Efficacy of MON 89034 Bt trait in conferring fall armyworm resistance in high yielding three-way and single-cross maize hybrids

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ABSTRACT

Maize production, especially in tropical sub-Saharan Africa, is hampered by the fall armyworm (FAW) posing a serious threat to food security and livelihoods. Many methods of control including pesticide use have been tried against FAW but without sustainable success. The main objective of this study was to investigate whether or not the Bt trait (MON89034) could be successfully integrated in high yielding tropical hybrids and confer effective resistance to FAW when deployed in three-way and single cross hybrids. The study was conducted under natural FAW hotspot conditions and under field conditions representative of farmer's situation. Conventional non-genetically modified (non-GM) tropical single cross hybrids and inbred lines were crossed to four WEMA Bt lines. The resultant three-way and single cross hybrids were evaluated at three sites, in South Africa. The results indicated adequate discrimination of hybrids according to FAW resistance and grain yield, under both FAW infestation and at two other sites with limited FAW pressure. The experimental Bt hybrids displayed high yields exceeding 5 t/ha and higher FAW resistance, which was comparable to standard genetically modified (GM) control hybrids. In sharp contrast, the conventional non-GM control hybrids recorded yield as low as 0 t/ha, under FAW infestation. They were highly susceptible to FAW which was indicated by high damage scores. Therefore, the event MON89034 was effective in conferring FAW resistance in both three-way and single cross hybrids. Although the environment main effects were highly significant ($P<0.001$) for grain yield, the three-way cross hybrids were relatively stable and showed non-significant ($P>0.05$) genotype x environment interaction (GxE) effects. In sharp contrast, GxE effects were highly significant ($P<0.001$) for grain yield of single cross hybrids, indicating that they were less stable than their three-way counterparts. New Bt hybrids with high cultivar superiority index and combining high yield potential and FAW resistance were identified. These included (H3WX3167Bt) (HSX5054Bt), (HSX5368Bt) and (H3WX3194Bt). The three-way experimental hybrid (H3WX3167Bt) had yield advantage of 64% above WEMA GM checks, 33% above local GM hybrid checks and 22% above conventional non-GM checks. The single cross experimental hybrid (HSX5368Bt) exhibited yield advantage of 127% above mean of conventional non-GM checks, 100% above mean of WEMA checks and 99% above mean of local GM checks, under FAW infestation. In addition, secondary traits, such as ear prolificacy and number of ears harvested per plot, which had significant direct and indirect effects for grain yield under FAW infestation were identified for use in construction of a viable selection index. Overall, the study was successful and showed efficacy of the Bt trait (MON89034) in conferring FAW resistance when deployed in tropical high yielding three-way and single cross hybrids. The best performing experimental Bt hybrids with high yield and high FAW resistance, and out-yielded both GM and non-GM standard commercial hybrids, would be advanced in the breeding program that targets the GM market segment in tropical Africa. A survey of the literature has not revealed prior studies on evaluation of FAW resistance in three-way cross hybrids. The trait is deployed predominantly in single cross hybrids, in the GM maize production lead countries, such as Argentina, Brazil, China, South Africa and USA. Therefore, this study formed a significant baseline for revealing useful information on the efficacy of the Bt trait in conferring FAW resistance in three-way cross hybrids which are predominantly deployed to smallholder farmers in tropical Africa.

DECLARATION

I, Pretty Nyaradzo CHINGOMBE, declare that,

1. The research reported in the dissertation is my original research except where indicated otherwise.
2. This dissertation has not been submitted for any degree or examination in any other university.
3. The dissertation does not contain graphics, text or tables that have been copied and pasted from the internet unless specifically acknowledged and referenced.
4. This dissertation does not contain other people's data, pictures, graphs or other information unless the person is acknowledged as the source of such information.
5. This dissertation does not contain another person's writing unless the source is specifically acknowledged. Where other written sources have been quoted, then
    a) Their words have been re-written but general information attributed to them has been referenced.
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Signed

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Pretty Nyaradzo Chingombe

(Candidate)

As the candidate's supervisor and co-supervisor, we agree to submission of this thesis.

……………………………………………………………………………………………….

Prof. John DERERA	Dr. Kwasi Sackey Yobo
(Supervisor)	(Co-Supervisor)

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To God, be all the thanks and glory, He made it all happen.

DEDICATION

I dedicate this thesis to my late father, my mother, my husband, my children and to everyone who supported me in this journey. I appreciate you all.

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CHAPTER ONE

1.1 General Introduction

This introductory chapter provides information on the importance of maize in southern Africa. This section highlights the impact of the fall army worm (FAW) on food security and the role that biotechnology can play in improving food security. It presents the problem statement, aim and objectives of the study, research questions, hypotheses, significance of the study and the structure of the dissertation.

1.2 Background

1.2.1 Significance of maize

Maize (*Zea mays* L.) is a crop of high importance all over the world. It does not only provide food for human consumption, but it also provides nutrition for animals and plays a role in industry (Abebe and Feyisa, 2017). Maize is processed and consumed in several ways. The grain is dried and milled to produce mealie meal which households prepare as porridge or thick porridge which is served with relish. The cobs can be consumed fresh on the cob, either boiled or roasted (NAFIS, Kenya, 2019).

Fall army worm (FAW) (*Spodoptera frugiperda* J. E. Smith, 1797) was first reported on the African continent in 2016. Since then, it has spread rapidly across the continent. Even though it favours mostly *graminaceous* crops, it can also feed on a wide variety of fruit and vegetables. Due to its ability as a strong flier as well as high reproductive potential, if not properly managed, it can cause severe yield losses (FAO, 2017).

1.2.2 The yield gap and production constraints

Low maize yields in farmers' fields could be due to many reasons. These include biotic and abiotic stresses, the use of outdated seed technology, and poor access to proper insect management and general crop management practices. Due to its impact on maize production, fall armyworm has become a significant biotic factor, contributing to the low yield in maize (Tufa and Ketema, 2016, Fischer *et al*, 2014, Kasoma, *et al.*, 2020 a).

1.2.3 Management and control of insect pests

Several species including, the maize stalk borer (*Busseola fusca* Fuller, 1901) and spotted stalk borer (*Chilo partellus* C. Swinhoe, 1885) have long been considered important pests in tropical Africa but now, the effects of fall armyworm on yields have been shown to be of economic importance (Assefa and Ayalew, 2019). The FAW is a new destructive pest in Africa and especially in Southern Africa. This is due to its ability to breed rapidly, migrate and feed on a wide range of host plants, all of which makes it difficult to control.

Farmers are encouraged not to depend on one mode of insect management in their fields to control insect pests. The use of cultural, chemical and biological methods to manage pests is an effective way to limit the effects of insect damage (United States Environmental Protection Agency 2019). Biological control involves the use of the target pest's natural enemies. Many methods exist on the effective use and introduction of natural enemies into an environment in which they are not native. These include importing natural enemies as well as mass production of the natural enemies to ensure a steady supply of the natural enemies (Kenis *et al.,* 2017). Monocropping presents insect pests with favourable conditions for them to thrive. The cultural control component of integrated pest management is important. Practices like intercropping and crop rotation are encouraged to reduce pest invasions, the likes of which pests such as FAW can do in a system where maize is mono cropped (FAO, 2018). Assefa and Ayelwa (2019) pointed out that other cultural control methods are also effectively used. These include farmers applying ash and hand-picking insect larvae. These methods are effective even without the extensive use of pesticides by farmers.

Even though the application of pesticides has been depended on in agriculture, it has its drawbacks. These include the pests developing resistance to pesticides, damage to the environment and implications on the health of farmers and farm workers. This makes the management of insect pests through pesticide use unsustainable. Fall armyworm has shown resistance to pesticides in America, where they originated (Abrahams *et al.,* 2019, Erasmus, 2017). Hence, new methods of managing fall armyworm are required.

1.2.4 Breeding for insect resistance in maize

The production of maize is constrained by various biotic stresses particularly insect pests. Infestation of insect pests on standing crop and stored grains not only reduces yield but also affects the quality of grains. The strategy for enhancing host plant resistance (HPR) is one of the cheapest, safest, and sustainable methods for managing insect pests. Being a leading contributor to the world cereal basket, maize is affected by various insect pests. Maize has undergone various improvements through diverse breeding tools starting from selection to the present transgenic approaches to minimize the losses due to insect pests (Karjagi *et al.,* 2017). In the current study an approach to improve maize for host plant resistance to the fall armyworm (FAW) was pursued.

1.2.5 Genetic modification for insect resistance in maize

Another way to deal with the FAW as well as other insects is the use of biotechnology. The Water Efficient Maize for Africa's (WEMA) Bt (*Bacillus thuringiensis*) varieties are being developed in a collaboration between the International Maize and Wheat Centre (CIMMYT) and government research institutions using gene technology donated by Bayer formerly Monsanto. WEMA maize shows resistance to the destructive fall armyworm pest. Initially, Bt maize was targeting stem borers but GM maize is also showing significant protection against FAW without the use of pesticides (Gakpo, 2018). Since every part of the maize plant is economically important, conferring resistance to the whole plant through genetic modification can provide the means to successfully and significantly increase the yield of maize crops. Bt technology has been used to control FAW with success in other countries. However, to protect the crop, it will still be important to scout for damage in the crop and have additional control strategies in place (Erasmus, 2017).

1.2.6 Benefits of considering GM maize for small holder farmers in Africa

GM technology has made major strides in increasing yields in agriculture. Some developing countries like China and India have embraced GM technology and have even surpassed the United States of America (USA) in growing Bt cotton (Pray and Huang, 2003). Developing countries in Africa, need to create policies to regulate this sector if they are to realize the benefits of GM technology (Mabaya *et al*., 2015). Those against GM technology cite the high profits that seed companies are set to gain, the destruction of traditional farming systems and biodiversity as farmers adopt GM technology, the evolution of insect resistance as well as raise doubt on the economic benefit to small scale farmers (Pray and Huang, 2003). However, the benefits of adopting GM crops including maize far outweigh the disadvantages.

The use of GM technology in maize through Bt maize has the potential to stabilize grain yield in the face of the vagaries of a changing climate. This is of great importance for Africa which is prone to droughts, new pest outbreaks, new diseases and food insecurity as a result of climate change. Not only is there potential for increased productivity and nutrition, but the environment also benefits from the decreased use of pesticides. Health-wise, farmers and farm workers would benefit immensely as they would have decreased contact with harmful chemicals (Qaim, 2010). The use of GM maize is also user friendly, such that small-scale farmers with limited technical know-how can easily use, as the protection is built into the seed. South Africa and Sudan are leading the way in Africa with the adoption of Bt maize and cotton. Small scale farmers in South Africa have experienced increase in productivity and they are now even able to grow maize and cotton for export, as they can harvest much more from small pieces of land. South Africa grows more than 2.3 million hectares of GM maize and it has been shown that 85% of maize grown in South Africa is GM maize (Venter, 2020). Adoption of GM crops has been cited to have led to reduction in pesticide use by 34% and increased farmer profits by almost 70% (Muzhinji and Ntuli, 2020).

The adoption of GM crops by smallholder farmers in tropical Africa should be encouraged. Studies have found that involvement of farmers in farmer organizations, level of education of the farmer, access to extension services and size of land holdings affect the GM adoption and intensity of adoption among small scale farmers (Ngcinela *et al*., 2019). Farmer organizations and extension services are a conduit of the flow of information. Extension officers help farmers learn about new varieties and how to effectively use the new innovations. Moreover, larger size of land holdings gives the farmer room to experiment with new varieties without risking their household food security (Ngcinela *et al*., 2019). By addressing these socio-economic concerns, more and more small-scale farmers would adopt GM maize at high intensity. The current study was designed to demonstrate that the Bt technology can be effectively delivered through the three-way cross hybrid that the farmers are currently growing across tropical Africa.

1.2.7 Role of secondary traits in conditioning yield

Grain yield is the most economically important trait in maize breeding programs. Traits such as plant height, ear height, ears per plant and anthesis-silking interval are also used by breeders for indirectly selecting for grain yield (Ertiro, *et al.,* 2020). Information about the relationships among grain yield and yield related traits is desirable for designing appropriate breeding strategies most especially for products to be deployed under stress conditions.

Several statistical tools have been employed in the study of relationship among traits. The most commonly used methods by breeders include path coefficient analysis. Path coefficient analysis has been widely used in crop breeding to determine the causal relationship between grain yield and its contributing components, and to identify those components with significant effects on yield for potential use as selection criteria (Oyekunle and Badu-Apraku, 2018). Due to the nature of grain yield in that it is difficult to breed for its improvement in isolation, selecting for secondary traits is then used effectively to improve grain yield (Oyekunle and Badu-Apraku, 2018, Badu-Apraku *et al*., 2012). Therefore, it was prudent to investigate the role of secondary traits in contributing to grain yield of hybrids under the FAW infestation in the current study. Such information has been scarcely reported in the literature.

1.3 Problem statement

Maize (*Zea mays* L.) is an important grain in sub-Saharan Africa. It is produced widely under a varying range of environments and altitudes. All parts of the maize plant are important, as they are used as food and feed as well have industrial purposes (NAFIS, 2019). However, successful maize production depends on factors that include the proper use of inputs like seed, fertilizers and crop and pest management, such that production does not negatively impact the environment (du Plessis, 2003, FAO, 2019). The main issue to be addressed by this study is whether the Bt gene used to genetically modify maize hybrids, is efficacious in conferring resistance to FAW in three-way and single cross hybrids. Smallholder farmers in Africa grow predominantly three-way cross hybrids because the seed is cheaper than that of single cross hybrids. Unfortunately, the Bt trait has been demonstrated to be effective in single cross hybrids in the lead production countries, such as Argentina, Brazil, China, South Africa and the USA. A survey of the literature indicates that its efficacy has not been investigated in the three-way cross hybrid. Breeding the trait in a three-way hybrid will enable farmers to have access to the technology in the type of varieties they are already growing with minimum impact change of seed prices, among other factors. This demonstration would encourage adoption of GM technology by the smallholder sector in Africa with a minimum change of culture and will go a long way in ensuring high yields in maize, in-spite of FAW pressure, which has increased due to global warming.

1.4 Significance of the study

Given the issues mentioned above, this study was significant in showing how breeding for insect resistance using GM technology could help to significantly increase maize grain yield under FAW pressure. This would have a profound impact on grain yield, food security and improvement of livelihoods for the communities whose economy is greatly influenced by maize production. Several studies have shown the relationship between grain yield and secondary traits under stresses, such as low N, drought and *Striga*, but not under the FAW infestation in a tropical or subtropical environment. This study could be instrumental in showing which secondary traits are important for grain yield under FAW pressure. This has implications for breeding and selection strategy to be employed in improving maize hybrids for tropical Africa. The study would also impact on policy positions regarding adoption of GM crops in Africa or not.

1.5 Research aim and goal

The main objective of this study was to investigate whether or not the Bt trait could be successfully integrated in high yielding tropical hybrids and thereby confer resistance to FAW when deployed both in a three-way and single cross hybrid design.

1.5.1 Research questions

The following research questions were answered.

1. How effective is the Bt trait (MON89034) in conferring resistance to FAW when deployed in a three-way and singe cross hybrid?
2. What is the yield gain of the three-way and single cross Bt hybrids relative to conventional non-GM hybrids?
3. What is the impact of direct and indirect effects of secondary traits on grain yield of three-way and single cross Bt hybrids under FAW infestation?
4. What is the cultivar superiority of the three- way and single cross Bt hybrids?

1.5.2 Research objectives

The specific objectives of the study were as follows:

1. To determine the efficacy of the Bt trait (MON89034) in conferring resistance to FAW when deployed in a three-way and single cross hybrid.
2. To determine the yield gain of the three-way and single cross Bt hybrids relative to conventional non-GM hybrids.
3. To determine the impact of direct and indirect effects of secondary traits on grain yield of three-way and single cross Bt hybrids under FAW infestation.

4. To determine the cultivar superiority of the three-way and single cross Bt hybrids over commercial hybrids.

1.5.3 Research hypotheses

The following hypotheses were pursued:

1. The Bt trait (MON89034) is effective in conferring resistance to FAW when deployed in a three-way and single cross hybrid.
2. The three-way and single cross Bt hybrids have higher grain yield relative to conventional or non-GM hybrids.
3. The secondary traits have significant direct and indirect effects on grain yield of three-way and single cross Bt hybrids under FAW infestation.
4. The three-way and single cross Bt hybrids have a higher cultivar superiority than the commercial hybrids.

1.6 Dissertation outline

The layout of the dissertation is as follows:

Chapter One: General introduction

- Provides the study background and outlines the scope, aim, objectives, problem statement, and significance of study and show the structure of the dissertation.

Chapter Two: Literature review

- Presents the theoretical framework of the study by reviewing literature pertaining to the importance of maize in African diets and economies, the effect of the FAW pest on maize production. Genetic modification of the conventional high yielding maize varieties with the MON89304 event is explored as the best control strategy.

Chapter Three: Research design and methodology

- Presents the layout and management of the field experiments used in the study. Field data collection methods and method of FAW infestation and data analysis are presented.

Chapter Four: Results

- Findings on the efficacy of the Bt trait in conferring resistance to FAW are presented. Results on the evaluation of FAW resistance in the three-way cross and single cross hybrids and yield data results of the three-way cross and single cross hybrids are also presented.

Chapter Five: General discussion

- It provides a general discussion of the findings in relation to the findings of existing research that informs the study.

Chapter Six: Conclusions, implications and recommendations

- Summarizes the key findings of the research chapters and presents the overall conclusions and recommendations for future breeding programs.

1.7 Conclusion and summary of research focus and limitations of the study

Maize is the most important staple cereal grown by smallholder farmers in sub-Saharan Africa. Infestation of maize crops by the FAW significantly reduces yield through damage to the leaves as well damage to the cobs. As maize is an important part of both the diet as well as income generation, it is therefore prudent to breed for resistance to FAW in maize. Genetic modification in maize for resistance to FAW is a viable option as it is cost effective and limits the use of pesticides, thereby being environmentally friendly. Currently, on the market there are no three-way cross hybrids that are genetically modified that farmers, especially subsistence farmers can access at affordable price. There is therefore a need to make these hybrids available to smallholder farmers in tropical Africa.

The major limitation of this study was that, even though the study seeks to find out the efficacy of MON89034 in conferring resistance to FAW in hybrids for use in tropical Africa, the study was carried under a subtropical environment in South Africa. This is mainly because many countries in tropical Africa are not yet open to the experimentation with or usage of GM maize. South Africa already has GM crops on the market and allows field trials of the deregulated traits such as MON89034 event; hence its choice for the three sites that were used in this study.

The following chapter provides a review of the relevant literature on FAW and its effects on maize yield and economic importance. The chapter also discusses the breeding progress of GM maize and general perceptions towards GM maize and existing strategies to manage FAW implemented to date. This is followed by Chapters 3 – 5 that covers the current research experimentation, findings and discussion.

CHAPTER TWO

Literature Review

2.1 General Introduction

The main objective of the study was to investigate whether or not the Bt trait could be successfully integrated in high yielding tropical hybrids and thereby confer resistance to fall armyworm (FAW) when deployed in a three-way and single cross hybrid design. The specific objectives were to determine the efficacy of the Bt trait (MON89034) in conferring resistance to FAW when deployed in a single cross hybrid and three-way cross hybrid design; determine the yield gain of the single cross and three-way cross Bt hybrids relative to conventional or non-GM hybrids; investigate the impact of direct and indirect effects of secondary traits on grain yield of single cross and three-way Bt hybrids under FAW infestation. Lastly, the study established the cultivar superiority of the single cross and three-way cross Bt hybrids over commercial hybrids. In this chapter the literature study was conducted to achieve the objectives. This review of the literature examined the origin and economic importance of maize, its grain yield potential and the production constraints. The origin and economic importance of FAW, and the yield reduction problems caused by FAW were also reviewed. It also showed the implications of FAW to food security and explored various approaches for breeding for FAW resistance. It is crucial to breed for FAW resistance in an adapted genetic background. For this reason, it was prudent to review pertinent literature on cultivar superiority index, genetic gains and the relationship between grain yield and secondary traits. This included reviews of the use of correlation and path coefficient analyses to determine the direct and indirect role of secondary traits for grain yield improvement. Lastly conclusions were drawn, and the identified knowledge gaps are highlighted. Implications for the adoption of GM hybrids in Africa are discussed.

2.2 Origin, social and economic importance of maize

Maize (*Zea mays* L.) is a diploid crop with ability to self and cross pollinate. Maize is produced all over the world and constitutes the main part of the diet for many people. It is valued as a good source of carbohydrates and is also a source of fats and some minerals and comes after rice and wheat in world production terms (Shiri *et al*., 2010).

Maize has its origins in the Americas where it was first domesticated. After its introduction in Africa, it quickly spread around the continent. It is now consumed more than any other grain crop on the continent (IITA, 2019). It is grown all over the world, even through to the tropics, even though it originated in the temperate climate. It is a highly adaptable crop, well able to be highly productive in a variety of environments and altitudes. Not only is it a source of food and feed for livestock, but maize is also used in industry (Kumar and Babu, 2016; Bouchet *et al*., 2013).

