

**ASSESSMENT OF AGRONOMIC PERFORMANCE AND HAPLOID
INDUCTION RATE OF TROPICALLY ADAPTED INDUCER MAIZE LINES**

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**A RESEARCH THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
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DECLARATION

This research thesis is my original work and has not been presented for a degree award in Kenyatta University/ or any other University.

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We confirm that the work reported in this research thesis was carried out by the candidate under our supervision as the University supervisors.

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DEDICATION

I would like to dedicate this work to my mother Rose Nzamu, my husband Peter Kyalo and my lovely daughter NardyaNdanu.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of variance
A.D	Days to 50% Anthesis
CFS	Committee on world food security
CIMMYT	International Maize and Wheat Improvement Center
C.V	Coefficient of variation
DH	Doubled haploid
FAO	Food and Agriculture Organization
GoK	Government of Kenya
HIR	Haploid induction rate
LSD	Least significant difference
RCBD	Randomized Complete Block Design.
S.D	Days to 50% Silking
SAS	Statistical Analysis Software
SSA	Sub Saharan Africa
TAILS	Tropically adapted inducer lines.
TTC	2,3,5Triphenyltetrazolium chloride
UNDP	United Nation Development Program

ABSTRACT

Hybrid maize varieties are developed using inbred lines. Conventionally, inbred lines are developed by self-pollination, which takes 6-8 generations. The technology of doubled haploid (DH) is new and the most efficient in the development of inbred lines since complete homozygosity is achieved in 2 crop seasons. Haploid inducers have been refereed variedly as genetic stock which through crossing to a diploid plant, result in a mix of haploid and diploid kernels. These progeny segregates to diploid (2n) and haploid (n) kernels due to anomalous fertilization. The International Maize and Wheat Improvement Center (CIMMYT) and Hohenheim University have developed several tropicalized inducer Lines (TAILs) that have not been tested for haploid HIR under the Kenya maize growing environment. A study was therefore set up in collaboration with CIMMYT at the DH facility located at Kiboko in Makueni County to test seven TAILs (first generation TAILs 7, 8, 9, Hybrid 9×7 and 9×8 and second generation TAILs 008 and 009) for HIR in Kenya. The objective was to improve the efficiency of hybrid maize development using haploid inducers with good agronomic performance and highest haploid induction rate. A split plot arrangement was used in the experiment in which the inducers were the main plots while the female populations were the sub plots. Randomized complete block design (RCBD) was adopted with three replications. Three female populations (ligule less tester and two normal populations) were crossed with the seven TAILs. The F₁ were germinated and the seedlings used to determine HIR of the Tails using their morphological plant characters. Pollen was harvested randomly from 10 plants of each haploid inducer line three days after pollen shed. TTC (2, 3, 5-triphenyltetrazolium chloride) solution was used as the staining technique of pollen at different times of day to determine pollen viability. Data collected was subjected to ANOVA using R- software version 3.2.3. Means of HIR and agronomic characters of the inducers were separated using the least significance difference (LSD) at 5% level of significance. The results showed that Tail 009 had the best plant and ear height, while Tail 7 had the best number of tassel braches and large tassel size. Pollen viability was high in the morning hours to noon and started to reduce in the afternoon hours. Despite this, pollen viability was above 90% in all the inducers, with TAIL 7 having the highest viability (98.45%) and TAIL 009 the lowest viability (92.77%). The ligule less tester and normal population 2 showed the highest HIR of 7.62-8.49% respectively; hence they can be used for the determination of HIR of target haploid inducer lines. Tail 8 and 9 had the highest HIR hence they can be used for haploid induction in target germ plasm. Plant height and HIR on the ligule less tester showed a positive correlation. The study was fundamental in identifying haploid inducer lines with high HIR for commercial development of homozygous lines and the results could lead to more efficient haploid induction in elite maize germplasm.

CHAPTER ONE: INTRODUCTION

1.1 Background information of the study

Maize is the third most traded cereal crop after wheat and rice. CIMMYT, (2000) ranked it top in terms of grain yield per unit area of land. Maize consumption worldwide is more than 116 million tons, with Africa consuming 30% and sub-Saharan Africa 21 % (FAOSTAT, 2011). In the developing world, maize demand will double by 2050. However, by 2025, maize will be the highly produced crop globally and in the developing world (Rosegrant *et al.*, 2008). In the current levels of maize harvests, its productivity growth still falls short of supply hence strong measures should be taken to increase growth in yield and production. This will lead to maize affordability for food to millions of maize consumers and reduced childhood malnutrition, deforestation, soil degradation, reduced biodiversity and accelerated depletion of concentrated fertilizer reserves (Smith and Haddad 2000).

In Kenya, the most important cereal crop is maize. Maize contributions in total agricultural production is more than 20 %, with 78 % of total cereal consumption, 25 % of employment, 44 % of total energy and 32 % of the total protein consumed by Kenyans (Hassan 1998). Exploitation of modern farming systems will require inputs from new technologies including new highly productive hybrids. Doubled haploid (DH) techniques are among such technologies that will increase the rapidity of developing such hybrids with better yields and other desirable characteristics of maize. Haploid breeding technology in maize has enabled rapid development of homozygous lines thus enhancing the speed at which introgressed desirable genes can get to the final breeding products thus increasing genetic gain (Eder and Chalyk, 2002; Röberet *et al.*, 2005; Chang and Coe, 2009; Geiger, 2009). The DH technology in

maize breeding is accepted as core to the enhancement of breeding efficiency worldwide. Haploid inducers with good agronomic performance and highest haploid HIR are desirable for commercial use (Röber *et al.*, 2005).

Apart from hybrid technology and off season nurseries, DH technology is contemplated as the third most important methodological achievement for maize breeding (Seitz 2004). For instance, CIMMYT has developed drought-tolerant DH lines for commercial exploitation in Kenya and other developing countries (Beyene *et al.*, 2013). In Europe, North America and more recently in China, inbred line development by DH technology has been adopted as a routine method in the private sector for hybrid maize breeding programs over the last decade (Schmidt, 2003; Seitz, 2005; Chen *et al.*, 2009). Nevertheless, maize breeding institutions in public and private sectors in several maize growing countries of tropics of Latin America, SSA and Asia have lagged in adopting the DH technology, yet these regions have the top most research priority for development of hybrid cultivars which have high yields and are drought tolerant (Prasanna *et al.*, 2010; Kebede *et al.*, 2011). The poor adoption of the DH technology could have resulted from various factors such as insufficient awareness about the DH technology, inaccessible tropicalized haploid inducers or lack of pertinent knowledge for integrating DH in breeding programs.

CIMMYT obtained haploid-inducing lines with temperate origin, which complicates their use in tropical conditions due to non-adaptation to tropical insect pests and diseases as well as photo periodic (Prigge *et al.*, 2011). Collaboration of CIMMYT with Hohenheim University developed haploid inducer lines adapted to the tropics (including TAIL 5, and TAIL 7 to TAIL 9, HIR ~ 5to 11%) referred as first generation TAILS, (Prigge *et al.*, 2012a). The second generation TAILS were solely

developed by CIMMYT, (Prasanna *et al.*, 2012). The accessibility of the TAILS can profoundly improve the proficiency of DH line production, thus enhancing seed set and haploid induction, while minimizing cost of haploid inducer line conservation and seed production. Seven TAILS were tested to determine their HIR and their agronomic performance in tropical environment in Kenya.

1.2 Problem Statement

Doubled haploid technology reduces the breeding cycle and improves selection efficiency in maize breeding (Szarejko and Forster, 2007; Chang and Coe, 2009; Geiger and Gordillo, 2009). To develop doubled haploids, a haploid inducer line is used for haploid induction in the target female population. Before development of tropically adapted haploid inducer lines (TAILS) by CIMMYT and Hohenheim University, the publicly available haploid inducer lines were of temperate origin, hence poorly adapted to the tropical conditions (Prigge *et al.*, 2012a). They displayed poor plant vigor, poor pollen production; poor seed set due to photoperiodic responses and were highly susceptible to tropical maize diseases when grown in tropical region. Both the first generation and second generation TAILS have not been tested for their agronomic performance and HIR in Kenya. This called for the need to test and document information on their suitability for growing and haploid induction in tropical Africa.

1.3 Objectives

1.3.1 General Objective

To improve the efficiency of hybrid maize development using haploid inducers with good agronomic performance and highest haploid induction rate.

1.3.2 Specific Objectives

- i. To assess the agronomic performance of seven tropically-adapted haploid inducer lines (TAILs).
- ii. To evaluate the haploid induction rate of the five tropically adapted inducer lines crossed with three female populations.
- iii. To determine correlation coefficients among agronomic characteristics and haploid HIRs of the TAILS.

1.4 Hypotheses

- (i) There are no significant differences on the agronomic performance among the seven maize haploid inducer lines.
- (ii) There is no significant difference in haploid HIR among the five haploid inducer lines.
- (iii) There is no correlation between haploid HIR and agronomic characters for the five haploid inducer lines.

1.5 Significance of the study

There has been no filed information on the TAILs for the haploid HIR and their agronomic characters in the sub- tropic and tropical climatic zones in Africa. The aim of the work was to document information on the haploid HIR of 7 TAILs and their agronomic performance such as pollen viability, plant height, ear height, tassel size, number of tassel branches, days to anthesis, and days to silking. This information would contribute to efficient application of DH technology to reduce cost and time of production of inbred lines for hybrid breeding programs. The maize hybrid varieties developed would have been incorporated with desirable genes for aspects like high yields, resistance to pest and diseases and drought tolerance hence contribute to food security.

