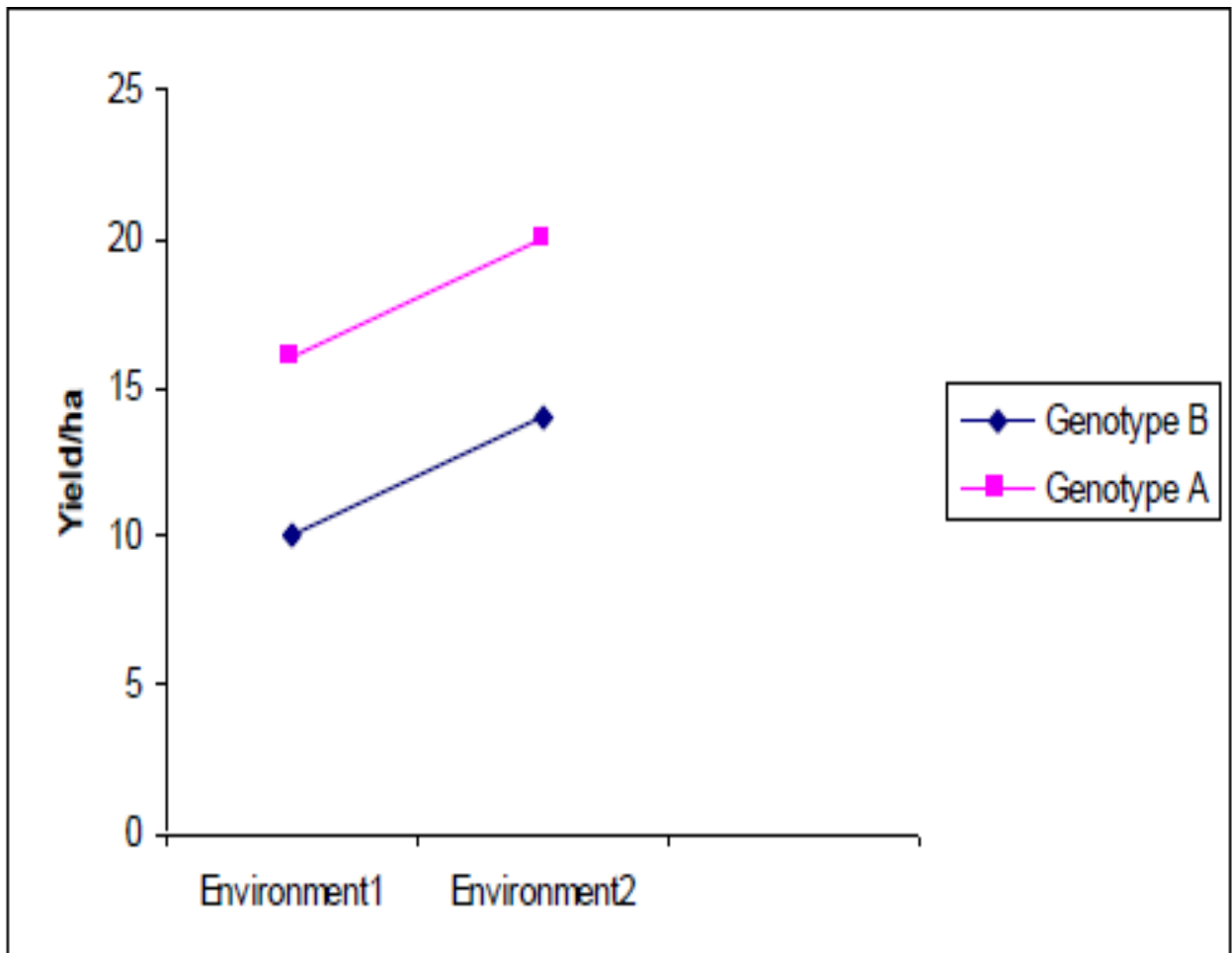


Figure 2.1 illustrating no interaction effects of GEI

2.7.2 Non Cross over GEI

Non cross over GEI happens when one genotype, genotype A (in Figure 2.2) responds better than genotype B, in all environments (*Gauch et al., 2009*). Genotype A and B respond differently in two environments but their ranks are unchanged. The response of two genotypes under different environments is non additive, the magnitude of inter-genotypic variance increases, and the environmental modifications of two genotypes are moving towards the same direction Figure 2.2 (*Magagane, 2011*).

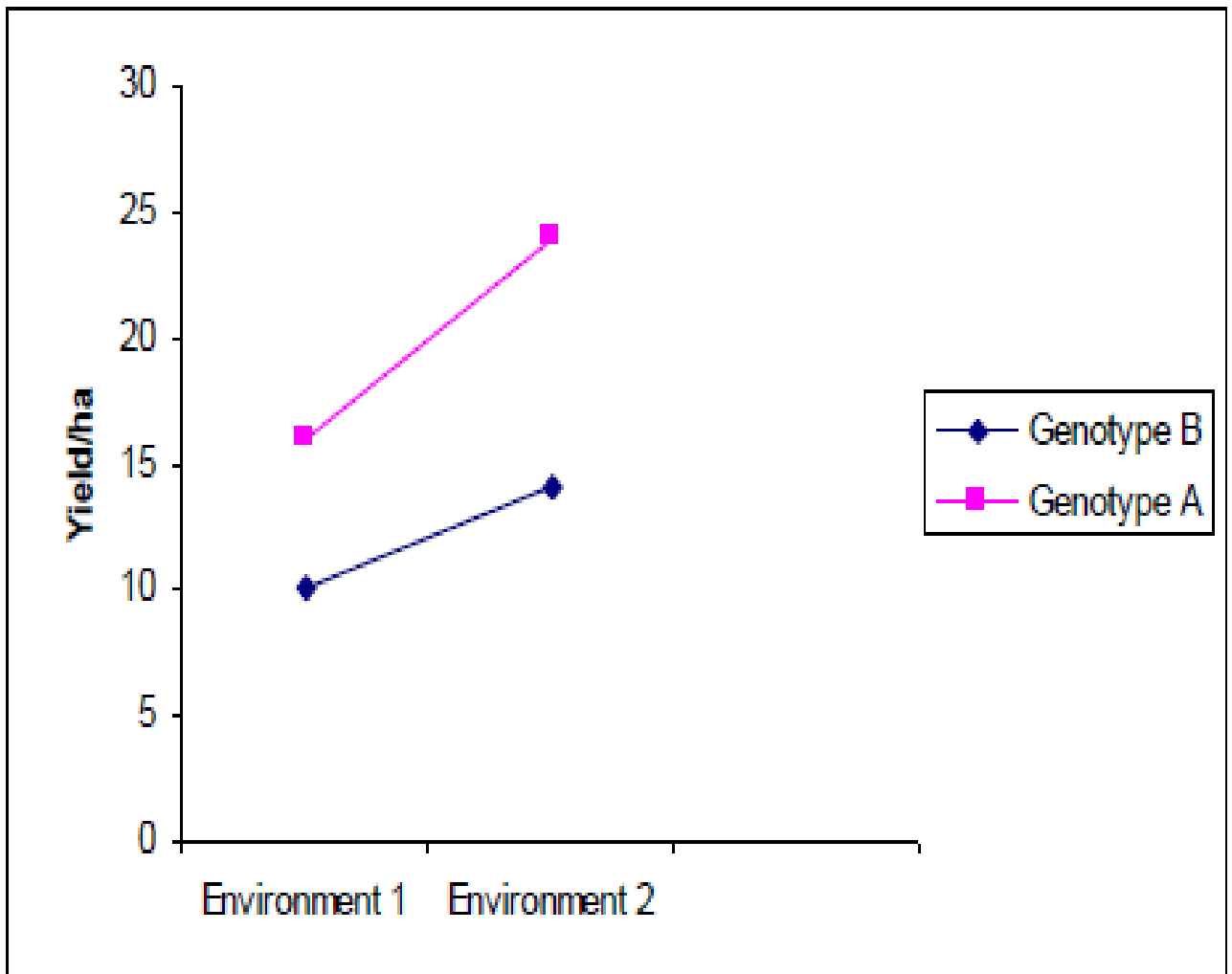


Figure 2.2 illustrating non cross over interaction effects of GEI

2.7.3 Cross over interactions

Cross over interaction vital to plant breeders, it occurs when one genotype example genotype (A) is outperforms other genotypes in one location, but when genotype B is more productive in the other location (Figure 2.3). Cross over interaction is differential and is not stable, thus, the basic test for cross over interaction is used for decision making on the evaluation of various genotypes in different locations (*Gauch et al., 2008*).

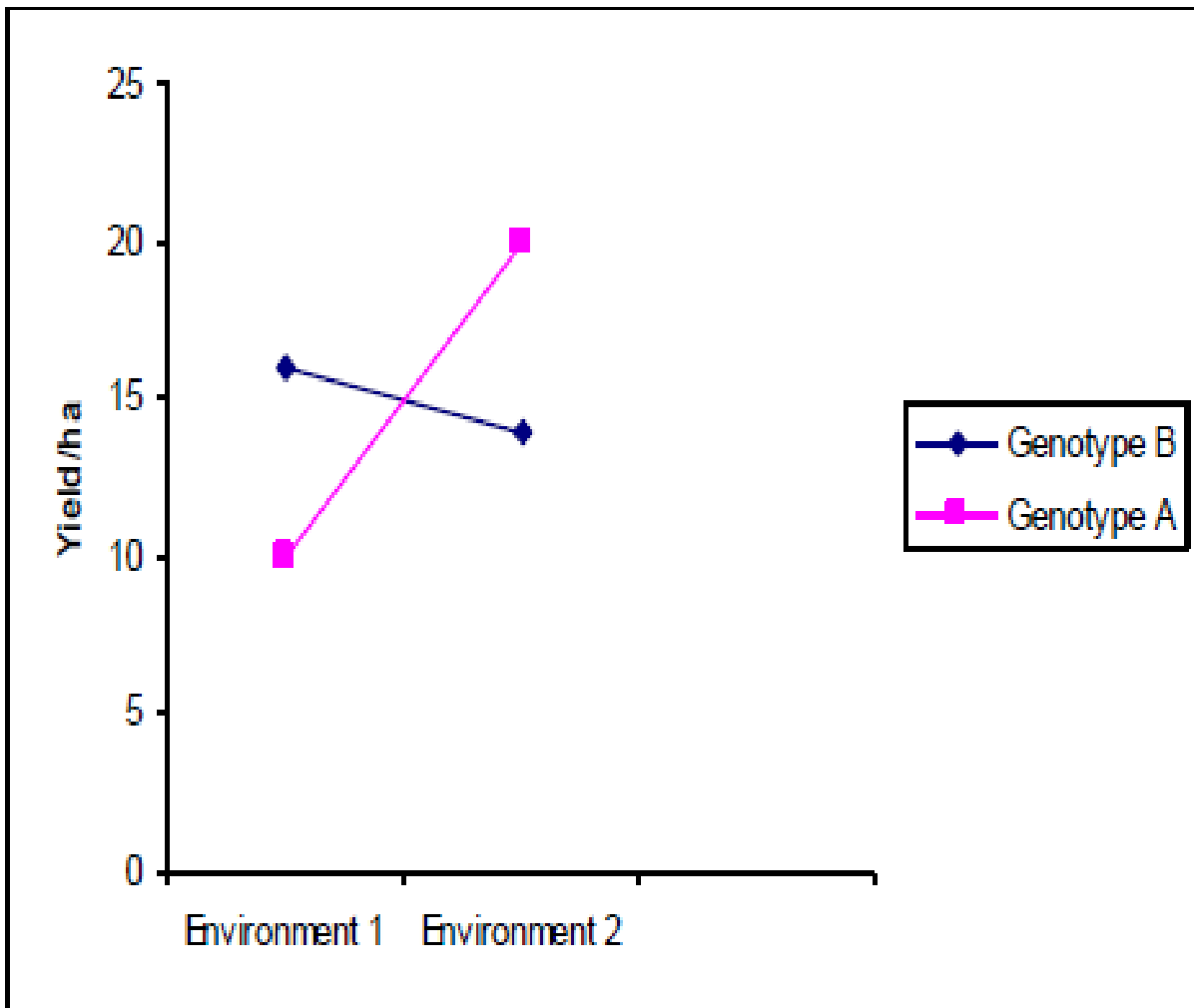


Figure 2.3 illustrating cross over interaction effects of GEI for yield variations