With the spread of maize, the world over, it has become the part of many people's daily meals. In Africa, maize based meals can be consumed several times a day in various forms. In the Zimbabwean context, maize in the form of maize meal porridge or cornflakes are consumed for breakfast, for lunch maize is consumed in the form of *sadza* which is a thick porridge made from maize meal. It is usually served with vegetables and meat. Supper can also consist of sadza served with relish. Statistics show that maize accounts for "*40% of the cereal production in Sub-Saharan Africa (SSA), where more than 80% is used as food* (FAOSTAT, 2016). *The crop provides at least 30% of the total calorie intake of people in Sub-Saharan Africa*".

Maize comes in a range of colours. White maize is most popular in southern Africa where it is grown mostly for food. Yellow maize is grown mostly for feed in America and some maize landraces have colours such as red, blue and black (IITA, 2019). Depending on region, maize processing for consumption differs. In Southern Africa, it is milled into maize meal and is used to make thin and thick porridge. Thin porridge is usually a part of breakfast while the thick porridge is consumed with relish for lunch or supper. Left over thick porridge can become part of a refreshing popular brew called *maheu* which is usually drunk while people work in their fields. Maize on the cob is enjoyed by many, either freshly roasted or boiled and sold readily especially at the start of the rainy season (Kumar and Babu, 2016).

Maize has become a great part of many economies in America, India, and China and in sub-Saharan Africa. This is due to its adaptability and wide consumption base. It is grown over large areas in sub-Saharan Africa, by both commercial and subsistence farmers. It has use both as a feed for animals and food for humans. Maize is produced both for consumption and for sale at household level, with many people using the money from sales to purchase items for household use. It is also a source of income through employment as people are employed as farm labourers as well through the sale of grain. Maize is so important so much that in years where the maize harvests are low, many governments must import maize in order to feed their people. The import and export of maize is a great part of many economies. Net exporters of maize sell to mostly African countries. Africa generally suffers from food insecurity because of harsh climatic conditions and events such as droughts and floods as well as political instability (Kornher, 2018).

Several African countries that depend on maize as a staple food crop, have adopted agricultural policies to maintain a steady supply of the commodity through increased production and productivity of the crop. Many governments work hard to protect the food security of their citizens by regulating the maize market. This is due to the strategic importance of maize. When maize production is low, the importation of maize becomes key in securing food security. Zimbabwe is an African country that has been experiencing significant deficits in its maize production. It has moved from its status as the breadbasket of Africa to being a net importer of maize to feed its population. This is supported by information that shows that not only Zimbabwe but other countries such as Kenya also import maize (Kornher, 2018).

2.3 Grain yield potential of maize

In maize breeding programmes, yield is the most important characteristic of the maize crop. With any improvement that breeders want to make to maize, the potential yield of the genotype is always considered. In the African context, there is a big difference between the yield potential of maize and what the farmer realizes at harvest on his farm. This is because many factors influence the farmer's ability to reach the yield potential on his field. Some of these factors have to do with field and crop management practices employed by the farmer. Some farmers do mixed cropping hence the yield potential is not reached because the farmer did not use the optimum planting density. Other factors such as poor germination, insect pests and diseases come in to significantly reduce yield. Most farmers do not have easy access to extension services and inputs such as fertilizer, which in turn severely affects the performance of the crop (Fischer, 2015).

Temperate zones achieve higher yields of maize than tropical zones. In tropical Africa, even though many farmers have adopted the use of hybrid seed, the yield realized by the farmers is significantly lower than in the developed world (World Maize Facts, 1993/1994). Subsistence farmers in Zimbabwe achieve yields of less than a tonne per hectare in comparison to neighbouring South Africa whose subsistence farmers even when using conventional hybrids achieve much higher yields. Sihlobo (2019) noted the government's desire to avail inputs in the form of seed and fertiliser to local farmers to enable farmers reach a target of 2.4 tonnes per hectare. A 2.4 tonne per hectare is a target yield which has only ever been achieved in 1973.

What the difference between potential yield and actual yield especially in tropical Africa shows is that even though maize is a crucial part of many diets and the economy, production of maize is hampered by many biotic and abiotic factors (Kiyyo and Kusolwa, 2017).

2.4 Maize Production Constraints

Even though many people the world over depend on maize for food and maize is grown on significant acreage, the production of maize is not without its own obstacles. Many factors come into play, to diminish the yields of maize (Adebayo *et al*., 2017). These factors are not only biotic but also abiotic in nature. Of late the issue of climate change has played a huge role in decreasing the maize production of maize. Particularly in Africa, the effects are felt strongly. Rainfall has become more and more erratic in sub-Saharan Africa; temperatures have increased significantly and more inclement weather events such as floods have played a role in destroying maize harvests. These unfortunate changes in climate further cause food security prospects of many individuals to be diminished. With these issues of low house-hold food security also come the issues of increased commodity prices. This leads to increased chances of malnutrition as food get more and more expensive for many. As mentioned, the factors that cause maize production constraints are also linked to soil fertility particularly nitrogen deficiency and salinity. Other factors include a lack of or adoption of technologies such as hybrid seed use, fertilizer application and good agronomic practices. With climactic changes, properly timed planting processes can become distorted. All these factors cause African maize production to fall behind that of other maize producers globally (IITA, 2019).

Farmers are not only having to contend with erratic weather patterns, inaccessible advanced technology or even issues to do with the fertility of their soils. They are also having to contend with insect pests and diseases. Maize is a crop that is attacked by many insect pests which include stem borers which have been linked with great yield losses in maize. Post -harvest pests such as maize weevils also decimate maize yields. These insects cause varying degrees of yield losses to farmers. In trying to deal with these pests, the production costs for the farmers also increase as they have to purchase pesticides to combat the effects of these pests on their yield (IITA, 2019).

Recently, since 2016, FAW has become a major biotic constraint, of economic importance in maize production affecting farmers' yields immensely. In view of these numerous constraints besetting maize production particularly in tropical Africa, and the threat of new invasive pest species compounding these production constraints, there is need to come up with innovative ways to eliminate production constraints. This needs to be done in a way that is sustainable, environmentally friendly, and safe for the farmers.

2.5 Strategies to improve maize productivity in Africa

Considering the prevailing stresses both biotic and abiotic, there is need to seek ways of improving maize productivity, especially in Africa. Africa's maize productivity is lagging behind the rest of the world at 1.5-2.0 t/ha (Adebayo *et al*., 2017). This is in spite of the widespread use of hybrids by small scale farmers. Increasing yield potential is a combination of genetics and management practices. Management practices include planting at appropriate time of the season, correct plant spacing, fertilizer application, and irrigation, pest and weed management. The environment is also pivotal in affecting maize productivity.

Strategies to increase maize productivity include improved measures on adoption of improved technologies and correct fertilizer applications by farmers in their fields, as well as increasing plant density (Tandzi and Mutengwa, 2019). Bearing in mind the high importance of maize in local diets and as a source of income for many farmers, there is need to increase productivity in maize. This is where GM technology comes in to provide a viable and sustainable way to boost maize production in tropical Africa.

2.6 Application of GM technology to manage production constraints

Genetic modification involves the transformation of organisms by inserting new genes into them (Zhang *et al*., 2016). GM crops are an important part of overcoming obstacles that developing countries face- the ever-increasing demand for food while focussing on quality and nutrition. Climate change is also posing challenges by creating biotic and abiotic stresses, previously non-existent. GM crops have the potential to increase cultivated potential by means of improved yield, quality and nutrition. Lastly crops can be genetically altered to handle environmental stresses, insect damage and herbicide applications better, without a negative impact on the crop plant, quality or yield.

Biotechnology has been evolving since the discovery of the double helix structure of DNA and the subsequent discovery that DNA codes for the production of proteins. Soon through the use of DNA ligases, it became possible to cut genes and introduce them into new organisms. Commercial production of GM crops began in the mid 1990's after being proved that they were safe for human consumption. *Bacillus thuringiensis* was used in the transformations to create

Bt cotton, Bt maize among others. Since then, commercial production of GM crops has been on large scale (Raman, 2017; Zhang *et al*., 2017).

Developing countries such as China, India and South Africa have adopted GM crops. South Africa implemented policies that allowed for the use of GM technology and in 1996 started growing Bt maize. Besides Bt maize, South Africa is also a big producer of Bt cotton. In terms of global production, production of GM soybean is higher than that of GM maize, although maize is the crop that has had the greatest number of transformations (Agaba, 2019; Pellegrino *et al*., 2018).

2.7 Benefits of GM Technology in Agriculture

2.7.1 General Overview

GM technology is highly beneficial, and this technology has been adopted quite readily by most farmers. This kind of technology offers solutions to farmers for several issues that they have had to deal with in times past. As mentioned earlier, the production of maize has been hampered by biotic and abiotic stresses and GM technology can help to manage these stresses. Issues such as drought and insect pests have been mitigated through the release of GM material that is drought tolerant, insect resistant and herbicide resistant. Elimination of these stressors has led to higher crop yields and also eliminates the farmers need to handle toxic pesticides. Increased food security and increased safety for the farmers is a huge bonus for farmers. Not only are the GM crops increasing productivity, but the environment is benefitting from lower pesticide use and lower greenhouse gas emissions (Raman, 2017). Currently on the market, there are various GM crops that have been engineered to be insect resistant, herbicide tolerant and drought tolerant.

2.7.2 GM Herbicide Resistant Crops

GM technology has provided farmers with Herbicide resistant maize. Herbicide resistant maize has the advantage of being resistant to broad spectrum herbicides. This allows farmers to spray herbicides on weeds without adverse effects on the crop. This means that the crops do not have to compete with weeds for survival. This competition has adverse effects on crop plants and can significantly affect crop yields. To combat the weeds, farmers traditionally incorporate weeding into their crop management systems. However, tilling has been found to loosen the topsoil and in turn cause significant loss of the fertile top soil. Besides weeding, farmers also use herbicides. Broad spectrum herbicides such as glyphosates and glufonisates are highly effective against weeds but they also harm the crop plants. To effectively deal with the issue of weeding, herbicide resistant crops were genetically engineered. Herbicide resistant commercial crops include maize, canola, soybean and cotton (ISAAA, 2020).

2.7.3 GM Drought tolerant crops

Out of the multitude of abiotic and biotic stresses that affect crop yields, drought appears to be the most potent stressor that can adversely affect crop yields. The changing climates, water pollution and generally scarce water resources around the continent all contribute to the water shortages the world currently faces. Breeding for drought tolerant crops has long been the focus in breeding programmes with early maturing varieties that can escape drought being developed. Biotechnology has also been working on drought tolerant crops. According to Monsanto- Company, 2009), in 2009 Monsanto released MON87460, the first GM drought tolerant maize variety. Water Efficient Maize for Africa has also been making strides in developing transgenic maize for Africa.

2.7.4 GM Insect Resistant Crops

Resistance to insect pests in plants is of paramount importance. Several insect pest species have been known to adversely affect yield on crop plants. Insect pests feed on the leaves, thereby reducing leaf area and photosynthesis. They also feed on the reproductive parts of crop plants, causing significant yield losses as well reducing quality of produce. Farmers have had to deal with insect pests with the use of chemical pesticides. These chemicals not only have health implications on the farmers, but they can affect the environment. Adoption of insect resistant maize for agriculture reduces chemical pesticide use. This significantly reduces the agricultural footprint on the environment. Not only that but the cost of production could be significantly lowered, and this gain passed on to the consumer. Food could potentially become cheaper and easily accessed by many people. The development of insect resistant GM crops has been made use of in cotton and maize. This is through the use of the Bt gene that is found in *Agrobacterium tumefaciens* (Gatehouse *et al*., 2011).

The introduction and use of transgenic crops have been a hotly debated topic, with those in support of biotechnology raising many points that show the advantages of biotechnology. The use of GM crops leads to lower pesticide use, which has health benefits for farmers and farm workers. This also impacts the environment positively. Production costs go down as the technology is built into the seed and so extra inputs like pesticides are not required (Azadi *et al*., 2015). Developing countries, particularly Africa could benefit from the increased productivity that GM technology potentially offers. Increased productivity under the difficult environments common to Africa, could lead to increased food security. In subsistence farming, small holdings are common, so GM crops could potentially increase productivity on the small pieces of land (FAO, 2011; Ali and Abdulai, 2010; Kathage and Qaim, 2012).

2.7.5 Advantages and concerns of GM crop use

Advantages of using GM technology to improve yield gains in maize include higher yields which result in better food security and nutrition and higher incomes for the farmers and their households. There is a cascading effect from the increased income as there is potential for the standard of living to go up for these households. With better standard of living comes improved access to education and services such as better healthcare (Gouse *et al*., 2005, 2006).

A lot of people have got negative impressions about GM technology stemming from a lack of proper understanding of what GM crops really are. This problem could be solved if the science community educate people in a manner that would help foster better understanding. On the other side, environmentalists also lobby against the use of GM products. They claim that many commercial seed companies pushing GM technologies do not conduct comprehensive environment impact assessments. These lobby groups accuse seed companies of pushing for their profit margins with no regard for the environment. Another issue is that particularly in Africa, there are no strong biosafety policies to govern the use of GM technology. Policies need to be drawn and enforced in order to ensure the proper use and management of this valuable technology. A clear biosafety framework and system is required before progress can be made in this sector. This has resulted in the failure by the continent at large to engage in GM trials or adoption of GM technology (Federoff, 2011; Mabaya *et al*., 2015; Raman 2017).

Lobby groups such as the Friends of the Earth are able to galvanize support and mobilize people to reject GM technology. They have successfully raised doubt in people’s minds about the potential advantages of GM technology particularly for small holder farmers. They speak to governments and push for policy makers to reject GM technology advancements. It is particularly true in Africa, where they have a sway. Friends of the Earth (2014) managed to get the Zambian government to reject food aid because it was genetically modified. This they did by convincing the government that this technology should be considered an additional risk to food safety.

The export of agricultural produce is a fundamental part of many economies. Many African countries export agricultural produce to the European Union, which is not favourable to GM products so, in order to maintain these markets, many governments do not allow GM technology within their borders. This then affects policy on GM technology which leads to African countries lagging behind in the adoption of GM crops (Cohen and Paarlberg 2002)

Another issue is that GM technology is patented technology, and as such intellectual property rights issues come up. However, it should be noted that patents do expire and with the expiration of such patents, a whole world of possibility opens up for the biotechnology sector particularly in Africa. Case in point is the WEMA Bt maize project. It is being pushed by the use of the formerly patented MON89034 event and it is creating opportunities for Africa to become more food self- sufficient. However, the lack of policies in the sector, makes Africa not so attractive to biotech companies. Biotech companies are concerned with the protection of intellectual property and in Africa, it is found to be lacking in laws to effectively protect the intellectual property rights (Adenle, 2012).

A potential threat to the full adoption of GM technology is the issue of the resultant pest resistance that could arise as a result of using GM technology. Insects breed and reproduce over very short periods of time as they have short life spans. This makes it easy for the pests to rapidly evolve resistance due to natural selection. Resistance in fall armyworm and stem borer have already been reported and documented. When insects begin to be resistant, it means that the trait is breaking down and will no longer be effective against the target species (Raman 2017). Resistance broke down in this manner due to the nature of the Bt events that were used in the transformation of the maize lines. GM technology has also since evolved to begin gene stacking in its transformations.

Many calls have been made for policy makers in African governments, to make inroads by drafting and implementing policies to manage the biotechnology sector. This is because in Africa, the lack of adoption is a policy issue. Not only is policy lacking, but funding and technical know-how are also an area that African governments need to invest in. This allows for proper research to be carried out and results in technology and knowledge relevant to Africa which leads to a more successful adoption of the technology. This will also lead to the demystification of GM technology. Evidence- based research have clearly demonstrated that genetically modified crops are drought resistant, increase productivity and can enhance Africa’s food security (Auda- Nepad, 2020).

Through the use of biotechnology insect resistant maize can be used in the control of the new invasive pest fall armyworm that has recently made its way into Africa. With proper information dissemination and proper policies in place, GM technology can prove very useful in assisting governments of developing countries become more food secure.

2.8 The origin and economic importance of the fall armyworm

2.8.1 Origin of fall armyworm

The fall armyworm (*Spodoptera frugiperda*, Smith) is an anthropod in the order of Lepidoptera and is the larval stage of a fall army-worm moth. Fall armyworm targets a variety of crops some of which are maize, rice, tobacco and even fruit such as orange, apple and many more. FAW is polyphagous and feeds on at least 100 plant species belonging to 27 families (Pogue, 2002). The FAW is a serious pest affecting mainly monocrop maize. It causes severe yield losses as it is able to destroy crops rapidly. In Africa, since its arrival, it has wreaked havoc on maize particularly and also other *graminaceous* crops, mainly because it was new and people were not aware of how to deal with it (Kasoma *et al*., 2020 b).

It arrived on the African continent in 2016, appearing in West Africa, but it has since spread to Southern Africa. It is a native pest to the Americas where farmers there have been dealing with it for a long time. Since its arrival in Africa, FAW is already showing itself to be a pest of economic importance. The fall armyworm is most destructive at the larva stage during which it feeds on the leaves, whorls and later the reproductive parts of the crop, thereby severely impacting yield (SANBI, 2018; Georgen *et al*., 2016).

2.8.2 Fall Armyworm Biology

The moth’s strong flying ability makes the control of FAW difficult. The female can fly very long distances over short periods, meaning that it can easily spread and establish quite rapidly (CABI, 2017). Not only is the female moth a strong flyer, she is also able to lay masses of eggs on host plants. These then hatch and the caterpillars are also able to migrate on to new host plants or begin feeding and causing damage to the plant on which they were laid (FAO, 2017).

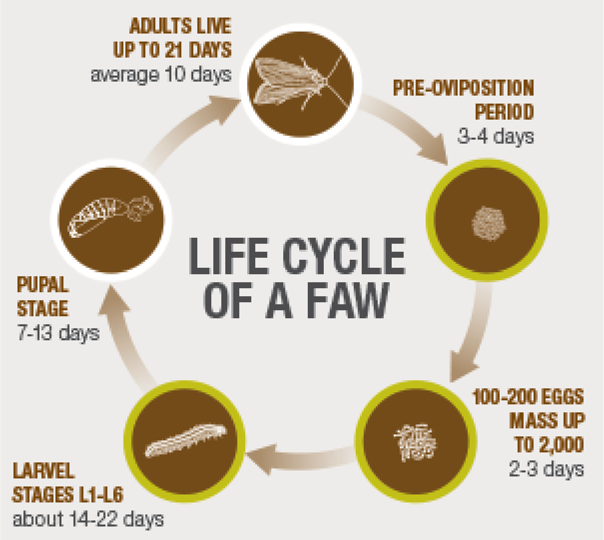
The egg masses laid by the female have eggs that up to 200 eggs per mass. During its lifespan the female moth can lay up to 2000 eggs in total. The larvae go through 6 instars before they mature and at each instar the larvae acquire distinct features that are different at each instar.

It is this distinctiveness that allow for the differentiation of FAW larvae from other insect pests. Duration of the larval is influenced heavily by temperature. The warmer temperatures of the summer months lead to the larvae maturing earlier than in the cooler temperatures of winter. This trait could also be linked with how FAW appears to be spreading and thriving in Africa. Temperatures are usually warm and the winters moderate, implying that they can breed and increase all year round (UF/IFAS Extension, 2017; Igyuve *et al*., 2018).

Adult moths exhibit differences between the sexes but they have a wingspan of up to 40mm. The male moth has distinct markings on its forewings which consist of triangular white spots on a grey/brown background. The female moth forewings are not distinctly marked, but are a generally brownish colour. Due to their nocturnal nature, the adult moths are very active at night (Igyuve *et al*., 2018).

Figure 2.1 shows the diagram of the life cycle of the fall armyworm (www.syngentaseedcare.com, 2018).

Figure 2.1. Life cycle of fall armyworm



2.8.3 Host plants for Fall Armyworm

Fall armyworm feed across a wide spectrum of host plants, but they tend to show preferences for certain species over others. Fall armyworm particularly feeds on the grass family with a huge preference for maize. Fall armyworm has been known to invade a field and cause significant damage to the crop overnight. When in numerous numbers they can behave the African armyworm by dispersing in large numbers and consuming all vegetation within their sight. The FAW mostly prefers *graminaceous* crops but it can also cause injury to vegetables and fruit trees. Crops like Sudan grass, soyabean, sugar cane, tobacco and wheat can also be injured by FAW (Casmuz *et al.* 2010, Igyuve *et al.*, 2018 Capinera, 2017). Fall armyworm can completely sever the stems of young plants and they also cause damage to leaves as they feed and they inflict damage on the reproductive parts of the plant. The larvae migrate to the whorl where they feed. The most danger they cause to the yield is when they feed on the developing kernels inside the ear. This results in cob damage which results in loss in both yield and quality. Cob damage is a very important factor in yield loss (Erasmus 2017, Ghidhiu and Drake, 1989; Williams and Davies, 1990; Marenco *et al.*, 1996).

2.8.4 Economic Importance of Fall Armyworm

Adding to the yield losses already caused by other biotic and biotic factors, FAW has shown that it can cause serious yield losses. With its nature to favour crops in the grass family especially maize, it has been shown to damage crops and inflict economic losses to maize growers. "*In a survey of 12 maize producing African countries, fall armyworm caused yield losses in a range of 8.3-20.6 million tonnes per annum, destroying 21-53% of the annual production of maize averaged over a 3- year period in these countries. The larvae eat so much of the plant and are very detrimental to crop survival and yield. They even burrow into the ear to eat the kernels during seed filling and maturity (UF/IFAS Extension,2017.*)". Seeing that FAW has become of great economic importance in maize producing countries, the management of this pest is of paramount importance. There is need to ensure that further decline in yield due to FAW is halted and the spread of the pest curtailed (CABI, 2017).

