INVESTIGATION OF ETHREL GAMETOCIDE IN FINGER MILLET (*Eleusine coracana,* L. Gaertn) HYBRIDS THROUGH GENETIC ANALYSIS

By

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DECLARATION

Declaration by the Candidate

This thesis is my original work and has not been presented for a degree in any other University. No part of this thesis may be reproduced without the prior written permission of the author and/or University of Eldoret.

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DEDICATION

This thesis is dedicated to my dear parents; Mr. John Kunguni Bateta and Mrs. Juliah Nasimiyu Kunguni.

ABSTRACT

Finger millet is a staple food crop of in Africa due to; desirable attributes like being highly nutritious, good grain storagability and strong cultural value. In Kenya, therefore, finger millet is on high demand for food, culture and trade. Low yield due to poor genetic potential varieties necessitate breeding for better varieties. Due to its floral architecture, finger millet is mostly self- pollinating with only 1% chances of cross pollination thus difficult to hybridize. Several plant emasculation techniques including mechanical, genetic and chemical exist, and applied depending on crop species, genetics and floral architecture. The study objectives were to determine optimal ethrel GL for effective emasculation and hybridization; determining the effect of ethrel on agronomic traits and identifying true F_1 hybrids from parent selfs using morphological and molecular techniques. This study investigated ethrel efficacy at three levels (1,500ppm, 1,750ppm and 2,000ppm) against 0ppm check in a 6x6 diallel crossing of six elite Kenyan varieties Okhale-1, Gulu-E, KACCIMI 72, IE 2872, IE 4115 and U-15 having contrasting traits. Ethrel was applied in a screen house at Zadoks plant developmental stage 45. The female parent harvested (F_1) seeds were planted and post emasculated generation screened in the field using morphological traits, and in the laboratory using molecular techniques, to identify hybrids. Field screening, involved evaluation of head to row plantsfrom female parent pollinated heads in parent pair blocks for successful crosses and possible gametocide effect on post emasculated generation. Morphological heterozygote success rate in the treatments was significantly highest at 2,000 ppm (63.98%), followed by 1,750 ppm (29.78%), 1,500 ppm (9.66%) and least at 0 ppm (1.65%). In the molecular screening, four different Single Nucleotide Polymorphism (SNP) markers were used to detect hybrids at F_1. Heterozygosity level in F_1s from selfs was effective with 2,000ppm (5.05%) being the highest followed by 1,750ppm and 1,500ppm (4.58%) and 0ppm having the least (4.27%). The optimal GL concentration that resulted in high hybrids was 2,000ppm. Ethrel gametocide affected some physiological growth factors of finger millet like plant height, ear exertion length and grain weight, but, has no effect on post emasculated and residual generation. Further studies should be done beyond 2,000ppm to see the difference in success rate. In order to check on progeny segregation F_2 and subsequent generations should be planted further.

TABLE OF CONTENTS

DECLARATION i
Declaration by the Candidate i
Declaration by Supervisors i
DEDICATION ii
ABSTRACT iii
LIST OF TABLES vii
LIST OF FIGURES viii
LIST OF PLATES ix
LIST OF APPENDICES x
LIST OF ACRONYMS AND ABBREVIATIONS xi
ACKNOWLEDGEMENT xii
CHAPTER ONE 1
INTRODUCTION 1
1.1 Background Information 1
1.2 Statement of the Problem and Justification 5
1.3 Objectives 6
1.3.1 General Objective 6
1.3.2 Specific Objectives 6
1.4 Hypotheses 6
CHAPTER TWO 8
LITERATURE REVIEW 8
2.1 Genetics of Finger Millet 8
2.2 Morphology and Cultivation 11
2.3 Global Importance 13
2.4 Production Constraints 16
2.5 Finger Millet Breeding 17
2.6 Diallel Analysis 18
2.7 Plant Emasculation Techniques 19
2.7.1 Mechanical Emasculation 19
2.7.2 Genetic Emasculation 20