2.8 Importance of genotype × environment interaction

Studies of genotype × environment interaction are vital in plant breeding as they give data on how locations influence variety performance (*Mohammadi et al., 2007*). Thus, this study seeks to support breeding programmes concerning decision making such as selecting appropriate environments for testing genotypes (*Kandus et al., 2010*). This helps in identifying stable and adaptable genotypes that are consistent in performance across diverse environments (Kang, 2005). Golbashy (2012) explains two types of stable genotypes, those that indicates stable average yield across environments, and those with high yield in specific environments, but with poor yield in non-targeted environments, these are genotypes with specific adaptability. Thus, this knowledge helps breeders in determining if there is need to

develop varieties for environments by identifying mega environments, specific as well as broad adapted cultivars.

2.9 Genotype × environment interaction studies from other countries in different crops

Genotype × environment interaction has been studied world-wide and in several crops. Mugagane (2012) found that minimum night temperatures determining physiological stages in maize are the major contributor of GEI. Genotype × environment interaction in durum wheat (*Purchase et al., 2000*), in water melon (*Smith et al., 2012*) have also been studied.

Perkins and Jinks (1968) did a research in sunflower and they found that the stability of yield performance of cultivars may be due to genetic control. He found that some inbred lines contribute more to stability of hybrids than other cultivars, thus, indicating that genotypes have a contribution to yield stability. To add, studies were also done in France on two sunflower networks. Yan et al (2007) have determined interactions for yield between genotypes growing length as well as the length of the season. A correlation between earliness of sunflower cultivars and earliness of season existed and found a positive yield correlation between the two factors. In Zimbabwe, studies of $G \times E$ were also conducted Chimene (2014) were maize inbred lines were evaluated and showed that genotype performance are limited to the environment and cultivars that were under test had difference in their performance across environments.

2.10 Heritability and its estimations

Heritability (H^2) measures variables caused by genetic effects and environmental diversity (*Yan et al., 2010*). Thus, the resemblance between phenotypic and breeding values can determine success in changing a character through selection and this can be measured by H^2 (*Yan et al., 2010*).

The H^2 of an individual can help breeders in nominating appropriate selection methods. Thus, when H^2 is high there is mass selection, but as the value of H^2 decreases more emphasis should be placed on pedigree and progeny test. Environmental factors such as, number of replications, plant density and plot size affect the magnitude of H^2 because, of such factors comparisons of estimates obtained in different experiments must be with caution.

Heritability comes in two types thus, broad sense and narrow sense heritability. Broad sense heritability comprises of total genetic variance or the share of total genetic variance to phenotypic variance and it explains the amount to which phenotype of an individual is controlled by its genetics (*Yan et al., 2010*). Narrow sense heritability an important quantitatively inherited trait is the ratio of additive genetic variance to phenotypic variance and explains how phenotypes are determined by the genes passed from one generation to the other. Thus, heritability plays a vital role as it aids selection in plant breeding programs by reducing the occurrence of $G \times E$.

2.11 Statistics tools that measure GEI

Genotypes differ in the way they respond to environmental variations, thus, it is very essential to evaluate genotypes in different locations (multi-environment trials) $G \times E$ interaction trials are a tool in advancing breeding as they can be used in decision making on which variety performs best in a given environment. Several methods have been used in identifying existence of $G \times E$ interaction in multi-location trials. Combined analysis of variance is the most popular analysis in interpreting Genotype \times environment interaction data, if GEI variance is found to be significant other methods can be used in measuring the stability of genotypes in different locations. Statistical tools used in the identifying stable genotypes can be classified into two, parametric and non-parametric approach measures. The

parametric approach is used only when there is continuous data while the non-parametric approach is used to analyze discontinuous data (Magagane, 2012)

2.11.1 Conventional analysis of variance

This is a method that is used to measure yield variations contained in GER observations where genotypic frequency environmental contribution and replications are used to come up with accurate results in experiments. Environment residual mean square is used to measure means that are found due to differences due to the varying environmental conditions in the experimental sites. GEI is measured in two dimensions thus, the additive and non additive (Magagane, 2012). The analysis of variance of combined data expresses the observed (y_{ij}) mean of the i^{th} genotype at the j^{th} location as :

$$Y_{ij} = \mu + G_i + E_j + GE_{ij} + e_{ij} \dots \dots \dots (1)$$

Where μ represent the general mean; G_i , E_j and GE_{ij} represent the effects of genotype, environment and the GEI and e_{ij} is the average of the random errors associated with the r^{th} plot that receives the i^{th} genotype in the j^{th} environment (Magagane, 2012). The non-additive interactions as defined in (1) imply that the expected value of the i^{th} genotype in the j^{th} environment (y_{ij}) depend not only on the levels of $G \times E$ but, on the combinations of levels of GEI (Crossa *et al.*, 2002).

The limitations with this statistical tool is that error variance over locations should be homogenous to attain different performance of genotypes. It should be noted that this method of measuring data only works on results that show significant difference. Analysis of variance of multi-location trials is functional for estimating the variance components associated to diverse sources of variation, together with genotypes and genotype \times environment interaction. Variance component methodology is vital in multi-location trials, since errors in measuring the yield performance of a genotype rise largely from $G \times E$ interaction . Hence, the information of the amount of relations is necessary to attain resourceful estimates of

genotypic effects and establish best recourse allocations in terms of the number of plots and environments to be incorporated in potential trials . The variance methodology is mostly used in breeding programs to measure genetic variability and estimation of heritability and predicted gain of a trait under selection (*Crossa et al., 1990*).

2.11.2 Stability analysis approach

This method gives information on how genotype responds to environmental variations, statistical tools have been developed in trying to understand GEI and the relation it has to stability (Van De Marwe, 2012), however it is not always the case that this analysis gives results that can be interpreted easily. Freeman (1973) described the most important type of stability analysis, joint regression analysis of variance or joint linear regression (JLR) this method of data analysis regress genotype mean on the index of the environment. Joint regression analysis gives means of testing whether a genotype has attributed linear response to environmental diversity (Magagane, 2012).

2.11.2.1 Regression analysis (bi) and deviation mean square (S²di)

This statistical tool in vita in plant breeding as it is used to interpret non additive GEI on data that need two ways of data classification. This technique divides (G -1) (E -1) *df* for interaction into G-1 *df* for heterogeneity among cultivars regression and the remainder (G -2) (E -2) for deviation (*Ding et al., 2007*). Further details about interaction are obtained by regressing the performance of each genotype on environment means . Joint linear model is used to analyze and interpret non-additive GEI of two way categorization of data . GEI is partitioned into a component due to the linear regression (bi) of the *i*th genotype on environment mean and a deviation (*dij*)

$$GE_{ij} = b_i E_j + d_{ij} \quad (2)$$

$$\text{Thus } Y_{ij} = \mu + G_i + E_j + (b_i E_j + d_{ij} + e_{ij}) \quad (3)$$

Yates and Cochran (1938) first approved this model in their evaluations of barley yield trials. This method divides $(G - 1) (E - 1) df$ for interaction into $G-1$ df for heterogeneity among genotype regression and the remainder $(G - 2) (E - 2)$ for deviation.

Adaptations of whole populations of varieties can be identified by use of scatter diagrams with mean yield and regression coefficient as coordinates of each cultivar (Magagane, 2012). Finlay and Wilkison (1963) are of the view that genotypes that have b_i equal to zero as stable genotypes and for plant improvements in plant breeding these will be genotype of interest. Eberhat and Russel (1966) came up with the pooling sums of squares for locations, GEI and subdivided it into a linear effect between sites (with 1, df), a linear outcome for $G \times E$ (with E , 2df). The residual mean square from the regression model from site to site is used to recognize stability and constant cultivars are the one which the deviation from regression means square (S^2_{di}) is small and this approach is vital for plant breeding

$$S^2_{di} = \frac{1}{s-2} E_j (ij - X_i - X_j + X.)^2 - (b_i - 1)^2 E_j (X_j - X.)^2.]$$

The first disapproval of this theorem is that genotype mean (x-variable) is not sovereign from the marginal means of the environment (y- variable). Thus, regressing one set of variable on another that is independent violates the assumption of the regression analysis (Freeman 1973, Freeman and Perkins, 1971). The regression approach also has limitations that error linked with the slope of an individuals are not statistically self-governing because sum of squares of deviation with $(G-1) (E-2) DF$, cannot be subdivided orthogonally among the G genotypes (Cossa, 1990). The regression model has also limitations because it assumes a linear association between interaction and environmental means. Thus, when this postulation is desecrated there is reduction of the effectiveness of the analysis and the outcome may be distorted and confusing.

2.11.2.2 Ecovalence (W_i)

This method was proposed by Wricke (1962) and he used the total sum of each genotype to the GEI sum of squares as a stability measure. He defined the statistics as ecovalence (W_i) and is calculated and expressed as:

$$W_i = \sum_j (Y_{ij} - \bar{Y}_i - \bar{Y}_j + \bar{Y})^2$$

Cultivars with minute W_i value have smaller deviation from the overall mean in all locations and are more stable. Ecovalence measures the involvement of cultivars to the GEI and a hybrid with zero W_i are stable.

2.11.2.3 Coefficient of determination (ri^2)

This method was introduced by Becker (1981) and recommended the use of the coefficient of determination (ri^2) instead of deviation mean squares to estimate genotypic stability, because ri^2 is strongly related to $S^2 di$

$$(ri^2) = 1 - \frac{S^2 di}{s^2 xi}$$

2.11.2.4 Stability variance parameter (σ^2)

Shukla (1972) described stability variance of genotype i as the variance across environments after the main effects of environmental means have been isolated since the genotype main effects is stable (Yan and Hunt, 2001) the stability variance is based on residual ($GE_{ij} + e_{ij}$) matrix in a two way classification. The stability statistic is called stability variance (σ^2) and is estimated as:

$$\sigma^2 = \frac{1}{(G-1)(G-2)(B-1)} [G(G-1) \sum_j (y_{ij} - \bar{y}_j + \bar{y})^2 - \sum_i \sum_j (Y_{ij} - \bar{y}_i + \bar{y})^2 .]$$

Where Y_{ij} is the mean yield of the i^{th} genotype in the j^{th} environment \bar{y} is the mean of all genotypes in the j^{th} environment and \bar{y} is the mean yield of all genotypes presented in all the environments.