2.8.5 Existing strategies to manage fall armyworm

As with any method to deal with insect pests, the major FAW control strategies to date have involved the chemical pesticides, the use of natural enemies, crop rotations and intercropping strategies as well as using germplasm that shows inherent resistance to fall army feeding and destruction (Prasanna *et al.*, 2018). In order to find more effective methods of dealing with this new pest, it is imperative to look at how farmers have been dealing with this pest in its native environment. Looking at control measures in its host environment could provide useful insights and ways in which to successfully deal with FAW. Moreover, the native range of the FAW is also the native range of maize and useful information can be gleaned from that association that they share (FAO, 2017).

FAO (2017) pointed out that some key lessons can be learned from investigating how the FAW has been managed to date in its region of origin- the Americas. Due to the fact that both the FAW and maize have coexisted in the same environment over a long period of time, it goes without saying that methods to effectively manage FAW can be found there. One method of control for FAW is natural enemies for FAW might be found in their natural environment. Fall armyworm has many natural enemies in the form predators, parasitoids and pathogens that provide a high level of natural control of FAW populations. This has been proven to be a highly effective form of control even where chemical control has not been applied (FAO, 2017).The use of natural enemies to FAW for its control is an option that is not yet available for use in sub-Saharan, but if it were it would provide farmers with a wider range of eco-friendly methods of control. Another strategy employed by farmers in the Americas involves scouting for FAW in the fields and the subsequent manual crushing of the scouted larvae. The strategy of intercropping is a viable option in sub-Saharan Africa as it is a concept that is not new especially to small scale farmers. Ash has also been applied to whorls, in order to deter FAW feeding and rainfall has been known to wash away the larvae from the plants. Many options from the farmers who have dealt with pest can provide small holder with choices for FAW that are not limited to the use of chemical pesticides. Pesticide use has got drawbacks that include pesticide resistance in crops, health implications for the farmers and the farm workers as well as negative effects on the environment (Abrahams *et al.,* 2017; Fatoretto *et al*., 2017, Prasanna *et al*., 2018).

2.9 Breeding for Insect Resistance using Classical Approaches

Even though fall armyworm is new on the African continent, it has proven itself to be of economic importance due to its ability to drastically reduce yields. Field and crop management practices can be beneficial in dealing with this new invasive pest. However, besides management practices, classical breeding can offer a solution through the breeding for host plant resistance in maize. Heavy reliance on pesticide use can prove detrimental to both the farmers and the environment. By providing seed with innate resistance already bred into it, host plant resistance could prove to be a long-term method of insect pest control that is cheaper for the farmer, better for the environment as well as sustainable. Such germplasm is ideal for small scale farmers especially in sub-Saharan Africa where maize productivity is severely compromised by many biotic and abiotic factors and where farmers do not have easy access to extension services and support (Kasoma *et al.,* 2020 b).

Plant breeding is the science of manipulating crops to increase the yield and other beneficial traits. It has been going on since people of old began consciously selecting plants for bigger fruit and other traits they found beneficial and keeping the seed to replant. It has been used extensively in maize because of its nature as a self-pollinated and cross-pollinated crop. By carefully selecting for particular traits, modern day breeders have been able to increase agricultural productivity. This is vital due to the exponential growth in population which requires that agricultural productivity also be increased to match (Mumm, 2013; Fischer, 2009).

Plant breeders always need to exploit genetic variation in order to maximize genetic gains. By crossing lines with desirable traits, new genetic combinations become available and subsequent trials allow for the release of varieties with increased productivity. Because of the constant need to exploit genetic variation, breeders are always looking for new variation to exploit. Sometimes the desirable trait is not naturally available and for that gap, biotechnology provides an answer. Biotechnology allows for traits that cannot naturally be introgressed into populations to be introduced. Once the crop has been transformed, the trait will be ready to exploit through plant breeding practices and techniques (Lorenz *et al*., 2011; Mumm, 2013).

Seeing that breeding relies a lot on variation for it to be successful and also noting that new and different challenges keep coming up such as new pests and diseases, a more efficient way to incorporate variation into maize genotypes is through the use of GM technology.

2.10 Integration of GM Technology to Manage Insect Resistance

It is possible to incorporate GM technology in the generation of hybrids that offer resistance to insect pests. This is carried out by crossing conventional hybrids with transgenic hybrids. The two methods of breeding can be used together to create superior hybrids that show resistance to biotic and abiotic stresses and are also high yielding. This method of breeding is gaining more and more importance in global agriculture as it is evident that biotechnology can feed into conventional breeding programmes and the results are hybrids that show improved yield and high genetic gains. Once the trait is introduced into the crop, breeders utilize the methods of engaging in field trials and assessments of yield and performance of the new hybrids under various environmental conditions (Pilacinski *et al*., 2011; James, 2007; James 2009; Mumm 2013). Breeding methods in today's world has come evolved over the years. New hybrids are being developed by integrating biotechnology and techniques such as molecular markers into conventional hybrids to produce superior hybrids that meet the needs of agriculture (Moose and Mumm, 2008).

As highlighted earlier, in dealing with new stresses, breeders tap into genetic variation in order for a breeding programme to be successful. However, tapping into genetic variation when breeding for FAW resistance can prove difficult in maize, where uniformity has been bred for over the course of many years. This has led to an erosion of variation that breeders so desperately need to tap into. The need to use genetic variation becomes more apparent due to the arrival of invasive species like FAW, where most of the varieties already on the market have shown to be susceptible to the fall armyworm (Kasoma *et al.,* 2020 b).

With the aim being to deploy maize varieties that are resistant to fall armyworm and with good adaptation to different agro-ecologies, finding novel sources of genetic variation is imperative. It is duly noted that maize plays a pivotal role in African agriculture and African economies as well as in providing food security. It has been highlighted that agriculture in tropical regions faces more challenges than in the temperate regions. This is due to the longer growing periods as well as the lack of very cold freezing winters. This poses major challenges for crop protection with regards to insect pests. With the advent of GM crops that could incredibly improve both quantity and quality of yields through improved insect resistance, it is imperative that Africa chooses genetic events that are suited to tropical Africa. These genetic events to be incorporated need to be chosen carefully as some have already shown signs of breakdown. Events of note to disintegrate are the single stack gene events such as MON 810 and TC1507.

2.10.1 Stacked traits in Biotech Crops and the need for gene stacking

In biotechnological terms, an event can either be single stacked or double or triple stacked. Stacking is when at least two genes of desirable traits are combined into single plant. Such stacking results in a much lower rate of disintegration due to resistance in target species Single stacked genes have shown to have a high rate of disintegration, rendering the trait ineffective against the target species (ISAAA 2020).

The need for gene stacking stems from the ability of target insects to develop resistance to the Bt trait proteins. When this happens, the resistance in the host plant will be shown to have broken down and the trait ineffective against the target species. Insects have the ability to evolve rapidly and thus gain resistance. Gene stacking makes it possible to have plants with multiple insect resistance and even crops which are resistant to insect and also herbicide tolerant. Single stacked genes require that refuges be planted together with the GM crop. This is to delay the onset disintegration. A refuge is made up of non-GM hybrids to provide the target insects with non-GM material to feed on. However, with stacked traits, the planting of a refuge is not necessary. In short, stacked events offer longer and greater productivity (Singh *et al*., 2018).

Several genetic events have been used to transform maize lines for resistance to various stresses. The first to be developed were the single stack events. These were followed by double stacked events. Double stacked and triple stacked events show greater resilience and are not readily broken down by resistance.

2.10 .2 Genetic Events

Several genetic events have been used in GM technology to transform maize for resistance to different stresses.

2.10.2.1 Event MON 810

MON810 is a product of Monsanto-Company. It was first commercially sold in 2009. The maize line was genetically modified to be resistant to lepidopteran insects. It is a single stacked event with Cry1Ab proteins. It conferred herbicide resistance, insect resistance and antibiotic resistance.

2.10.2.2 Event MON863XMON810

This is a product of Monsanto and it is double stacked event. The maize line was genetically modified to be resistant to lepidopteran insects. It also confers resistance to coleoptera and antibiotic resistance.

2.10.2.3 Event TC1507: A case study of Brazil

According to Bernadi *et al.* (2015), the following about transgenic maize was reviewed. Brazil is a major producer of maize in the world. The country started growing Bt maize in 2007 and had reached high GM adoption levels by farmers. The Bt trait that was used was a single stack event TC1507. Huge success with the Bt trait was experienced in controlling fall armyworm, which had replaced the extensive use of chemical pesticides to control the pest. However due to the single stack nature of the trait, field evolved fall armyworm began to emerge, rendering the trait ineffective against fall armyworm. This scenario is not unique to Brazil. It occurred also in South Africa, which is the leading producer of GM crops in Africa.

Other than Bayer (formerly Monsanto Company), other commercial seed companies have put GM hybrids on the commercial market. According to the African Centre for Biodiversity (2019), currently Corteva has got three genetically modified maize lines that it wants to introduce into South Africa; these are :2,4D herbicide tolerant maize with the event DAS-40278-9, the stacked 2,4D and glyphosate herbicide tolerant maize with the event NK603XDAS-40278-9 and the stacked 2,4D and glyphosate and glufosinate herbicide tolerant maize and Bt insecticidal maize with the event MON89034 X TC150S X NK603 X DAS-40278-9

2.10.2.4 Event MON89034, its deployment and target organisms

MON89034 is genetically engineered Bt maize by Monsanto, which is a double stack event because it expresses two proteins that are toxic to insects in the lepidoptera family. These two proteins are known as: Cry1A.105 and Cry2Ab2. The toxins have been proven to be effective against European corn borer and fall armyworm. Previous transgenic crops with a single stacked event such as MON810 which were used extensively against these insect species, have broken down and are no longer effective against the target species. Cry1A.105 and Cry 2A2b are not the only events that are available for exploitation, other constructs are also available. These include Cry 1Ac, Cry 2Ae and Vip3A. These can be exploited to bring about transformations that immensely benefit agricultural productivity. Since the inception of *Agrobacterium tumefaciens* mediated transformations in maize in 1996, Bt maize has been seen to be effective in controlling insect pests in all the countries where these GM crops have been grown (Test Biotech, 2019; Yang *et al*., 2017).

2.11. Types of hybrids grown in Tropical Africa and its implications for GM Integration

In sub-Saharan Africa, the preferred colour of maize for human consumption is white, while in America yellow maize is predominantly grown for feed. Even though maize hybrid seed has been in production for a long time, small holder farmers in sub-Saharan Africa use outdated maize hybrid varieties some as old as 15 years, as well as open pollinated maize varieties (OPV's). These maize varieties have low yield potential and are no longer adapted to the current environmental challenges faced on the continent. This is a stark contrast of the level of hybrid use in developed countries. In developed countries, hybrid seed technology not older than 5 years is used. The farmer's lack of access to new and improved hybrids is part of the reason why maize production in Africa lags behind that of the developed countries. There is need to improve farmer's access to improved hybrid seed by improving the current seed systems and marketing opportunities (Abate *et al*., 2017).

In a bid to improve the realised yields by small holder farmers private-public partnerships have been implementing the Water Efficient Maize for Africa project since 2008 in order to supply farmers with drought tolerant and insect protected maize royalty free. Private seed companies have also released GM maize hybrids especially in South Africa. They have deployed the Bt trait in mostly single cross hybrids, although some three- way cross hybrids are on the market.

2.12 The Evolution of Hybrid Technology

The commercial production of maize in America has followed an upward trend with regards to yield since the beginning of the use open pollinated varieties to hybrid production up till now where GM hybrids are on the market. African agriculture is still using open pollinated varieties, local varieties as well as hybrid varieties. It has been noted that the uptake of hybrid seed varieties has been slow. Most of the small holder farmers use obsolete maize varieties which lead to low yields (Abate *et al*., 2017).

Maize has great genetic variability, making it possible for plant breeders to manipulate it for increased yield, better insect and disease resistance and drought tolerance. It can be self - pollinated and cross pollinated has led to it being improved upon immensely. In America, maize production began to increase with the introduction and uptake of open pollinated varieties. As advancement in breeding techniques grew, there was a progression from open pollinated varieties to double cross hybrids. The production of hybrid maize has enabled maize production to increase and play a role in increasing food security at household level. From double cross hybrids, maize breeding techniques moved on to the production of single cross hybrids and subsequently three-way hybrids. The latest additions to modern day commercial hybrids are the GM hybrids that have been on the market since the mid 1990's (Khan *et al*., 2018; Sesay *et al*., 2017).

Although the widespread use of open pollinated maize varieties has been overshadowed by hybrids, they have their advantages, the major one being that there is no need to purchase new seed every planting season. Farmers can keep their seed from the previous to grow again the next season. The seed production cycle can therefore be continued indefinitely, from generation to generation. Another advantage is that OPV's can be grown under less favourable with lower crop inputs and still produce a good harvest for the farmer (Eosta.com)

By crossing two inbred lines, single crosses are generated. In spite of the increased productivity of single cross hybrids, for them to express their full potential, single cross hybrids require that farmers employ high standards in their cultural practices and that they be grown under favourable environmental conditions (Demari *et al*., 2016). According to Beyene (2016), the use of single cross hybrids in agriculture has a number of advantages. These include the relative ease of the production and maintenance of the inbred lines, better expression of heterosis, suitability for high yield environments, uniformity of the plants in terms of plant and ear height, tasselling and silking as well as pollen shedding. Lastly due to the availability of excellent inbred lines, single cross hybrids constitute a great proportion of the hybrid seed on the maize seed market. Some of the disadvantages associated with single cross hybrids are that are that the female parental line, being an inbred line, is not very high yielding, and resulting in low seed production. This in turn results in high cost of seed production, which then leads to the seed price being expensive.

By crossing a single cross hybrid and an inbred line, three-way hybrids are generated. Unlike the single cross hybrids, three-way cross hybrids exhibit better adaptability to various environments, even the not so favourable ones. Again, when compared to single cross hybrids, three-way hybrids have lower cost of production and hence lower seed price because of greater seed production at crossing. There are disadvantages associated with three-way hybrids. Three-way hybrids do not produce uniformity in plant height as well as other traits and in producing three-way hybrids comes the task of maintaining three parental inbred lines (Singh *et al*., 2012; Beyene, 2016).

Breeding programmes have to contend with the effects of environment on genotypes as they try to find high yielding and stable genotypes across different environments. Genotypes do not yield the same across the different environments. Genotype by environment interactions cannot be ignored and their presence requires testing of the genotypes across different environments over a number of years before they can be released for sale (Szareski, *et al*., 2018; Ribeiro, 2012).

In addition to OPV's, single cross and three-way maize hybrids on the market, there are GM maize hybrids. The use, adoption and consumption of GM maize hybrids has been on the rise, as the land under the production of GM maize has increased. In developing countries like South Africa small holder farmers have now been growing GM crops such as Bt maize for almost a decade. This shows that commercial production of GM crops is no longer limited to commercial farmers (Gouse *et al*., 2016).

2.13 The Impact of Secondary Traits on Selection for Grain Yield

In any breeding programme, the yield gain with enhanced traits is always the goal. This shows that yield is the most important trait. However, yield is a complex trait which is highly dependent on other agronomic traits. By breeders selecting for improvement in these traits, improved yield becomes possible. In order to do this successfully, it is imperative to determine the relationship between yield and its secondary traits (Grafius 1960; Fellahi *et al*., 2013).

2.13.1 Correlation, Regression and Path Coefficient Analyses

Due to the complex nature of grain yield inheritance, breeders use a few tools to help them to select for high yielding maize varieties in their breeding programmes and calculate subsequent genetic gains. These are correlation, regression and path coefficient analyses. These tools serve various purposes. Correlation indicates the intensity of association between any two characters. Correlation between traits can either be positive or negative. Positive correlation means that as one trait increases, the other one also increases. When correlation is negative, it means an inverse relationship exists between the traits. As one increases the other decreases. Positive correlations between desirable traits are most desired by breeders because improvement in trait, leads to the simultaneous improvement in the other trait. Negative correlation, however, means that both traits cannot be simultaneously improved (Soumya and Kamatar, 2017; Saidaiah *et al*., 2008).

In breeding programmes, whose focus is insect resistance, improvement for pest resistance depends on the correlation between the traits for insect resistance and agronomic traits, particularly yield. Selection for low damage scores on damage parameters could lead to higher yields. In relation to fall armyworm damage, some studies found leaf damage to be negatively associated with grain yield and cob damage also showed a negative correlation with grain yield (Kasoma *et al.,* 2020; Oloyede Kamiyo, 2015).

Path coefficient analysis is used to determine which components have effect on yield. It shows the role of direct and indirect effects of secondary traits on yield. Path coefficient analysis helps the breeder know which traits have the highest effects on yield, so that preference in selection is given to the traits with significant effects (Soumya and Kamatar, 2017; Hefny, 2011).

2.14 Cultivar Superiority

In order for a maize breeding programme to be of effective use, it needs to identify the genotypes that show high yields and that are stable across different environments. Environmental factors have been known to interact with the genotype and affecting the yield potential of the genotype in the different environments. Yield is used as a measure for cultivar superiority. This is because yield is considered the most important agronomic characteristic of the crop. Releasing superior germplasm which is high yielding and stable across environments is an important key to raising productivity of maize (Gauch, 2006; Cornelius and Crossa, 1999; Perkins and Jinks, 1971).

2.15 Conclusion and implications

The literature review was effective in addressing the objectives of the current study. It also exposed knowledge gaps which this current study can fill. The review of literature sought answer the research questions of this study and to identify knowledge gaps. The questions were: How effective is the Bt trait (Mon89034) in conferring resistance to fall armyworm when deployed in a three-way and single cross hybrid design? It has been shown in the literature that the event MON89034 has been effective for controlling fall armyworm, where it has been deployed in maize for insect resistance against stem borer, however how it will perform when deployed in a three-way and single cross hybrid design to control fall armyworm was a knowledge gap. Secondly, what is the yield gain of the three-way and single cross Bt hybrids relative to non-GM or conventional hybrids? The yield gain of the three-way and single cross Bt hybrids relative to non- GM or conventional hybrids is yet unknown and presented a knowledge gap. Thirdly, what is the impact of direct and indirect effects of secondary traits on grain yield of three-way and single cross Bt hybrids under fall armyworm infestation? Literature has shown that leaf damage and cob damage are negatively impactful on grain yield. The last question was, what is the cultivar superiority of the three- way and single cross Bt hybrids compared to conventional non-GM hybrids and local GMO hybrids already on the market? The cultivar superiority of the three-way and single cross Bt hybrids is yet unknown and presented a knowledge gap that this study aimed to fill.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction and research design

The overall research was divided into two experiments. The first experiment (Experiment 1) entailed generation and subsequent evaluation of the genetically modified (GM) three-way cross hybrids. The second experiment (Experiment 2) was devoted to generation and evaluation of single cross GM hybrids. The study was conducted under natural hotspot fall armyworm (FAW) conditions, while yield evaluation was conducted under field conditions which represent the farmer's situation. The chapter presents the germplasm, research design and methodology employed to address the study's aims and objectives. It explains the experimental design and management of field sites. It describes the method used to screen hybrids for FAW in the fields. The chapter also presents the methods for the collection of data on agronomic traits and how the data was analysed using various models that fitted to the data.

3.2. Germplasm and mating design

The conventional germplasm used in the study comprised tropical single crosses and inbred lines which were sourced from Seed Co Ltd in Zimbabwe. The single cross hybrids were used to generate three-way crosses and the parental inbred lines were used to generate the single cross hybrids, in combination with GM maize inbred lines. The seed of genetically modified maize inbred lines were obtained from the African Agricultural Technology Foundation (AATF) WEMA Bt lines project. The WEMA project is a partnership of the AATF and Bayer (formerly Monsanto). The WEMA Bt lines used in the study were: WMB4105, WMB4810, WMA2001 and WMA3306. These are all white germplasm lines of medium physiological maturity group. Conventional (non- GM) commercial hybrids for use as the standards or controls for the experiments were obtained from Seed Co Ltd. Table 3.1 shows the list of the commercial hybrid checks which were used in the study. The four WEMA Bt lines were crossed with 30 Seed Co single cross hybrids at the Makhathini Research Station in the winter of 2019. All the single cross hybrid parents are white grain and tropical adaptation. The WEMA Bt lines were used as males and the Seed Co hybrids were used as the females. The resultant GM were used for Experiment 1. The experimental hybrid list is shown in Appendix A and B. As a result, 110 hybrid entries were used as these had sufficient seed for planting in trials. Experiment 2 had 208 entries.