2.7.3 Chemical Emasculation 25

2.8 Zadoks Developmental Stages in Cereals 27

2.9 Detection and Selection of True F_1 Hybrids 28

2.9.1 Morphological Markers 29

2.9.2 Biochemical Markers 30

2.9.3 Molecular Markers 30

2.9.3.1 Microsatellites 31

2.9.3.2 SNP Markers 32

2.10 High resolution melting (HRM) Analysis 34

CHAPTER THREE 37

MATERIALS AND METHODS 37

3.1 Study Site 37

3.2 Plant Materials 37

3.3 Screen House Study 38

3.3.1. Parent Arrangement and Ethrel Treatment 38

3.3.2 Plant Hybridization 41

3.3.3 Data collection 42

3.3.3.1 Days to Heading 42

3.3.3.2 Days to Flowering 42

3.3.3.3 Days to Maturity 42

3.3.3.4 Plant Height (cm) 42

3.3.3.5 Number of Productive Tillers per Plant 43

3.3.3.6 Ear Exertion Length (mm) 43

3.3.4.7 Plant Grain Weight (Gms) 43

3.4 Field Screening of F_1s 43

3.4.1 Site Climatic and Edaphic Conditions 43

3.4.2 Field Lay-out 43

3.5 Molecular Screening for F_1 45

3.5.1 Laboratory Screening for True F_1s from Parents 45

3.5.2 DNA Extraction Protocol by CTAB 45

3.5.3 Genomic DNA, Quality and Quantity 48

3.5.4 SNP Primer Design 49
3.5.5 Finger Millet SNP Markers..49
3.5.6 Polymerase Chain Reaction (PCR) 50
3.5.7 PCR Run on High Resolution Melting Analysis for Screening F_1s 52
3.6 Data Analysis 53
CHAPTER FOUR 54
RESULTS..54
4.1 Screen House Ethrel Application and Agronomic Traits Study. 54
4.1.1 Production of Controlled Crosses 54
4.1.2 Preclusive study on Ethrel gametocide..54
4.1.3 Effect of Ethrel on Finger Millet Agronomic Traits 55
4.2 Field Study on Post Emasculation Generation Plants..60
4.2.1 Field F_1 Screening from Selfs 60
4.3 Laboratory Screening for True F_1s from Parents. 62
4.3.1 Genomic DNA Quality and Quantity. 62
4.3.2 Polymerase Chain Reaction. 63
4.3.3 High Resolution Melting Analysis (HRMA). 65
CHAPTER FIVE..69
DISCUSSION..69
5.1 Screen house and Field Study 69
5.2 Molecular Analysis..74
CHAPTER SIX 77
CONCLUSIONS AND RECOMMENDATIONS 77
6.1 Conclusions 77
6.2 Recommendations 77
REFERENCES 79
APPENDICES 104

LIST OF TABLES

Table 2.1: Nutritive content of finger millet as compared with other cereals 14
Table 2.2: Developmental stages in cereals 28
Table 2.3: Chronological evolution of molecular makers 31
Table 3.1: Finger millet varieties used in the study 38
Table 3.2: Field arrangement of plants in a block 45
Table 3.3: Total number of samples used in DNA extraction 48
Table 3.4: Four finger millet SNP primers 50
Table 3.5: Concentrations and volumes for each PCR reaction for SNP primer 51
Table 3.6: Master Mix preparation (1x volume) 52
Table 4.1: Percent pair success rate of true F_1 crosses 55
Table 4.2: Analysis of variance mean squares for finger millet agronomic traits as influenced by gametocide level 56
Table 4.3: Screen house agronomic trait means under different gametocide levels (GL) across six finger millet varieties per plant 57
Table 4.4: Grain weight means for the 15 crosses generated from six finger millet genotypes 58
Table 4.5: Fischer's protected least significant differences for comparing means of finger millet post ethrel treated generation 62
Table 4.6: Genetic analysis of the 120 progenies alongside six finger millet varieties across GLs and their recipricals 66