1.6 Conceptual Framework

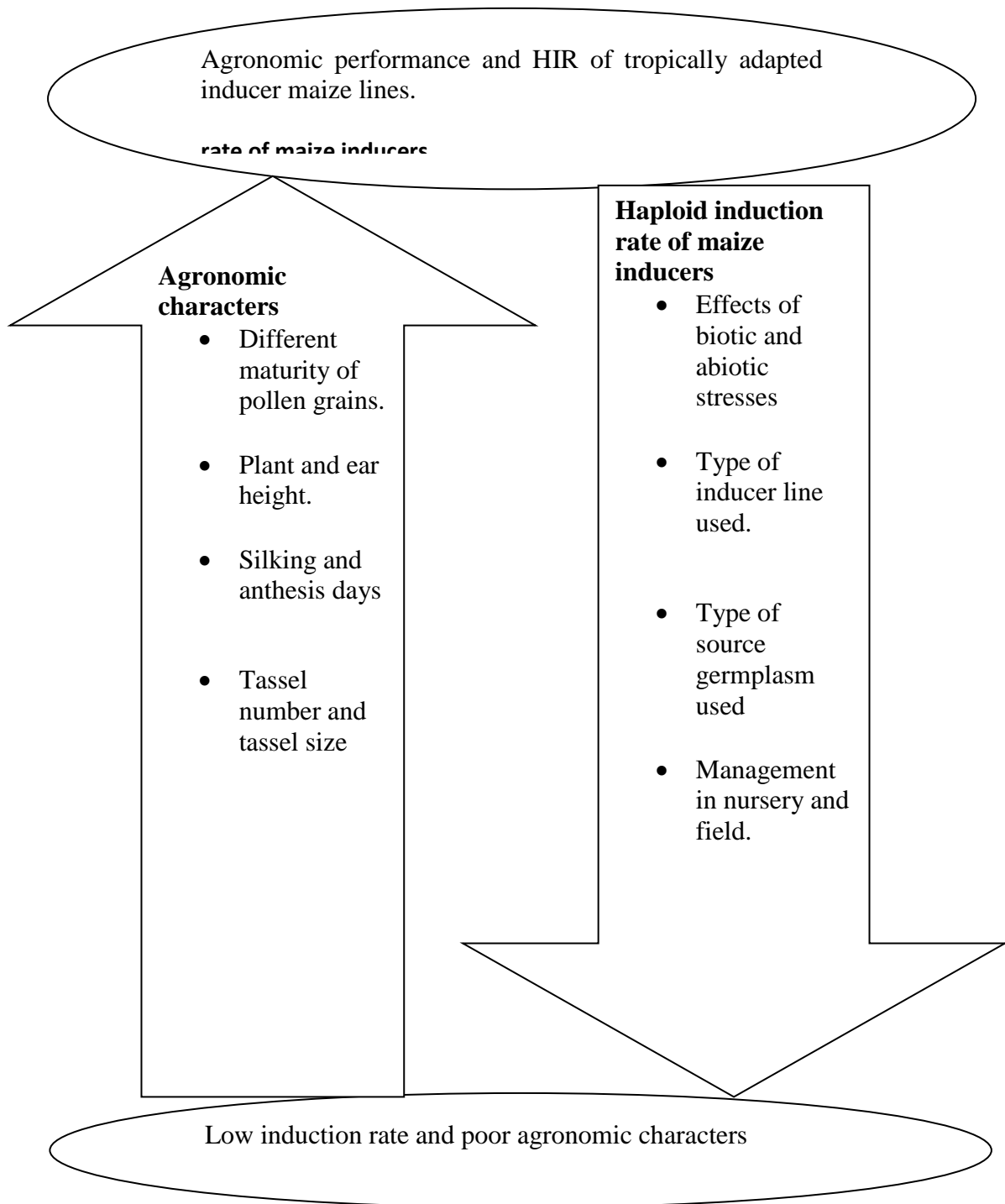


Figure 2.1:

2.1 Background information of the study

DH technology by use of haploid inducer lines is a tool used by breeders to shorten the breeding cycle for developing maize inbred lines. To form a “doubled haploid” (DH) variety, the haploid (n) cells undergoes either spontaneous or artificial doubling of the chromosome successfully (Chase 1949, 1952a, 1952b, and 1969). The haploid inducer lines which have been in use were developed in temperate climatic zones. First generation TAILS were developed by CIMMYT and Hohenheim University, with HIR of 5.5-8% (Prasanna *et al.*, 2012). The second-generation TAILS were developed by CIMMYT exclusively (Prasanna *et al.*, 2012). These haploid inducer lines have been tested for HIR in sub-tropical regions of Mexico but not in Africa, specifically Kenya where there is a functional DH facility. Plant aspects of haploid inducer lines play a crucial role in the general performance of the TAIL. Some of the plant traits include plant height, ear height, number of tassel branches, pollen viability, anthesis and silking days.

2.2 Food Security Globally

Food security has been defined in different ways by several organizations worldwide. Food security is basically the ability of individuals to obtain enough food daily. Across nations, it is referred to as the ability of people to secure adequate food. Anderson (1990) distinguished food security at levels of the nation and households. The approach to assess food security in the two categories is not the same making the difference important. Nationally, food security is the condition where the nation can produce enough food for its people. At the community and household level, people should be able to access affordable and nutritional food at all times (Anderson 1990).

Worldwide, agriculture is the backbone of food security (FAO 2004). It plays a major role to alleviate poverty through food prices reduction, being source of employment, improving farm income and increasing wages. This makes agricultural activities crucial part of government planning and policy approaches towards reduction of food insecurity and accelerates economic growth.

Increased investment in agriculture will help to conquer the current inequalities. Food security in the country can be achieved by strengthening the production mechanisms in the community. Agricultural sectors play a crucial role towards food security in a country (FAO 2008c). Rural areas have majority of the people in direct need of food security. These people living in rural areas had access to land but have inadequate skills and access to recourses to farm adequately.

Approximately, 850 million people worldwide are under nourished (CFC report 2005). This number has barely shifted since 1990-1992 base period for the world summit and millennium development goal commitment on minimizing hunger by half by 2015. As of May 2006, 39 countries were in serious food insecurity situation and required external aid. Majority of these were in Africa and Asia. Reports by FAO (2010) indicate that approximately 240 million people in sub-Saharan Africa lack access to nutritionally suitable food. In the sub-Saharan Africa, agricultural yields remain lower than other developing regions. Technologies, techniques and inputs in agriculture are required to enhance production as it lags far behind the rest of the developing world.

In Kenya, suitable land for agricultural utilization is less than 20 percent; hence the country is vulnerable of food shortages (Langinger, 2011). In terms of Gross Domestic Product (GDP), the importance of agricultural sector is declining despite being the backbone of the economy (Waithaka, *etal.*, 2013). Kenya has most of its

population living in the rural areas, hence depends on agriculture for their livelihoods. Agricultural sector in Kenya depends heavily on rainfall, thus greatly been impacted by the current trends of climate change. For example, in Kenya, droughts are often and they last longer and the rainfall patterns became unreliable (Patel *et al.*, 2012). Long-lasting and periodic droughts faced in the past lead to enduring food crisis. Climate change and volatile energy prices have powered the present food crises acting as the key factors affecting concept of food affordability. They are altering the habits of food consumption, production and food market. Due to these factors, resource poor communities are forced to reduce their intake of sufficient nutritional products (GoK, 2011).

2.3 Origin of Maize and Ancestry

Watson and Dallwitz, (1992) described origin of *Zea mays* to be the Mesoamerican region (Mexico and Central America). Domestication of maize according to archeological records began at least 6000 years ago independently in regions of the southwestern United States, Mexico, and Central America (Mangelsdorf, 1974). There have been various hypothesis proposed on maize domestication (OECD, 2003). Maize taming process has frequently been described by Teosintes (*Z. diploperennis* and *Z. mays* sp. mexicana) and *Tripsacum* species, (Mangelsdorf, 1974; Galinat, 1988).

Some studies suggest that *Z. mays* may be a product of hybridization between *Tripsacum* and *Zea* with further crossings of Teosinte producing the present varieties (Mangelsdorf 1974). Inter generic hybridization of either *Z. diploperennis* or *Tripsacum* with extinct varieties could probably have been origin of maize (Raduet *al.*, 1997; Purseglove, 1972). Eubanks (1997a, 2003) proposed that domesticated

maize may have arisen through human selection of natural hybrids between *Tripsacum* and perennial teosinte.

2.4 Ecology of maize and taxonomy

The genus *Zea* is classified in the tribe Maydeae of the grass family Poaceae (OECD 2003; USDA 2005). Genus *Zea* has five species of which *Zea mays* L. is of economic importance. The chromosomal number in *Zea mays* is $2n=20$ (Doebly, 1990). ICAR, (2006) described maize crop to be a warm weather crop grown in a wide range of climatic conditions. Altitude range from 0 to 3800 meters above sea level with growing seasons of 42 to 400 days. Maize in the tropics grows well between 30 degrees north to 30 degrees south, while sub-tropical maize between 30 to 34 degrees both North and South respectively. Temperate maize grows on latitude beyond 34 degrees (Paliwal 2000b; Farnham *et al.*, 2003). Rainfall per annum of 60cm and well distributed throughout the growing season is suitable for growing maize. After tasseling, maize requires above 50% of its total water requirements in about 30 to 35 days. Poor yields and shriveled grains can result in case of inadequate soil moisture at grain filling stage. Maize cannot withstand frost at any stage. Soil texture in maize cultivation is crucial as it controls moisture and nutrient capacity. Maize can be grown on a variety of soils. However, ideal soils for cultivating maize are loam or silt loam surface soil and brown silt clay loam having permeable sub soil. The soils should also be deep, well drained and rich in organic matter with optimum pH of 7.5-8.5 (Shaw, 1988)

2.5 Maize morphology

Tropical maize plant is a tall (1-4m) annual grass which forms a seasonal root system bearing a single erect stem made up of nodes and internodes (Esau, 1977b). The plant

has nodes that narrow top wards and the leaves are broad with single leaflets arranged in two opposite directions. The leaf has a sheath and a blade. Maize gradually developed under tropical conditions hence a C4 plant and therefore more efficient utilizing carbon IV oxide than C3 plants (Esau, 1977a).

The reproductive parts of maize are partitioned into separate pistillate (ear) which is the female flower and staminate (tassel) been the male flower. Its habit of growth is determinate, with the shoot terminating into the inflorescences bearing staminate or pistillate flowers (Dhillon and Prasanna, 2001). Mangelsdorf, (1974) described the reproduction in maize in which a single plant has male and female reproductive organs. The terminal flowers been the tassels which develop the male spikelet's only and they grow in pairs with one being sessile (having no stalk) and the other pedicellate, and a single blossom on a lean stalk. More than one thousand potential kernels are contained in each ear. With only one of the flowers developing, each ear bears a spikelet just like the male tassels. Each of these flowers has one ovary terminated by a long style known as the "silk". Silks are covered by fine hairs made to catch pollen blowing in the wind. The silk should develop into one kernel and if it fails to get pollinated, the kernel does not appear. The pollen grains trapped by the silk are about 1/250th of an inch in diameter and barely visible to the naked eye (Baltazaret *al.*, 2005)

2.6 Pollination of maize

Maize is a cross-pollinating species. The pollen grains are small and have lightweight enabling them to be carried by wind (Sleper and Poehlman, 2006). Pollen germinates on the silk and the pollen tube descends down with two generative nuclear; one fuses with the egg to produce the embryo and the other fuses with a double nucleus to form

endosperm of each kernel. This results in the process of *xenia* in which the two breeds of corn combine, generally with the kernels taking on the characteristics of the male pollen (Watson and Dallwitz, 1992). New varieties of maize continue to appear after the cross pollination.

2.6.1 Pollen viability

Success of haploid induction depends on pollen viability. Viability of pollen is basically regarded as the ability of the pollen grain to perform its work of delivering sperm nuclear to the embryo sac following compatible pollination (Shivanna *et al.*, 1991). Evaluation of pollen viability is tiresome, takes a lot of time, and it is not always practicable (Heslop-Harrison *et al.*, 1984). Pollen viability can be tested by various techniques such as staining, germination test of *in vivo* and *in vitro* or by final seed set analysis (Adhikari and Campbell, 1998; Dafni and Firmage, 2000). Reliable methods of estimating pollen viability have been reported in some species of monocots such as; maize, rice and sorghum (Tuinstra and Wedel, 2000; Luna *et al.*, 2001).