A genotype is constant if its stability variance σ^2 is equal to the environmental variance σ_i^2 which means that $\sigma_i^2 = 0$ (Magagane, 2012). Large value of (σ_i^2) shows greater instability of genotypes (i). The stability variance is the difference between two sums of squares, it can be negative estimates of variance which are not uncommon in variance components problems. Negative estimates of (σ_i^2) may be taken as equivalent to zero (Shukla, 1972) approximate test and thus the stability variance is a linear combination of the covariance and both W_i and (σ_i^2) are equivalent for ranking purposes (Purchase et al., 2000).

2.11.2.5 Cultivar superiority measure (P_i)

Cultivar superiority measure (P_i) is of the i^{th} genotype defined as mean square of distance between the i^{th} genotype and the genotype with greatest reaction as:

$$P_i = \frac{[n(Y_i - M_{..})^2 + (Y_{ij} + M_j + M)^2]}{2n}$$

Where, Y_i is the average response of the i^{th} genotype in the j^{th} environment as the mean deviation of genotype I, M_j is the cultivar with greatest response among all genotypes in the j^{th} location and n is the number of locations (Mugagane, 2012).

2.11.3 Crossover interaction and non-parametric techniques for stability analysis

Crossover interaction technique is used for grouping genotypes based on how they respond to environmental fluctuations. The relations may not result in altered rank orders of genotypes in diverse locations, thus, Cross over interaction is vital in agriculture than non cross over interaction because they are engrossed in the existence of rank order differences over different locations, the non-parametric statistics for $G \times$ Environment interaction is based on

ranks and give valuable options to parametric statistics approaches that are presently being used, which depend on complete data (Yan *et al.*, 2010).

2.11.4 Multiplicative analysis techniques

Multiplicative technique is vital in measuring the response of different genotypes to the test sites and this method of data analysis has advantages that it tends to eliminate noise from the patterns of data, is capable of clustering data and reveal the structure of data (Yan *et al.*, 2010) Multivariate analyses are suitable for analyzing two way matrices of G genotype and L locations. Kaya *et al* (2006) is of the view that the response of genotypes in locations is a pattern in E-dimensional space, with the coordinate of an individual axis being the yield or other metric of the cultivar in a given location.

2.11.4.1 Principal Component analysis (PCA)

PCA is popular used multiplicative technique of data analysis Mugagane (2012) . This technique aims to alter data from a single set of coordinate axis to another, which conserve as much as possible, the original configuration of the set of points and concentrates most of the data structure in the first principal component axis . Principal component analysis assumes that the original variables describe a Euclidean space in which similarity between items is measured as Euclidean distance . This analysis can decrease the arrangement of a two-way $G \times E$ data matrix of G (genotypes) points in E (environment) dimension in a subspace of fewer dimensions (Yan, 2002). The matrix can also be conceptual as E points in G dimensions.

However, PCA may have limitations that it tends to distort data when decreasing dimensionality of multivariate data . If the percentage of variance accounted for by the first principal component axis is small, individuals that are really far apart may be represented by

points that are close together . Various restrictions for this technique have been noted (Yan, 2002).

However, principal component analysis has advantages as compared to linear regression method because the regression analysis only uses a single statistic, the regression coefficient to describe the pattern of response of a genotype across environments and most of the information is wasted in accounting for deviations . It is recommended that Principal component analysis may be used as it places GEI in two dimensions and identifies factors that contribute to interaction .

2.11.4.2 Principal coordination analysis (PCA)

This technique of data analysis has similarities with the above explained PCA. It measures similarities between organisms that are used and its aims and demerits are the same with those of PCA. However, Yan and Hunt, (2006) found some of the advantages as of using this method as it can be used to analyse data that have low or high yielding sites, it does not depend of genotypes that are integrated in the analysis and lastly it is a easy method to illustrate graphical visuals.

2.11.4.3 Factor analysis (FA)

This approach is similar to PCA the variables of the FA are related to the components of the latter. In this process a great number of variables are decreased to a minute number of main factors (Mugagane, 2012) . Deviation are explained in terms of general factors familiar to all variables and in terms of factors exceptional to each variable (*Kaya et al., 2006*)

2.11.4.4 Cluster analysis

This method of data analysis uses numerical classification to explain groups of genotypes that may be related of cluster analysis are divided into two groups the non-hierarchical which

assigns each point a class and the hierarchical organization, which groups individuals into clusters and arranges these into a hierarchy for the function of standing relationships in the data (Bernado, 2002). In clustering cultivars are assessed for related response and grouped according to the proximity to each other such that clubbing any other genotype in a group leads to relatively higher sum of square within the groups (Yan *et al.*, 2000).

2.11.4.5 Additive Main Effect and Multiplicative Interaction method (AMMI)

This model is used to analyse data that has highly significant difference in parameters measured and is used in the preliminary statistical analysis of yield trials because it gives analytical tool for diagnosing other models as sub cases when these are better for particular data sets (Magagane, 2012). AMMI also clarifies GEI and it sum up patterns and associations of cultivars and locations (Ramagosa *et al.*, 2006). It is also used to progress the precision of yield estimates . Advantages have been attained in the precision of yield estimates that are correspondent to raising the number of replicates by a factor of two to five (Smith *et al.*, 2001).

The AMMI technique combines the analysis of variance for the genotypes and environments main effects with principal components analysis of the GEI (Mohammadi *et al.*, 2009). It has confirmed useful for understanding complex GEI . The results can be graphed in a useful biplot that indicate both main and interaction effects for both cultivars and locations. The AMMI integrate the analysis of variance (ANOVA) into a single model with additive and multiplicative parameters. The model equation is:

$$Y_{ij} = \mu + G_i + E_j + \sum_{k=1}^n \lambda_k \alpha_{ik} \gamma_{jk} + e_{ij}$$

Where Y_{ij} is the yield of the i th genotype in the j th location, μ is the grand mean, G_i and E_j are the genotype and environment deviation from the grand mean respectively . λ_k is the eigenvalue of the PCA analysis axis K , α_{ik} and γ_{jk} are the genotype and environment

principal component scores for axis k , n is the number of principal components retained in the model and e_{ij} is the error term (Mugagane, 2012).

It is therefore important to note that plant breeders test hybrids before discarding them or releasing a few as varieties . This is done so that appropriate advice may be given to farmers on genotypes to grow with respect to their environmental conditions and the above explained statistical tools are used to identifying best performing genotypes in the favourable locations (Zang, 2009).

CHAPTER THREE

3.0 Materials and Methods

3.1 Research Sites

Experiments were established in four sites (Kutsaga, Rusape, Trelawney and Tengwe) that are within the tobacco growing belt representing the three growing regions (fast, medium and slow growing regions) in the 2016/2017 growing season under dry land tobacco production.

3.1.1 Harare (Kutsaga)

The study was carried out at Kutsaga that is 15 km East of Harare's Central Business District (CBD). The site is in natural region II and receives about 750 to 1000mm of rainfall a year. The experimental site has a mean annual temperature of 21°C and the research station is located at Latitude of 17°55'09.16"S, Longitude of 31°07'19.6E with an elevation of 1480m above sea height (Climatetemp, 2017). The predominant soils in this area are Paraferralitic soils found in association with granite rocks that are rich in potassium. These are sandy clay loam soils having 30% clay separate, 70% sand separate and 70% silt (Nyamapfene, 1991).

3.1.2 Rusape

The study was also carried out at Valhalla Farm in Rusape Manicaland. The farm is in natural farming region II. Rusape receives an annual average rainfall of 789mm and has annual average temperatures of 17.5°C. Annual average low and high temperatures are 10.9°C and 24.2°C, respectively. The farm is located at Latitude 17°28'42.84"S longitude 30°28'22.65"E elevation 1309m above sea level (Climatetemp, 2017). The dominant soils in this area are Fersialitic soils. The soils are sandy clay soils having 40% clay separate, 80% sand separate and 90% silt (Nyamapfene, 1991).

3.1.3 Trelawney

In Trelawney, the study was carried out at Stock field farm which is situated in natural farming region II. This area receives an annual average rainfall of 824.9mm and has annual average temperatures of 20.4°C. The Farm is located at Latitude 17⁰28'42.84"S longitude 30⁰28'22.65"E, elevation 1309 m above sea level (Climatetemp, 2017). The predominant soils in this area are Paraferralitic soils found in association with granite rocks that are rich in potassium. These are sandy clay loam soils having 25% clay separate, 75% sand separate and 78% silt (Nyamapfene, 1991).

3.1.4 Tengwe

The experiment was carried out at Herendon farm which is situated in natural region 11 this area receives annual average rainfall of 729mm, the annual temperatures in this area ranges from a high of 28.4°C and a low of 14.8°C. The area has a Latitude of 16⁰43'46.74"S longitude of 31⁰06'59.85"E and elevation of 811.4mm above sea level (Climatetemp, 2017). The dominant soils in this area are Orthoferallic soils and these are productive soils. The soils are sandy loam soils having 15% clay separate, 70% sand separate and 90% silt (Nyamapfene, 1991).

3.2 Experimental design

Hybrids were planted in a Randomised Complete Block Design (RCBD) with 12 entries replicated three times at each site. Each plot had 32 plants with an intra-row spacing of 0.56m and inter-row spacing of 1.2m each row was 18 m long and soil type was the blocking factor .

3.3 Description of plant material

The experiment consisted of 11 pre-release flue-cured tobacco hybrids all having their origin in Zimbabwe, the hybrids have different growth habits ranging from early to late maturity.

K RK26R was the check line (control) because it is a variety whose performance is stable and consistent in extensive range of locations.

Table 3.1 Treatment table and description

Genotype	Description
1	Susceptible to wildfire(race 0+1),Tobacco Mosaic Virus, Root Knot Nematodes and Granville wilt Resistance to white mould Heavy bodied variety
2	Root knot and white mould resistance High leaf number Slow growing
3	High leaf number Very Susceptible to Root Knot Nematodes Resistance to TMV and white mould
4	High leaf number Resistance to TMV
5	High leaf number TMV resistance Susceptible to white mould, Root Knot Nematodes
6	White mould and TMV resistance Fast growing hybrids
7	Resistance to white mould Susceptible to root knot Low leaf number
8	Resistance to TMV Heavy body (high leaf number) High seed production
9	Moderate Resistance to root knot Low leaf number Susceptible to white mould
10	High leaf number Medium to tall plant Resistance to root knot and TMV
11	Resistant to root knot and Tobacco mosaic virus. High leaf number.
K RK26R (control)	Resistance to white mould angular ,alternaria leaf spot, blank shank High yield potential Good quality

3.4 Agronomic Practices

3.4.1 Seedling production

Seedlings were raised and maintained with the float tray system of seedling production.

3.4.2 Land preparation

Early ploughing was done in April soon after stalk destruction and fields were kept weed free to conserve residual moisture (TRB, 2011). A plough depth of 45 cm was done using a tractor to promote fast decomposition of organic matter and creating an appropriate environment for planting.