Table 3.1 List of commercial hybrid checks

Entry number	Designation	Type of hybrid	Type of cross
96	SC419	Conventional/Non-GM	Three-way cross hybrid
97	SC633	Conventional/Non -GM	Single cross hybrid
98	SC301	Conventional/Non -GM	Single cross hybrid
99	SC403	Conventional/Non- GM	Single cross hybrid
100	PAN5R-891BR	GM	Single cross hybrid
101	DKC75-65BR	GM	Single cross hybrid
102	WE6207B DKC	GM	Single cross hybrid
103	WE6210B	GM	Single cross hybrid
104	WE6208B	GM	Single cross hybrid
105	DKC68-58BR	GM	Single cross hybrid
106	LG	GM	Single cross hybrid
107	33H54BR	GM	Single cross hybrid
108	P1184BR	GM	Single cross hybrid
109	P2553WBR	GM	Single cross hybrid
110	P1690BR	GM	Single cross hybrid

GM: genetically modified

3.3 Experimental sites

The experimental three-way cross and single cross hybrids were evaluated in three growing environments at three locations in South Africa, during the summer of 2019/20. Makhathini Research Station is located on the Makhathini Flats. It is situated 27° 39’S, 32° 10’E Elevation is at 73 metres and annual rainfall is 569mm. Dundee Research Station is located in the uMzinyathi District Municipality. It is situated S28° 08’S, 30° 18’E. Elevation is 1247m and it receives an average rainfall of 765mm per annum. Potchefstroom Research Station is situated S26° 47’46.1’’ E27° 05’26.0’’. Elevation is 1340m and it receives an average rainfall of 615mm per annum.

3.4 Experimental design and management

At each site both, three-way cross hybrids and single cross hybrids were evaluated in separate trials which were designated as Experiment 1 and Experiment 2. The three-way hybrid trial was laid out as a 10x11 alpha lattice design at all sites. Each of the 11 blocks contained 10 hybrids. Single cross hybrid trial design was 13 by 16 alpha lattice design at all sites. Each of the 16 blocks contained 13 hybrids. Both experiments were replicated twice at each site. At all the sites 6 m long plots were planted except at Potchefstroom which had 5m long plots. Inter row spacing was 0.9m and interrow spacing was 0.25m, while interrow spacing was 0.75m at Potchefstroom. Basal fertilizer (NPK) was applied (75 kg N, 50 kg P, 25 kg K per hectare) at planting. Top dressing of 120 kg per hectare in the form of Limestone Ammonium Nitrate LAN (28% N) was applied four weeks after emergence. All the sites were under irrigation. Pre-emergence and post-emergence herbicides were used and manual hand-weeding was done at all three sites. Insecticide was not used at all at any of the sites to allow FAW infestation on the growing maize.

3.5 Data Collection

3.5.1 Agronomic traits

Data for agronomic traits such as field weight, grain weight, plant height and ear height were measured as described as follows: Plant height was measured between the base of the plant to the first tassel branch using a metre ruler. Ear height was measured between the plant to the first insertion of the top ear of the same plant using a meter ruler. Ear prolificacy was calculated as ratio of number of ears in a row to number of plants of plants in a row at harvest. Grain moisture was calculated as percentage of water content in the grain measured at harvest. Field weight was calculated as weight of the de-husked ears harvested from each plot using a scale. Grain weight was calculated as the weight of the grain after shelling using a scale. Grain yield was calculated from weight of ears adjusted to yield per ha calculated from field weight and adjusted to 12.5% moisture content, 80% shelling percentage and plot size. Number of plants was the number of plants in a row at harvest. Ears harvested was number of cobs harvested from each row. Ear -plant ratio was the number of ears harvested expressed as a ratio in relation to the number of plants harvested per plot. Cob length was the length of cobs averaged over three representative cobs post-harvest. Kernel rows per cob was number of kernel rows on a cob averaged over three representative cobs post-harvest.

Grain yield was calculated from the field weight measured as cob weight per plot to 12.5% moisture and 80% shelling percentage using the following formula adapted from Lauer (2002):

$$GY = \frac{\text{Field weight (kg)}*1000(\text{m}^2)*(100-\text{MOI})*\text{shelling}\%}{1000(\text{kg})*\text{plot area}(\text{m}^2)*(100-12.5\%)}$$

where GY is grain yield and MOI is grain moisture content.

3.5.2 Screening for FAW damage

Makhathini Research Station is a natural hotspot for FAW therefore, the hybrids were screened for FAW resistance at the station. Damage for FAW damage was also assessed on maize also grown at Dundee. Screening for FAW damage was done on both the leaves and cobs. On the leaves visual assessment of damage was done and rated on a scale of 1-9, where 1 is no FAW damage and 9 represents the worst FAW on the leaf. Cob damage was assessed visually and rated on a scale of 1-9, where 1 is no FAW damage on the cob and 9 represents the worst FAW damage on the cob. Leaf area damage percentage was a visual assessment of how much of the leaf area had been damaged due to fall armyworm feeding as a percentage.

3.5.3 Screening for diseases

Maize streak Virus, Turcicum Leaf Blight and rust scores for disease severity were made on a scale of 1-5 using visual assessment. Where 1 indicated no damage on the leaf to 5 indicating very severe leaf damage.

Phaesphaeria Leaf Spot scores for disease severity were made on a scale of 1-9 using visual assessment. Where 1 is no leaf damage to 9 indicating severe damage.

3.6 Data Analyses

The analysis of data involved general analysis of variance, cultivar superiority index and analysis of relationships among traits.

3.6.1 General ANOVA and Mean separation Test

Data was analysed using the following fixed model (Singh and Chaudhary, 1985):

$$B_{ijk} = \mu + G_j + E_i + (G_j * E_i) + E_i(r_k)(b) + \varepsilon_{ijk}$$

B_{ijk} = observed response

μ= grand mean

G_j = the effect of the jth genotype

E_i= the effect of the ith environment

G_j*E_i = genotype X environment interaction

$E_i(r_k)(b)$ = error associated with the kth replication in blocks in the ith environment

ε_{ijk} = random error

Hybrid means were separated by Fischers protected LSD at $p \leq 0.05$ significance level.

Analysis of variance data was analysed using the nlme package in R software. R software is a program and routine for computing statistical analyses and plotting graphs. The software is operated by a command line using the R language. It runs on all major computer operating systems. Nlme: Linear and non- linear mixed effects model the nlme package is used to fit and compare Gaussian linear and non-linear mixed effects data hosted in the R software. Liner models refer to the relationship between two variables that exhibit a constant rate of change. In contrast non-linear equations model the relationship between two or more variables that are related by a non- constant rate of change between the parameter and dependent variables. In breeding, many quantitative traits exhibit linear and non- linear relationship with grain yield such that linear and non- linear mixed effects models are used (R Core Team, 2014).

3.6.3 Cultivar Superiority Index

The performance of genotypes was analysed according to the model (Lin and Binns, 1988). This was conducted using BMS version 16 software

$$P_i = \frac{\sum(X_{ij} - M_j)}{2n}$$

P_i= mean square between the cultivar's yield and maximum yield in each environment

X_{ij} = the yield of the ith genotype in the jth environment

M_j = the maximum yield in the j^{th} environment

N = number of environments

3.6.4. Relationships between Grain yield and secondary traits

The relationship between grain yield and secondary traits was investigated using three different approaches as follows: Correlation analysis, regression analysis and path coefficient analysis. The R software using the Agricolae package were employed in the data analysis for all three analyses.

3.6.4.1. Correlation analysis

Correlations were performed following the method of Payne *et al*. (2007) based on Pearson's correlation analysis.

$$R= \frac{\sum(x_i-x)\ (y_i-\bar{y})}{\sqrt{\sum(x_i-x)^2}\ \sum(y_i-\bar{y})}$$

R= correlation coefficient

X_i= values of the x variables in a sample

X= mean of the values of the x variable

Y_i= values of the y variable in a sample

$\bar{y}$= mean of the values of the y variable

3.6.4.2 Regression analysis

In the regression analysis, yield and agronomic traits were treated as response and independent variates respectively using the following model:

$$Y = \alpha+\beta X+\varepsilon$$

Y= yield response of the genotype (dependent variable)

α = yield response when the independent variable X=0

β = rate of change for Y for each unit of X

X = the value of the independent variable

$\mathcal{E}$ = error associated with prediction of Y from X

Regressions with a coefficient of determination less than 10% were considered negligible.

3.6.4.3. Path coefficient analysis

Path analysis is a statistical technique based on stepwise regression analysis to evaluate causal relationships between a respondent variable and two or more independent variables. Path coefficient analysis was computed as suggested by Dewey and Lu (1959). It was performed using Agricolae package in R software to deduce direct and indirect effects of secondary traits on grain yield of the hybrid.

$$R_{ij}=P_{ij}+\sum r_{rk}P_{kj}$$

R_{ij}= mutual association between the independent character *i* (yield related trait) and dependent character *j* (grain yield)

P_{ij}=componentsnof direct effects of the independent character *i* on the dependent character *j*

$\sum r_{ik}P_{kj}$= summation of components of indirect effects of a given independent character *i* on a given dependent character *j* via all other independent character *k*.

The contribution of the remaining unknown characters is measured as the residual as given by:

$P_R = \sqrt{(1-\sum P_{ij}r_{ij})}$

3.7 Conclusion

This chapter described the germplasm, crossing, experimental design, management, data collection and data analysis in detail. The findings from the study are presented in Chapter 4.

CHAPTER 4

RESULTS

4.1 Introduction

This chapter presents the outcomes of the completed study. The overall research was divided into two experiments (Chapter 3). Experiment one was the three-way cross hybrids and experiment two was the single cross hybrids. Consequently, the results are also presented in two parts following the same order. First part presents the results from the three-way cross hybrids trial (Experiment 1). The second part presents results from the single cross hybrid trials (Experiment 2) in line with the objectives of the study which are presented in Chapter 1.

4.2 EXPERIMENT 1-THREE-WAY CROSS HYBRIDS STUDY

4.2.1 Efficacy of the Bt trait in conferring resistance to fall armyworm

4.2.1.1 Analysis of Variance for agronomic traits and fall armyworm damage parameters

The Experiment 1 which is the three-way hybrid crosses evaluation was conducted at Makhathini Research Station under the fall army worm (FAW) infestation. The station is a hotspot site for the FAW. This enabled the assessments of the hybrids for efficacy of the Bt trait in conferring resistance to FAW in the three-way cross hybrid. Table 4.1 shows that number of plants was a highly significant ($P<0.001$) as a covariant for grain yield, field weight, number of ears harvested, leaf area damage score, cob damage score, leaf damage score 1, leaf damage score 2 and significant ($P<0.01$) for cob damage score. Entry (Genotype) was a highly significant ($P<0.001$) source of variation for leaf area damage, cob damage score, cob damage percentage, leaf damage score 1, leaf damage score 2 and number of damaged cobs. Entry (Genotype) was significant ($P<0.05$) for ears per plant and number of ears harvested.

Table 4.1 ANOVA for three-way experimental hybrids with number of plants as covariate for grain yield and secondary traits at Makhathini Research Station

Source of variation	Df	Grain yield	Ears per plant	Number of ears harvested	Leaf area damage %	Cob Damaged Score	Cob damage %	Leaf Damage Score 1	Leaf damage Score 2
Rep	1	16.5411	0.5189	728.15	2363.9	0.29378	3.7	0.01892	0.6425
Rep*block	20	5.599	0.35827	300.35	1734.2	0.2797	1695.4	0.47599	0.4938
NP	1	10.0953***	0.00926	830.18***	7881.2***	0.63155**	7920.9***	6.56116***	2.613***
Entry	98	0.8438	0.07208*	48.62*	1517.5***	0.19623***	1741.6***	0.60657***	0.4223***
Residual	94	0.6561	0.05145	34.7	630.7	0.06967	437	0.06536	0.1709
Total	214	1.3498	0.09137	74.66	1181.9	0.15471	1215.3	0.38025	0.3291
LSD		1.687	0.4577	12.27	50.91	0.5526	43.54	0.5159	0.8343
CV		58.28	45.84	40.97	90.33	40.22	50.52	35.94	84.98
SE		0.81	0.2268	5.89	25.11	0.264	20.91	0.2557	0.4134

LSD = least significance difference at 5%, CV = coefficient of variation *, **, ***= level of significance at p≤0.05%, p≤0.01%, p≤0.001%

4.2.1.2 Mean performance of three-way experimental hybrids at Makhathini

The genotypes of the three-way experimental hybrids were ranked according to yield for Makhathini. Table 4.2 presents the means and ranking of the experimental hybrids as well as the conventional hybrid checks. The highest yielding three-way experimental hybrid under FAW pressure was entry 31 (H3WX3194Bt) which had a yield of 3.4 t ha^{-1} and 3.2t ha^{-1} adjusted grain yield. The cob damage score was 3.1 indicating little damage to the cobs by the FAW. Incidence of cob damage was 40.2% while the number of damaged cobs was 8.4. Leaf area damage percent was 0.7 while leaf damage score was 1.0 indicating that the leaves were not damaged by FAW feeding.

The conventional GM checks had yields as low as 0 t ha^{-1}. This showed that there was significantly high FAW pressure to drastically reduce the yields of the conventional non-GM hybrid checks. SC403 showed that no cobs were harvested due to there not being any cobs on the plant. It also had a leaf damage score of 8, indicating heavy leaf damage due to feeding by FAW. This shows that SC403 was adversely affected by the FAW pressure in the field.

WEMA check hybrid (WE6210) had a yield of 2.8 t ha^{-1}. It had a cob damage score of 2.5 and incidence of leaf feeding as measured by leaf area damage percentage was 18.2%. Leaf damage score was 1 showing that it did not suffer injury from leaf feeding by fall armyworm. WEMA checks are GM hybrids that have been availed royalty free to the small-scale farmers in South Africa and are being tested for release in other tropical Africa countries that may adopt GM hybrids.

Even though entry 85 (H3WX3254Bt) was ranked fifth, it had a cob damage score of 1.1, indicating that there was no significant damage to the cobs. The leaf damage score of 1.5 showed that there was no significant leaf feeding by FAW.

Table 4.2

ENTRY_NO	Genotypes	Pedigrees	Rank	Grain Yield	No of plants	Cob damage score	No damaged cobs	Damaged cobs %	Ears harvested	Ears/plant	% Leaf area damaged	Leaf damage score 1
31	H3WX3194Bt	Proprietary information	**1**	3.4	29.8	3.1	8.4	40.2	23.5	0.7	0.7	1.0
33	H3WX3198Bt	Proprietary information	**2**	2.9	33.8	3.6	8.6	31.0	27.4	0.8	-0.1	1.0
103	WE6210B	Proprietary information	**3**	2.8	26.1	2.5	2.5	18.2	23.9	1.0	-1.5	1.0
101	DKC75-65BR	Proprietary information	**4**	2.8	30.7	1.8	5.4	14.6	24.9	0.8	-1.8	1.0
85	H3WX3254Bt	Proprietary information	**5**	2.9	28.4	1.1	0.2	-0.3	23.4	0.7	-0.1	1.5
75	H3WX3242Bt	Proprietary information	**6**	2.6	30.5	2.9	0.3	13.2	21.1	0.7	-1.9	1.0
89	H3WX3258Bt	Proprietary information	**7**	2.6	26.7	1.3	1.8	2.8	19.6	0.7	-0.1	1.0
92	H3WX3261Bt	Proprietary information	**8**	2.9	35.0	0.8	1.0	1.6	28.0	0.6	1.0	1.5
107	33H54BR		**9**	2.7	31.7	2.7	9.3	74.3	21.3	0.6	23.8	7.5
26	H3WX3188Bt	Proprietary information	**10**	2.6	28.3	2.2	2.0	13.9	16.9	0.6	-2.8	1.0
80	H3WX3249Bt	Proprietary information	**11**	2.4	28.5	2.2	2.6	6.2	25.3	0.9	-0.2	1.0
102	WE6207B DKC		**12**	2.6	32.7	3.9	3.2	11.1	22.8	0.7	4.2	2.0
110	P1690BR		**13**	2.3	29.5	2.9	10.3	43.8	17.7	0.3	22.9	8.0
41	H3WX3206Bt	Proprietary information	**14**	2.3	30.5	1.7	1.1	11.2	22.8	0.7	9.9	1.0
81	H3WX3250Bt	Proprietary information	**15**	2.3	31.7	1.7	2.5	5.1	16.3	0.5	1.5	1.0
36	H3WX3201Bt	Proprietary information	**16**	2.1	27.5	0.7	2.1	-0.2	19.0	0.5	32.9	1.0
44	H3WX3209Bt	Proprietary information	**17**	2.0	25.8	1.7	1.2	4.8	15.3	0.6	0.7	1.0
73	H3WX3240Bt	Proprietary information	**18**	2.1	28.8	0.9	-0.4	-1.2	13.7	0.4	-2.5	1.5
76	H3WX3243Bt	Proprietary information	**19**	2.0	27.9	1.2	1.5	4.6	15.5	0.5	2.5	1.5
29	H3WX3191Bt	Proprietary information	**20**	2.0	26.0	2.2	5.2	15.1	24.0	0.5	-1.4	1.5
Conventional non-GM check hybrids												
99	SC403		**99**	-0.5	22.2	4.0	3.9	62.2	0.0	0.0	6.4	8.0
106	SC419		**96**	0.0	28.9	4.4	2.5	87.2	2.0	0.1	73.1	8.0
105	SC301		**98**	-0.1	27.2	6.1	7.3	95.6	4.5	0.2	83.2	8.0
97	SC633		**97**	1.0	24.8	7.7	11.5	86.7	12.6	0.4	86.6	8.0
	Mean			1.4	27.1	3.4	5.4	41.4	14.4	0.5	27.8	4.7
	CV			60.8	14.9	33.5	63.3	44.6	39.9	43.9	77.8	12.7
	Heritability			0.3	0.1	0.8	0.6	0.8	0.4	0.4	0.7	1.0
	MeanLSD			1.9	8.3	2.5	7.6	39.1	12.8	0.5	43.8	1.2
	Pvalue			0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Negative grain yield was due to adjustments to the data for experimental error

4.3 Impact of secondary traits on grain yield under fall armyworm infestation

The association between grain yield and secondary traits varied depending on trait. The association can be explained by correlation, regression and path coefficient analyses.

4.3.1 Correlation, Path and Regression Coefficient Analyses

Correlations of traits measured at Makhathini under FAW pressure are presented in Table 4.3. Strong, positive and significant (P<0.001) correlation coefficients were found between grain yield and field weight, cobs per plant and number of ears harvested. This showed that as field weight, ears per plant and number of ears harvested increased so did grain yield. Weak, negative and highly significant (P<0.001) correlations were found between grain yield and leaf area damage percentage, damaged cobs percentage, leaf damage score 1 and 2. This showed that as leaf area damage percentage, damaged cobs percentage and leaf damage scores increased, yield decreased.

The path coefficient analysis model accounted for 85% of the secondary traits impact on grain yield of three-way cross hybrids under the FAW infestations as indicated by the residual of 15%. Table 4.4 shows direct and indirect effects of secondary traits on yield of three-way cross hybrids at Makhathini. The most important direct effect was number of ears harvested as it had a positive impact on yield of more than 83%. This means number of ears harvested had the greatest impact on yield. Number of damaged cobs had the second highest positive direct contribution on grain yield of 20%. This is because the lower the number of damaged cobs, then grain yield would increase. Leaf damage score 2 had a negative direct effect on yield.

The number of ears per plant (ear prolificacy) had the highest positive indirect effect of 63% on yield via number of ears harvested. Number of plants had the second highest positive indirect effects on yield via number of ears harvested of 32%. Number of damaged cobs had 18% positive effects on yield via number of ears harvested. Cob damage had 19% negative indirect effect on yield number of ears harvested. Cob damage had 16% positive effect on yield via number of damaged cobs. Leaf damage score 1 had 22% negative effect on yield via number of ears harvested. Leaf damage score 2 had 22% negative effect on yield via number of ears harvested. Leaf area damage percentage had 21% negative effect on yield via number of ears harvested.

The regression data for Makhathini is presented in Table 4.5. The R squared value indicated that the regression model accounted for 74% of the direct effects. Number of ears harvested, on grain yield was highly significant (P<0.001), while all the other traits were non-significant (P>0.05).