LIST OF FIGURES

Figure 2. 1: Tablet screen shot showing SNP location..33
Figure 3.1: Parent arrangement in the screen house for 6×6 diallel mating 40
Figure 4. 1: Mean gametocide effect frequency across seven parameters for the six genotypes crossed 60
Figure 4.2: Gametocide level percent proportion on Post ethrel treated generation showing number of recorded F_1s 61
Figure 4.3: Agarose gel (2%) zymogram of parental DNA PCR amplification products for SNP 2(1458-1992 L and 1458-1992 R) run under a PCR touch-down program with annealing temperature ranging from 60°C - 55°C 63
Figure 4.4: Agarose gel (2%) zymogram of parental DNA PCR amplification products for SNP 3(1925-2474 L and 1925-2474 R) run under a PCR touch-down program with annealing temperature ranging from 65°C - 60°C 64
Figure 4.5: Agarose gel (2%) zymogram of parental DNA PCR amplification products for SNP 4(2837-3585 L and 2837-3585 R) on the left and SNP 5(3502-4102 L and 3502-4102 R) on the right run under a PCR touch-down program with annealing temperature ranging from 63°C 64
Figure 4.6: Fluorescence change over increasing temperature range for GE, KA-72 and heterozygote PCR amplification products 67
Figure 4.7: Melt profile for GE, KA-72 and heterozygote PCR amplification products from HRM analysis..68

LIST OF PLATES

Plate 2. 1: Finger millet ear shapes: (a) open, (b) fist and (c) incurved 12
Plate 2. 2: Finger millet grain colours: (a) white, (b) red and (c) light brown..................12
Plate 4. 1: Ethrel emasculated and pollinated finger millet head.....................................54

LIST OF APPENDICES

Appendix I: Field planting layout 104
Appendix II: Preparation of TBE buffer..106
Appendix III: Grain weight across the 15 cross replicates generated from the six genotypes..107
Appendix IV: Observed morphological traits for the six genotypes used in the screen house study 109
Appendix V: Plant height across the 15 cross replicates generated from the six genotypes 110
Appendix VI: Ear exertion length across the 15 cross replicates generated from the six genotypes..112
Appendix VII: Number of productive tillers across the 15 cross replicates generated from the six genotypes...114
Appendix VIII: Nanodrop© readings for all the samples...116

LIST OF ACRONYMS AND ABBREVIATIONS

AFLPs	Amplified Fragment Length Polymorphism
CHA	Chemical Hybridizing Agent
CRD	Completely Randomized Design
CTAB	Cetyl trimethylammonium bromide
dNTPs	Deoxynucleotide triphosphates
DS	Developmental Stage
ETHREL®	2-chloroethylphosphonic acid
FAOSTAT	Food and Agricultural Organization Statistics
GL	Gametocide Level
ICRISAT	International Crops Research Institute for Semi-Arid Tropics
KALRO	Kenya Agricultural and Livestock Research Organization
ng	nanogram
ISSR	Inter-Simple Sequence Repeat
PCR	Polymerase Chain Reaction
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
SSR	Simple Sequence Repeat
SNPs	Single Nucleotide Polymorphisms
U	Units
µl	Micro litre
UV	Ultra Violet
HRMA	High Resolution Melting Analysis

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CHAPTER ONE

INTRODUCTION

1.1 Background Information

Archeological and linguistic evidence suggests that farmers in eastern Africa were cultivating finger millet before the last 5,000 years (Klichowska, 1984). The exact area of domestication is not yet known but there are suggestions that it is either Uganda or the Ethiopian highlands (Bennetzen et al., 2003). Nevertheless, there is proof that finger millet originated from, and was first domesticated in the eastern African sub-humid uplands (Dida et al., 2008). It was then introduced to South Asia (India) from its centre of origin by sea in the third millennium B.C (Bennetzen et al., 2003). From here, the spread of finger millet cultivation extended to other parts of the world.

Finger millet (*Eleusine coracana,* L. Gaertn) is widely grown in environments having different ranges of rainfall and an annual temperature range of 11°C to 27°C in regions of Africa and Asia and is reported to tolerate soils with pH of 5.0-8.2 (ICRISAT/FAO, 1996). In Africa it is cultivated mainly in central, southern and eastern parts (Obilana & Manyasa, 2002), where it serves as a subsistence and a food security crop given its nutritive and cultural value, and good storability (Holt, 2000). Currently, finger millet is gaining popularity among communities in both Africa and Asia because of its nutritional importance (Falcon & Naylor, 2005).