Haploid HIR depends on pollination time and the method, (Rotarenko *et al.*, 2009). To obtain optimum outcome, manual pollination is preferred as opposed to open pollination. Haploid plant tassels in most cases have anthers that produce limited pollen or none, although few plants may have tassels producing abundant pollen (Prasanna *et al.*, 2012). The uncertainty of pollen production calls for efficient utilization of pollen grains during pollination. Pollen viability is an important variable to test in DH maize lines.

2.6.2 Pollen management

Management of pollen has been investigated as a method to limit trans-gene flow in maize (Luna *et al.*, 2001). Development of various methods of gene flow management is enabled by the knowledge of pollen viability. To get improved or hybrid vigor genotypes and a good fruit fixation, pollen viability becomes an essential precondition. The study of pollen viability is of importance in breeding programs especially in controlled pollination experiments (Borém and Miranda, 2007). Depending on the temperature and several other environmental factors, pollen grains release take place throughout the day from morning to evening.

Pollen viability can be assessed using various techniques. The most common technique for testing pollen viability is germination test of *in vivo* and *in vitro* and chemical dyes test (Dafni and Firmage, 2000). The stain ability is a simple procedure which is less expensive and it provides quick results, making its adoption easy for works involving pollen grains. Correlation can then be estimated through counting of the aborted and non-aborted pollen grains (Tuinstra and Wedel, 2000). Various dyes can be used to test pollen viability, among them: acetic carmine, triphenyltetrazoliumchloride, aniline blue and malachite green with acid fuchsine. In the study, information of pollen viability is of great importance as it would show the best time of the day to do pollination when pollen grains are more viable.

2.7 Importance of Maize in the world

Large differences in maize yields are recorded in maize growing areas worldwide. The total world production of maize was estimated to be 875 tones with the United States, China and Brazil harvesting 31%, 24% and 8% of the total production of maize, respectively (FAO 2012). Analysis of the maize usage reveals that it has been

used mainly as feeds for animals, human consumption and alcohol production. Nevertheless, maize usage for fuel production has significantly increased (Wallington, *et al.*, 2012). Maize crop is among the three most important cereals for human consumption hence its production is important. Various breeding techniques have been employed to ensure good agronomic variables such as yield, resistant to pests and diseases, drought tolerant among others have been obtained. DH technology in maize production is among the breeding techniques for hybrid development.

Maize is the leading staple food in Kenya. An average Kenyan consuming the crop gets 40 to 45 percent calories (GoK, 2003). Rural households heavily depend on maize for subsistence food and source of income (MoA, 2004). About 26 million bags (23 million metric tons) of maize per annum are produced under an area of 1.5 million hectares. Consumption of maize is estimated at 98 kilograms per household which approximates to roughly 27 to 31 million metric tons per year. Maize is also important as it accounts for 28 percent of gross farm output (Jayne *et al.*, 2001). The demand of maize has been on the increase, outstripping its domestic supply hence making the country a net maize importer.

With the country's population estimate to reach 43.1 million by 2020 (Jayne *et al.*, 2001), this implies that the country will more be relying on imports to meet the deficits. DH technology in maize breeding can be adopted for efficiency hybrid production. Through this, high yields as an agronomic variable among others can be achieved. The DH technology through use of maize haploid inducer line with high haploid HIR would be more efficient.

2.8 Development of Maize varieties

The adoption of enhanced maize genotypes to meet the market demand is growing in the developing countries. In Mexico, less than 20% of varieties grown in the 1950s remain today in cultivation (World Watch Institute 2000). CIMMYT is the leading organization in preservation of maize germplasm. It has the world's largest collection of maize accessions, with over 17,000 lines (CIMMYT, 2000).

2.8.1 Classical way of developing inbred lines

Development of hybrid varieties in maize and other crops involves use of homozygous inbred lines. The inbred lines have conventionally been produced by breeders and researchers by selfing heterozygous genotypes for 5 to 6 generations. This method is costly and takes a lot of time (Eder and Chalyk, 2002). Inbreeding in maize involves mating of individuals with relatives. This may include; self-pollination, sib-mating and parent-offspring mating (Kent, 2001). Application of doubled haploid technology in breeding lead to creation of fixed, pure breeding varieties in fewer seasons (Szarejko and Forster, 2007; Chang and Coe, 2009; Geiger and Gordillo, 2009). Practical application of DH technology has been a challenge over the past decades due to the low rates of haploid induction.

2.8.2 Hybrid Maize

Maize hybridization was first completed by a group of professional breeders in USA in early 1900s (Lee and Tollenaar, 2007). The practice involves crossing of two inbred lines to produce a single cross hybrid. To produce a hybrid, the breeder should control the source of pollen in the crosses. This can be accomplished by either use of cytoplasmic male sterile lines as the female parent or detasseling the female parent in combination with a male fertile restorer line. Hybridization involves consideration of

combining ability of individual inbred lines in order to identify results in superior hybrids.

The efficiency of hybrid lines developments can be hastened by application of DH technology, which results to the development of inbred genotypes within 2-3 seasons. Hybrids have hybrid vigor with good agronomic characters such as high yield, drought tolerant, resistance to pest and diseases among others.

2.8.2.1 Types of Hybrids

i) Single cross hybrid

This involves crossing of two inbred lines (line $A \times B = A \times B$). The resulting plants from the hybrid reveal improved vigor and yields (Duvick and Cassman 1999).

ii) Double cross hybrid

Four unrelated inbred lines are involved in the cross to produce two parents. The resulting two parent lines are then crossed to get double cross hybrid seeds $[(A \times B) \times (C \times D)]$ (Duvick and Cassman 1999).

iii) Three way crosses

The cross involves three unrelated inbred lines to produce the hybrid seed $[(A \times B) \times C]$.

Over years, single cross hybrids have been widely used in maize breeding. In the developed countries, maize hybrid serves a key role in the maize production. Duvick and Cassman (1999) estimated 5 years for the whole procedure from the first hybrid cross to commercial release. Selection principle in developed countries for hybrids are yield and stability making them to have relatively short time on the commercial market (< 10 years on average). This occurs as a result of their replacement by higher yielding hybrids, rather than disease problems or insect susceptibility (Duvick and

Cassman 1999). Other characteristics of maize such as grain starch, protein ratio, decreased tassel size and reduction in barrenness at high density among others have changed significantly in association with the selection efforts for increased yields (Duvick and Cassman 1999).

2.9 Doubled haploid production in maize

Maize breeding programs in Kenya is surveyed as a success story (Olwande and Smale, 2012; Mathenge *et al.*, 2014). Over the years, an increase in the number of improved maize varieties, especially hybrids has been released by several maize breeders. However, increase in maize yields has not been matched (Faostat, 2012). This could be due to some factors such as low adoption in some zones, unpredictable weather, poor agricultural practices and expensive inputs (Gitu, 2006; Ogada *et al.*, 2014). Higher yields are the key factors for breeding hybrid varieties. Application of DH technology in production inbred lines which are used for development of hybrid maize varieties becomes an essential tool for breeding.

Spontaneous chromosome doubling for haploid maize plants can occur at different rates in the male and female inflorescence. A male inflorescences that produces normal pollen grains can have spontaneous chromosome doubling range of 2.8 to 46 percent and be genotype specific (Liu and Song, 2000; Wei and Chen, 2006; Han *et al.*, 2006). Spontaneous chromosome doubling for the female inflorescence of a haploid plant can be shown by seed set which range from 25 to 94 percent (Chalyk *et al.*, 1994; Liu and Song, 2000; Han *et al.*, 2006). Occurrence of spontaneous doubling may be in various ways such as somatic cell fusion, endo-reduplication, endomitosis among others (Jensen, 1974; Testillano *et al.*, 2004). Protoplast fusion (somatic cell fusion) occurs in several steps which include; digestion of cell wall with

cellulase enzymes. The protoplasts then undergo fusion where the two nuclei fuse into a single nucleus. At the end of the process, hormones to trigger the growth of a cell wall around the newly-formed cell are released. The cell is now diploid and the chromosome doubling process complete at that point (Testillano *et al.*, 2004).

Scanlon and Takaes, (2009) described end reduplication or end mitosis to occur when the mitotic process declines. This starts around 10 to 14 days after pollination (Kowles and Phillips, 1985), at which time, the endo reduplication process begins. A repeated cycle of alternating DNA replication and non-replication characterizes the process. This takes place in the absence of chromatid separation, nuclear division and cytokinesis (Scanlon and Takaes, 2009). Kowles *et al.* (1990) submitted flow cytometry findings to play a crucial role in the spontaneous chromosome doubling in the entire genome during end reduplication cycle.

Artificial chromosome doubling is a reliable asset of doubled haploid production (Geiger, 2009). Crop breeding programs benefits significantly from the doubled haploid (DH) technology which produces inbred lines effectively (Dunwell, 2010). DH lines usage improves genetic gains and enhances breeding effectiveness in maize breeding through simplification of the logistics and cost reduction for line development and maintenance (Schmidt, 2003; Melchinger *et al.*, 2005; Lee and Tracy, 2009).

DH technology in maize basically entails *in vivo* or *in vitro* induction using a haploid inducer line, identification of the haploids, doubling the haploid chromosome complement and the production of seed from fertile DH plants (Prasanna *et al.*, 2012; Prigge and Melchinger, 2012). Doubled haploid (DH) lines in maize can be produced by use of various methods. One of the efficient methods productions of double

haploid is through colchicine treatment of anther-derived maize callus (Wan, *et al.*, 1989). Several colchicine-based protocols suitable for large-scale chromosome duplication have been developed for *in vivo* and *in vitro* production of maize DH lines (Barnabás *et al.*, 1999). Most of the breeding companies have adopted artificial chromosome doubling treatments which are less toxic to plant tissue and pose minimal danger to the humans handling them (Geiger and Gordillo, 2009). Colchicine was considered as unreliable asset before 1990's due to its effects which were highly genotype precise when applied to haploid maize seedlings. Besides this, colchicine needed higher concentration which proved to be toxic to treated seedlings (Jensen, 1974), as it has a low affinity for plant microtubules (Eigsti and Dustin, 1955). Gayen *et al.* (1994) tested three rates of colchicine, 0.03 percent, 0.06 percent and 0.1 percent, in a solution of 0.50 percent DMSO (dimethyl sulfoxide). The coleoptile tips were removed and the seedlings placed in cotton wads that were moistened at intervals of one hour with the colchicine solutions for 6, 12, and 24 hours at 18 degrees Celsius. The highest chromosomal doubling rate as per the results was 18.05 percent yielded by the coleoptile tip removed and treated with 0.06 percent colchicine solution for 12 hours.