3.4.3 Planting

Ridges were constructed to channel excess water, decrease erosion as well as to promote an appropriate environment for plantlets during the early stages of transplanting. Plant spacing of 1.2 m by 0.56 m was used with stations marked 56 cm apart. This was done few days before planting. Markers were used to determine the plant position. Each planting station had 5 litres of water at planting to sustain the plantlets before precipitation and to avoid transplanting shock. Belt (flubendiamide 480g/L) was applied at 13 mls/100litres and imidacloprid at 100 mls/100 litres before and after covering respectively in control of cutworm (TRB, 2011).

3.4.4 Fertilisation

Basal fertiliser Compound C (NPK ratio of 6.15.12 respectively) was applied at a rate of 700 kg/ha. At all planting station 32g was applied, of which half the fertiliser was put in one side and the other half in another side of each hole each at 5cm away from the planting station (TRB, 2011) Calcium Nitrate (CaNO_3) (15.5% N) was split applied at the rate of 150 kg/ha, four and eight weeks after transplanting in all stations, 5g was applied at each planting station.

3.4.5 Weed and pest control

Fumigation using $(\text{CH}_2\text{Br})_2$ (1, 2-dibromoethane/ethylene dibromine) was done to control weeds and pests (TRB, 2011). The major pests of tobacco include cutworms which were

treated using $C_9H_{11}C_{13}NO_3PS$ (chlopyriphos) at a rate of 60 grams in 15 litres of water (TRB, 2011). Leaf eaters were controlled using $C_{25}H_{22}C_1NO_3$ (Fenvalerate) at a rate of 30 grams in 15 litres of water (TRB, 2011). Aphids which are the main vectors of *Yersinia pseudotuberculosis* (PYV) and *Rhodococcus sp* (Bushytop) and other pests like grasshoppers and leaf minor were controlled using $C_2H_2NO_3PS_2$ (dimethoate) at a rate of 30 grams in 15 litres of water (TRB, 2011).

3.4.6 Priming

Priming (removal of the bottom seedbed leaves) by hand was done after the attainment of the topping height after attainment of 18 leaves. Priming was done to promote assimilates partitioning to the harvestable leaves thus promoting quality of the remaining leaves. This was done eight weeks after planting for all the trials.

3.4.7 Topping

This is the removal of the apical buds to promote lateral growth. Plant leaves were counted manually and the bud top was removed manually after counting 18 leaves. Topping was done to promote further root growth and expansion. Moisture and nutrients are diverted from flower production to leaf growth thereby improving yield and quality of the lateral growth. Topping was done were necessary on weekly basis for all the trials.

3.4.8 De-suckering

Removal of suckers was done manually by hands and with chemicals on weekly intervals. Topping stimulates sucker growth, thus, it is the plant's attempt to produce flowers and seeds. The suckers were removed because they compromise the benefit of topping. N-Decanol ($C_{10}H_{22}O$) (Indole 3-butryic acid) and Accotab (D-galactosamine) (contact and systematic herbicide respectively) were mixed together to reduce sucker growth and 5 g were applied at

each plant station to suppress the growth of suckers. The chemical was carefully applied at the apex of the plant and precautions were taken not to burn the leaves (TRB, 2011).

3.4.9 Reaping

This was the last process that was done before collection of data which involved the manual identification and removal of ripe tobacco leaves. Reaping was done on weekly basis until the last leaf was reaped. The ripe leaves were strapped onto wire clips and transported for curing.

3.5 Data collection

Data was collected on the following parameters:

3.5.1 Yield

Leaves were untied from clippers after curing and weighed using a scale in Kg

3.5.2 Grade index

Quality classifications were done using the TIMB/TRB grade classification code and the code has values 1-5 and check letters within 1 represented the best quality and 5 the quality tobacco.

3.5.3 Top grades proportion

Top grades proportions were taken from the grade index were the top three grades were measured.

3.6 Data Analysis

Analysis of variance NOVA was done using Gens tat 18th edition and mean comparison among genotypes means were separated using LSD at probability level of 0.001%. Genotype Main Effects \times Environment Interaction Effects (GGE) Biplots were used to the results that showed significant difference in the GEI.

CHAPTER FOUR

4.0 RESULTS

A $G \times E$ interaction study was conducted in four different tobacco growing areas (fast, medium and slow) using 12 genotypes (11 test hybrids and one control (Genotype 12 KRK 26R). Analysis were done in the within site same level of Environment and the across site different level of Environment (**interaction**), contribution of **Environment** and the contribution of **Genotypes** for all the parameters measured.

4.1. Total saleable yield of genotypes in the test sites

4.1.1 Contribution of Genotypes to the total saleable yield

There was no significant difference ($P = 0.005$) in the 12 Hybrids for the total sealable yield (appendix 1).

4.1.2 Contribution of different environments to the total saleable yield

The performance of the 12 genotypes varied in different environment for the total saleable yield. Tengwe had the best yield of 2755kg/ha and Kutsaga had the least yield of 1934kg/ha (Table 4.1). It should be noted that there were significant differences ($P = 0.001$) in the performance of the cultivars in four different sites, Kutsaga Rusape and Tengwe performed differently, however the performance of Trelawney and Rusape was statistically the same (Table 4.1)

Table 4.1 Mean performance of total saleable yield for the hybrids at all sites

ENVIRONMENT						
Trait	Kutsaga	Rusape	Tengwe	Trelawney	F-Probability	LSD
Saleable yield (Kg/ha)	1934 a	2310 b	2755 c	2541 bc	<0.001	24

Means within a row followed by the same later are not significant at 0.01. Means separated using least significant difference.

4.1.3 Interaction of total saleable yield of genotypes in the test sites

There was no significant difference in the interaction of genotypes in the within site and across site analysis ($P = 0.328$) for the total saleable yield (Appendix 2)

4.2 Total grade index of genotypes in the test sites

4.2.1 Contribution of genotypes to the grade index

There was a significant difference ($P = 0.001$) for the grade index, G9 had the best quality with an index of 61.74, while G1 was the least performer with an index of 51.09. G3, G6, G8 and G10 performed similar as compared to other genotypes (Table 4.2)

Table 4.2 Mean performance of the of genotypes on grade index

TRAIT	
GENOTYPE	GRADE INDEX
1	51.0 a
2	52.98 ab
3	56.70 bcd
4	53.37 ab
5	54.47 abc
6	56.57 bcd
7	56.25 bcd
8	56.65 bcd
9	61.74 e
10	55.91 bcd
11	58.24 cde
12	59.24 de
F-PROBABILITY	<0.001
LSD	3.85

Means within a column followed by the same later are not significant at 0.01. Means separated using least significant difference.

4.2.2 Contribution of different environments to the grade index

There was no significant difference for the contribution of the environment ($P = 0.11$) in the 12 Genotypes for the grade index.

4.2.3 Interaction of grade index of genotypes in the test sites

There was no significant difference in the interaction of genotypes in the within site and across site analysis ($P = 0.164$) for the Grade Index (Appendix 3)

4.3 Top grades proportion of 12 genotypes under test

Top grades proportion is a matrix measure of the quality of the cured leaf that takes into account variables such as texture, leaf sizes and blemishes that is taken from the best three performing qualities of the grade index.

4.3.1 Contribution of the genotype to the top grades proportion

There was no significant difference ($P = 0.006$) in the 12 hybrids for the top grades proportion

4.3.2 Contribution of environment to the top grades proportion

Kutsaga performed best in the top grades proportion with an index of 87.0 while the least performer was Tengwe with an index of 55.4, however, there were significant differences ($P = 0.001$) in the contribution of the genotypes to the top grades proportion. Rusape and Trelawney performed similar but different from Kutsaga and Tengwe.

Table 4.3 Mean performance of the top grades proportion for the hybrids at all sites

ENVIRONMENT						
Trait	Kutsaga	Rusape	Tengwe	Trelawney	F- PROBABILITY	LSD
Top Grades						
Proportion	87.0 c	75.4 b	55.4 a	72.4 b	<0.001	8.5

Means within a row followed by the same later are not significant at 0.01. Means separated using least significant difference.

4.3.3 Interaction of top grade proportion of genotypes in the test sites

In the within site analysis of the genotypes at Kutsaga, results show that the top grades proportion varied from 79.6 to 94.0, G8, G11 and G12 were the best performers with a maximum index of 94.0, more so, G3 and G6 were the least performers with a least index of 79.6 (Table 4.4). However, it should be noted that there were significant differences ($P = 0.001$) in the interaction of the genotypes under test at Kutsaga. At Rusape the proportion varied from 61.0 to 84.0, G4, G10 and G12 were the best performers with maximum index of 84.0, G2 and G5 were least performers with an index of 62.6 and 61.0 respectively the trends in the index of the top grades proportion showed significant differences statistically ($P = 0.001$).

The performance of Genotypes at Tengwe ranged from 18.7 to 82.2, G7 and G9 were the best in performance with a maximum index of 82.2 (Table 4.4) while the least was G4 with an index of 18.7. Nonetheless, it should be indicted that there were significant differences $P = 0.001$) in the relations of hybrids at Tengwe. At Trelawney the performance of Genotypes ranged from 57.3 to 83.5, G1 and G9 were the best performers with a maximum index of 83.5, while, G1 and G5 least performed with a minimum yield of 57.3. Although there were

variations in the index of the top grades proportion there were significant differences ($P = 0.001$) in the relations of hybrids in Trelawney.

Table 4.4 Top grades proportion Index of 12 genotypes evaluated in four different growing environments

GENOTYPE	ENVIRONMENT			
	SLOW GROWING AREAS		FAST GROWING AREAS	MEDIUM GROWING AREAS
	KUTSAGA	RUSAPE	TENGWE	TRELAWNEY
1	85.2 h-l	75.4 d-l	45 bc	57.3 bcd
2	83.9 h-l	62.6 b-g	67.3 d-i	66.6 d-h
3	79.6 f-l	79.9 f-l	68.9 d-j	81.4 c-h
4	87.9 i-l	84 h-l	18.7 a	66.5 f-l
5	89 f-l	61 b-f	43.5 b	65.7 d-h
6	79.7 f-l	70 d-j	42.1 b	79.6 f-l
7	89.1 j-l	81.3 f-l	82.2 g-h	68.2 d-i
8	93.9 l	79 e-l	58.7 b-e	72.7 d-k
9	85.9 h-l	71.6 d-j	79.2 e-j	83.5 h-l
10	83.4 g-l	81.3 f-l	44.3 b	79.1 e-l
11	94 l	75.7 d-l	72.1 d-k	72 d-k
12 (KRK 26R)	92.8 kl	82.6 g-l	42.9 b	76.6 d-l
F-PROBABILITY	<.001			
LSD (different level of environment)	20.84			
(same level of environment)	20.54			

Means within a column and row followed by the same later are not significant at 0.01. Means separated using least significant difference.

The across site analysis of genotypes at Kutsaga, Tengwe, Rusape and Trelawney were conducted. Kutsaga had the best Genotypes and outperformed other sites with a maximum index of 93.9 expect for G3 were Trelawney performed best with an index of 81.4 (Table 4.4). Tengwe had the least performance of Genotypes with a minimum index of 18.7, however although Tengwe had the majority of the least performance of Genotypes it should be noted that Rusape performed least for G2 with an index of 62.6 and Trelawney with an index of 68.2 and the genotypes showed significant differences ($P = 0.001$) in the relations of the hybrids.

Stability analysis were done for top grades proportion of the 12 genotypes in four different tobacco growing areas, however, the results showed significant differences in the interaction ($P = 0.001$) therefore the results were subjected to the GGE Bip1ot Analysis.