Finger millet is highly valued by most farmers because it serves as a reserve food in times of famine and a staple food crop in the semi arid tropics of Africa (ICRISAT/FAO, 1996; Obilana & Manyasa, 2002). The food products of finger millet include fermented and

non-fermented porridge, pancake-like flat breads and fermented alcoholic and non-alcoholic beverages (ICRISAT/FAO, 1996). Finger millet contains high levels of vitamin B, folic acid, phosphorus, iron, potassium and 16 times much more calcium than maize (Saturni et al., 2010). When produced and consumed together with rice, finger millet can solve anaemia deficiency especially in women (Dida & Devos, 2006). In terms of malting qualities, finger millet can be used in solving malnutrition that kills millions of babies throughout the tropics because it is cheap and nutritious (Pawar & Dhanvijay, 2007). It is superior to other cereals and ranks second to barley in malting quality (National Research Council, 1996). According to Riley et al. (1992), finger millet has the potential to improve resource management and serve as a staple food, weaning food for infants, or a cash crop which provides income generating opportunities for most families in rural Africa.

Although consumption demand for finger millet is increasing, yield on farmer's field is low, at about 15-16% of its potential in Kenya (Oduori, 1998). The low yield is attributed to low research priority, limited uses, difficulty in crop management, lack of improved varieties, poor crop husbandry, competition from other crops like maize (*Zea mays*) and sorghum (*Sorghum bicolor*), lack of commercial food products, poor technology in processing, pests and diseases including *Striga*, lodging and moisture stress in dry areas (Mitaru et al., 1993; Oduori, 1993; Mushonga et al.,1993).

Cross-pollination of inbred lines resulting in the creation of hybrids has been used successfully in other cereals including rice (Evenson & Gollin, 2003), maize (Duvick, 2001), wheat (Waines & Hegde, 2003) and in sorghum (Rosenow et al., 1998) to significantly increase their yield. However, the genetic improvement of finger millet by

breeders has been limited partly due to difficulties associated with hybridization, particularly emasculation (Hilu & De Wet, 1980; CAB International, 2005; Oduori et al., 2008). Finger millet is mostly self-pollinating, with only 1% cross pollination aided by wind, which limits hybrid development (Purseglove, 1972; Jansen & Ong, 1996). This is due to its floral architecture (Hilu & De Wet, 1980; CAB International, 2005) that makes it very difficult to emasculate and hybridize. Most emasculation techniques suggested for use in finger millet breeding are mechanical and seem to be labour intensive, time consuming and require long term experience to come up with successful hybrids, yet still very inefficient (Riley et al., 1989). The use of chemicals such as Ethrel® (2-chloroethyl phosphonic acid) as a male gametocide was suggested because it saves on time and labour compared to hand emasculation which is requires more technical skills (Berhe & Miller, 1978).

Ethrel is one of the plant growth regulator hormones that has been used for increasing yield, promoting fruit maturity, improving colour, and advancing harvest timings (Nickell, 1978) and is known to induce flowering (Mohan & Saiswal, 1972). Brown & Earley (1973) reported the use of ethrel to promote male sterility in wheat (*Triticum aestivum*) with one foliar application of 1000-2000ppm. Ethrel was also evaluated for emasculation of sugarbeet (*Beta vulgaris*) (Fendal, 1967). It was used in the hybridization of finger millet at concentrations 700ppm, 1,000ppm, 1,500ppm and 2,000ppm with varying but promising results (Oduori et al., 2008).

Once hybridization is achieved, one major challenge would be to correctly and more efficiently select crosses from selfs among the resulting progeny. Conventionally, morphological traits have been used to compare F_1 progenies with parental lines in order to select for likely successful crosses. To be certain, the selected F_1 progenies have to be advanced to F_2 in order to observe segregation of morphological traits between the two parents. This process is slow and tedious. A promising technique, used by Graner et al. (1994) and Siedler et al. (1994) applies molecular markers to detect successful crosses at F_1.

Extraction of genomic DNA is an important step for all studies involving molecular markers. Molecular markers are small and discrete entities that are carried forth from generation to generation, and are often used to determine genetic relatedness in several crop species (Schulman et al., 2004). The most promising molecular marker for use in identifying hybrids is Single Nucleotide Polymorphism (SNP) markers (Nasu et al., 2002). They have the advantage of being biallelic in nature (two possible alleles at a target site) and thus easily assayed (Sachidanandam et al., 2001). They have been used successfully to detect hybrids in many cultivated crops such as rice (Feltus et al., 2004; Monna et al., 2006), maize (Batley et al., 2003; Bi et al., 2006) and recently in polyploid rapeseed (*Brassica napus*) (Trick et al., 2009). These markers would therefore be the markers of choice in finger millet for the detection of hybrids compared to other DNA markers.