Table 4.3. Correlations among traits of three-way experimental hybrids under fall armyworm infestation at the Makhathini Research Station

Traits	Grain yield	Leaf area damage %	Cob Damage Score	Ears per plant	Cob damage %	Leaf Damage Score 1	Leaf Damage Score 2	Number of Damaged Cobs	Number of Ears Harvested
Grain yield									
Leaf Area Damage %	-0.17***								
Cob Damage Score	-0.05	0.65***							
Ears Per Plant	0.83***	-0.07	0.09						
Cob Damage %	-0.24***	0.71***	0.82***	-0.12					
Leaf Damage Score 1	-0.30***	0.73***	0.72***	-0.15***	0.80***				
Leaf Damage Score 2	-0.17***	0.97***	0.66***	-0.06	0.72***	0.75***			
Number of Damaged Cobs	0.20***	0.60***	0.70***	0.36***	0.75***	0.59***	0.61***		
Number Ears Harvested	0.89***	-0.09	0.06	0.96***	-0.16***	-0.21***	-0.09	0.33***	

***= level of significance at p≤0.001%

Table 4.4. Direct (Diagonal in bold) and indirect effects (above and below diagonal) of secondary traits on grain yield of three way cross hybrids, under fall armyworm infestation, at Makhathini Research Station

Traits	NP	EPP	NEH	NDC	DC%	CDS	LDS1	LDS2	LAD%	total effects on GY	P value
Number of plants (NP)	**0.045093**	-0.02592	0.320242	-0.01143	0.02431	0.001828	0.049327	0.059408	-0.01715	0.445699	0
Ears Per Plant (EPP)	0.009262	**-0.126210**	0.63542	0.065803	0.004865	-0.00032	0.020899	0.017418	-0.00474	0.622389	0
Number of ears harvested (NEH)	0.017263	-0.095880	**0.83649**	0.04381	0.023111	0.000598	0.035734	0.047023	-0.01368	0.894479	0
Number of damaged cobs (NDC)	-0.00253	-0.040760	0.179844	**0.203772**	-0.08428	-0.00582	-0.09489	-0.12453	0.03731	0.068117	0
Damaged cobs % (DC%)	-0.0108	0.006050	-0.19047	0.169215	**-0.10149**	-0.00655	-0.11677	-0.14619	0.043457	-0.35355	0
Cob damage score (CDS)	-0.01119	-0.005460	-0.06791	0.160998	-0.09026	**-0.00737**	-0.10645	-0.13274	0.039611	-0.22076	0.648
Leaf damage score 1 (LDS1)	-0.01644	0.019500	-0.22098	0.142944	-0.08761	-0.0058	**-0.13527**	-0.14895	0.043855	-0.40875	0
Leaf damage score 2 (LDS2)	-0.01534	0.012586	-0.2252	0.14528	-0.08495	-0.0056	-0.11536	**-0.17466**	0.052215	-0.41103	0
Leaf area damage percentage (LAD)	-0.01464	0.011332	-0.21649	0.143885	-0.08347	-0.00552	-0.11227	-0.1726	**0.052839**	-0.39694	0.25

Residual = 15%

Table 4.5. The regression of secondary traits on grain yield of three- way experimental hybrids under fall armyworm infestation at Makhathini Research Station

Parameter	Estimate	Standard error	t value	P value
Intercept	0.045018	0.483213	0.093	0.926
Number of plants	0.007444	0.015442	0.482	0.631
Ears per plant	-0.069723	0.371481	-0.188	0.850
Number of ears harvested	0.104414	0.015516	6.729	0.00***
Number of damaged cobs	-0.021957	0.021174	-1.037	0.302
Cob damage %	0.003745	0.004341	0.863	0.390
Cob damage score	-0.008749	0.052638	-0.166	0.868
Leaf damage score 1	0.033022	0.029279	-1.128	0.262
Leaf damage score 2	-0.077763	0.074785	-1.040	0.301
Leaf area damage%	0.002372	0.006671	0.356	0.723

***= level of significance at p≤0.001%

Adjusted R^2 0.7358

4.4 Genetic gains assessment

4.4.1.1 Analysis of Variance Across Sites for three-way Cross Experimental hybrids

According to Table 4.6, the analysis of variance showed that site (environment) was highly significant source of variation for grain yield. Number of plants was also a highly significant source of variation for grain yield.

Table 4.6: ANOVA for grain yield with NP as covariate across three sites

Source of Variation	Degrees of freedom	Grain Yield
Site	2	532.446***
Rep	1	0.474
Rep*block	20	4.196
Number of Plants	1	126.101***
Entry	98	1.318
Site*Entry	194	1.722
Residual	322	1.443
LSD		1.387
CV		37.09
SE		1.201

LSD = least significance difference at 5%, CV = coefficient of variation ***= level of significance at p≤0.001%

4.4.1.2. Cultivar Superiority Across sites for Three-way Cross Experimental Hybrids

The genotypes were ranked according to their cultivar superiority index. The top 20 and the four conventional non-GM hybrid checks are presented in (Table 4.7). The highest yielding three-way experimental hybrid was (H3WX3167Bt) which had a yield of 4.6 t ha^{-1} and the lowest cultivar superiority index of 0.5 (a low cultivar superiority index is desirable as it indicates that the hybrid combines high yield potential with dynamic stability). It was 22% above mean of conventional hybrids, 64% above mean of WEMA GM checks and 33% above mean of local GM checks.

The conventional non-GM hybrid checks SC403 had yield of 2.3 t ha^{-1}, a superiority index of 6.3. It had negative 39% above mean of conventional checks, negative 18% above mean of WEMA GM checks and negative 34% above mean of local GM checks.

Table 4.7 Cultivar Superiority Analysis Across three sites for three-way cross hybrids

ENT	Genotypes	Pedigree	GY	Cultivar Superiority Index	% above mean of conventional checks	% above mean of WEMA checks	% above mean of Local GMO checks
1	H3WX3161Bt	Proprietary information	**1,9**	5,1	-21%	-43%	-45%
2	H3WX3162Bt	Proprietary information	**2,8**	2,9	15%	-17%	-21%
3	H3WX3163Bt	Proprietary information	**2,4**	4,9	-2%	-29%	-32%
4	H3WX3164Bt	Proprietary information	**2,5**	3,0	4%	-25%	-28%
5	H3WX3165Bt	Proprietary information	**3,2**	2,6	34%	-4%	-8%
6	H3WX3167Bt	Proprietary information	**4,1**	0,5	72%	24%	18%
7	H3WX3168Bt	Proprietary information	**2,8**	4,3	16%	-16%	-20%
8	H3WX3169Bt	Proprietary information	**3,1**	3,1	28%	-7%	-11%
9	H3WX3171Bt	Proprietary information	**3,0**	4,1	23%	-11%	-15%
10	H3WX3172Bt	Proprietary information	**2,8**	2,3	16%	-17%	-20%
11	H3WX3173Bt	Proprietary information	**2,8**	3,0	18%	-15%	-18%
12	H3WX3174Bt	Proprietary information	**2,1**	4,7	-13%	-37%	-40%

13	H3WX3175Bt	Proprietary information	**3,3**	2,9	39%	0%	-4%
14	H3WX3176Bt	Proprietary information	**2,7**	4,5	14%	-18%	-22%
15	H3WX3177Bt	Proprietary information	**3,0**	3,8	26%	-9%	-13%
16	H3WX3178Bt	Proprietary information	**2,7**	4,4	13%	-19%	-22%
17	H3WX3179Bt	Proprietary information	**2,8**	3,4	16%	-16%	-20%
18	H3WX3180Bt	Proprietary information	**2,9**	3,6	18%	-15%	-18%
19	H3WX3181Bt	Proprietary information	**2,8**	1,9	17%	-16%	-20%
20	H3WX3182Bt	Proprietary information	**2.5**	1.5	4%	-25%	-28%

4.4.1.3 Cultivar Superiority of three-way hybrids under Fall Armyworm infestation

This analysis for cultivar superiority was done for the experimental three-way hybrids at Makhathini under FAW pressure. The genotypes were ranked according to their mean yield at Makhathini. According to Table 4.8, the most superior three-way experimental hybrid was entry 31 (19CSA3194Bt) with 147% above mean of conventional non-GM check hybrids, 47% above mean of WEMA GM check hybrids and 85% above mean of local GM hybrids checks.

Among the conventional non-GM checks, which all suffered adversely under fall armyworm pressure, SC403 had negative 136% above mean of conventional Non-GM check hybrids, 122% above mean of WEMA GM check hybrids and negative 127% above mean of local GM hybrids checks.

The top 20 hybrids and the four conventional non-GM hybrid checks are presented in Table 4.8.

Table 4.8 Cultivar Superiority Analysis for three-way cross experimental hybrids at Makhathini Research Station

ENTRY_NO	Genotypes	Pedigrees	Rank	Grain Yield	% above mean of conventional checks	% above mean of WEMA checks	% above mean of Local GM checks
Best 20							
31	H3WX3194Bt	Proprietary information	**1**	3.4	147%	47%	85%
33	H3WX3198Bt	Proprietary information	**2**	2.9	109%	25%	57%
103	WE6210B	Proprietary information	**3**	2.8	100%	20%	50%
85	H3WX3254Bt	Proprietary information	**4**	2.9	108%	24%	56%
101	DKC75-65BR	Proprietary information	**5**	2.8	105%	23%	54%
75	H3WX3242Bt	Proprietary information	**6**	2.6	89%	13%	41%
92	H3WX3261Bt	Proprietary information	**7**	2.9	113%	27%	60%
89	H3WX3258Bt	Proprietary information	**8**	2.6	86%	11%	39%
26	H3WX3188Bt	Proprietary information	**9**	2.6	90%	13%	42%
107	33H54BR		**10**	2.7	94%	16%	45%
102	WE6207B DKC		**11**	2.6	87%	12%	40%
80	H3WX3249Bt	Proprietary information	**12**	2.4	75%	4%	31%
41	H3WX3206Bt	Proprietary information	**13**	2.3	68%	0%	26%
110	P1690BR		**14**	2.3	66%	-1%	24%
104	WE6208B		**15**	2.4	71%	2%	28%
81	H3WX3250Bt	Proprietary information	**16**	2.3	71%	2%	28%
82	H3WX3251Bt	Proprietary information	**17**	2.1	56%	-7%	17%
79	H3WX3247Bt	Proprietary information	**18**	2.2	58%	-5%	19%
29	H3WX3191Bt	Proprietary information	**19**	2.0	44%	-14%	8%
73	H3WX3240Bt	Proprietary information	**20**	2.1	233%	-9%	-3%
Conventional non-GM check hybrids							
97	SC633		**97**	1.0	-24%	-55%	-43%
96	SC419		**96**	0.0	-101%	-100%	-100%
98	SC301		**98**	-0.1	-107%	-104%	-105%
99	SC403		**99**	-0.5	-136%	-122%	-127%

4.5 EXPERIMENT 2-SINGLE CROSS HYBRIDS STUDY

4.5.1 Analysis of Variance for agronomic traits and fall armyworm damage parameters

Table 4.9 shows that number of plants was a highly significant ($p<0.001$) source of variation for ears harvested per plant. Entry (Genotype) was a highly significant ($P<0.001$) source of variation for grain yield, ear height, plant height, ears harvested per plant, ear-plant ratio, leaf damage score 1 and leaf area damage percentage and significant ($P<0.01$) for leaf damage score 1 and plant height.

Table 4.9 ANOVA for grain yield and secondary traits with number of plants as covariate at Makhathini Research Station

Source of variation	DF	Grain Yield	Plant Height	Ear Height	Ears Harvested Per plant	Ear Plant Ratio	Leaf Damage Score1	Leaf Damage Score2	Leaf Area Damage %
Rep	1	148.8616	57725.5	7106.3	2464.8	0.122661	0.53056	0.4837	2502.5
Rep*block	14	6.6164	50502.2	12149.7	195.33	0.199776	2.88013	3.4923	14490.4
NP	1	1.0241	79.7	84.2	473.55***	0.008076	0.10437	0.0012	21
Entry	185	2.2612***	114788.8***	74194.3***	94.9***	1.959635***	93.74441***	76.1473**	321999.4***
Error	209	0.7256	413.8	190.4	40.04	0.005262	0.06721	0.1417	623.2
LSD		1.667	39.93	27.09	12.42	0.1424	87.13	0.7388	49
CV		40.67	10.06	11.47	30.86	12.09	0.26	147.42	152.23
SE		0.8518	20.34	13.8	6.328	0.07254	1.409	0.3764	24.96

LSD = least significance difference at 5%, CV = coefficient of variation **, ***= level of significance at, p≤0.01%, p≤0.001%

4.5.1.2 Mean Performance of Single Cross Hybrids at Makhathini

Due to the disruptions caused by the Covid 19 lockdown, collection of data for the single cross experimental hybrids was hampered for traits such as cob damage score and damaged cobs percentage.

According to Table 4.10, entry 175 (HSX5368Bt) was the highest yielding single cross hybrid at Makhathini with a yield of 5.0 t $ha^{-1}$and 4.9 t ha^{-1} adjusted grain yield, under the FAW infestation. This hybrid had a leaf damage score of 1.5, indicative of very little feeding by the fall armyworm. Even though entry 105 (HSX5227Bt) had a lower yield than entry 175, with a yield of 4.3 t $ha^{-1}$and 4.1 t ha^{-1}adjusted grain yield, it was a very clean crop. It was free from fall armyworm feeding shown by the score of 1.0 in terms of leaf damage and showed 0% incidence of feeding.

The conventional non-GM hybrid checks were shown to be adversely affected by fall armyworm pressure at Makhathini. SC301 and SC419 had yields of 0 t ha^{-1}. Leaf damage scores in the range of 8 and incidence of feeding at 90% were indicating heavy leaf damage due to fall armyworm feeding. In spite of the high number of plants, low numbers for ears harvested were recorded. This is shown by SC301 which had 33.3 plants but only 8.3 ears harvested. The fall armyworm pressure drastically reduced the reproductive capabilities of the conventional hybrids leading to low or zero grain yields.

Entry 110 (HSX5239Bt) was ranked 20th and it had a yield of 3.4 t ha^{-1}and 3.5 t ha^{-1}adjusted grain yield. It had a score of 1 for leaf damage score and 0 percent leaf area damage. These scores indicated that no leaf feeding occurred on this experimental hybrid.

Table 4.10 Mean Performance of Single Cross Experimental Hybrids at Makhathini Research Station

ENTRY NO	Genotypes	Pedigrees	Rank	Grain Yield	Number of plants	Ears harvested	Ears per plant	Leaf Area damage %	Leaf Damage 1
Best 20 hybrids									
175	HSX5368Bt	Proprietary information	**1**	5.0	32	33.8	1.0	0	1.5
107	HSX5232Bt	Proprietary information	**2**	4.4	35.3	27.3	0.8	0	1.0
99	HSX5202Bt	Proprietary information	**3**	4.3	30.6	29.4	1.0	0	1.0
105	HSX5227Bt	Proprietary information	**4**	4.3	30.8	18.3	0.6	0	1.0
191	HSX5419Bt	Proprietary information	**5**	4.2	33.9	31.5	0.9	0	1.0
112	HSX5244Bt	Proprietary information	**6**	3.9	36.1	23.8	0.7	0	1.5
185	HSX5404Bt	Proprietary information	**7**	3.9	33.1	35.1	1.1	0	1.0
193	HSX5426Bt	Proprietary information	**8**	3.9	32.7	25.5	0.8	0	1.0
171	HSX5350Bt	Proprietary information	**9**	3.9	34.8	27.8	0.8	0	1.0
140	HSX5288Bt	Proprietary information	**10**	3.9	34.4	24.3	0.7	0	1.0
130	HSX5278Bt	Proprietary information	**11**	3.9	38.4	27.7	0.7	0	1.0
80	HSX5156Bt	Proprietary information	**12**	3.7	33.2	27.1	0.8	0	1.0
91	HSX5178Bt	Proprietary information	**13**	3.7	37.5	27.1	0.7	0	1.0
192	HSX5421Bt	Proprietary information	**14**	3.5	33.1	29.8	0.9	0	1.5
119	HSX5259Bt	Proprietary information	**15**	3.5	39.3	33.3	0.9	0	1.0
10	HSX5019Bt	Proprietary information	**16**	3.5	35.7	27.9	0.8	0	1.5
116	HSX5248Bt	Proprietary information	**17**	3.5	35.7	25.7	0.7	0	1.0
1	HSX4989Bt	Proprietary information	**18**	3.4	39.7	26.9	0.7	0	1.0
109	HSX5237Bt	Proprietary information	**19**	3.4	33.4	21.0	0.7	0	1.0
110	HSX5239Bt	Proprietary information	**20**	3.4	39.0	23.1	0.6	0	1.0
Conventional non-GM check hybrids									
194	SC419		**207**	-0.3[#]	34.9	9.5	0.3	90	4.5
196	SC301		**206**	-0.2	33.3	8.3	0.3	90	8.0
195	SC633		**198**	0.3	31.7	7.9	0.2	90	8.0
197	SC403		**190**	0.8	30.2	9.1	0.3	90	8.0
	Mean			2.1	11.6	58.5	59.2	0.7	
	CV			43.1	15.6	2.8	3.2	163.8	
	Heritability			0.6	0.1	0.6	0.5	0.3	
	Min			0.0	0.9	53.0	53.0	-2.0	
	Max			6.5	18.6	69.0	69.0	9.0	
	MeanLSD			1.8	3.6	3.3	3.8	2.3	
	Pvalue			0.0	0.1	0.0	0.0	0.0	

[#] Negative grain yield values were as a result of adjustments of the data for experimental error. Otherwise negative values show that yield was zero for those entries; since the plants were completely destroyed by FAW.

4.6 Impact of secondary traits on grain yield under fall armyworm infestation

4.6.1 Correlation, Path and Regression Analysis

Correlations of traits measured at Makhathini for the single cross experimental hybrids are presented in Table 4.11. Ears harvested per plant (ear prolificacy) showed a strong, positive and highly significant (P<0.001) correlation with grain yield. Plant and ear height showed a moderately strong, positive and highly significant (P<0.001) correlation with grain yield. Leaf area damage percentage, leaf damage score 1 and 2 and ear-plant ratio showed weak, negative and highly significant (P<0.001) correlation with grain yield.

Table 4.12 shows that the path coefficient analysis model accounted for 72% of the secondary traits impact on yield as indicated by the residual of 28%. Ears harvested per plant had the highest positive direct effects on yield (Table 4.9). Plant height had the second highest positive direct effect on grain yield, followed by ear-plant ratio. Leaf damage score 1 had a positive direct effect on grain yield. Ear height had the highest negative direct effect on grain yield followed by leaf damage score 2. Number of plants had negative direct effect on grain yield. Table 4.9 showed that ear height had 16% positive effect on yield via plant height. Ear height had 21% positive impact on yield via ear-plant ratio. Plant height had 15% negative effect on yield via ear-plant ratio. Plant height had 21% positive effect on yield via ears harvested per plant. Ear-plant ratio had 20% negative effect on yield via ear height. Ear-plant ratio had 18% negative effect on yield via plant height. Leaf damage score 1 had 18% negative effect on yield via ears harvested per plant. Leaf damage score 1 had 20% negative effect on yield via leaf damage score 2. Leaf damage score 2 had 22% negative effect on yield via ears harvested per plant. Leaf area damage percentage had 23% negative effect on yield via ears harvested per plant. Leaf area damage percentage had 24% negative effect on yield via leaf damage score 1.

The regression data for Makhathini is presented in Table 4.13. The R squared value indicated that the regression model accounted for 42% of the direct effects. The regression of, ears harvested per plant on grain yield was highly significant (P<0.001). The regression of leaf damage score 2 and leaf area damage percentage on grain yield was significant (P<0.05).

Table 4.11 Correlation between grain yield and secondary traits for single cross experimental hybrids at Makhathini Research Station

Traits	Grain Yield	Ear Height	Ears Harvested per Plant	Ear Plant Ratio	Leaf Area Damage %	Leaf Damage score 1	Leaf Damage score 2	Number of Plants	Plant Height
Grain Yield									
Ear Height	0.35***								
Ears Harvested per Plant	0.80***	0.33***							
Ear Plant Ratio	-0.11***	0.50***	-0.04						
Leaf Area Damage %	-0.40***	-0.43***	-0.41***	-0.18***					
Leaf Damage score 1	-0.39***	-0.45***	-0.42***	-0.18***	0.82				
Leaf Damage score 2	-0.39***	-0.44***	-0.40***	-0.18***	0.99	0.83			
Number of Plants	-0.04	0.02	0.12***	0.06	0.02	-0.02	0.02		
Plant Height	0.51***	0.55***	0.41***	-0.39***	-0.30***	-0.33***	-0.30***	-0.04	

*, **, ***= level of significance at p≤0.05%, p≤0.01%, p≤0.001%

Table 4.12 Direct (diagonal in bold) and Indirect Effects (above and below diagonal) of secondary traits on grain yield of single cross experimental hybrids at Makhathini Research Station

Traits	NP	EH	PH	EPR	EPP	LD1	LD2	LAD%.	Total effects on GY	P value for total effects
Number of Plants (NP)	**-0.14643**	-0.01614	0.02179	0.003011	0.111411	-0.0061	0.012294	-0.00023	-0.02039	0
Ear Height (EH)	-0.00691	**-0.34199**	0.16043	0.212174	0.106784	-0.05955	0.084677	-0.00188	0.153726	0
Plant Height (PH)	-0.00756	-0.13005	**0.421879**	-0.15719	0.217916	-0.04767	0.067115	-0.00146	0.362979	0
Ear Plant Ratio (EPR)	-0.00125	-0.20565	-0.18795	**0.352839**	-0.04335	-0.02282	0.029959	-0.00069	-0.07891	0
Ears per plant (EPP)	-0.02211	-0.0495	0.124616	-0.02073	**0.737741**	-0.03883	0.074175	-0.00174	0.803621	0
Leaf damage score 1 (LD1)	0.005877	0.134008	-0.13232	-0.05298	-0.18847	**0.151974**	-0.20884	0.004559	-0.28619	0
Leaf damage score 2(LD2)	0.007402	0.119063	-0.11641	-0.04346	-0.22499	0.130488	**-0.24322**	0.005396	-0.36573	0.013
Leaf Area Damage %. (LAD%)	0.006081	0.118768	-0.11345	-0.04464	-0.23626	0.127669	-0.24181	**0.005427**	-0.37822	0.954

Residual Effect = 0.288022

Table 4.13 The regression of secondary traits on grain yield for single cross experimental hybrids at Makhathini Research Station

Parameter	Estimate	Std Error	t value	P value
Intercept	0.054403	0.907613	0.060	0.95226
Number of plants	0.005909	0.013409	0.441	0.65993
Ears harvested	-0.001573	0.006482	-0.243	0.80846
Plant height	0.005676	0.004098	1.385	0.16761
Ear plant ratio	1.684286	1.004798	1.676	0.09526
Ears harvested per plant	0.110490	0.011706	9.439	0.00***
Leaf damage score 1	0.059259	0.035509	1.669	0.09672
Leaf damage score 2	-0.642093	0.216489	-2.966	0.00339**
Leaf area damage%	0.054459	0.018930	2.877	0.00446**

, *= level of significance at, p≤0.01%, p≤0.001%

Adjusted R^2 =0.4234

4.7 Genetic gains assessment

4.7.1 Analysis of Variance across sites for Single Cross Hybrids

According to Table 4.14, site (Environment) was shown to be a highly significant (P<0.001) source of variation for grain yield, ear height and plant height. Number of plants was shown to be a highly significant (P<0.001) source of variation for grain yield, ear height and plant height. Entry (genotype) was shown to be a highly significant (P<0.001) source of variation for ear height and plant height. Site (Environment) by Entry (Genotype) was shown to be a significant source of variation for grain yield.