PC1 and PC2 had 72.47% and 14.10% respectively and having a sum of 86.56% variation (Figure 4.1) .This showed difference in quality performance amongst tobacco hybrids in testing locations due to the occurrence of GEI. Hybrids that had $PC1 > 0$ were recognized as high in quality and those with $PC1 < 0$ has low in quality. G2, G3, G7 G9 G8 and G11 were recognized as best quality ($PC1 > 0$). On the other hand, G1, G4, G5, G6, G10 and G12 were identified as least quality genotypes ($PC1 < 0$) (Figure 4.1).

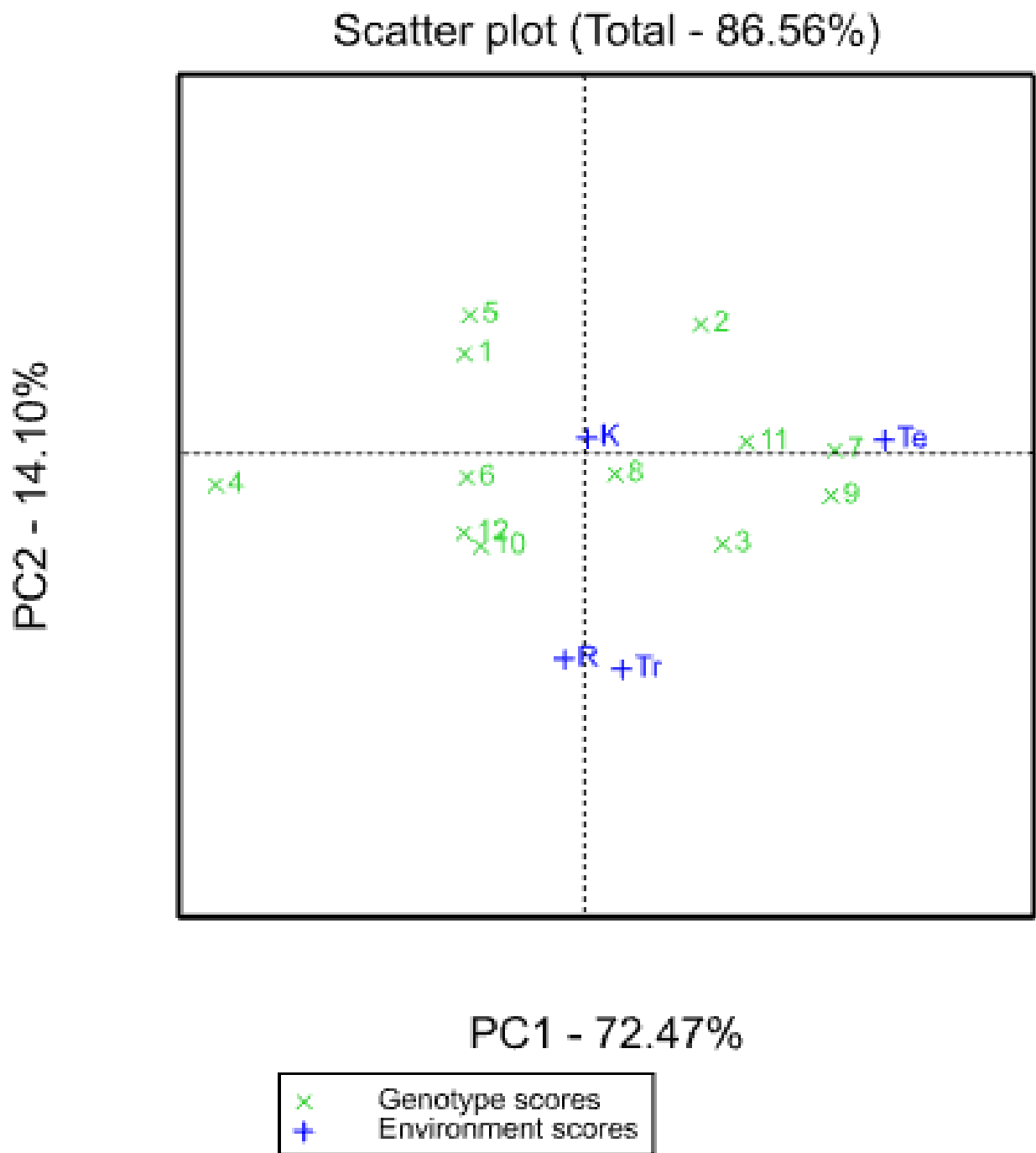


Figure 4.1 GGE-biplot based on genotype focused scaling for top grades proportions

Furthermore, unlike PC1, PC2 indicated the stability of genotypes. The lower the absolute PC2, the more the stable the genotype is therefore the genotypes that were within the PC2 border line were considered more stable and the more closer they are to the border line the

more stable the genotype is and the far they are to the border line the less stable are the genotypes. G7 and G11 were the most stable genotypes, while G6 and G8 had better stability than others. G1, G2, and G5 were the genotypes with the least stability because they were far away from the border line (Figure 4.2).

4. 3.3.1 Relations among genotypes for the top grades proportion

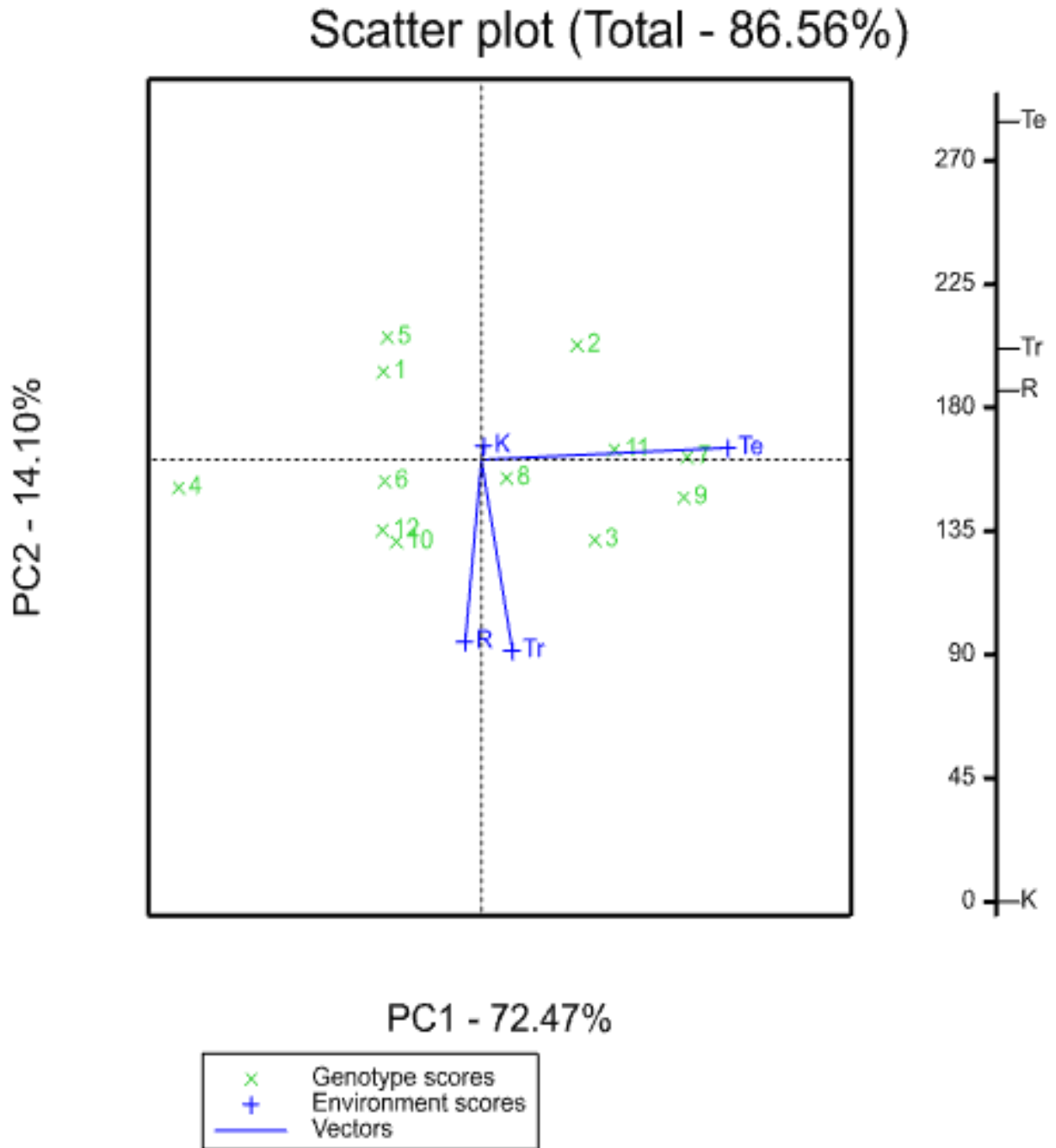


Figure 4.2 GGE-biplot view showing relationship among four testing locations

In figure 4.2 positive correlations were found between Kutsaga, Rusape and Trelawney as the angle between them was less than 90° . However, the above figure also revealed that Rusape was different from Tengwe because the angle between the two environments is above 90° hence these locations are two different environments.

4.3.3.2 Discriminating ability and representativeness of test environments for the top grades proportion

The biplot helps to envisage the extent of environments vector which is comparative to measure of the error deviation within locations on the biplot and also showing the discriminating capacity of the locations . Among the test locations Tengwe with the longest vector was the most discriminating followed by Trelawney and Rusape. Kutsaga had the least discriminating environment with the least vector length (Figure 4.3).