Eder and Chalyk, 2002, tested the procedure further across many genotypes. Germination of the seedlings was done for coleoptiles length of about one centimeter. The coleoptile tips were then removed and the seedlings submerged in 0.06 percent colchicine solution containing 0.50 percent DMSO for 12 hours at room temperature in a dark room. The success rate of nearly 50 percent was shown when the artificial doubling rates were averaged across all tested genotypes.

Zabirova *et al.* (1996) utilized colchicine in another method as a comparison study. Plant seedlings grown to three or four leaf stage were injected with colchicines solution containing 0.125 percent colchicine and 0.50 percent DMSO three to five millimeters above the apex. Across all the studied genotypes, a doubling rate of 16 percent was achieved (Eder and Chalyk, 2002). In conclusion, the authors found both methods effective in doubling haploid seed chromosome numbers.

Alternative methods include treatment of haploids with nitrous oxide (Kato, 2002). The initial experiment done to develop standardized method used several time periods, gas pressures and plant stages. The most effective method was achieved by treating haploid maize plants with nitrous oxide at the flower primordial stage for two days at 600 kPa. The method revealed an average of 44 percent chromosomal doubling rate across the eight varieties on further test using four inbred lines and four F₁ hybrids. The donor genotypes were also found to have a big influence on the effects of the treatment with a doubling range of 17 to 90 percent across the eight genotypes. Despite being labour intensive, the approach also needed use of special equipment to accommodate the pressures exerted by the gas. Hitherto, this approach is not easily adapted to a project that requires a large number of plants to be treated. Regardless of the method, artificial chromosome doubling of maize haploids is costly, requires special facilities, and involves noxious substances (Kleiber *et al.*, 2012). A companion study on search of artificial treatments (Melchinger *et al.*, 2016) has been done and a portion of the research has investigated the options for exploitation of haploids' natural fertility for DH line production (Hantzschel and Weber, 2010).

2.10 Double haploid technology

The technology of doubled haploid (DH) has provided significant economic advantage to crop breeding for its efficient production of inbred lines (Dunwell, 2010). The DH technology essentially involves *in vivo* induction using a haploid inducer stock, identification of haploids, doubling the haploid chromosome complement, and the production of seed from fertile DH plants (Prasanna *et al.*, 2012; Prigge and Melchinger, 2012).

Crossing of the source germplasm with pollen from maternal haploid inducers that have the capability to induce haploid embryos is done to achieve *in vivo* haploid induction. DH is adopted worldwide in routine maize breeding programs. This technique seems to be solution to many problems like slow hybrid development, in Agriculture research sector (Forster *et al.*, 2005). DH technology accelerates inbred line development. The technology also reduces the costs for nursery and maintenance of breeding work (Röber *et al.*, 2005). All the existing haploid inducer lines were of temperate origin that would never be successful in extreme environmental conditions. These inducers display poor vigor, poor pollen production, poor seed set, and high susceptibility to tropical maize diseases when grown in tropical region (Prasanna *et al.*, 2012). DH technology application enhances the efficiency of hybrid development in maize. This is enabled by identification of haploid inducer line with high haploid HIR and good agronomic characters.

2.10.1 Haploid identification

Efficient screening system to differentiate between haploid and diploid kernels resulting from induction cross is a major issue to apply in the *in vivo* haploid induction approach on a commercial scale. Haploids can be distinguished from

diploids based on the phenotypic markers or differences in plant characteristics at the adult stage (Xu *et al.*, 2013; Weber, 2014; Wu *et al.*, 2014). Melchinger *et al.*, (2016) recommended haploid identification at the seed or seedling stage for the large scale production of DH lines, as the chromosomal doubling is generally achieved by treating the haploids at the early seedling stage with mitotic inhibitors such as colchicines.

Geiger,(2009) described a haploid kernel resulting from induction cross using inducers equipped with *R1-nj* marker to have a purple crown on regular triploid endosperm and a non-pigmented scutellum, whereas a regular F_1 kernel shows coloration of both the aleurone and scutellum. The ‘red crown’ or ‘navajo’ kernel trait encoded by the dominant mutant allele *R1-nj* of the ‘red color’ gene *RI* is the most efficient haploid identification marker. In the presence of the dominant pigmentation genes *A1* or *A2* and *C2*, *R1-nj* conditions deep pigmentation of the aleurone layer (endosperm tissue) in the crown (top) region of the kernel. In addition, it causes pigmentation of the scutellum (embryo tissue).

In the haploid induction experiments, the first red crown mutant was used as selectable marker, (Nanda and Chase 1966; Greenblatt and Bock, 1967). Effective results were achieved when the donor had colorless seeds and the inducer homozygous for *R1-nj* and the dominant pigmentation genes. A haploid induction kernel has a red crown (regular triploid endosperm) and a non-pigmented scutellum, whereas a regular F_1 kernel displays pigmentation of both the aleurone and scutellum (Geiger, 2009). If only the egg cell but not the central cell is fertilized, the kernel has a pigmented (diploid) embryo and a non-pigmented, diploid maternal endosperm and aborts during early kernel development. Kernels resulting from (unintended) selfing or out crossing with other colorless donors do not show any pigmentation.

The “red crown” marker does not work for dominant anthocyanin inhibitor genes such as *CI-I* or where the donor genome is homozygous for *RI*. Belicuas *et al.*, (2007) found that the genes occur quite frequently in European flint or tropical materials. This makes haploid identification possible in the early seedling stage if the inducer is homozygous for the genes *BI* and *PII* which in conjunction condition with light-independent pigmentation of the coleoptile and root of the F₁ seedlings. Haploid identification method can also be done by determination of oil concentration of the kernel (Rotarenko *et al.*, 2007). The authors observed that kernels with haploid embryos have a significantly lower oil concentration than those with a diploid F₁ embryo. This is due to the reduced size of haploid embryos when compared with diploid embryos.

2.10.2 Advantages of DH in maize breeding

DH technology has quite many merits in maize genetics and breeding; salient among these are:

- i. Reduces the cycle of breeding by developing complete homozygous lines in 2-3 generations.
- ii. Effectiveness and precision of selection is greatly enabled, more so when used together with molecular markers and year-round nurseries (Röber *et al.*, 2005; Geiger and Gordillo, 2009).
- iii. Simplifies logistics such as time, labour and reduces costs, in developing new breeding varieties (Geiger and Gordillo, 2009).
- iv. Reduces efforts for genotype perpetuation (Röber *et al.*, 2005).

- v. When used with molecular makers, it facilitates access to germplasm within either the female or the male parental lines of hybrid varieties (Heckenberger *et al.*, 2005).

2.10.3 DH development

Most haploid inducers reported, were developed in temperate environments and were mainly assessed there for agronomic performance and HIR. Prigge *et al.*(2011) reported that temperate inducers are less adapted to the agro-climate prevalent at (sub) tropical research stations. Prasanna *et al.* (2012), described five steps on DH Lines development;

The first step involves generation of haploid seed through induction crosses of female (F₁ or F₂ populations) x male haploid inducer line to generate haploid kernels. This is followed by screening of all kernels to identify haploid kernels based on embryo and endosperm color marker gene (R1-nj) or any other system. Chromosome doubling of haploid kernels using colchicine or any other treatment to produce D0 plant is done. The delicate treated seedlings are planted in pots or directly in field nurseries. Self-pollination of each plant (D0) that shed pollen to produce (D1) doubled haploid line marks the final stage of the DH development.

2.10.4 Haploid induction rate

Spontaneous haploids induction was observed in US Corn-Belt germ plasm at a rate about 0.1 % (Chase, 1952). For any commercial application, the value was very low. An inbred line called “stock 6” with an HIR of 1 to 2% was later discovered (Coe, 1959). The variety served as the ancestor of all subsequently haploid inducer lines developed. Scientists working in India, the former Soviet Union, France and Germany (Sarkar *et al.*, 1994 and Röber *et al.*, 2005) made remarkable improvement in the

HIR. The University of Hohenheim presently developed RWS inducer line which is most effective, (Röber *et al.*, 2005). This inducer is adapted to the temperate climate of Central Europe but is still effective in tropical environments (Röber *et al.*, 2005). The inducer line also has an HIR of about 8% on average across a wide range of donors and environments. A sister line of RWS, RWK-76, developed from the reciprocal cross (WS14 × KEMS) provided an average HIR of 9 to 10%.

Based on the first inducer of maternal haploids in maize (*Zea mays* L.), Stock 6 (Coe, 1959), several new haploid inducer lines have been created (Tyrnovet *et al.*, 1984; Lasharmes *et al.*, 1988; Sarkar *et al.*, 1994; Shatskaya *et al.*, 1994; Chalyk, 1999; Roberet *et al.*, 2005). The inducers posse's dominant anthocyanin marker genes allowing haploids to be identified at different stages (dry seeds, seedlings and mature plants), and their haploid-inducing rate was significantly increased in comparison with the initial inducer (Stock 6).

Overestimation of the HIR can be avoided by use of homozygous donor genotypes (females) for recessive markers such as the mutant's *ligule less* or *glossy*. Röber *et al.*, (2005) detected a remarkable difference on HIR between donor genotypes. However, the range of variation was small compared with that reported for *in vitro* culture techniques (Spitkó *et al.*, 2006). Environmental factors can also lead to considerable variation in HIR (Röber *et al.*, 2005). Above this, the HIR may depend on the method and time of pollination (Rotarenko *et al.*, 2009). To obtain the best results, manual pollination compared with open pollination in an isolated plot, three days after silk appearance. The information of HIR would help farmers to adopt the best haploid inducer line with highest HIR and good agronomic characters which will be used for hybrid development.

2.10.5 Characteristics of haploids

Haploid plants are smaller and less vigorous than corresponding diploid homozygous lines (Auger *et al.*, 2004). Geiger *et al.* (2006) documented that most haploids display a certain level of female fertility but haploids lack male fertility generally (Chalyk, 1994). Nevertheless, certain donor genotypes have been detected to provide haploid plants producing traces of pollen which could successfully be used for selfing (Chalyk, 1994).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Location of the study

The study was carried out in Kiboko DH facility in Makueni County. The facility is located along Nairobi-Mombasa 200km south east of Nairobi with an altitude between 900m and 1000m above sea level. The area receives rainfall of 150mm-650mm per year. Temperature ranges from 12⁰C to 28⁰C. There are two rainfall seasons, the short and long rains, which occurs during October-December and March-May respectively (www.meteo.go.ke). The soils are well drained, very deep, dark reddish brown to dark red, friable sandy clay to clay (Acric-Rhodic Ferrassols) developed from undifferentiated basement system rocks, predominantly banded gneisses. The study was carried out in two seasons between the months of September to December 2015 and January to March 2016.