Table 4.14 ANOVA across three sites with number of plants as covariate for single cross experimental hybrids

Source of variation	Degrees of freedom	Grain Yield	Ear Height	Plant Height
Site	2	557.8***	44276***	100618***
Rep	1	80.3	5209	43875
Rep*block	1	4.5	269	5063
NP	1	186.3***	460733***	1267916***
Entry	185	2.3	617***	974***
Site*Entry	369	2.5*	254	505
Residuals	663	2.1	232	472
LSD		2.096	30.99	48.81
CV		43.19352	13.85249	10.62733
SE		0.433	6.53	10.3

LSD = least significance difference at 5%, CV = coefficient of variation *, ***= level of significance at p≤0.05%, p≤0.001%

4.7.2 Cultivar Superiority Across sites for Single Cross Hybrids

The genotypes were ranked according to their cultivar superiority index and mean yield across the three sites and the top 20 and the four conventional non-GM hybrid checks are presented in Table 4.15 .(HSX5054Bt) which was an experimental hybrid had the lowest cultivar superiority index of 2.7 and a mean yield of 4.5 t ha^{-1}. The small value for cultivar superiority index indicates that the hybrid combines high yield potential with dynamic stability. It was 6% above mean of conventional checks, 32% above mean of WEMA GM checks and 37% above mean of local GM hybrid checks.

Among the conventional non-GM hybrids, SC403 had a superiority index of 13.5 and yield of 1.9 t/ha. It had negative 54% above mean of conventional hybrids, negative 43% above mean of WEMA GM checks and negative 41% above mean of local GM hybrid checks.

Table 4.15 Cultivar Superiority Analysis across three sites for single cross experimental hybrids (data ranked by CSI)

ENT	Genotypes	Pedigree	Rank	Grain Yield	Cultivar Superiority Index (CSI)	% above mean of conventional checks	% above mean of WEMA checks	% above mean of Local GM checks
26	HSX5054Bt	Proprietary information	**1**	4.5	2.7	6%	32%	37%
93	HSX5180Bt	Proprietary information	**2**	3.8	3.1	-11%	11%	15%
199	DKC75-65BR	Proprietary information	**3**	1.6	3.3	-63%	-54%	-52%
95	HSX5183Bt	Proprietary information	**4**	3.7	3.7	-14%	8%	12%
146	HSX5301Bt	Proprietary information	**5**	2.9	4.0	-32%	-16%	-12%
139	HSX5287Bt	Proprietary information	**6**	2.8	4.2	-35%	-19%	-16%
149	HSX5305Bt	Proprietary information	**7**	2.3	4.3	-46%	-32%	-30%
188	HSX5409Bt	Proprietary information	**8**	2.2	4.4	-49%	-36%	-34%
94	HSX5181Bt	Proprietary information	**9**	3.1	4.4	-27%	-10%	-6%
144	HSX5298Bt	Proprietary information	**10**	2.3	4.6	-45%	-32%	-29%
116	HSX5248Bt	Proprietary information	**11**	3.3	4.7	-22%	-3%	1%
138	HSX5286Bt	Proprietary information	**12**	3.1	4.8	-26%	-8%	-5%
165	HSX5340Bt	Proprietary information	**13**	2.7	4.8	-36%	-21%	-18%
6	HSX5002Bt	Proprietary information	**14**	5.3	4.9	24%	55%	61%
92	HSX5179Bt	Proprietary information	**15**	3.2	4.9	-24%	-6%	-2%
145	HSX5299Bt	Proprietary information	**16**	3.1	5.1	-28%	-10%	-6%
91	HSX5178Bt	Proprietary information	**17**	3.3	5.1	-23%	-4%	0%
32	HSX5062Bt	Proprietary information	**18**	4.9	5.2	14%	42%	47%
110	HSX5239Bt	Proprietary information	**19**	2.9	5.2	-33%	-16%	-13%
130	HSX5278Bt	Proprietary information	**20**	3.2	5.3	-26%	-8%	-4%
	Conventional non-GM checks							
195	SC633		**137**	1.7	10	-61%	-52%	-50%
196	SC419		**196**	1.9	10.3	-56%	-46%	-44%
197	SC301		**144**	1.9	10.7	-54%	-43%	-41%
194	SC403		**153**	1.9	13.5	-54%	-43%	-41%

4.7.3 Cultivar superiority of single cross hybrids Makhathini under FAW infestation

This analysis for cultivar superiority was done for the experimental single cross hybrids at Makhathini under FAW pressure. The top 20 hybrids and the four conventional non-GM hybrid checks are presented in Table 4.16.

The genotypes were ranked according to their mean yield at Makhathini. According to Table 4.16, the most superior single cross experimental hybrid under FAW pressure was entry 175 (HSX5368Bt). It was 127% above mean of conventional non-GM hybrids, 100% above mean of WEMA GM hybrid checks and 99% above mean of local GM hybrids.

The new experimental hybrids in the top 20 outperformed all the GM hybrid checks used in this study. The best ranked GM hybrid check (33H54BR) was ranked number 29 with a grain yield of 3.3 t ha^{-1}and the lowest ranked GM hybrid check was (PAN5R-891BR) with a yield of 1.3 t ha^{-1}.

The conventional non-GM hybrids performed poorly under FAW pressure. SC419 had negative 112% above mean of conventional Non-GM hybrids, negative 110% above mean of WEMA GM hybrid checks and negative 110% above mean of local GM hybrids.

Table 4.16 Cultivar Superiority Analysis for Single Cross hybrids Makhathini

ENTRY NO	Genotypes	Pedigrees	GY	Rank	% above mean of conventional checks	% above mean of WEMA checks	% above mean of Local GM checks
175	HSX5368Bt	Proprietary information	5.0	**1**	127%	100%	99%
107	HSX5232Bt	Proprietary information	4.4	**2**	101%	76%	75%
99	HSX5202Bt	Proprietary information	4.3	**3**	96%	72%	71%
105	HSX5227Bt	Proprietary information	4.3	**4**	95%	71%	70%
191	HSX5419Bt	Proprietary information	4.2	**5**	91%	68%	67%
112	HSX5244Bt	Proprietary information	3.9	**6**	79%	57%	56%
185	HSX5404Bt	Proprietary information	3.9	**7**	79%	57%	56%
193	HSX5426Bt	Proprietary information	3.9	**8**	78%	57%	56%
171	HSX5350Bt	Proprietary information	3.9	**9**	77%	56%	55%
140	HSX5288Bt	Proprietary information	3.9	**10**	75%	54%	53%
130	HSX5278Bt	Proprietary information	3.9	**11**	75%	54%	53%
80	HSX5156Bt	Proprietary information	3.7	**12**	70%	50%	49%
91	HSX5178Bt	Proprietary information	3.7	**13**	66%	46%	45%
192	HSX5421Bt	Proprietary information	3.5	**14**	61%	41%	41%
119	HSX5259Bt	Proprietary information	3.5	**15**	60%	41%	40%
10	HSX5019Bt	Proprietary information	3.5	**16**	59%	40%	39%
116	HSX5248Bt	Proprietary information	3.5	**17**	57%	38%	37%
1	HSX4989Bt	Proprietary information	3.4	**18**	55%	36%	36%
109	HSX5237Bt	Proprietary information	3.4	**19**	54%	35%	35%
110	HSX5239Bt	Proprietary information	3.4	**20**	54%	35%	34%
Conventional non-GM check hybrids							
197	SC403		0.8	**190**	-65%	-70%	-70%
195	SC633		0.3	**198**	-85%	-87%	-87%
196	SC301		-0.2	**206**	-108%	-107%	-107%
194	SC419		-0.3	**207**	-112%	-110%	-110%
GM hybrid checks							
205	33H54BR		3.0	**29**	38%	22%	21%
207	P2553WBR		3.3	**31**	49%	31%	30%
202	WE6208B		3.0	**44**	35%	18%	18%
208	P1690BR		2.8	**47**	25%	10%	10%
199	DKC75-65BR		3.0	**54**	35%	19%	18%
201	WE6210B		2.9	**55**	31%	16%	15%
204	LS8541BR		2.8	**61**	28%	12%	12%
200	WE6207B		2.4	**72**	8%	-5%	-6%
206	P1184BR		2.4	**73**	11%	-3%	-3%
203	DKC68-58BR		2.0	**132**	-9%	-20%	-21%
157	PAN5R-891BR		1.3	**157**	-42%	-49%	-49%

Negative grain yield was due to adjustments to the data for experimental error

4.7.4 Performance of single cross experimental hybrids under disease pressure at Potchefstroom

4.7.4.1 Analysis of variance for Single Cross Hybrids at Potchefstroom

Entry (Genotype) was shown to be a highly significant (p<0.001) source of variation for maize streak virus, phaeosphaeria leaf spot, rust and turcicum.

Table 4.17 ANOVA for single cross hybrids without NP as covariate for diseases at Potchefstroom Research Station

Source of variation	Df	Grey Leafspot	Maize streak virus	Phaeosphaeria leaf spot	Rust	Turcicum
Rep	1	5.1421	1.5024	0.11476	96.0409	17.779
Rep*block	14	2.2093	0.8252	0.2894	3.078	5.68
Entry	185	0.6342	0.489***	0.11132***	0.7876***	2.177***
Residual	212	0.4521	0.2696	0.08499	0.7354	1.158
LSD		1.314	1.0091	0.5681	1.667	2.091
CV		41.02	41.3	26.53	29.07	39.19
SE		0.6724	0.5192	1.430.2915	0.85753	1.076

LSD = least significance difference at 5%, CV = coefficient of variation *, **, ***= level of significance at p≤0.05%, p≤0.01%, p≤0.001%

4.7.4.2 Genetic Gains Assessment for Single Cross Hybrids at under disease pressure at Potchefstroom

The assessment for mean performance of the experimental hybrids was only done for the single cross hybrids at Potchefstroom due to logistical issues.

4.7.4.2 Mean Performance of Single Cross Experimental Hybrids at Potchefstroom

This analysis of mean performance was done for the experimental single cross hybrids at Potchefstroom under disease pressure. The genotypes were ranked according to their yield. At Potchefstroom, according to Table 4.18 the highest yielding single cross hybrid was an experimental single cross hybrid entry 156 (HSX5318Bt) with a yield of 7 t ha^{-1}. This new experimental hybrid showed impressive yield potential under disease pressure environment. The top 20 hybrids are presented in Table 4.18.

Table 4.18 Mean Performance of Single Cross Experimental Hybrids at Potchefstroom

ENTRY _NO	Genotypes	PEDIGREE	Grain Yield	Rank	RUST	Turcicum	Grey Leaf Spot	Phaeosphaeria Leaf Spot
156	HSX5318Bt	Proprietary information	6.8	**1**	1.5	2.9	3.4	4.4
81	HSX5157Bt	Proprietary information	6.6	**2**	1.0	1.5	2.5	2.0
206	P1184BR		6.4	**3**	1.1	1.1	1.9	2.2
202	WE6208B		6.5	**4**	1.0	1.2	2.1	2.1
148	HSX5304Bt	Proprietary information	6.6	**5**	1.4	2.7	3.6	4.1
19	HSX5041Bt	Proprietary information	7.0	**6**	1.0	2.5	3.2	4.3
197	SC403		6.6	**7**	1.0	1.0	3.6	2.5
152	HSX5310Bt	Proprietary information	5.9	**8**	1.0	1.0	1.6	2.3
17	HSX5037Bt	Proprietary information	6.2	**9**	1.0	1.0	3.5	1.0
108	HSX5234Bt	Proprietary information	5.9	**10**	1.0	1.5	2.6	2.4
150	HSX5307Bt	Proprietary information	6.3	**11**	1.1	1.1	2.4	1.2
47	HSX5088Bt	Proprietary information	6.0	**12**	1.0	1.2	3.3	1.7
183	HSX5401Bt	Proprietary information	6.1	**13**	2.4	2.4	3.7	3.9
177	HSX5371Bt	Proprietary information	5.9	**14**	1.1	2.0	3.2	3.6
157	HSX5320Bt	Proprietary information	6.4	**15**	1.0	1.6	2.0	2.4
120	HSX5260Bt	Proprietary information	6.1	**16**	1.0	1.6	4.5	2.4
137	HSX5285Bt	Proprietary information	5.6	**17**	1.0	1.3	3.0	1.3
70	HSX5131Bt	Proprietary information	6.0	**18**	1.0	1.3	3.0	2.8
92	HSX5179Bt	Proprietary information	5.7	**19**	1.0	0.9	3.0	2.3
133	HSX5281Bt	Proprietary information	6.0	**20**	1.0	0.9	2.5	2.9
22	HSX5046Bt	Proprietary information	3.0	**204**	1.4	1.2	3.1	2.6
194	SC419		2.4	**205**	1.0	1.6	2.6	3.1
90	HSX5177Bt	Proprietary information	2.7	**206**	1.0	1.0	1.6	1.9
185	HSX5404Bt	Proprietary information	2.5	**207**	1.0	2.0	2.9	4.0
73	HSX5137Bt	Proprietary information	2.1	**208**	1.0	2.6	3.9	4.1
	Mean		4.5		1.1	1.6	2.9	2.7
	CV		29.6		26.3	38.8	29.3	37.3
	Heritability		0.0		0.2	0.4	0.0	0.5
	Min		1.0		1.0	1.0	1.0	1.0
	Max		9.0		3.0	4.0	6.0	7.0
	Mean LSD		2.7		0.6	1.3	1.7	2.1
	P-value		0.5		0.0	0.0	0.3	0.0

4.7.4.3 Cultivar Superiority of Single Cross Experimental Hybrids at Potchefstroom

This analysis for cultivar superiority was done for the experimental single cross hybrids at Potchefstroom under disease pressure. The genotypes were ranked according to their yield. According to Table 4.19, the most superior single cross experimental hybrid under disease pressure was (HSX5041Bt). It had 131% above mean of conventional checks, 158% above mean of WEMA checks and 174% above mean of local GM hybrid checks. SC403 performed very well with a 117% above mean of conventional Non-GM checks, 143% above mean of WEMA GM checks and 159% above mean of local GM hybrid checks. The worst performing hybrid was (HSX5137Bt). It had negative 32% above mean of conventional checks, negative 24% above mean of WEMA GM checks and negative 19% above mean of local GM checks.

The top 20 hybrids and the four conventional non-GM hybrid checks are presented in Table 4.19.

Table 4.19 Cultivar Superiority Analysis for Single Cross hybrids at Potchefstroom

ENTRY _NO	Genotypes	PEDIGREE	Grain Yield	Rank	% above mean of conventional checks	% above mean of WEMA checks	% above mean of Local GM checks
19	HSX5041Bt	Proprietary information	7.0	**1**	131%	158%	174%
156	HSX5318Bt	Proprietary information	6.8	**2**	123%	150%	166%
197	SC403		6.6	**3**	117%	143%	159%
148	HSX5304Bt	Proprietary information	6.6	**4**	117%	142%	158%
81	HSX5157Bt	Proprietary information	6.6	**5**	116%	141%	157%
202	WE6208B		6.5	**6**	113%	137%	153%
206	P1184BR		6.4	**7**	111%	136%	151%
157	HSX5320Bt	Proprietary information	6.4	**8**	108%	133%	148%
150	HSX5307Bt	Proprietary information	6.3	**9**	107%	131%	146%
17	HSX5037Bt	Proprietary information	6.2	**10**	102%	125%	140%
183	HSX5401Bt	Proprietary information	6.1	**11**	101%	124%	139%
120	HSX5260Bt	Proprietary information	6.1	**12**	100%	123%	138%
100	HSX5208Bt	Proprietary information	6.1	**13**	99%	123%	137%
91	HSX5178Bt	Proprietary information	6.0	**14**	96%	119%	133%
47	HSX5088Bt	Proprietary information	6.0	**15**	96%	119%	133%
70	HSX5131Bt	Proprietary information	6.0	**16**	96%	119%	133%
133	HSX5281Bt	Proprietary information	6.0	**17**	96%	118%	133%
161	HSX5331Bt	Proprietary information	6.0	**18**	96%	118%	133%
152	HSX5310Bt	Proprietary information	5.9	**19**	93%	116%	130%
108	HSX5234Bt	Proprietary information	5.9	**20**	93%	116%	130%
	Worst 5						
22	HSX5046Bt	Proprietary information	3.0	**204**	1%	5%	15%
194	SC419		2.4	**205**	-20%	-11%	-5%
90	HSX5177Bt	Proprietary information	2.7	**206**	-12%	-8%	0
185	HSX5404	Proprietary information	2.5	**207**	-19%	-9%	-3%
73	HSX5137Bt	Proprietary information	2.1	**208**	-32%	-24%	-19%

4.8 Conclusion

This chapter described the outcomes of this study and highlighted the patterns observed. Results revealed that FAW resistance was conferred by the Bt trait in the experimental hybrids as evidenced by little to zero feeding on the leaves, little to no damage on the cobs and significant high yields of the Bt experimental hybrids under FAW pressure. The marked difference in damage scores and grain yield between the Bt experimental hybrids and the conventional non-GM hybrid checks show that fall army pressure did discriminate between the two. The data on the effects of secondary traits on yield under FAW pressure were presented. The effects of secondary traits showed the traits that had direct and indirect effects on yield. Indicating that these traits can be focussed on for selection, for increased grain yield. The experimental hybrids which were superior for yield when compared to conventional non-GM checks, WEMA GM checks and local GM hybrids and under both disease and no disease pressure were identified. The discussion and implications of these findings for maize breeding and policy are presented in the next chapter.

CHAPTER FIVE

GENERAL DISCUSSION

5.1 Introduction

This chapter presents the discussion of the findings and interpretation of findings from the study. The experiment was divided into Experiment 1 which is three-way hybrid crosses and Experiment 2 which is the single cross hybrids. In each section, the discussion begins with an analysis of the efficacy of the Bt trait in conferring resistance to fall armyworm (FAW) in three-way and single cross hybrid design and its implications for future breeding programs. It also discusses the relationship between cob damage scores, leaf damage score and grain yield. This is followed by genetic gains and cultivar superiority analysis of the experimental hybrids. It is followed by analysis of the performance of the single cross experimental hybrids under disease pressure. A conclusion on the chapter is drawn.

5.2 Efficacy of the Bt trait in conferring resistance to fall armyworm

5.2.1 Analysis of variance for agronomic traits and fall armyworm damage parameters

The analysis of variance for three-way cross hybrids at Makhathini revealed that number of plants differed significantly ($P<0.001$) and affected discrimination of hybrids for grain yield and field weight. This indicates that FAW or other factors such as stand establishment could have affected the grain yield. Chimweta *et al*. (2020) found that under heavy fall armyworm infestation, cut stems in young maize plants due to fall armyworm feeding could result. This could affect plant stand. Number of plants also differed significantly for the number of ears harvested, leaf area damage percentage, cob damage score, cob damage percent, leaf damage score 1 and leaf damage score 2. Therefore, a covariance analysis was used with the number of plants per plot as the covariate.

Entry (Genotype) main effects were highly significant ($P<0.001$) for leaf area damage, cob damage score, cob damage percentage, leaf damage score 1, leaf damage score 2 and number of damaged cobs. This indicates that fall armyworm had a significant impact on the hybrids and also indicated the discriminating effect of the fall armyworm infestation. Entry (Genotype) main effects were significant ($P<0.05$) for cobs per plant and number of ears harvested. The presence of significant differences among the different genotypes could be attributed to the presence of discriminating capacity provided by the fall armyworm pressure at Makhathini.

The coefficient of variation (CV) illustrates the quality of the data especially reliability. A low coefficient of variation indicates high level of uniformity. In this study, the coefficient of variation for the fall armyworm traits were high, with 90.33% for leaf area damage percentage. High coefficient of variation values has been shown to be a result found under stress conditions, of which Makhathini with FAW pressure, was. This is corroborated by the findings of Mugo *et al.* (2011) under infestation by *Buseola fusca*. The high coefficient of variation for leaf area damage score and leaf damage score could indicate that feeding was not uniform among the genotypes. It was a natural infestation, so the FAW might not have been uniformly distributed for them to attack the field uniformly.