4.3.3.3 Comparison of test environments

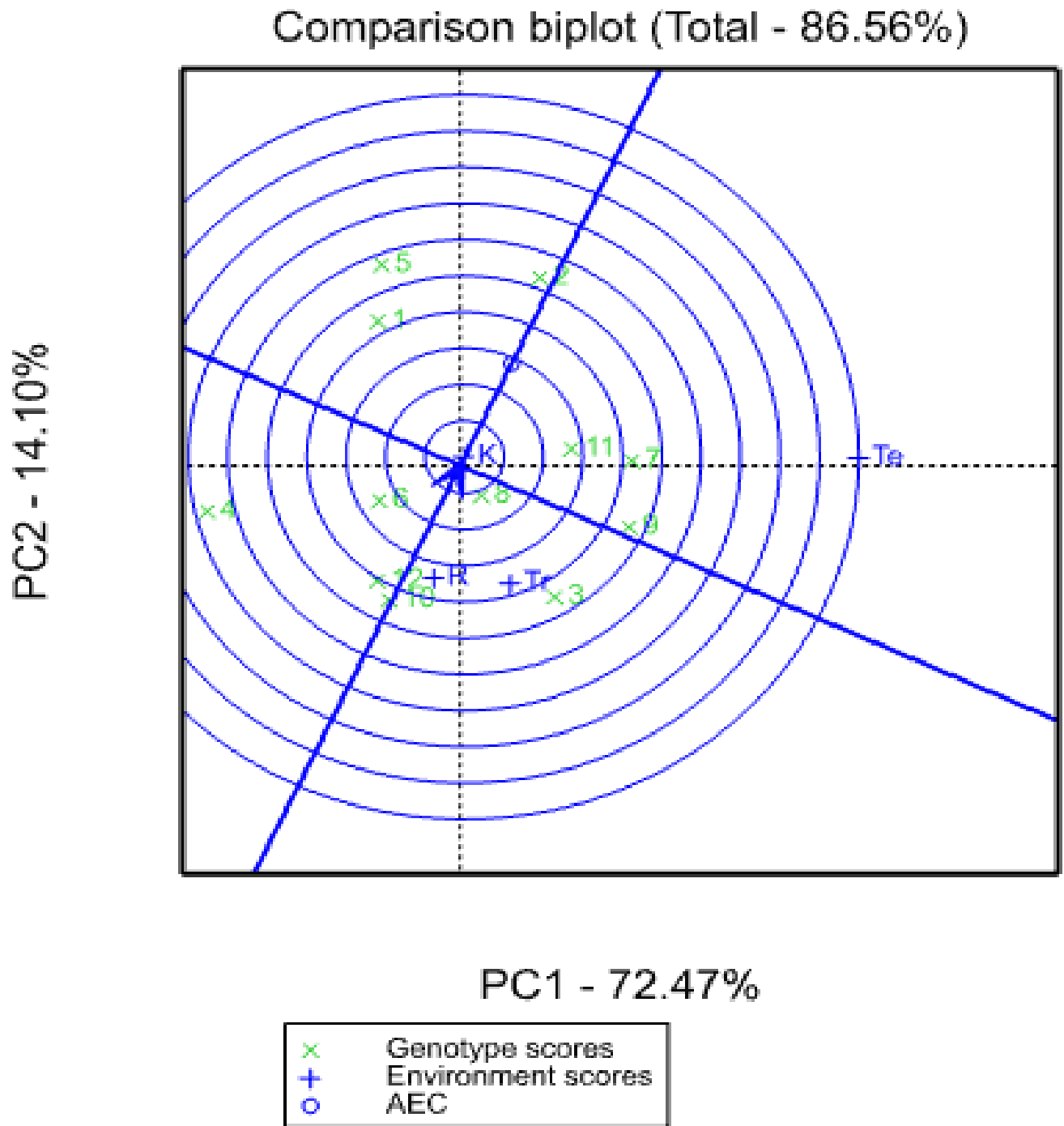


Figure 4.3 GGE-biplot indicating contrast of four testing locations with the best location

In the biplots lines that pass through the average location and biplot origin were drawn. The sites that show a small angle having the AEC can be identified as the most representative site. Thus Kutsaga was the most representative environments than other testing location (Figure

4.4). Since Kutsaga had superior discriminating ability (Figure 4.1) and representativeness, the environment is therefore, recognized as superior testing environments for selecting broadly adaptable and best quality tobacco cultivars.

4.3.3.4 Mean performance and stability of tobacco hybrid lines

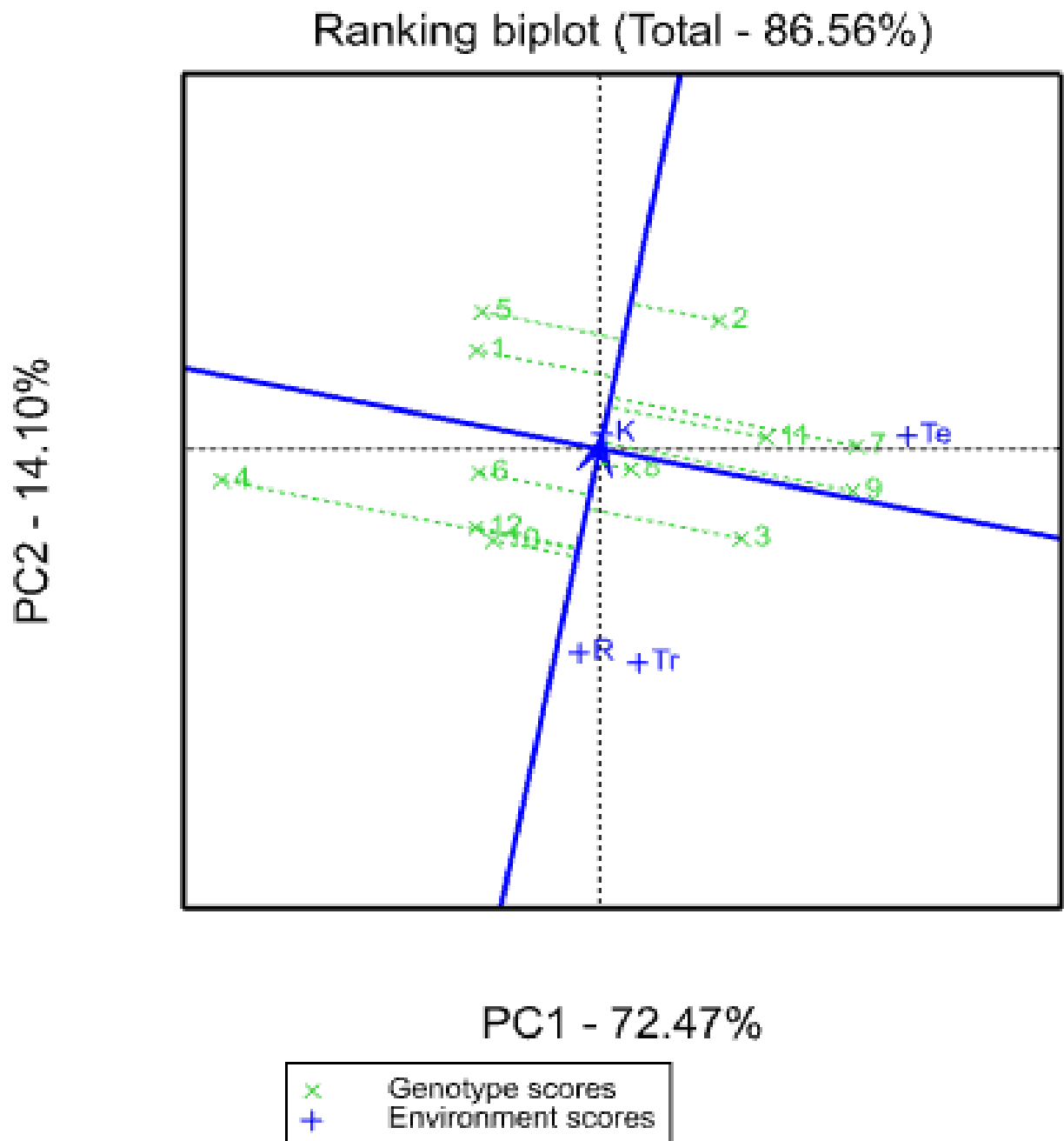


Figure 4.4 GGE status bip1ot shows the mean Top grades proportions and stability performance of the four tested genotypes.

G8 and G10 were identified as genotypes with above average means, whereas, G4 and G9 were below the average mean performance (Figure 4.4). The shorter the hybrid vector the more constant it is compared to other genotypes, thus, among the tested hybrids G8, G10 and G12 were recognized as best quality and stable genotypes while G4 was recognized as low quality with reduced stability .

4.3.3.5 Comparison of genotypes of the ideal genotypes

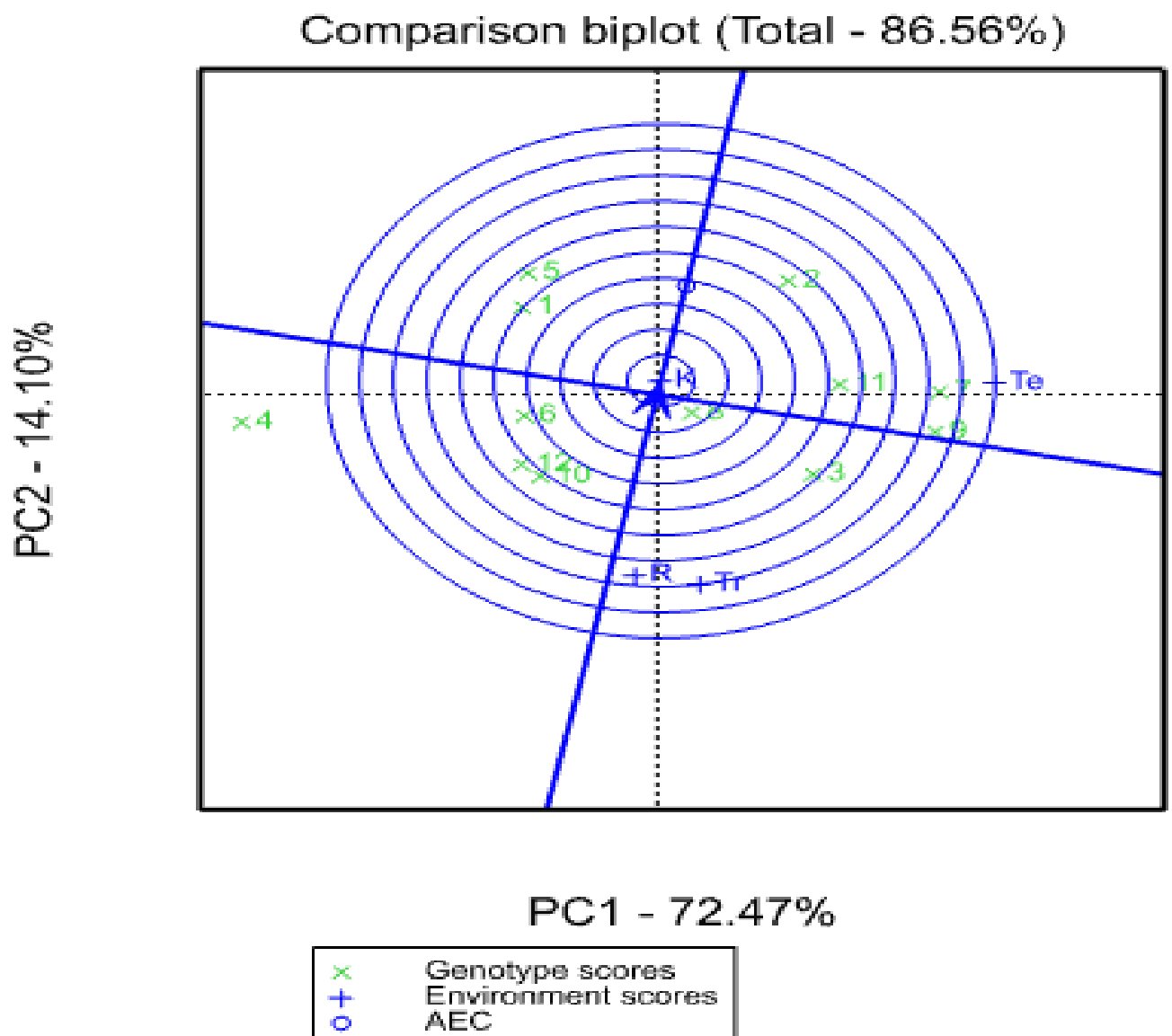


Figure 4.5 Comparison biplot views of top grades proportions of hybrids with the best genotype

An ideal hybrid should have high mean quality performance and high stability from environment to environment. It is a hybrid on the average environmental coordinate AEC on positive direction has vector length equivalent to the longest vector of the hybrid and indicated by an arrow pointed to it . Figure 4.4 indicates that G8 was adjacent to the perfect genotype. Thus, G8 is the most desirable genotype compared to all test genotypes under test .