3.2 Materials of the study

Seven TAILS were tested for agronomic performance. Five of the TAILS (first generation Tail 7, 8, 9, second generation Tail 008, 009) and two hybrid haploid inducers (Hybrid Tail 9×7, and Hybrid Tail 9×8) were used to test the HIR with three female maize populations ((Liguleless tester, Normal population 1(D1187-444), and Normal population 2 (D1187-453)). The materials used in the study were offered by CIMMYT under Global maize program.

Table 3.1: Field Layout

Rep	S/no	Treatment (Main plot Inducer lines)	Sub plot 1 (Female populations)	Sub plot 2 (Female populations)	Sub plot 3 (Female populations)
1	1	5(Hybrid 9×8)	Ligule less tester	Normal population 1	Normal population 2
1	2	2(1GT 8)	Normal population 1	Normal population 2	Ligule less tester
1	3	3(1 GT 9)	Normal population 2	Ligule less tester	Normal population 1
1	4	4(Hybrid 9×7)	Normal population 1	Normal population 2	Ligule less tester
1	5	1(1GT 7)	Normal population 2	Ligule less tester	Normal population 1
1	6	7(2GT 008)	Normal population 1	Normal population 2	Ligule less tester
1	7	6(2GT 009)	Normal population 2	Ligule less tester	Normal population 1
2	8	6(2GT 009)	Ligule less tester	Normal population 2	Normal population 1
2	9	5 (Hybrid 9×8)	Normal population 1	Ligule less tester	Normal population 2
2	10	7(2GT 008)	Ligule less tester	Normal population 2	Normal population 1
2	11	2(1GT 8)	Normal population 1	Ligule less tester	Normal population 2
2	12	1(1GT 7)	Ligule less tester	Normal population 2	Normal population 1
2	13	4 (Hybrid 9×7)	Normal population 1	Ligule less tester	Normal population 2
2	14	3(1 GT 9)	Normal population 2	Normal population 1	Ligule less tester
3	15	3(1 GT 9)	Ligule less tester	Normal population 1	Normal population 2
3	16	4 (Hybrid 9*7)	Normal population 1	Normal population 2	Ligule less tester
3	17	2 (1GT 8)	Normal population 2	Ligule less tester	Normal population 1
3	18	5(Hybrid 9*8)	Normal population 1	Normal population 2	Ligule less tester
3	19	1(1GT 7)	Ligule less tester	Normal population 1	Normal population 2
3	20	6(2GT 009)	Normal population 2	Ligule less tester	Normal population 1
3	21	7(2GT 008)	Normal population 1	Normal population 2	Ligule less tester

Seven TAILs were crossed with three female populations (Table 3.1). The experiment was carried out in a split plot arranged in a randomized complete block design (RCBD) with three replicates. The main plot had seven haploid inducer lines (First generation TAILs 7, 8, 9, hybrid 9×7 and 9×8, second generation 008 and 009) while the sub plots were the three female populations (ligule less tester and two normal maize inbred). Each sub plot had four rows of 5 meter length and a plant population of 100 plants. The isolation distance between the plots was one meter. The female populations were planted with the first stagger of the haploid inducer lines to ensure nicking at pollination. The main plot of haploid inducer lines had 12 rows and plant population of 792 plants. Inducer planting was staggered at weekly intervals for three weeks to ensure nicking with females at pollination.

Detasseling of the female populations was carried out and ears shoots covered with shoot bags to prevent open pollination. The tassels of the inducer lines were covered for good management of the pollen grains shed. Controlled manual pollination was done between the haploid inducer lines and the female populations. All F₁ seeds in each female population were harvested per plot and haploid seeds determined following the seedling morphological characteristics as per Melchinger *et al.* (2016). Five haploid inducer lines were sampled for pollen harvesting.

3.3 Data collection on Agronomic characters

Data on the agronomic characters was scored on plants of seven haploid inducer lines provided by CIMMYT for testing. The agronomic characters scored were plant height, ear height, tassel size, number of tassel branches, days to anthesis and silking, and pollen viability.

3.3.1 Plant height and ear height.

Plant and ear height were measured as per the guidelines of UPOV (2009) protocol. Twenty plants were sampled randomly for data collection in the main plot (Appendix 4). Plant height was measured from the soil surface to the base of the flag leaf. Ear height was measured from soil surface to the upper ear attachment. Data collected was recorded and average determined from the 20 sampled plants.

3.3.2 Anthesis days and silking days

Days to anthesis was recorded as the number of days from sowing to the time when 50% of the plants released pollen within a haploid inducer line. Days to silking was captured as the number of days from sowing to the time when 50% of the population of haploid inducer line had silked.

3.3.3 Number of tassel branches and tassel size

Twenty plants on each haploid inducer line were sampled randomly. The number of primary tassel branches was counted on the anthers and recorded (Appendix 4). Tassel sizes were calculated from the number of tassel branches and grouped into three categories, Large, medium and small.

3.3.4 Pollen viability

Pollen viability study was carried out using TTC (2, 3, 5-triphenyl tetrazolium chloride) stain based on a modified procedure from Dafni (1992) and Pio (2004), (Appendix 5). Five inducer plants per main plot were sampled for pollen harvesting after all tassels flowered. Pollen grains were harvested in a span of two hours at 8 am, 10 am, 12 noon, 2 pm and 4pm. The use of TTC staining dye was in reference to the colour change of the tissue in the presence of the dye. The TTC salt reduces

dehydrogenase respiratory enzyme in live tissue resulting in a red carmine coloured compound called formazan, while the dead or non-viable tissues are not dyed (Beyhan and Serdan, 2008).

Three drops of 1% TTC solution were placed on three microscope slide. Sufficient pollen grains were sprinkled on these micro slides to just cover the surfaces. A cover glass was placed over each slide, taking care to exclude most air bubbles. The slides were placed in a petri dish on glass rods over water. The petri dishes were covered and placed in the dark in an oven at 55° c. The slides were removed after 90 minutes and wiped free of moisture. The slides were examined with binocular microscope at X 100 magnification, using blue filtered reflected light to provide contrast. The mechanical stage was moved randomly, and the pollen grains counted in a predetermined sector of the field to a total of 25. The random movement was done sixteen times to count 400 hundred grains in each slide, giving a total of 1200 from each sample. The pollen which stained red or pink in color was considered viable, while the unstained (colorless) considered unviable. The procedure was repeated for all the 7 haploid inducer lines and data recorded.

3.4 Haploid induction rate

This study was conducted to test 7 TAILs for high HIR in (sub) tropical climate. This was achieved by crossing three (sub) tropical CIMMYT maize lines as female populations with 7 tropicalized inducers Lines. In response to sub (tropical) climate, the study led to identification of a haploid inducer line with high HIR and good agronomic characters in tropics. Haploid HIR was determined from the first filial generation crosses of haploid inducer lines (Tail 7, 8, 9, Hybrid 9×7 and 9×8) crossed with the three-female population which included F₂ ligule less tester (PHD3*PHD8),

Normal population 1 (D1187-444) and Normal population 2 (D1187-453). Seedling morphological characteristics were used for putative haploid identification including seedling height, leaf size and color and plant vigor.

3.4.1 Procedure to determine Haploid induction rate on liguleless tester

For the liguleless tester, 960 seeds of each F₁ inducer cross were germinated in green house with three replicates of 320 seeds each (Appendix 6). The seed germination was done in trays filled with volcanic cinder. Watering was done regularly. After germination of the seedlings, morphological markers of ligule less tester were phenotyped 14 days after sowing, in growth stage of three leaves, Abendroth *et al.*, (2011) procedure. Emerson *et al.*, (1935) guidelines were also incorporated to classify putative haploids as plants that did not display a ligule and auricle in the three-leaf stage with their leaves oriented vertically. The induction rate was defined as the number of seedlings with haploid characters divided by all seedlings investigated (De André Harpes 2009) as follows;

Percent (%) HIR = $(\#HP/\#TP) \times 100$; where HIR = Haploid HIR, #HP = Number of haploids, and #TP = Total number of plants

3.4.2 Haploid induction rate on Normal populations 1 and 2

Random sampling of 1200 seeds from the F₁ seeds harvested from the cross of haploid inducer lines and the two female populations (Normal 1 and 2). Field layout was set covering an area of 1.2 ha with spacing of 75 cm by 25 cm. Planting was done in three replicates each with 400 seeds. The isolation distance between the plots was 1 meter. Agronomic management included watering and weeding done manually for 45 days. Data on the total number of plants which survived after 45 days was collected and recorded (Appendix 7). The putative haploids were determined from the whole

population using the morphological markers such as seedling vigour, plant height, leaf size and leaf colour.

3.5 Data Analysis

The data collected was subjected to analysis of variance (ANOVA) using R software version 3.2.3. Means of HIR and the agronomic characters were separated using the least significance difference (LSD) at 5% level of significance. The correlation coefficients among HIR and agronomic characters were done. The correlation coefficients were worked out using the CORR procedure (SAS Institute Inc., 2008) and the corresponding Pearson Correlation. Coefficients, simple statistics, and p-values were obtained. All correlation co efficiency tests were analyzed at $\alpha = 0.05\%$ significance level.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Agronomic performance on the seven TAILS

The results from morphological characters revealed significant difference at $P \leq 0.05$ level on most of the morphological agronomic characters tested (Table 4.1).

Table 4.1: Agronomic performance of haploid inducer lines

Inducer	Plant Height (cm)	Ear Height (cm)	NTB	Tassel size	DTA	DTS
First generation TAIL 7	120.16b	29.67d	12.43a	Large	50c	50b
First generation TAIL 8	125.40ab	38.00bc	10.95b	Large	51b	50b
First generation TAIL 9	117.67b	43.33b	8.50d	Medium	49d	48c
Second generation TAIL 008	96.92c	36.42c	10.78b	Large	54a	54a
Second generation TAIL 009	131.70a	68.67a	6.57e	Small	54a	54a
Hybrid Tail 9×7	119.92b	38.75bc	9.95bc	Large	48e	47d
Hybrid Tail 9×8	123.08ab	44.00b	9.47cd	Medium	49d	48c
MEAN	119.26	42.69	9.81		50.71	50.14
C.V	0.09	0.29	0.19		0.05	0.05

Means with same letter(s) within the column are not statistically different at $P \leq 0.05$
NTB-number of tassel branches, DTA-days to anthesis and DTS-days to silking

The agronomic results obtained were similar to field study carried out by Chaikam *et al.* (2016) on development and validation of red root marker of haploid inducers in maize. Their agronomic performances were well suited to tropical and sub-tropical climatic zones with better flowering, pollen production, resistance to tropical diseases and insects, hence making management less expensive in tropical environments. The study carried out in Kenya led to identification of the best haploid inducer line with the best agronomic performance of the variables tested. The seven TAILS tested

showed agronomic variations in some aspects which could have resulted from the different environmental conditions.