For the single hybrids experiment, number of plants provided a highly significant source of variation for number of ears harvested. This was similar to the results from the three-way cross hybrids experiment. Entry (Genotype) main effects were highly significant ($P<0.001$) for grain yield, plant height, ear height, ears harvested per plant, ear plant ratio, leaf damage score 1 and leaf area damage percentage. Entry (Genotype) was significant ($P<0.01$) for leaf damage score 2. The presence of significant differences among the genotypes could be attributed to the presence of discriminating capacity provided by FAW pressure at Makhathini. The adapted and high yielding genotypes were able to express their potential in this environment.

5.2.2 Mean performance of experimental hybrids at Makhathini

Under FAW pressure at Makhathini, in the three-way hybrid experiment, the top 20 hybrids in terms of yield were GM material. This means they out-yielded their non- GM counterparts under FAW infestation. This implies that the Bt trait was effective in controlling fall armyworm. The first and second high yielders were Bt experimental hybrids- (H3WX3194Bt) with 3.2 t ha^{-1}and (H3WX3198Bt) with a yield of 2.9 t ha^{-1}. Commercial GM hybrids checks (WE6210Bt) and (DKC-65Br) came third and fourth with 2.8 t ha^{-1}. This indicates that the new experimental hybrids were superior to the current commercial hybrids in South Africa, which is an indication of genetic gains by the programme. The Bt trait was highly effective for the control of FAW and revealed significant differences between the GM and non-GM hybrids. Conventional, non-GM hybrids such as SC403, SC301, SC633 and SC419 performed poorly in this environment with yields as low as 0t/ha, indicating that FAW pressure was high and the non- GM hybrids succumbed. The low yield of the conventional non-GM hybrids is a stark contrast to the high yields exhibited by the experimental hybrids. This implies that new experimental hybrids with high yield under FAW infestation could be advanced. The cobs of the conventional non-GM hybrids experienced up to 95% damage due to FAW, indicating that yield and grain quality will be compromised when conventional hybrids are grown under FAW infestation. As a result, farmers will not be able to get premium price when they sell their grain after it has been graded.

Cob damage is very important to the farmers as this affects both yields and quality of grain in a very clear manner that they can see easily. Under FAW pressure the quality of the cobs is greatly reduced. GM hybrids should be recommended to smallholder farmers who have limited access to chemical control. By availing to them seed that has resistance already built in will not only result in higher yields, but lower costs of production as they will not need to buy insecticides.

The study indicated that significant gains were achieved over non-GM commercial checks, indicating that the Bt MON89034 was effective at controlling the FAW under natural conditions, at the Makhathini hotspot, in KwaZulu-Natal, South Africa. The high performance shown by the Bt experimental hybrids in comparison to the local checks, especially non-GM material, means that in areas under fall army worm infestation, they can be recommended in place of conventional non-GM hybrids with an expected increase in productivity. The identified entries can be selected for possible further development toward FAW resistance. Experimental hybrids (H3WX3197Bt) and (HRWX3198Bt) were highly desirable in terms of grain yield and low scores for FAW parameters, indicating that they combined high level of FAW resistance with high yield potential. These hybrids could be advanced in the breeding programme and work towards their release in tropical Africa, in areas of high FAW infestation.

In the single cross hybrids experiment, all the top twenty hybrids were Bt experimental single cross hybrids. This means that the new experimental hybrids outperformed both the GM hybrid checks as well as the conventional non-GM hybrids. The new experimental hybrids' outperformance is also what was found under Experiment 1 for the three-way cross hybrids. The major difference is that only experimental hybrids are in the top twenty in experiment 2. This means that the new experimental hybrids were superior to the current commercial GM and non-GM hybrids on the market in South Africa. The highest yielding experimental hybrid was (HSX5368Bt) with 4.9 t ha-^{1}. This Bt experimental hybrid experienced very little feeding on the leaves. This indicated that the Bt trait was highly effective for the control of FAW and revealed significant differences between the new experimental hybrids and the GM and non-GM conventional hybrids checks. Even though the experimental hybrid (HSX5227Bt) had a lower yield than the highest yielding experimental hybrid, with a yield of 4.1 t ha-^{1}, it was a very clean crop. It was free from FAW feeding. This shows that at times resistance can occur in a low yielding genetic background. The conventional non-GM hybrids such as SC419 and SC301 had 0t t ha-1 yield, showing that they were adversely affected by the presence of FAW pressure. They experienced high levels of feeding on the leaves as shown by high leaf damage score and a high incidence of feeding occurring. This implies that at Makhathini, FAW pressure was high and the non-GM conventional hybrids succumbed. This shows that conventional non-GM hybrids are not well suited for use under FAW pressure.

Significant gains were achieved over non-GM commercial checks as well as GM checks. The high performance shown by the Bt experimental single cross hybrids compared to both the GM checks and conventional non-GM hybrids means that in areas affected by FAW, they can be recommended in place of conventional non -GM hybrids with expected increase in productivity. The identified entries can be selected for possible further development towards breeding for FAW resistance. Experimental hybrids (HSX5368Bt) and (HSX5232Bt) were highly desirable in terms of grain yield and low scores for FAW parameters. These hybrids could be released in tropical Africa.

5.3 Impact of secondary traits on grain yield under fall armyworm infestation

5.3.1. Correlation Coefficient Analysis

For the three-way cross hybrids, the presence of correlations between grain yield and some of the secondary traits shows that those traits can be exploited in yield improvement, under FAW infestation. Any improvement in these characters with positive correlation with yield, simultaneously might lead to an increase in yield. Grain yield and ears per plant (ear prolificacy) was shown to be very important as evidenced by a strong positive correlation of 83%. This is corroborated by findings by Musundire *et al.* (2019). Number of ears harvested had a strong positive correlation with grain yield of 89%. This indicated that a hybrid that had a high number of ears harvested would be ideal for increasing grain yield. This implies that yield can be improved by selecting for ears harvested. This agrees with findings from Jatto *et al.* (2015) and Adu *et al*. (2016) in their work on correlation among yield and yield components in maize. Therefore, prioritizing selection for ear prolificacy and ears harvested can most likely result in a marked increase in grain yield.

A negative correlation was found between grain yield and cob damage percentage, leaf damage score 1, leaf damage score 2 and leaf area damage percentage. This finding was found for both the three-way and single cross hybrids. This shows an inverse relationship between grain yield and these traits. As leaf damage and incidence of feeding increased, yield decreased. This indicated the role of insect feeding on leaf in reducing grain yield. This corroborates the findings of Kasoma *et al*. (2020 a) and Kumela *et al.* (2018). This showed the negative impact that feeding on both leaf and cob has in reducing grain yield. This negative correlation indicates that damage by the FAW was partly responsible for the variation of hybrids for grain yield under the FAW infestation. The negative correlation between grain yield and leaf and cob damage suggested that it is possible to identify high yielding genotypes by considering genotypes with low scores for FAW related traits. The results underline that effective control of FAW would be obtained by minimising leaf and cob damage, and that this can be achieved by a combination of chemical control with host plant resistance.

Leaf damage can potentially reduce grain yield by affecting area available for photosynthesis. Baudron *et al.* (2019) found that there would be need for the whole whorl to be destroyed for leaf damage to have a significant impact on yield, therefore leaf damage alone cannot be used as a predictor for grain yield loss. There is a need to further investigate the relationship between leaf damage and grain yield. Kasoma *et al*. (2020 b) highlighted that cob damage represents a more direct indicator of the impact of FAW on grain yield. However, cob damage can only be measured at the end of the growing season, when it is too late to implement mitigation strategies. Damaged cobs are also prone to be infected by fungi which produce harmful mycotoxins that are dangerous to humans as well as livestock, thereby reducing the quality of the grain (Miller et al., 2003). The current study looked at both the direct and indirect effects of these traits on the yield of maize hybrids under the FAW infestation. This is discussed in the next section.

The presence of correlations between grain yield and some of the secondary traits shows that those secondary traits can be exploited to increase yield in breeding. This is because if the traits that are positively correlated to grain yield are expressed highly in the genotypes, then yield can consequently be improved. The correlation between grain yield and ears harvested is very important as shown by the value of 80% for correlation. Under Experiment 1, with the three-way hybrids, ears harvested was also found to be very important for grain yield. This means that selecting for hybrids that have high number for ears harvested can increase grain yield. Grain yield and plant height had a positive 51% correlation. This finding is corroborated by Sesay et al. (2017) and Devasree (2020) who in their studies also found positive correlations between grain yield and plant height. Ear height and grain yield had 35% correlation. This corroborates the findings by Musundire et al. (2019). Ears harvested and plant height can be selected for to increase yield.

5.3.2 Path coefficient analysis

The study shows that the secondary traits had a significant direct and indirect impact on the grain yield of hybrids under the FAW infestation hence they would not be ignored during selection of hybrids for advancement in the breeding programme. This study categorizes path coefficient values suggested by Lenka and Mishra (1973) were used as: negligible 0.00-0.09, low 0.01-0.19, moderate 0.20-0.29 and high 0.30-0.99. The high direct and indirect effects of number of ears harvested on the grain yield of three-way cross hybrids, this is corroborated by the findings of Adu *et al*. (2016), This indicates that this was the most important trait for improvement of grain yield of the hybrids. Number of ears harvested had 83% direct effect on yield, indicating that has a high impact on grain yield performance of the hybrids under the FAW infestation and should therefore be given high priority during selection. This is because. the greater the number of ears harvested, the higher the yield. Moderate direct effects came from number of damaged cobs at 20%, indicating that damage to the cobs caused a decrease in yield. This is corroborated by Kasoma *et al*. (2020 b) if the number of cobs that are damaged are high, yield goes down. Therefore, selecting for cobs with low cob damage scores could lead to increase in yield. Cob damage is a very important trait to the farmer as it affects the yield in a manner that the farmers understands and also impact on the quality of grain. Decrease in cob quality is because damaged cobs are prone to infection by fungi, which produces mycotoxins which have harmful effects on both humans and livestock (Miller *et al*., 2003). Therefore, improving this trait is something the farmer will greatly appreciate.

The analysis suggests that indirect selection for grain yield is possible through selection for ear prolificacy (number of ears per plant). The number of ears per plant had the highest positive indirect effect on yield via number of ears harvested of 63%. Musundire *et al*. (2020) and Adu *et al*. (2016) also found the same findings. High indirect effects on grain yield was from number of plants via number of ears harvested of 32%. This means that by selecting for number of ears harvested, then ears per plant and number of plants would also be selected for simultaneously. Leaf damage score 1, leaf damage score 2 and leaf area damage percentage had negative, moderate indirect effects of 22% on grain yield via number of ears harvested. When leaf damage occurs this reduces photosynthetic area, which in turn reduces the yield. So, in order of importance, breeders would pay attention to ear prolificacy, cob damage score and number of ears harvested.

Under the single cross hybrid study, the high direct and indirect effects of ear prolificacy (ears harvested per plant) of 73.7% indicated that it was the most important trait for grain yield improvement. This is corroborated by Adu *et al*. (2019). Plant height came second after ears harvested per plant with high direct effects on yield of 42%. These results are similar to those found under experiment 1. Plant height was found to be important for grain yield by Munawar *et al*. (2018) and Pavan (2011).

The high negative direct effects of ear height on grain yield were the highest of 34%. Munawar *et al*. (2018) also found similar findings. This indicated that ear placement is an important trait to select for. This is because as the height of the cob on the plant increased so did its negative impact on grain yield. This is because higher placement of the ear results in lower rate of pollination as well as delayed grain filling due to the lengthened vegetative stage as the plant grows taller. Leaf damage score 2 had the moderate negative direct effects on yield. This implied that increased leaf damage led to lower grain yield. Hence selecting for genotypes that exhibit lower ear placement and low leaf damage scores can increase yield.

Moderate positive indirect effects on grain yield came through ear height via ear-plant ratio and plant height had positive moderate indirect effects via ear prolificacy. By selecting for ear height and ear prolificacy, ear-plant ratio and plant height will be selected for. Moderate negative indirect effects on grain yield came through leaf area damage percentage via leaf damage score 2 of 24%, moderate negative indirect effects came through leaf area damage via ear prolificacy, leaf damage score 2 had moderate indirect effects via ear prolificacy, leaf damage score 1 had low indirect effects via ear prolificacy and ear-plant ratio had moderate indirect effects via plant height and plant height had low indirect effects via ear height. Indirect selection for grain yield is possible through selection for ear prolificacy, ear height and plant height.

5.3.3 Regression Analysis

Regression analysis helps the breeder to determine which correlations between traits are of significant importance. The regression analysis showed that number of ears harvested, were significantly important in affecting grain yield. Musundire *et al*. (2019) found significant regression coefficient for ear prolificacy. The significance of the regression analysis showed that their relationship with grain yield was linear in nature, therefore, the regression analysis was consistent with the path coefficient analysis findings.

The regression analysis for the single cross hybrids showed that ears per plant (ear prolificacy) were highly significant. Leaf damage score 2 and leaf area were also significant in affecting grain yield. Musundire *et al*. (2019) found significant regression coefficients for plant height and ear prolificacy. The significance of the regression analysis for these traits showed that their relationship with grain yield was linear in nature, therefore, the regression analysis was consistent with the path coefficient analysis findings.

5.4 Genetic Gains Assessment

5.4.1. Analysis of variance across sites for the experimental hybrids

The analysis of variance for both the three-way cross hybrids and single cross hybrids across the three sites (Makhathini, Dundee and Potchefstroom) revealed that site (Environment) main effects were highly significant ($P<0.001$) for grain yield. This means that yield of hybrids for grain yield differed greatly across the three sites. The wide range of grain yield shows that the genotypes reacted differently across the three sites, hence it would be difficult to select hybrids without paying attention to the site or the environment that the site represents. There was no FAW at Potchefstroom and Dundee. The sites also differed in altitude, from lowland at Makhathini to mid altitude environments at Dundee and Potchefstroom. Number of plants were shown to have revealed highly significant ($P<0.001$) differences among the three-way and single cross hybrids for grain yield showing that across the three sites, the number of plants varied greatly. This could be attributed to the effect of FAW pressure at Makhathini, where some plants could have been damaged by FAW or other factors such as vermin at the seedling stage. The analysis of variance showed that entry (Genotype) was not significant for grain yield across the three sites as well as no site by entry (Genotype*Environment) interaction was present. This revealed the absence of substantial amount of variation among the hybrids evaluated or genetic effects were masked by the experimental error. This was exhibited by high CVs and low heritability estimates, especially at Dundee. Therefore, a nonparametric test such as the Cultivar Superiority Index was used to discriminate the hybrids according to grain yield across sites. The results of the Cultivar Superiority index are discussed in the next section.

Genotype main effects were not significant for grain yield. This means that there was no variation among the genotypes for grain yield. This was a different result for the three-way hybrids in Experiment 1. Site by entry (GEI) were significant ($P<0.05$) for grain yield. The three environments used in this study were different from each other. There was no FAW infestation at Dundee and Potchefstroom and also there was a difference in altitude. The significant genotype by environment interaction shows that the genotypes showed variability across the different environments because the environment influenced the expression of the genotype. Significance of site by entry interaction for grain yield revealed that genotypes interacted significantly with environments indicating that macro environmental differences were present under all three environments studied. Significant differences in environments and site by entry interaction was also found by Arunkumar *et al*. (2020). The presence of highly significant Genotype by environment interaction for grain yield confirmed the need for extensive testing of the new hybrids in multiple environments over years before making cultivar recommendations (Badu-Apraku *et al*., 2012).

5.4.2 Cultivar superiority across sites for three-way cross hybrids.

The genotypes were ranked according to their cultivar superiority index and mean yield across the three sites. This was effective for discriminating the hybrids according to performance and enable identification of superior hybrids that combined high yield with stability. The hybrid check (DKC75-65Br) had a cultivar superiority of 0.5 but did not have the highest mean yield. This shows that it was highly stable but not high yielding. This means that it would least disappoint the farmers and it is suitable for the low input environments where farmers are risk averse. However, the ideal hybrid must combine high yield potential with high level of stability. In contrast the experimental hybrid, (H3WX3167Bt), an experimental Bt hybrid had an index of 0.5 and the highest mean yield of 4.6t ha^{-1}. It was 64% above mean of WEMA GM checks, 33% above mean of local GM hybrid checks and 22% above mean of conventional non-GM hybrids. This means that it was both stable and high yielding which is the expectation of every farmer. It showed superiority over the current hybrids on the market in South Africa and could be recommended for advancement. Overall, across sites, only three entries in the top 20 were not part of the Bt experimental three-way cross hybrids. The Bt experimental hybrids were FAW resistant, showing that the Bt trait was exhibited in a high yielding background, which is the ideal situation for the farmers in tropical Africa. This means that these experimental hybrids have potential to be recommended for environments represented by the three sites with expected high productivity. They would be advanced in the programme.

(DKC65-Br) which had high superiority index among the three-way cross experimental hybrids came third place among the single cross experimental hybrids. in the single cross hybrids experiment, ranked first was an experimental hybrid (HSX5054Bt). It had superiority index of 2.7 and 4.5 t ha^{-1}grain yield. This means that this experimental hybrid showed stability and high yield. It was 37% above mean of local GMO checks, 32% above mean of WEMA checks and 6% above mean of conventional non-GM hybrids. This indicates that it was superior to the GM hybrids and conventional non-GM hybrids on the South African market and it can be recommended for advancement. Amongst the top twenty hybrids only one was not an experimental hybrid. The rest were the Bt experimental hybrids. This means that these experimental hybrids have potential to be recommended for environments represented by the three sites with expected high productivity.

5.4.3 Cultivar Superiority of three-way hybrids under fall armyworm pressure

Makhathini provided fall armyworm infestation, and the genotypes were ranked according to their mean yield. The hybrid ranked first in this environment was an experimental hybrid (H3WX3194Bt). It had a yield of 3.2 t ha^{-1}adjusted grain yield, with 147% above mean of conventional checks, 85% above mean of local GMO checks and 47% above mean of WEMA checks. The second highest yielding was also an experimental hybrid, (H3WX3198Bt). Both these experimental hybrids performed better than the WEMA check (WE6210Bt) which came third. This means that they were both superior to the GM and non -GM hybrids presently on the South African market. Among the top 20 only 5 were not experimental hybrids. This showed the superiority of the experimental hybrids. The Bt trait was effective in conferring resistance as the experimental hybrids had high yields under FAW infestation. The conventional hybrids did poorly in this environment. Three of them had 0 t ha^{-1}yield. This means that Makhathini had sufficient FAW pressure and the susceptible hybrids failed to be productive in this environment. This means that these experimental hybrids have potential to be recommended for environments under FAW pressure and increase productivity for farmers as they showed superiority under FAW pressure.

Under the single cross hybrids experiment, within the top 20, only experimental hybrids were found. This shows that the experimental hybrids were superior to local GM hybrids and non-GM hybrids that are on the market in South Africa. In Experiment 1, not all the experimental hybrids were in the top 20, however in Experiment 2, all the best performers were experimental single cross hybrids. The hybrid ranked first in this environment was (HSX5368Bt) with a yield of 4.9 t ha^{-1}. It was 127% above mean of conventional non-GM checks, 100% above mean of WEMA checks and 99% above mean of local GMO checks. This shows this experimental hybrid to have been superior to all the GM hybrids checks as well as the conventional non-GM hybrid checks. This hybrid can be put forward for advancement in further breeding programmes to provide tropical Africa with high yielding superior hybrids with FAW resistance. The experimental hybrids managed to produce high yields while the conventional non-GM hybrids suffered the adverse effects of FAW pressure. This was reflected in their very low yield, even as low as 0t/ha. The environment at Makhathini had FAW pressure that impacted conventional non-GM hybrids. It is evident that non-GM hybrids are not the best option for farmers under FAW threat as they risk having no harvest. The high performance shown by the experimental hybrids means they can be recommended for use in environments under FAW infestation as they showed superiority under FAW pressure.

5.5 Performance of single cross hybrids under disease pressure at Potchefstroom

5.5.1 Analysis of variance for single cross hybrids at Potchefstroom.

The analysis of variance showed that Entry (Genotypes) was highly significant ($P<0.001$) for maize streak virus, phaeosphaeria leaf spot, rust and turcicum leaf blight but not significant for grey leaf spot. This means that genotypes differed greatly among themselves except for grey leaf spot. This result was different from the findings by Wegary *et al.*, (2008) who found that the maize genotypes were indicating inherent differences for resistance to grey leaf spot. However, the variability in the genotypes for maize streak virus, phaeosphaeria leaf spot, rust and turcicum leaf blight can be further exploited in breeding for hybrids that do well under FAW as well as disease pressure.

5.5.2 Mean performance of single cross experimental hybrids at Potchefstroom

The hybrids were ranked according to their mean yields. The best performing experimental single cross hybrid in this environment was (HSX5318Bt), with a grain yield of 7.0 t ha^{-1}. In second place was another experimental hybrid (HSX5157Bt), with a yield of 6.8 t ha^{-1}. Its disease scores were low, with rust at 1.5, turcicum 1.5, Grey leaf spot 2.5 and phaeosphaeria leaf spot 2.0. It showed the ability to yield high under this environment. It was not susceptible to diseases and showed high yield. This highly desirable to farmers. Two conventional non-GM hybrids were in the top 20, meaning that in a similar environment, farmers can use them, even though they are not as high yielding as the experimental hybrids. The rest in the top 20 were Bt experimental hybrids. The high performance of these experimental hybrids shows that they can also be used in further research in breeding programmes and they be recommended in similar environments to Potchefstroom and yield quite productively.