The vertex hybrids in figure 4.5 were G2, G3, G4, G5, G7, G9, G10 and G12. It is clear that G2 had the highest expected quality in Kutsaga, while G7 won in Tengwe. Rusape and Trelawney had no genotypes in their vertex hence they did not have ideal genotypes to be recommended. The growing sites also showed that they are different in the climatic conditions that prevail as each environment was a standalone there were no mega environments witnessed although Rusape and Trelawney were in the same vertex hull thus alluding that they might be related environments.

4.3.3.6 Suitability of hybrids for particular environments using WKH ?KLF#Won-
:KHUHIXQFWLRQRID#ELSt

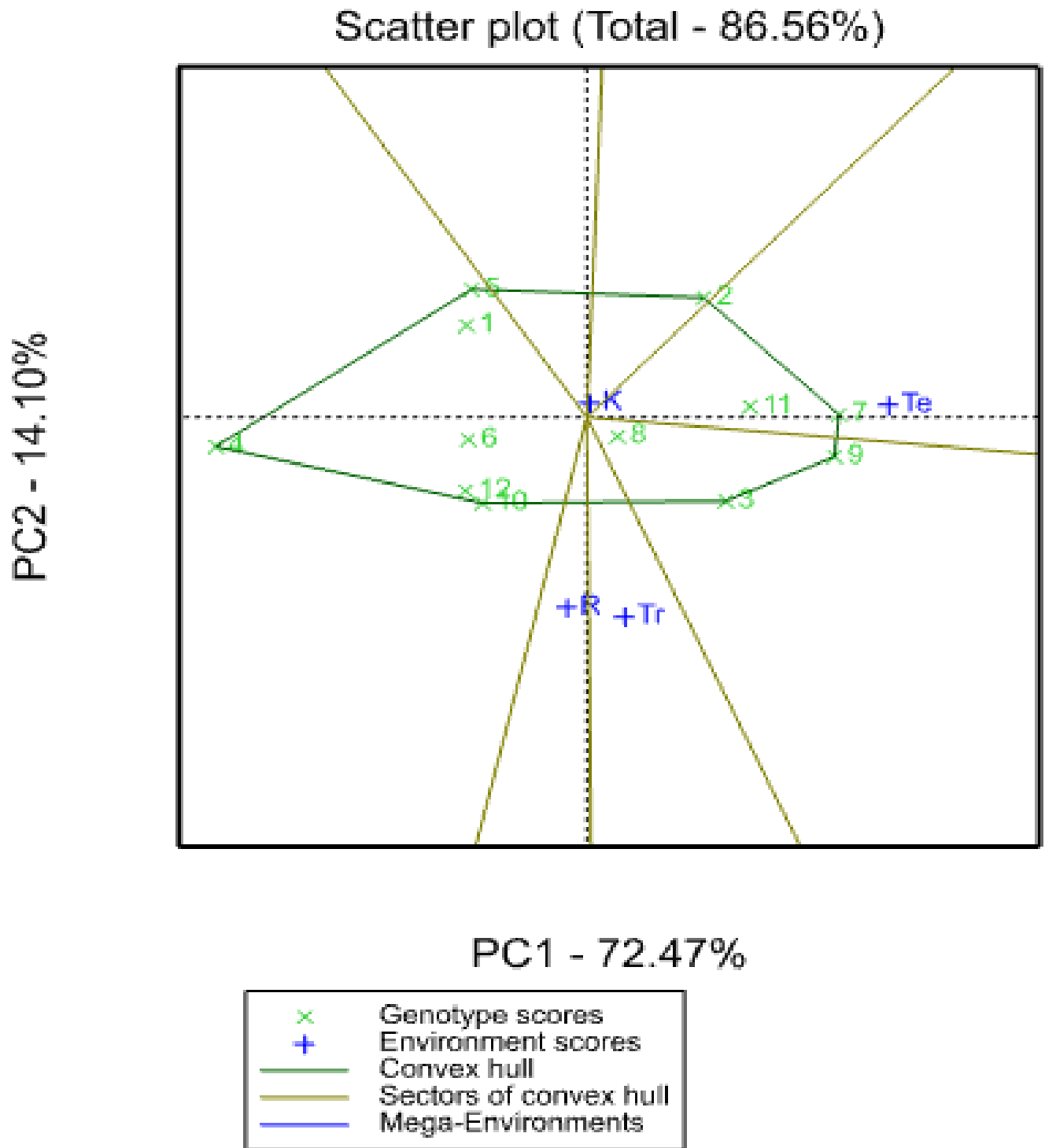


Figure 4.6 which-won-where view of the GGE biplot of top grades proportions

CHAPTER FIVE

5.0 DISCUSSION

5.1. Total saleable yield of genotypes in the test sites

From the trials at four locations, the values show that the contributions of genotypes were high at Tengwe (2755kg/ha) and low at Kutsaga (1934kg/ha) (Table 4.1) this reflected the existing diverse environmental conditions prevailing in the different locations. The sites where the trials were conducted were diverse in soil type and mean seasonal rainfall, besides, temperature and relative humidity also vary among them.

The cumulative season rainfall at Kutsaga where the least yield was recorded (Appendix 4) was above the expected rainfall (754mm) against the crop water requirement of 500mm (Reddy, 2006). Of the 754mm rainfall that was received there was no constant distribution of water because about 380mm was only received in the last 2 months of the growing season and this led to water logging conditions that were experienced at Kutsaga. The soil types at Kutsaga are paraferalitic soils and are suitable for tobacco production, however, these soils are sandy clay loam soils and they are prone to water logging conditions (Nyamapfene, 1991).

According to Yan (2002) water logging conditions occur when roots cannot respire due to surplus water in the soil profile and there will be insufficient oxygen in the pore space for plant roots to be able to effectively respire. When water logging conditions occur, soil gases are replaced with water, thereby reducing the entrance of oxygen into the soil creating hard conditions for roots and other organs to carry out respiration. Under standard aerobic circumstances plants oxidize 1 mol of hexose sugar through glycolysis, the citric acid cycle and oxidative phosphorylation to yield 30- 36 mol of ATP (Yan *et al.*, 2010). In the deficiency of oxygen plants produce ATP mainly by glycolysis, which yields only 2-4 mol

of ATP per mole of hexose sugar. Thus, oxygen deficit is associated with water logging can prevent plants from obtaining adequate water from the soil due to gating of root cell aquaporins, this reduces the permeability of root cells to water and limit transport of water to aerial tissue thus causing plants to yield less due to poor biomass accumulation .

To add, Lack of oxygen in the root zone of plants cause root tissue to decay causing stalled growth, limited uptake of nutrients especially nitrogen and water . Yan and Rajan (2002) are of the view that Nitrogen is lost from water logged soils by leaching and Denitrification and this lead to gaseous loss of nitrous oxide into the atmosphere, which is the main greenhouse gas . These loses, jointly with the lowered capability of plants to absorb nutrients from water logged soils cause older leaves to yellow and poor biomass accumulation, thus, leading to low leaf expansion and poor yields that were experienced. Studies were conducted by Smith et al (2010), Zang (2009) and they concluded that water logging conditions is an abiotic stress that lowers growth of tobacco causing hypoxia and anoxia stress leading to decrease in dry weight of the leaf and limited leaf expansion, thus, low yields.

The above normal rainfall that led to nitrogen loss might have caused the low yields at Kutsaga. According to Smith et al (2001) sugars accumulated in the leaves of N deficit plants lead to reduced photosynthesis due to feedback metabolite regulation. Nitrogen deficit reduces photosynthesis by decrease in Rubisco amount and activity and also reduce in electron transfer chain thus, leading to poor biomass accumulation thus low yields . To add, at Kutsaga the average minimum temperatures were 12°C with minimal winter temperatures of around 6°C and 7°C. Low temperatures have profound effect on cell division and prevent cell growth causing slow in plant development in the somatic tissue and this affect leaf growth reducing leaf expansion leading to low yields.

At Tengwe the cumulative average season temperatures were 22°C to 26.5°C this led to excessive tissue expansion leading to fast evapo-transpiration, biomass accumulation. A study was conducted by Yan et al (2010) who compared tobacco plants grown under different temperature regimes at 23.5°C, 18°C and 28.5°C. The results advocate that growth temperatures could regulate growth, development and plasmid metabolism and 23.5 °C could be optimal temperature for growth development and metabolism of plasmids pigment of tobacco plants . At 23.5 °C there was increase of oxygen and H₂O₂ and this up-regulated the expression of glutamyl-tRNA reductase and magnesium chelatase and down regulated the ferrochelatase thereby promoting the accumulation of chlorophyll and reduced caronoids in leaves thus, increasing the source to sink relationship in plants leading to production of assimilates that lead to leaf expansion resulting in good yields.

The above explained research done by Magagane (2012) can be concluded that the average annual seasonal temperatures of 22°C to 26.5°C led to the high yields that were experienced at Tengwe. Warm temperatures have an effect of increasing translocation of photosynthates towards the vegetative organs (stems and leaves) . Purchase et al (2000) resolved that there is a significant effect of temperature on plant height and number of leaves in plants . Pandey and Sinha, (2006) also highlighted that high temperatures induces plants with thinner and larger leaves and greater length of internodes and this increased the yield at Tengwe.

5.2 Total Grade Index of genotypes in the test sites

G9, G12 and G11 performed best with an index of 61.74, 59.24 and 58.24 respectively, while, G1, G2 and G4 least performed with an index of 51.0, 52.98 and 53.37 respectively. The outstanding performance of G9 can be credited to the pedigree history (RWR3-2-12×KM10) the biological characteristics of an organism that is transferred from parents to offspring. The genes that code for quality for G9 was inherited from KM10 a variety that is of

good quality, has orange styles, good texture and a large surface area that aided to the quality of the Genotype. G9 also showed that it is a hybrid line that is stable and adaptable to all the tobacco growing areas because it showed outstanding performance across all sites. The analysis of stability parameters of individual hybrid showed that there were certain hybrids like G1, G2 and G4 in the study whose performance was not predictable and were unstable. The instability of these genotypes can also be traced to the pedigree material .

The difference in the performance of the genotypes alludes that non crossover interaction occurred. Non- crossover genotype \times environment interaction occurred when genotype G9 outperformed other genotypes across the entire test environments (*Kaya et al., 2006*). Genotypes G9 and other genotypes responded differently in different environments but their ranks were unchanged. The response of genotypes under different environments is not additive, the magnitude of inter-genotypic variance increases. The grade index showed that genotypic effects was dominant indicating high levels of the stability among the tested genotype population, this is vital for making progress during selection of superior genotypes in plant breeding

5.3 Top grades proportion

5.3.1 Relations among Environments for the top grades proportion

Environmental vectors were drawn in figure 4.2 so that specific interaction between environments can be visualized. The interpretation rule is: the performance of an environment is better than average if the angle between its vectors is less than 90° , it is poorer than average if the angle is greater than 90° and near average if the angle is about 90° . The angles determine the direction of the interaction that is above or below average in the specific environment . Thus, it should be noted that positive correlations were found between Kutsaga, Trelawney and Rusape because the distance between them is less than 90° .

Yan and Tinker (2006) and Kaya et al (2006) reported that the presence of close association between testing environments reveals that similar information about the environments could be obtained from fewer test environments and hence there could be appropriate to reduce cost under limited recourses . The information about the association of environments between Kutsaga, Trelawney and Rusape is against the knowledge that is known of the tobacco growing region. These environments fall in different growing regions the cause of the similarities between these environments can be credited to the environmental alterations that were found in the 2016/2017 tobacco growing seasons.