4.1.1 Plant height

Second generation Tail 009 was the tallest inducer with a height of 131.7cm, that was significantly ($P \leq 0.05$) different from all the others except first generation Tail 8 (125.40 cm) and Hybrid 9×8 (123.08), (Table 4.1). Second generation Tail 008 was the shortest with plant height of 96.92 cm, which was significantly ($P \leq 0.05$) different from the other six Tails. First generation Tails 7, 8, 9 and hybrids 9×8 and 9×7 were not significantly ($P \leq 0.05$) different from each other.

Variation in plant height could be attributed to differences existing in the genetic constitution of the TAILS maize varieties as earlier renowned by Ali *et al.* (2006). From the study, the first-generation Tails developed in collaboration with CIMMYT and Hohenheim University did not show variation of plant height with the hybrids tested. The second-generation Tails developed by CIMMYT had variation in plant height (Table 4.1). The results of haploid inducer lines to have small plant size concurred with the finding of Rotarenco *et al.* (2010). Due to the small plant size, most of the inducer lines cannot be used to produce haploids by open pollination in isolated fields, as they would not release enough pollen to pollinate the females. To overcome that, hybrids between inducers are often used. However, problems may appear in hybrid inducers, such as reducing the frequency of haploid induction and changing the expression of marker genes.

The call for breeders to focus on breeding taller haploid inducer lines with high HIR still needs to be emphasized as noted by Rotarenco *et al.* (2010). Tall plants have their tassels posed higher and this gives advantage of pollination as isolation blocks can be

used for open pollination rather than manual. This could reduce costs of expensive manual pollination as observed by Chaikamand Mahuku (2012). Haseeb-ur-Rehman *et al.* (2010) reported that increased plant height provides more green area for increased photosynthetic activities and assimilates needed for grain filling.

4.1.2 Ear height

The finding on table 4.1 revealed that first generation TAIL 7 (29.67 cm) was significantly ($P \leq 0.05$) different from the other Tails hence the shortest ear height. Second generation Tail 009 (68.67 cm) was significant ($P \leq 0.05$) different from the other six inducers and exhibited the highest ear height. First generation Tail 8, 9, hybrid 9×7 and hybrid 9×8 were not significantly ($P \leq 0.05$) different. The results from the study were comparable to finding of De AndréHarpers *et al.*, (2009) and Chaikam *et al.* (2016).

For the ear height, first generation Tails and hybrids showed variation except Tail 7. Attempts are currently being done at CIMMYT to develop better adapted inducer genotypes that can successfully be used for *in vivo* haploid induction in tropical environments. From the study, key values of a good haploid inducer line include adequate pollen production and good agronomic characteristics such as plant and ear height to facilitate maintenance, agreeing with the conclusions of De AndréHarpes (2009). The better adapted inducer genotypes can then be successfully used for *in vivo* haploid induction, improving maize breeding in tropical zones.

Height of the main ear is a vital characteristic for breeding (Zsuzsanna *et al.*, 2002). Higher ear height gives higher chances for more ears to develop from the nodes below. However, too high ear height can makes the weight of the ear to bend the stalk or even break it. Lower ear height is unfavourable for yield and makes harvesting a

challenge; but it does protect the stalk from excessive weight. Breeding in both direction has been attempted, but practical experience shows that the ideal height is somewhere in between neither too high nor too low. It is of great importance for the ears to be at the same height within a population.

Guidelines on the morphological characters of maize (*Zea mays* L.) are contained in UPOV TG/2, which includes plant height in the 1st group, as a genetically complex and environmentally stable trait. Both characters are measured together, in the middle of the waxy stage. From the study, the variations of plant height and ear height could have been influenced by the genetic background of the varieties and the many environmental effects. Competition for resources like light may influence plant height and ear height concurring with results of Mason *et al.* (1974) and Park *et al.* (1989). Habitat also has a considerable effect on the plant and ear height (El-Sherbieny *et al.*, 1991).

The information on plant height and ear height from the study offers additional knowledge that can be used by breeder to breed new cultivars of TAILS as formerly reported (Dwivedi *et al.*, 2007, Mba *et al.*, 2012, Collard *et al.*, 2008). These agronomic beneficial traits can further be exploited through markers and genomic regions associated with them for breeding better cultivars (Dwivedi *et al.*, 2007 and Varshney *et al.*, 2009). Plant height is considered during morphological descriptions and development of other trait because it is closely connected with such important characters as flowering date and yield.

4.1.3 Number of Tassel branches

First generation Tail 7 was the best haploid inducer line with the highest number of tassel branches (12.43), that was significantly ($P \leq 0.05$) different from all the other

Tails (Table 4.1). Second generation Tail 009 had the least number of tassel branches (6.57), that was significant ($P \leq 0.05$) different from the other six inducers. For the hybrids 9×7 and 9×8 there was no statistical difference observed on the number of tassel branches. Second generation Tails 008 (10.78) and 009 (6.57) had statistical difference at $P \leq 0.05$ with Tail 009 showing the least number of tassel branches. The number of tassel branches ranges from 6.57 to 12.43.

As observed by De André Harpes (2009), a good haploid inducer line should produce adequate pollen grains. Pollen grains production is related to the number of tassel branches and the capacity of all the branches to generate pollen grains. Many tassel branches gives an advantage of more pollen grain production by the haploid inducer lines. The study confirmed finding of Chaikam *et al.* (2015) in that, not all branches of the haploid inducer lines produce pollen grains hence the more the branches, the more likely hood of more branches producing pollen.

4.1.4 Tassel sizes

From the study, tassel size was determined from the number of tassel branches (Table 4.1) and grouped into three categories such as large, medium and small. Second generation Tails 009 had the least number of tassel branches and grouped to have “small” tassel size. The other 6 Tails had Tassel sizes ranging from Large to medium.

The study on morphological tassel traits in maize breeding programs are of importance as the inbred lines development aims at reducing the size and number of branches and maintaining enough pollen production, concurring with the finding of Duwick (2005), Fischer and Edmeades (2010). Traits of the tassel affect grain yield either physiologically by competing for photo- synthetics or physically by forming shade over the plant (Guei and Wasson, 1996). Scores from the study implied that the

more the number of tassel branches the larger the tassel size of the TAIL. However, when the tassels are large, they act as a drain for photo assimilates which could be directed toward grain production and also restricting the passage of solar radiation through the canopy (Edwards, 2011). Additionally, inhibitory effect on ear development can be caused by smaller tassels as they produce lower levels of auxins and decrease apical dominance agreeing with the findings of Sangoi *et al.*, (2006). In addition to the environment, it permits the production of one or more ear per plant. Number of tassel branches is considered vital for pollen yield (Vidal- Martínez *et al.*, 2001b).

Inducers with many tassel branches increases chances of getting enough pollen to pollinate the source population hence good seed set in the cobs, agreeing with conclusions of Khunsa *et al.* (2015). In maize hybrid breeding programmes, an ideal male parent is supposed to have large tassels that can produce large amounts of pollen whereas an ideal female should partition more assimilates towards big ears and hence should possess small tassels (Upadyayula *et al.*, 2006). This justifies the need for Haploid inducer lines used as male parents to have many tassels branches making it to have large tassel size hence increase chances of producing more pollen for pollination hereafter good seed set in the cobs. For production, larger tassels are selected to ensure sufficient and extended pollen availability in case of certain situations such as stress environments like drought or nutrients deficiency and maize variety (Sofi, 2007).

4.1.5 Anthesis days and Silking days

Days to anthesis ranged from 48 to 54 days (Table 4.1). Hybrid Tail 9×7 (48 days) had the least days to anthesis, that was significantly ($P \leq 0.05$) different from the other

Tails hence the best haploid inducer line. Second generation Tails 008 and 009 had no significant difference ($P \leq 0.05$) on anthesis day (AD) as they took the longest period (54 days) to reach anthesis compared to the other 5 haploid inducer lines. The results of maize plants scope of anthesis days earlier than the silking days collaborates with those of Harper *et al.* (2009). This implies that the pollination days can be done immediately silks appear hence promote good seed set in the cobs.

Findings of silking days agreed with those of Chaikam *et al.* (2016). Among the seven haploid inducer lines, silking days range from 47 days to 54 days (Table 4.1). Hybrid 9×7 (47 days) had the least days to silking while second generation Tail 008 and 009 had the longest days to silking (54 days).

In Maize, male flowering occurs first and silking can be delayed especially under unfavourable conditions. In the study conducted, most inducers had a day interval between anthesis and silking, while others had same anthesis and silking. Silking days when in rhyme with anthesis days is an advantage as pollination could be done when pollen grains are highly viable hence promoting good seed set in the cobs. The anthesis days and silking days are quite important when selecting a haploid inducer line as they determine the pollination time and seed set in cobs. De AndréHarpes (2009) observed anthesis-silking interval is an indicator of stress adaptation. The shorter the interval, the better the plant tolerates the stress. Drought affects maize production in most parts of Kenya considering that over 80% of the land is arid and semi-arid. Edmeades *et al.* (2000) consistently showed that a short ASI is a strong measure of drought tolerance in maize genotypes. Betrán *et al.* (2003) reported that under drought stress, grain yield had a low heritability but was positively correlated with ASI suggesting that these secondary traits could be applied in breeding for

drought stress tolerance. This implies that breeding haploid inducer lines which have short ASI in tropic climate zones makes them to be tolerant to drought. Under water limiting conditions, selection of a shorter ASI would therefore result into increased yield, concurring with finding of Bänziger and Lafitte (1997).

4.1.6 Pollen viability as determined by time factor and haploid inducer lines

Pollen viability analysis was done on different haploid inducer lines at different time of the day (Table 4.2) on the 3rd anthesis day.

Table 4.2: Pollen viability by time of the day

Time	% viability
8.00 am	98.45a
10.00 am	97.89a
12.00 noon	98.77a
2.00 pm	94.17b
4.00 pm	95.47b
Mean	96.95
CV	0.02

Means with same letter(s) within the column are not statistically different at $P \leq 0.05$

Higher pollen viability was obtained in the morning hours (8.00 am to 12.00 noon), as compared to those in the afternoon hours (2.00 pm to 4.00 pm). At 12.00 noon pollen viability was at its peak (98.77%), though not significantly ($p \leq 0.05$) different to those at 8.00 am and 10.00 am (Table 4.2).

Table 4.3: Pollen viability of haploid inducer line as influenced by TAIL

Inducers	% viability
First generation TAIL 7	98.45a
First generation TAIL 8	96.88ab
First generation TAIL 9	96.77b
Second generation TAIL 008	94.40c
Second generation TAIL 009	92.77d
HYBRID 9×7	97.21ab
HYBRID 9×8	97.11ab
Mean	96.22
CV	0.02

Means with same letter(s) within the column are not statistically different at $P \leq 0.05$

Different haploid inducer lines showed variation in pollen viability tested on day 3 of pollen shed (Table 4.3). First generation Tail 7 exhibited the highest percentage of pollen viability (98.45%), that was significantly ($P \leq 0.05$) different from the other haploid inducer lines except first generation Tail 8, hybrid 9×7 and 9×8. Second generation Tail 009 had the lowest percentage pollen viability (92.77%), that was significantly ($P \leq 0.05$) different from all the other Tails. The 7 haploid inducer lines tested had pollen viability of more than 90%.