5.5.3 Cultivar Superiority Analysis for Single Cross hybrids at Potchefstroom

The genotypes were ranked according to their yield for their performance under disease pressure. The highest yielding hybrid was an experimental hybrid (HSX5041Bt), with a yield of 7.0t/ha, 174% above mean of local GMO checks, 158% above mean of WEMA checks and 131% above mean of conventional non-GM checks. It showed superiority over the GM and conventional non-GM hybrids in this environment as it was high yielding. SC403 which is a conventional non-GM hybrid came third, this means that farmers can still make use of this hybrid, even though it is not as high yielding as the experimental hybrids. Out of the top 20 only three were not Bt experimental hybrids. This points to the genetic gains in this programme. Not only are the experimental hybrids effective under FAW infestation, but they also perform very well under disease prevalence. The high performance by the experimental hybrids shows that they can also be used in further research in breeding programmes and they be recommended in similar environments to Potchefstroom and yield quite productively.

5.6 Conclusion

This chapter discussed the outcomes observed in the study and correlated them with findings from previous studies on the subject. The study revealed that there was sufficient FAW pressure at Makhathini as the susceptible genotypes performed poorly under FAW infestation. This shows that there was discrimination between susceptible and resistant genotypes. The study revealed that the Bt trait was effective in conferring resistance when deployed in three-way cross and single cross hybrids. This is evidenced by the high yields and low desirable scores for fall armyworm parameters exhibited by the new experimental three-way and single cross hybrids. The study revealed that in both Experiments 1 and 2 the new experimental hybrids exhibited superiority over the GM hybrids and non-GM conventional hybrids through higher yields. This shows that there were genetic gains in this study. The impact of secondary traits on grain yield under fall armyworm infestation revealed ears harvested as the most important trait to select for to increase yield in Experiment 1 and ear prolificacy as the most important trait to select for in Experiment 2 to increase yield. The overall conclusions for the study, implications and recommendations for future breeding programs are drawn in the next chapter.

CHAPTER SIX

CONCLUSION, IMPLICATIONS AND RECOMMENDATIONS

6.1 Introduction

Previous chapters have highlighted the economic importance of fall armyworm (FAW) damage on maize yield in maize hybrids (Chapters 2, 3, 4 and 5). Conversion of tropical maize hybrids to transgenic hybrids by integrating the “MON89034” event was pursued to confer resistance to FAW. This led to the formation of Bt three-way and Bt single cross experimental maize hybrids that were evaluated in three environments. The objective of this chapter is to provide an overview of the outcomes from the study. This includes highlighting the major objectives and findings from the literature review (Chapter 2) and the completed research (Chapters 3-5) and challenges of the study for breeding. Conclusions are drawn and the recommendations for future studies have been laid.

6.2 Summary of objectives and research approach

The main objective of this study was to investigate whether or not the Bt trait could be successfully integrated in high yielding tropical hybrids and there-by confer resistance to fall armyworm when deployed both in a three-way and single cross hybrid design. The questions which this study aimed to answer were: How effective is the Bt trait (MON89034) in conferring resistance to FAW when deployed in a three-way and single cross hybrid design? Secondly, what is the yield gain of the three-way and single cross Bt hybrids relative to non-GM or conventional non-GM maize hybrids? Thirdly, what is the impact of direct and indirect effects of secondary traits on grain yield of three-way and single cross Bt hybrids under FAW infestation? The last question was, what is the cultivar superiority of the three- way and single cross Bt hybrids compared to conventional non-GM hybrids and local GM hybrids which are on the market in South Africa? These questions were answered by conducting field experiments in South Africa. The study was divided into two experiments. Experiment 1 was the three-way cross hybrids and Experiment 2 was focused on the single cross hybrids’ assessment. Due to the current restrictions on GM products in tropical Africa, the study was conducted in South Africa but using tropical maize hybrids.

6.3 Summary of the Major Findings

6.3.1 Efficacy of the Bt trait in conferring resistance to fall armyworm

For both the three-way and single cross hybrids, it can be concluded that the environment, at Makhathini provided adequate discrimination among the genotypes due to sufficient FAW pressure. This is supported by the analysis of variance which showed that there were significant differences among the genotypes, at Makhathini. The genotypes which were not adapted to FAW pressure showed lower yields and higher scores for FAW damage parameters, such as leaf damaged and cob damage scores. Their yield was as low as 0t/ha with scores for leaf and cob damage as high as 8 for the susceptible hybrids. The new Bt experimental hybrids showed higher yields and higher scores for FAW resistance parameters. The new Bt experimental hybrids were ranked within the best 20 performing hybrids. Among the three-way cross experimental hybrids, (H3WX3194Bt) and (H3WX3198Bt) were highly desirable in terms of grain yield and higher ranking for FAW resistance parameters. Within the single cross trial, all the top 20 best performers were the new Bt single cross experimental hybrids. The hybrids (HSX5368Bt) and (HSX5232Bt) were highly desirable in terms of grain yield and higher resistance scores for FAW resistance parameters. These high performing experimental hybrids can be considered and recommended for release in FAW infested areas in tropical Africa. It can be concluded that MON89034 was effective in conferring resistance to FAW when deployed in both a three-way and single hybrid design.

6.3.2 Impact of secondary traits on grain yield under fall armyworm pressure

Both the three-way and single cross experimental hybrids showed a very strong, positive and highly significant correlation between grain yield and ear prolificacy. This showed that selecting for ear prolificacy will also cause an increase in yield. Fall armyworm parameters showed negative correlations with grain yield, indicating that the FAW impacted on yield in susceptible hybrids which were not converted to the Bt trait. By selecting for genotypes that have low scores (resistance) for fall armyworm traits, grain yield can be improved. The three-way cross hybrids showed that number of ears harvested had high direct and indirect effects on yield showing it to be a very important trait for improving grain yield of hybrids. Cob damage score had high direct effects on yield, affecting yield in a negative way. High cob damage scores resulted in low grain yield. Cob damage represents a more direct indicator of the effect of FAW on grain yield. Indirect selection for grain yield is possible through ear prolificacy. Within the single cross hybrids, ears harvested per plant (ear prolificacy) had the highest direct effect on yield, followed by plant height and ear-plant ratio. This showed it to be a very important trait to select for. Ears harvested and leaf damage score had negative direct effects on yield. The highest indirect effect came through leaf area damage indirect selection is possible through leaf area damage percentage. It can be concluded that the impact of secondary traits on grain yield of hybrids under FAW pressure were identified and would be taken into account when improving yield of hybrids.

6.3.3 Genetic gains assessments

6.3.3.1 Analysis of Variance

The three-way hybrids assessment in Experiment 1 showed that environments main effects were significant for yield across the three sites. Genotypes and genotype by environment interactions effects were not significant across the three sites. This was shown through the analysis of variance. Absence of genotype by environment interactions meant that there was no noise coming from the interaction between genotype and environments. This shows that there is a high correlation between the phenotype and the genotype, and that hybrids would be consistently ranked in each of the three environments which were represented by these sites. The single cross hybrids assessment in Experiment 2 showed that environments were significantly different across the three sites and that genotype by environment interaction was present and significant. Genotype by environment interaction makes genotypes perform differently in different environments. This interaction contributes to noise and reduces the heritability of the trait as well as affecting breeding progress due to inaccurate selection. The genotypes can be further studied in future breeding programmes.

6.3.3.2 Cultivar Superiority across three sites

The small value for the cultivar superiority index indicates that the hybrid combines high yield potential with dynamic stability. This is desired for hybrids which are targeted for deployment in tropical Africa. Using cultivar superiority index and mean grain yield, the genotypes which had the lowest cultivar superiority index and highest yield across the three sites were identified. Within the three-way hybrids experiment, the hybrids (H3WX3167Bt) and (H3WX3189Bt) were identified as having superiority over the conventional non-GM hybrids checks, WEMA hybrid checks and the local GM hybrid checks which are current on the market, in South Africa. This indicates that there are three-way cross hybrids with potential for advancement in the GM market segment in tropical Africa. Within the single cross hybrids experiment, the hybrids (HSX5054Bt) and (HSX5180Bt) were identified as having superiority over the conventional non-GM hybrid checks, WEMA hybrid checks and the local GM hybrids checks which are on the market in South Africa, qualifying them as candidates for advancement in the hybrid program that aims to identify new hybrids for the GM market segment in tropical Africa.

6.3.3.3 Cultivar Superiority under fall armyworm pressure

The genotypes were ranked using grain yield and the genotype which had the highest yield was identified. Within the three-way hybrids experiment (H3WX3194Bt) was identified as the best performing three-way experimental hybrid under fall armyworm pressure. The single cross experimental hybrid identified as the best performing was (H3WX5368Bt). These were identified as having superiority over the conventional non-GM hybrids checks, WEMA hybrid checks and the local GM hybrid checks which are already on the market. They can be considered and recommended for advancement under fall armyworm pressure in tropical Africa.

6.4 Performance of single cross experimental hybrids under disease pressure at Potchefstroom

The experimental hybrids were assessed under disease pressure at the Potchefstroom Research Station. The genotypes showed significant differences for maize streak virus, phaeosphaeria leaf spot, rust and turcicum leaf blight. This shows there is variability among the hybrids for disease resistance that can be exploited in further breeding programs. The genotypes were ranked according to grain yield for the assessment of their mean performance. The highest yielding genotypes were single cross experimental hybrids, outperforming local GM checks, WEMA checks and conventional non-GM hybrids. These hybrids were (HSX5318Bt) and (HSX5157Bt). These have potential to do well under disease pressure. It can be concluded that the genetic gains and cultivar superiority of the Bt experimental hybrids has been revealed.

6.5 Recommendations

In light of the above conclusions, the following recommendations were made:

- It is recommended that the identified best performing hybrids in this study (H3WX3194Bt, H3WX3198Bt, HSX5368Bt, HSX5232Bt, H3WX3167Bt, H3WX3189Bt, HSX5054Bt and HSX5180Bt), be moved further in the breeding program so that Bt hybrids suitable for tropical Africa can be identified and released for use under FAW pressure.
- It is recommended that there is need to repeat these experiments. This is because the FAW parameters need multiple scouting for a much more comprehensive set of data. The timing of the scouting also needs to be improved. This was curtailed by the Covid19 lockdown restrictions during the current study.
- It is recommended that the experiments need to be repeated to confirm the performance of the genotypes across the different environments, because significant genotype x environment interactions were observed especially for the single cross hybrids.
- It is recommended that selection through ear prolificacy needs to be emphasised in order to increase grain yield under FAW infestation need to be emphasised.
- It is recommended that Makhathini and Potchefstroom must not be discarded as test sites as they represent stress environments, which are useful in identifying stress tolerant genotypes. Makhathini showed great capacity to discriminate the genotypes under FAW pressure, while Potchefstroom was capable of discriminating the hybrids under disease pressure.
- It is recommended that since these hybrids need to be introduced into tropical Africa, then further work needs to be done in countries with tropical environment. The current study was carried out in South Africa as it has policies open to work on genetically modified crops, which other African countries do not allow at this stage.

  On the overall, this study must be repeated for another season to confirm their performance and reaction to fall armyworm under both rainfed and irrigation conditions in SA and tropical Africa. this could not be accomplished due to time limitations of 1 year for MSc study.

5.3 Overall Conclusion

Overall, the study was successful in showing the efficacy of the Bt trait “MON89034” in conferring resistance to FAW when deployed in high yielding three-way and single cross hybrid design. It was also successful in identifying the Bt three-way and single cross experimental hybrids with potential for use in FAW infested areas. This was shown by the gains over the local GM and non-GM hybrids under FAW as well as disease pressure. The main goal is to provide genotypes which are resistant to FAW and have good agronomic traits. The experimental hybrids (H3WX3194Bt, H3WX3198Bt, HSX5368Bt, HSX5232Bt, H3WX3167Bt, H3WX3189Bt, HSX5054Bt and HSX5180Bt) which displayed high yield potential and FAW resistance would be advanced in the programme. Further research and improvement need to be done until the hybrids which are high yielding and FAW resistant are readily available to the farmer in tropical Africa.

The study answered the research questions as it was shown that the Bt trait (MON89034) was found to be effective in conferring resistance to FAW when deployed in a three-way and single cross hybrid design. The yield gain of the Bt three-way and single cross experimental hybrids was established to be above that of the conventional non-GM hybrids, WEMA checks and the local GM hybrids on the market. The impact of direct and indirect effects of secondary traits on grain yield of three-way and single cross Bt experimental were established. Lastly the cultivar superiority of the three-way and single cross Bt experimental hybrids was established to be higher compared to conventional non-GM hybrids, WEMA checks and local GM hybrids on the market in South Africa.

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Appendix A Single Cross Hybrids Genotypes

ENTRY_NO	Genotypes
1	HSX4989Bt
2	HSX4990Bt
3	HSX4993Bt
4	HSX4999Bt
5	HSX5001Bt
6	HSX5002Bt
7	HSX5005Bt
8	HSX5013Bt
9	HSX5018Bt
10	HSX5019Bt
11	HSX5020Bt
12	HSX5021Bt
13	HSX5023Bt
14	HSX5024Bt
15	HSX5026Bt
16	HSX5036Bt
17	HSX5037Bt
18	HSX5040Bt
19	HSX5041Bt
20	HSX5043Bt
21	HSX5044Bt
22	HSX5046Bt
23	HSX5047Bt
24	HSX5050Bt
25	HSX5051Bt
26	HSX5054Bt
27	HSX5055Bt
28	HSX5056Bt
29	HSX5057Bt
30	HSX5058Bt
31	HSX5060Bt
32	HSX5062Bt
33	HSX5063Bt
34	HSX5064Bt
35	HSX5065Bt
36	HSX5072Bt
37	HSX5076Bt
38	HSX5078Bt
39	HSX5079Bt
40	HSX5080Bt
41	HSX5081Bt
42	HSX5082Bt
43	HSX5083Bt
44	HSX5084Bt

45	HSX5085Bt
46	HSX5086Bt
47	HSX5088Bt
48	HSX5089Bt
49	HSX5091Bt
50	HSX5092Bt
51	HSX5093Bt
52	HSX5094Bt
53	HSX5095Bt
54	HSX5096Bt
55	HSX5097Bt
56	HSX5099Bt
57	HSX5102Bt
58	HSX5103Bt
59	HSX5104Bt
60	HSX5105Bt
61	HSX5111Bt
62	HSX5113Bt
63	HSX5114Bt
64	HSX5116Bt
65	HSX5117Bt
66	HSX5123Bt
67	HSX5124Bt
68	HSX5126Bt
69	HSX5127Bt
70	HSX5131Bt
71	HSX5133Bt
72	HSX5134Bt
73	HSX5137Bt
74	HSX5139Bt
75	HSX5141Bt
76	HSX5145Bt
77	HSX5148Bt
78	HSX5154Bt
79	HSX5155Bt
80	HSX5156Bt
81	HSX5157Bt
82	HSX5158Bt
83	HSX5161Bt
84	HSX5166Bt
85	HSX5169Bt
86	HSX5170Bt
87	HSX5171Bt
88	HSX5174Bt
89	HSX5175Bt
90	HSX5177Bt
91	HSX5178Bt
92	HSX5179Bt

93	HSX5180Bt
94	HSX5181Bt
95	HSX5183Bt
96	HSX5184Bt
97	HSX5188Bt
98	HSX5199Bt
99	HSX5202Bt
100	HSX5208Bt
101	HSX5210Bt
102	HSX5214Bt
103	HSX5219Bt
104	HSX5220Bt
105	HSX5227Bt
106	HSX5230Bt
107	HSX5232Bt
108	HSX5234Bt
109	HSX5237Bt
110	HSX5239Bt
111	HSX5242Bt
112	HSX5244Bt
113	HSX5245Bt
114	HSX5246Bt
115	HSX5247Bt
116	HSX5248Bt
117	HSX5249Bt
118	HSX5258Bt
119	HSX5259Bt
120	HSX5260Bt
121	HSX5261Bt
122	HSX5264Bt
123	HSX5265Bt
124	HSX5267Bt
125	HSX5268Bt
126	HSX5272Bt
127	HSX5273Bt
128	HSX5274Bt
129	HSX5276Bt
130	HSX5278Bt
131	HSX5279Bt
132	HSX5280Bt
133	HSX5281Bt
134	HSX5282Bt
135	HSX5283Bt
136	HSX5284Bt
137	HSX5285Bt
138	HSX5286Bt
139	HSX5287Bt
140	HSX5288Bt

141	HSX5289Bt
142	HSX5291Bt
143	HSX5297Bt
144	HSX5298Bt
145	HSX5299Bt
146	HSX5301Bt
147	HSX5302Bt
148	HSX5304Bt
149	HSX5305Bt
150	HSX5307Bt
151	HSX5308Bt
152	HSX5310Bt
153	HSX5312Bt
154	HSX5313Bt
155	HSX5317Bt
156	HSX5318Bt
157	HSX5320Bt
158	HSX5323Bt
159	HSX5324Bt
160	HSX5325Bt
161	HSX5331Bt
162	HSX5332Bt
163	HSX5334Bt
164	HSX5337Bt
165	HSX5340Bt
166	HSX5341Bt
167	HSX5342Bt
168	HSX5343Bt
169	HSX5345Bt
170	HSX5348Bt
171	HSX5350Bt
172	HSX5360Bt
173	HSX5366Bt
174	HSX5367Bt
175	HSX5368Bt
176	HSX5369Bt
177	HSX5371Bt
178	HSX5374Bt
179	HSX5391Bt
180	HSX5395Bt
181	HSX5396Bt
182	HSX5399Bt
183	HSX5401Bt
184	HSX5402Bt
185	HSX5404Bt
186	HSX5405Bt
187	HSX5406Bt
188	HSX5409Bt

189	HSX5410Bt
190	HSX5415Bt
191	HSX5419Bt
192	HSX5421Bt
193	HSX5426Bt
194	SC419
195	SC633
196	SC301
197	SC403
198	PAN5R-891BR
199	DKC75-65BR
200	WE6207B DKC
201	WE6210B
202	WE6208B
203	DKC68-58BR
204	LS8541BR
205	33H54BR
206	P1184BR
207	P2553WBR
208	P1690BR

Appendix B Three-way Cross Hybrids Genotypes

ENTRY NO	Genotype
1	H3WX3161Bt
2	H3WX3162Bt
3	H3WX3163Bt
4	H3WX3164Bt
5	H3WX3165Bt
6	H3WX3167Bt
7	H3WX3168Bt
8	H3WX3169Bt
9	H3WX3171Bt
10	H3WX3172Bt
11	H3WX3173Bt
12	H3WX3174Bt
13	H3WX3175Bt
14	H3WX3176Bt
15	H3WX3177Bt
16	H3WX3178Bt
17	H3WX3179Bt
18	H3WX3180Bt
19	H3WX3181Bt
20	H3WX3182Bt
21	H3WX3183Bt
22	H3WX3184Bt
23	H3WX3185Bt
24	H3WX3186Bt
25	H3WX3187Bt
26	H3WX3188Bt
27	H3WX3189Bt
28	H3WX3190Bt
29	H3WX3191Bt
30	H3WX3193Bt
31	H3WX3194Bt
32	H3WX3195Bt
33	H3WX3198Bt
34	H3WX3199Bt
35	H3WX3200Bt
36	H3WX3201Bt
37	H3WX3202Bt
38	H3WX3203Bt
39	H3WX3204Bt
40	H3WX3205Bt
41	H3WX3206Bt
42	H3WX3207Bt
43	H3WX3208Bt
44	H3WX3209Bt
45	H3WX3210Bt

46	H3WX3211Bt
47	H3WX3212Bt
48	H3WX3213Bt
49	H3WX3214Bt
50	H3WX3215Bt
51	H3WX3216Bt
52	H3WX3217Bt
53	H3WX3219Bt
54	H3WX3220Bt
55	H3WX3221Bt
56	H3WX3223Bt
57	H3WX3224Bt
58	H3WX3225Bt
59	H3WX3226Bt
60	H3WX3227Bt
61	H3WX3228Bt
62	H3WX3229Bt
63	H3WX3230Bt
64	H3WX3231Bt
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66	H3WX3233Bt
67	H3WX3234Bt
68	H3WX3235Bt
69	H3WX3236Bt
70	H3WX3237Bt
71	H3WX3238Bt
72	H3WX3239Bt
73	H3WX3240Bt
74	H3WX3241Bt
75	H3WX3242Bt
76	H3WX3243Bt
77	H3WX3245Bt
78	H3WX3246Bt
79	H3WX3247Bt
80	H3WX3249Bt
81	H3WX3250Bt
82	H3WX3251Bt
83	H3WX3252Bt
84	H3WX3253Bt
85	H3WX3254Bt
86	H3WX3255Bt
87	H3WX3256Bt
88	H3WX3257Bt
89	H3WX3258Bt
90	H3WX3259Bt
91	H3WX3260Bt
92	H3WX3261Bt
93	H3WX3262Bt

94	H3WX3263Bt
95	H3WX3264Bt
96	SC419
97	SC633
98	SC301
99	SC403
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101	DKC75-65BR
102	WE6207B DKC
103	WE6210B
104	WE6208B
105	DKC68-58BR
106	LS8541BR
107	33H54BR
108	P1184BR
109	P2553WBR
110	P1690BR