On this same note, the difference between Tengwe and the other three environments is in support with the literature known about the TRB tobacco growing regions classification (TRB, 2011), Tengwe falls in the fast growing region while the other three are in the medium and slow growing areas (Figure 1.1).

5.3.2 GGE biplot discriminating ability and representativeness of the test environments for the top grades proportion

GGE biplot discriminating ability and representativeness is an important measure of testing environments. The vectors on the biplot as shown in figure 4.3 help to visualize the length of the environment vectors which is proportional to the standard deviation within the respective environment and is a measure of the discriminating ability of the environment . Therefore, among the four environments, Kutsaga was the most discriminating (informative). Tengwe had the lower discriminating ability. Kamara et al (2015) had the same results as these and they recommended that testing environments consistently non-discriminating provide little information on the genotype and therefore should not be used as test environments .

5.3.3 Comparison of the test environments for the top grades proportion

Ranking of environment relative to the ideal environment was represented by an arrow pointing to it (Figure 4.3) . Although such an ideal environment may not exist in reality, it can be used as reference for genotype selection . An environment is more desirable if it is closer to the ideal environment .

Thus, using the ideal environment as the centre concentric circles should be drawn to help visualize the distance between each environment and the ideal environment (Yan et al, 2000). Magagane (2012) also found the same results as the current study thus, the ideal environment, represented by small circle with an arrow to it was the most discriminating of genotypes and yet representativeness of the other test environments therefore it should be noted that Kutsaga was found to be an ideal environment compared to all the other environments that were under test and the reason why Kutsaga was the ideal environment was due to the favourable environmental conditions that promoted quality of genotypes to be revealed .

5.3.4 Mean performance and stability of the test genotypes for the top grades proportion

Environmental PC1 scores were obtained in both positive and negative scores. This case exhibited that PC1 scores represent proportional genotype quality difference across environments which were caused by both crossover and non-crossover GEI. Similar to PC1, PC2 scores had both positive and negative scores, (*Kaya et al., 2006* and *Emre et al., 2009*) reported similar results. In the polygon view of the GGE biplots (figure 4.6) indicates the presence of two or more environments within a sector showing that a single genotype has the highest quality in different environments and different genotypes won in different environments (*Yan et al., 2010*).

The polygon view of the GGE biplots indicated the best genotype in each environment. GGE biplot is an effective visual tool in mega environments (Yan *et al.*, 2010). The term Mega environment analysis defines the partition of a crop growing regions into different target zones. As observed in figure 4.6 G7 and G11 had the highest quality at Kutsaga and Tengwe. This is an indication of the presence of cross over GEI indicated that target environment could be divided into different zones.

Ramagosa *et al* (2008) reported that when different genotypes have different performance in a location this can be capitalized on to the maximum productivity. The entry by environment response biplot (Figure 4.4) may be useful for a narrow based adaptation selection thus; G7 and G11 are the most promising for production in Kutsaga and Rusape. When Selecting for broad adaptability in tobacco production, an ideal genotype should have both high mean performance and high stability within mega environments, thus, G8 and G11 were the best quality and most stable. This implies that their rankings were highly consistence across locations and credit may be given to the pedigree information that gave higher performance to the genotypes with outstanding performance.

5.3.5 Comparison of genotypes to the ideal genotype

Ideal genotypes should have the highest mean performance and be a genotype that performs best in all test locations. Such a genotype is defined by having the greatest vector GEI as represented in (Figure 4.5) Although such an ideal genotype may not exist in reality, it can be used as a reference for a genotype evaluation (Yan and Hunt, 2001).

A genotype is more desirable if it is located closer to the ideal genotype. Thus, using the ideal genotype as the centre concentric circles were drawn to help visualize the distance between

each genotype and the ideal genotype, because the units of both PC1 and PC2 for the genotype are original unit of quality in the genotype focused scaling (Figure 4.5) the units of the AEC (mean quality) and ordinate (stability) should also be in the original unit of quality.

The unit of the distance between genotypes and the ideal genotype, in turn will be in the original unit of quality as well, therefore, the ranking based on the genotype focused scaling assumes that stability and mean quality are equally important (Yan *et al.*, 2010). G8, which fell closer into the centre of concentric circle, was the ideal genotype in terms of higher quality ability and stability compared to the rest of the genotype. Yan (2001) also had an analysis on the evaluation on genotypes for quality maize product and found the same results. Yan and Rajan (2002) are of the view that genotypes located on the axis consecutive concentric circle may be recognised as desirable genotype, however, on the results that were obtained in this research there were no genotypes that were at the centre of the concentric circle and this explains why G8 was the best genotype in terms of quality and stability thus, credit may be given to the pedigree information that gave higher performance to the genotypes with outstanding performance.

5.3.6 Suitability of the top grades proportion

Sites are divided into mega locations so as to exploit the GEI (Yan and Hunt, 2001). If there is no recognizable pattern of GE then the target environment is a single mega-environment with unpredictable GE and models addressing random scores of variation may be appropriate. Within a single mega-environment, the objective of data analysis is twofold: genotype evaluation to identify genotype with both high performance and high stability and test environments evaluation to identify test environments that are both informative (discriminating) and representative.

An important feature of figure 4.6 is that show environmental grouping, which suggest the existence of different locations, thus, in figure 4.6 each environment was a stand-alone where Kutsaga and Tengwe were classified into different environments while, Rusape and Trelawney were closer to each other although they were classified into different environments and this information is in alignment with the tobacco growing regions where there are fast, medium and slow growing areas (TRB, 2011).

Based on Cooperative Cultivar Trials (MET experiments) done at TRB proposed that tobacco growing regions consist of three mega-environments TRB (2011). It should be noted that similar results were also obtained by Yan and Hunt (2001) were 2 mega-environments were found, however different from this study, they proposed that multi-year data is required to confirm if the patterns can be repeatable across years.

To add, a convex hull was drawn on cultivars relatively remote from the bip1ot origin so that all other cultivars are continued within the convex hull. Kutsaga and Tengwe had G2, G7 and G11. Trelawney and Rusape had no genotypes that fell in their convex quarters, also G1, G10 and G12 had no best environments indicating that these cultivars were not best in any environment and it indicates that they were the poorest cultivars in some or all the environments. Information like the above help in breeding programs to indentify better adaptable genotypes for specific environments leading to success of plant improvement.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

It was with this aim that this research was conducted in four different tobacco growing areas of Zimbabwe to identify stable and adaptable hybrids with superior agronomic performance for commercial production in Zimbabwe during the 2016/2017 growing season.

There was significant difference ($P = 0.01$) in the contribution of the environment total sealable yield to which brought about variations to the contribution of the genotypes. Tengwe had the best yield of 2755kg/ha and Kutsaga had the least yield of 1934kg/ha, however, Kutsaga, Rusape and Tengwe performed differently, however the performance of Trelawney and Rusape was statistically the same. More so, for the grade index the genotypic performance contributed much to the variations of the genotypes. G9 had the best quality with an index of 61.74, while G1 was the least performer with an index of 51.09. G3, G6, G8 and G10 performed similar as compared to other genotypes.

From this study, GEI of 11 pre-realise hybrid evaluated interaction was found to be significant for top grades proportion and insignificant for the total sealable yield and grade index. The presence of large genetic variability for top grades proportion quality indicated that good progress can be made in selecting for quality under different environments. Although variability among genotypes was highly significant within and among the testing environments, locations were found to contribute greatly to the variations in hybrids performance. This indicates that, unpredictable environmental conditions are one of the major players in selecting superior and widely adaptable tobacco hybrids under Zimbabwean conditions.

G3, G8, G9 and G11 had the best quality these genotypes have potential for production in Kutsaga and Tengwe and other locations within the same agroecological zones. Hence, these hybrids can be considered as candidate varieties for commercial production for they outperformed the check variety KRK26R. G1, G4 and G6 were the poorest in performance and they performed poorer than the check and therefore they are not good candidates for commercial variety production and can be discarded for future progress. Kutsaga was identified as the best testing environment while, Tengwe was the poorest in performance.

- It is therefore recommended that tobacco yields best in fast growing areas such as Tengwe which gave the highest yield of 2755kg/ha and also that environment influence the way genotypes perform from location to location.
- More so, for the Grade Index it is recommended that G9 should be adopted by famers who want to maximize the tobacco quality. The top grades proportion revealed that Kutsaga was the best performing location compared to all the other locations in terms of giving the best styles in tobacco leaf, while, G8 and G9 were identified as the most stable and adaptable genotypes.
- It is recommended that genotypes that had PC1 and PC2 positive relations should be adopted as varieties because they showed high quality performance (G7, G8 and G11).
- It is suggested that famers who have environmental conditions similar to Kutsaga or in Kutsaga should adopt G2, for the Genotype had best quality and had a promising performance for such environments.
- Farmers in Tengwe or those who share environmental conditions similar to Tengwe should adopt G8 and G11 for these genotypes had best quality there.
- It is advised that, there should be adoption of new technology in analysing multiple location experiments like the AMMI model. AMMI is more appropriate in the initial

statistical analysis of yield and quality, clarifies $G \times E$ interaction and it summarizes patterns and relationships of genotypes and lastly also can be used to improve the accuracy of yield and quality estimates.

- The study should be repeated for two to three seasons because information based on a single season is biased and the season in which the experiment was conducted had the above normal rainfall that need verification of replicating the study across sites.

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Appendices

Appendix 1 Analysis of Variance for Total Sealable Yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
BLOCK stratum	2	423317.	211658.	1.16	
BLOCK.ENVIRONMENT stratum					
ENVIRONMENT	3	13316729.	4438910.	24.30	<.001
Residual	6	1095964.	182661.	0.76	
BLOCK.ENVIRONMENT.GENOTYPE stratum					
GENOTYPE	11	7074480.	643135.	2.69	0.005
ENVIRONMENT.GENOTYPE	33	8854340.	268313.	1.12	0.328
Residual	88	21041705.	239110.		
Total	143	51806535.			

Appendix 2 analysis of variance for the Grade Index

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
BLOCK stratum	2	20.13	10.06	0.21	
BLOCK.ENVIRONMENT stratum					
ENVIRONMENT	3	1331.93	443.98	9.40	0.011
Residual	6	283.41	47.23	2.10	
BLOCK.ENVIRONMENT.GENOTYPE stratum					
GENOTYPE	11	1105.66	100.51	4.47	<.001
ENVIRONMENT.GENOTYPE	33	967.62	29.32	1.31	0.164
Residual	88	1977.21	22.47		
Total	143	5685.95			

Appendix 3 analysis of Variance for the Top Grades Proportion

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
BLOCK stratum	2	739.4	369.7	1.69	
BLOCK.ENVIRONMENT stratum					
ENVIRONMENT	3	18416.5	6138.8	28.09	<.001
Residual	6	1311.3	218.5	1.36	
BLOCK.ENVIRONMENT.GENOTYPE stratum					
GENOTYPE	11	4681.9	425.6	2.66	0.006
ENVIRONMENT.GENOTYPE	33	11951.7	362.2	2.26	
Residual	88	14100.2	160.2		
Total	143	51201.0			