Study of pollen viability was of great importance as it gave information on the best time to carry out pollination and the haploid inducer line with the highest pollen viability. Viability of pollen was witnessed to reduce with time after release under natural condition, agreeing with conclusions of Ferreira *et al.* (2007). This could have been due to intrinsic factors such as state of physiological maturation of the pollen grain, its origin, genetic characteristics and plant nutrition. Extrinsic factors which could have contributes to loss of viability with time include composition of the culture medium, pollen density in the medium, temperature and incubation time, collection

period, environmental conditions such as temperature and humidity concurring with findings of Stanley and Linskens (1974). Costa *et al.* (2012) when studying the effect of collection time on the viability of maize pollen, reported that regardless of the cultivar, the first hours of release of maize pollen grains occur in the morning (8:00 to 12:00 a.m.) hence being more suitable for pollination. From the study, pollen viability was highest at 12.00 noon. These results were similar those of Maeda *et al.* (2012) on *in vitro* assessment of the viability of maize pollen, achieving the best results at 10:00 a.m. Reduction in pollen viability from morning to afternoon hours could have resulted from high temperature stress during pre-anthesis, which could lead to decreased seed set as observed by Prasad *et al.*(2008). Pollination done from morning to noon could lead to better seed set on the female population than pollination in after noon hours. These results revealed that pollination could be timed to be done at different times of the day, with morning to noon hours giving the highest percentage of viable pollen grains compared to after noon hours. More viable pollen increases the chances of fertilization hence good seed set.

4.2 Haploid HIR on TAILS and the female populations

Table 4.4: Haploid induction rate recorded in different TAILS

Haploid inducer line	% HIR
First generation TAIL 7	6.17c
First generation TAIL 8	8.48a
First generation TAIL 9	7.77ab
Hybrid TAIL 9×7	7.37abc
Hybrid TAIL 9×8	6.81bc
Means	7.32
CV	0.12

Means with same letter(s) within the column are not statistically different at $P \leq 0.05$

The five haploid inducer lines evaluated had haploid HIR variation from 6.17 to 8.48% (Table 4.4). First generation Tail 8 had the highest haploid HIR of 8.48%, that was significantly ($P \leq 0.05$) different from the other Tails except first generation Tail 9 and hybrid 9×7, while first generation 7 had the least HIR of 6.17%, that was significantly different ($P \leq 0.05$) from the other Tails except hybrid Tail 9×7 and 9×8.

Table 4.5: Haploid HIR on female population

Female population	% HIR
Normal population 2 (D1187-453)	8.49a
Ligule less Tester	7.62a
Normal population 1 (D1187-444)	5.86b
Means	7.32
CV	0.18

Means with same letter(s) within the column are not statistically different at $P \leq 0.05$

For the three female populations, HIR varied from 5.86% to 8.49%. Normal population 2(D1187-453) had the highest HIR of 8.49%, that was significantly ($P \leq 0.05$) different from the other female populations except ligule less tester (Table 4.5). Normal population 1(D1187-444) had the least HIR of 5.86%, that was significantly different ($P \leq 0.05$) from all the other female populations.

Initially, 7 haploid inducer lines which include first generation Tails (Tail 7, 8 and 9), hybrids Tail (9×7 and Tail 9×8) and second generation Tails (008 and 009) were to be evaluated for HIR. After crossing with the three female populations, the second-generation Tails did not produce enough seeds for HIR evaluation. This was probably caused by longer days to anthesis (54 days) which did not match with silking days of

the female populations as they were shorter with around 5 days (49 days) leading to poor seed set in the cobs.

The results obtained on HIR for the five haploid inducer lines evaluated (1GTAILS 7, 8, 9, hybrid 9×7 and 9×8) were similar to findings of (Chaikamet *et al.*, 2016). From the study, the haploid seedlings exhibited reduced plant vigor, whereas diploid individuals resembled the donor plant and were vigorous concurring with findings of Chase (1952) and Auger *et al.* (2004). Haploid seedlings had narrow erect leaves which were pale green while diploids had broad drooping leaves that were deep green. Haploid seedlings were also shorter than the diploids ones.

The difference on the haploid HIR could have been brought about by a number of factors. The inducer line used as a pollinator could have influenced haploid HIR as difference inducer lines have different capacities of induction. Environmental conditions during the growing period of inducer lines such as drought, pest and diseases and infertile soils could have influenced haploid HIR as they affects the general plant growth, concurring with findings of Kebede *et al.*, (2011). Other factors which could have influence the haploid HIR were method used to cross pollination inducer lines and the female population as observed by Rotarencu, Dicu and Sarmaniuc (2009). The type of source germplasm used to determine the haploid HIR also affect induction rate (Prigge *et al.*, 2012; Wu *et al.*, 2014).

The most reliable system of haploid identification is based on morphological characters displayed by recessive genes on female population of ligule less or glossy tester (Neuffer *et al.*, 1997). In this study, ligule less tester was used with two other normal populations to evaluate the HIR. The normal population 2 and ligule less tester gave relatively higher HIR than normal population 1 which showed least HIR. This

could have resulted from the different germ plasm of the female population (source germ plasm) used as a maternal parent, as it could have affected the HIR, in accord with the results of Kebede *et al.* (2011), Prigge *et al.* (2012) and Wu *et al.* (2014). Studies of genotypic differences in the source germplasm for its response to haploid induction are crucial for HIR increase, because both parents have an effect HIR in maize as seeing in the conclusions of (Chase 1952, 1969, Coe 1959, Lashermes and Beckert 1988, Eder and Chalyk 2002).

4.3 Correlation coefficients among morphological characteristics and HIR of TAILS

Correlation coefficient analysis was carried out between haploid HIR for three female population and the morphological characteristics of the TAILS, which included plant height, ear height, the number of tassel branches, anthesis days and silking days (Table 4.6). Correlation coefficient on HIR and pollen viability with respect to time factor was also done (Table 4.7).

4.3.1 Correlation coefficient on HIR of ligule less tester and plant height

Table 4.6: Correlation between HIR and 5 Agronomic variables among 3 female populations

Female population	Plant height	Ear height	NTB	DTA	DTS
Ligule less tester	0.539*	0.396 N/S	-0.017 N/S	0.618*	0.395N/S
NP1	-0.496 N/S	0.069 N/S	-0.436 N/S	-0.363 N/S	-0.473N/S
NP2	0.196 N/S	0.317 N/S	-0.387 N/S	0.028 NS	-0.145N/S

*Correlation is significant at $p \leq 0.05$ level. N/S not significant at $P \leq 0.05$. NTB-number of tassel branches, DTA-days to anthesis and DTS-days to silking

The results revealed that HIR can be affected either positively or negatively by an agronomic character. The type of female population used to determine HIR can also have an impact on the correlation coefficient of agronomic characters. Out of the 18 variables analysed in the three female populations, three variables had moderate positive linear relationship and one factor moderate negative linear relationship which were statistically different at $p \leq 0.05$.

There was a positive and significant correlation between HIR and plant height (0.539) at $p \leq 0.05$ for ligule less tester (Table 4.6). The significant correlation coefficient was only witnessed in the ligule less tester and not the other two normal populations. This could have been due to presence of the recessive genes conferred by the morphological characteristics of ligule less tester or glossy tester hence suitable source population for haploid identification assenting with conclusions of Neuffer *et al.* (1997). Positive correlation means that if the value of one variable increases, the value of the other variable also increases and vice versa. The higher the coefficient of correlation, the stronger the relationship. From the study, the positive significant correlation on HIR and plant height implied that increase of plant height led to increase in HIR. Generally, haploid inducer lines display short plant heights and this has been an area of focus by many researchers to improve their heights, concurring with results of Rotarengo *et al.* (2010). Inducer with good plant height makes management practices such as pollination among others easier. Plant height can also impact mode of pollination, replacing hand pollination with open pollination in isolated blocks hence reduce cost of labour, bagging materials among other inputs, agreeing with findings of Chaikam *et al.* (2016). Taller haploid inducer lines showed higher HIR on ligule less tester.

4.3.2 Correlation on HIR of Ligule less tester and Anthesis Days

There was positive and significant ($P \leq 0.05$) correlation between HIR and days to anthesis (0.618) (Table 4.6). Anthesis days are crucial in determination of HIR as pollen production influences seed set in the cobs once pollination is done in good time. Increase in anthesis days showed increase in HIR. This could have led to pollen grains production spreading for longer period increasing chances of pollination of the source population. For the normal population 1 and 2, HIR and some morphological character revealed positive correlation while others showed negative correlation but there was no significant ($P \leq 0.05$) difference (Table 4.6).

4.3.3 Correlation of HIR of Normal population 1 (D1187-444) on pollen viability per time

Table 4.7: Correlation between HIR and pollen viability against time among 3 female populations

Female	8.00 am	10.00 am	12.00 noon	2.00 pm	4.00 pm
Ligule less tester	-0.099N/S	-0.457N/S	-0.305N/S	-0.259N/S	-0.211N/S
NP1	-0.184N/S	-0.399N/S	0.569*	-0.351N/S	-0.032N/S
NP2	-0.209N/S	-0.162N/S	-0.254N/S	0.045N/S	-0.623*

*. Correlation is significant at $p \leq 0.05$ level. N/S: Not significant at $p \leq 0.05$

There was a positive and significant ($P \leq 0.05$) correlation (0.569) between HIR and pollen viability at 12.00 noon (Table 4.7). The release of pollen grains can start from dawn to noon, depending on the temperature, humidity and genetic constitution of the plant concurring with results of Ferreira *et al.* (2007). Pollen viability increases chances of fertilization hence higher seed set in the cob. This would have a positive impact during evaluation of seedlings after planting for HIR as more plants would give precise numbers for evaluation. From the analysis of pollen viability against

time, results revealed viability been high in the morning hours till noon than the afternoon hours, hence pollination done at that time leads to good seed set in the cobs.

4.3.4 Correlation of HIR of Normal population 2 (D1187-453) on pollen viability per time

There was a negative and significant ($P \leq 0.05$) correlation (-0.623) between HIR and pollen viability at 4.00 pm (Table 4.7). This implied that decrease in pollen viability per time had an impact in HIR. This could have resulted from poor seed set in the cobs due to poor fertilization as most of the pollen grains had denatured at 4.00 pm. Pollen morphology can be interfered with by high temperature stress during floret development and results in an abnormal exine wall, degeneration of tapetum cells, and membrane damage, leading to pollen sterility in grain sorghum, which is a C4 plant of maize as observed by Hammer *et al.* (1993), Djanaguiraman *et al.* (2014), wheat, Prasad and Djanaguiraman,(2014), and soybean Djanaguiraman *et al.* (2013a, b). High temperatures and low relative humid could have caused the denaturing of pollen grains hence the negative correlation at 4.00pm. From analysis of pollen viability with respect to time, viability decreases in the afternoon hours from 2.00 pm hence this can lead to decrease in HIR.

CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

There were significant differences on most of the agronomic characters scored among the seven haploid inducer lines. The second-generation Tail 009(131.7 cm) was the tallest while Tail 008 (96.92 cm) was the shortest. The second generation Tail 009 had the highest ear height while first generation Tail 7 the least ear height. Second generation Tail 009 had the least number of tassel branches hence “small” tassel size, while Tail 7 had the best number of tassel branches hence “large” tassel size. The second generation Tails 008 and 009 had equal DTA and DTS and were also the latest (54 days) to flower. The others (Tail 7, 8, 9, hybrid 9×7 and 9×8), anthesis and silking days ranged between 47 to 50 days. On pollen viability, it was concluded that the best time to carry out pollination was morning hours before noon (8 am to 12 noon) as viability at that time is high compared to afternoon hours (2pm to 4 pm). First generation Tails (7, 8, 9) and their hybrids (9×7 and 9×8) had relatively higher pollen viability percentage compared to second generation Tails (008 and 009). Generally, pollen viability was above 90% for the TAILS tested.

There were significant differences among the five haploid inducer lines tested on the three female populations for HIR. For the female populations, ligule less tester (7.62%) and normal population 2 (8.49%) had the best HIR compared to normal population 1(5.86%). First generation Tail 8 was the best haploid inducer line with highest HIR of 8.48%. 1GTail 7 had the least HIR of 6.17%.

On correlation of HIR on the female populations and agronomic characters of the TAILS, ligule less tester had positive significant ($P \leq 0.05$) correlation with plant height and anthesis days. The other two populations (Normal 1 and 2) did not show a

significant correlation on HIR and agronomic characters such as plant height, ear height, number of tassel branches, anthesis and silking days. Among the three female populations, HIR for normal population 1 revealed a positive significant ($P < 0.05$) correlation on pollen viability at 12 noon. HIR for normal population 2 showed negative significant correlations on pollen viability at 4 pm. The ligule less tester did not show significant correlation on HIR with pollen viability. The findings from the experiment rejected the study hypotheses.

5.2 Recommendations

From the analyses and discussion of the results, the following are recommended:

- i. Ligule less tester and normal population 2 can further be used for assessing other inducers lines for haploid induction.
- ii. Haploid inducer line with the highest HIR such as first generation Tail 8 can be used widely for commercial production of homozygous lines.
- iii. More studies to be done on adaptability of the tropicalized haploid inducer lines to tropical conditions of Africa.
- iv. Other methods need to be employed on HIR studies and their results compared to identify the most efficient haploid inducer lines which do not give false positives.

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APPENDICES

Appendix 1:

Means, 95% confidence intervals, and ranges associated with haploid HIRs of seedling morphology in first generation Tails and hybrids haploid inducer lines assessed based on a ligule less tester

Inducer	No. of testcross seedlings evaluated	HIR mean	HIR confidence interval	HIR range
First generation TAIL 7	659	6.28	6.25-6.31	5.85-6.90
First generation TAIL 8	761	10.92	10.76-11.08	7.97-13.29
First generation TAIL 9	802	7.01	6.69-7.31	6.51-7.86
Hybrid 9×7	847	6.86	6.38-7.34	6.47-7.43
Hybrid 9×8	822	7.02	7.03-7.02	6.93-7.12

Appendix 2:

Means, 95% confidence intervals, and ranges associated with haploid HIRs of plant morphological characters in first generation Tails and hybrid haploid inducer lines assessed based on normal population 1(D1187-444)

Inducer	No. of Testcrossseedlings evaluated	HIR mean	HIR confidence interval	HIR range
First generation TAIL 7	1043	5.24	5.20-5.28	4.60-5.90
First generation TAIL 8	1021	5.04	4.94-5.14	3.50-6.73
First generation TAIL 9	972	7.09	7.03-7.15	6.01-8.02
Hybrid 9×7	1032	6.52	6.50-6.54	6.34-6.83
Hybrid 9×8	944	5.38	5.29-5.47	4.18-7.19

Appendix 3

Means, 95% confidence intervals, and ranges associated with haploid HIRs of plant morphological characters in first generation Tails and hybrids haploid inducer lines assessed based on Normal population 2 (D1187-453)

Inducer	No. of testcross seedlings evaluated	HIR mean	HIR confidence interval	HIR range
First generation TAIL 7	929	6.98	6.28-7.08	5.56-8.83
First generation TAIL 8	978	9.48	9.47-9.49	9.38-9.58
First generation TAIL 9	941	9.22	9.09-9.35	7.74-11.72
Hybrid 9×7	1018	8.72	7.88-9.56	7.44-10.17
Hybrid 9×8	968	8.04	7.95-8.13	6.57-9.56

Appendix 4

Data collection on Plant height, ear height and count of tassel branches



Plate 4: (A) Measuring plant height, (B) measuring ear height (C) counting number of tassel branches

Appendix 5: Determination of pollen viability;

Plate 5: (A) Pollen harvesting in field, (B) Staining pollen grains with TTC solution, (C) Drying pollen in oven, (D) Samples of dried pollen grains and (E) Viewing under light microscope and recording pollen viability scores.

Appendix 6 Assessment of Haploid plants of ligule less tester

Plate6: (A) Planting ligule less seeds in trays, (B) Field survey prior to data scoring, (C) Haploid and diploid Seedling identification, (D) Sampled haploid and diploid seedlings

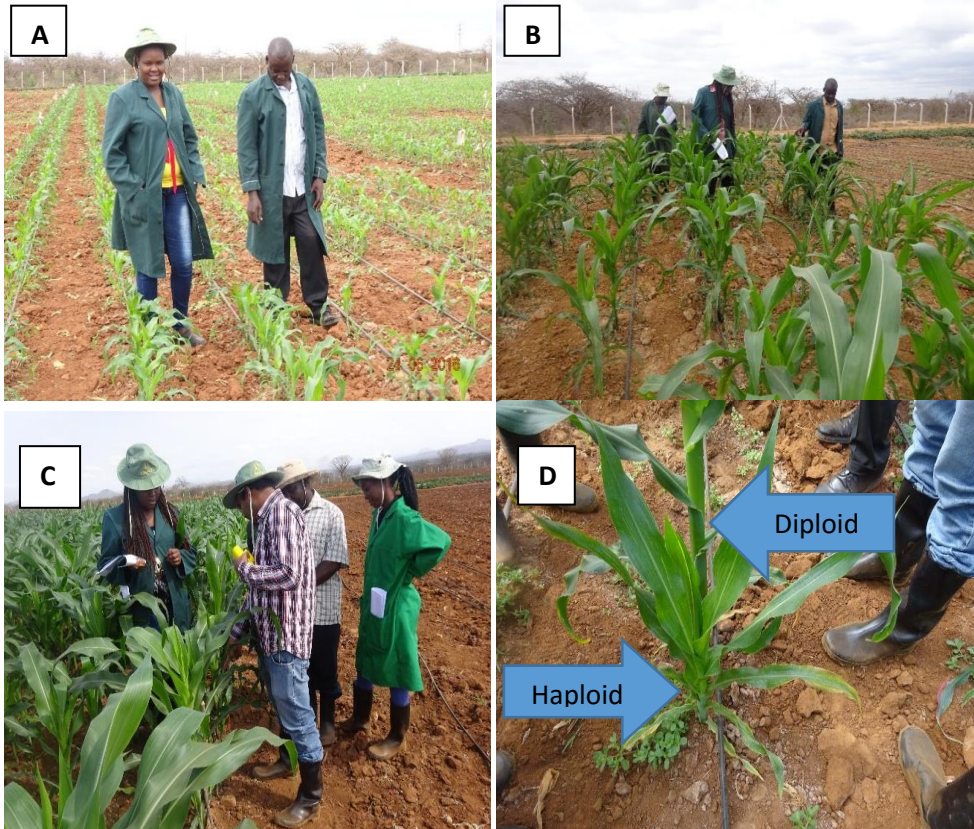
Appendix 7:**Evaluation of haploid plants on normal population 1 and 2:**

Plate 7: (A) Field survey 2 weeks after planting, (B) Data collection on 45th day after planting, (C) Data scoring on haploids and diploids and (D) Haploid and diploid plants.

Appendix 8: Approval Letter by Graduate School



KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke

Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 810901 Ext. 57530

Internal Memo

FROM: Dean, Graduate School

DATE: 4th February, 2016

TO: Janet Mwendu Nzamu
C/o Agricultural Science and Technology

REF: A143/27689/2013

SUBJECT: APPROVAL OF RESEARCH PROPOSAL
=====

This is to inform you that Graduate School Board, at its meeting of 27th January, 2016 approved your Research Proposal for the M.Sc Degree Entitled, "Assessment of Pollen Viability and Haploid Induction Capacity of Tropicalised Inducer Lines in Makueni County, Kenya".

You may now proceed with your Data Collection, subject to clearance with Director General, National Commission for Science, Technology and Innovation.

As you embark on your data collection, please note that you will be required to submit to Graduate School completed Supervision Tracking Forms per semester. The form has been developed to replace the Progress Report Forms. The Supervision Tracking Forms are available at the University's Website under Graduate School webpage downloads.

Thank you.

EDWIN OBUNG'U
FOR: DEAN, GRADUATE SCHOOL

c.c. - Chairman, Department of Agricultural Science and Technology

Supervisors:

1. Dr. Wilson M. Thagana
C/o Department of Agricultural Science and Technology
Kenyatta University
2. Dr. Stephen N. Mugo
Principal Scientist/Maize Breeder
International Maize and Wheat Improvement Center (CIMMYT)
C/o Department of Agricultural Science and Technology
Kenyatta University

EO/rwm

Appendix 9: Research Authorisation Letter by Graduate School**KENYATTA UNIVERSITY
GRADUATE SCHOOL**E-mail: dean-graduate@ku.ac.keWebsite: www.ku.ac.keP.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 8710901 Ext. 57530

Our Ref: A143/27689/2013

DATE: 4th February, 2016Director General,
National Commission for Science, Technology
and Innovation
P.O. Box 30623-00100
NAIROBI

Dear Sir/Madam,

**RE: RESEARCH AUTHORIZATION FOR JANET MWENDE NZAMU- REG. NO.
A143/27689/2013**I write to introduce Ms. Janet Mwendu Nzamu who is a Postgraduate Student of this University. She is registered for M.Sc degree programme in the **Department of Agricultural Science and Technology**.Ms. Nzamu intends to conduct research for a M.Sc thesis Proposal entitled, "**Assessment of Pollen Viability and Haploid Induction Capacity of Tropicalised Inducer Lines in Makuani County, Kenya**".

Any assistance given will be highly appreciated.

Yours faithfully,


MRS. LUCY N. MBAABU
FOR: DEAN, GRADUATE SCHOOL

EO/rwm