The study herein reported used ethrel in crossing six finger millet genotypes in a 6 x 6 diallel design including reciprocals based on using morphological means (Griffing, 1956). True F_1 progenies were identified using both morphological and molecular methods. Effects of the use of ethrel in the resulting progenies were determined through morphological and genetic comparison of treated and untreated parents and progenies.

1.2 Statement of the Problem and Justification

Finger millet is a cereal crop that plays an important role in food and nutritional security due to its high nutritional value and good storage quality (Gomez, 1993; Dida et al., 2007). Despite its importance, its productivity is limited to between 400 and 2,000 kg/ha (Dida et al., 2007) and yet the demand is high. This is attributed to a number of factors including; limited or lack of suitable varieties for different agro-ecologies, *Striga* infestation, pest and disease damage, drought, poor agronomic and water management practices and declining soil fertility (Oduori, 1998; Holt, 2000).

Research and improvement efforts are needed to significantly increase finger millet productivity, crop diversification and a better nutritional environment for farmers. Cross bred varieties would help bridge the gap of yield between farmers and research stations. Hybridization process needs to be more efficient to realize better cross bred varieties by the use of ethrel (Oduori et al., 2008).

It is against this background that studies to determine the efficacy of ethrel gametocide at varying concentrations for successful emasculation and hybridization were initiated. This study was conducted in a screen house, field, and in the laboratory. Determination of efficient ethrel concentrations for hybridization will facilitate and enhance hybridization

breeding in finger millet for generation of better adapted varieties for farmers. This will contribute to improved livelihoods of the resource poor farmers and open market opportunity to generate income.

1.3 Objectives

1.3.1 General Objective

To contribute to increased finger millet production through optimization of hybridization using ethrel for enhanced breeding for superior varieties.

1.3.2 Specific Objectives

The specific objectives of the research were as follows:-

1. To determine optimal ethrel gametocide concentration for effective emasculation and hybridization of finger millet.
2. To determine the effect of Ethrel® on agronomic traits of parental finger millet varieties.
3. To identify true F_1 hybrids from parent selfs using both morphological and molecular (SNPs) methods.

1.4 Hypotheses

The study was based on the following hypotheses:-

1. H_0: There is no ethrel gametocide concentration that causes high emasculation without damaging female fertility.

2. H_0: Ethrel does not affect agronomic traits of parental finger millet varieties.

3. H_0: True F_1 hybrids cannot be distinguished from parent selfs using morphological and molecular markers (SNPs).

CHAPTER TWO

LITERATURE REVIEW

2.1 Genetics of Finger Millet

Finger millet (*Eleusine coracana*) belongs to the Chloridoideae sub-family (Philips, 1972; Clayton & Renvoize, 1986) that includes tef (*Eragrostis abyssinica)* the only other crop in the sub-family (Bennetzen et al., 2003). It falls under the genus *Eleusine* that comprises nine species, eight of them being wild African grasses (Werth et al., 1994) and one cultivated *Eleusine coracana species*. Studies have indicated that *Eleusine coracana species* exists in two forms that is; cultivated form (*Eleusine coracana* subsp. *coracana*) and a wild form (*Eleusine coracana* subsp. *africana*), which is an aggressive colonizer (Dida et al., 2006). Finger millet is believed to have originated through selection and further cultivation of a large grain mutant of *Eleusine africana* (Neves et al., 2005).

Eleusine coracana and its likely progenitor *E. africana* have a common allotetraploid genome of AABB (Dida et al., 2006). It has x = 9 and 4x= 36 chromosomes (Dida & Devos, 2006). The cultivated *E. coracana* has morphological similarity to both *E. indica* (2n=18) and *E. africana* (2n=36). Basing on homology, Dida et al. (2008) regarded *E. africana* as a sub species of *E. coracana.* Hybridization and chromosomal doubling between diploid *E. indica* and unknown diploid led to evolution of cultivated finger millet and the wild progenitor *E. africana* (Dida et al., 2006). Fertile allotetraploids that produce viable gametes show diploid meiosis and with almost 100% self-pollination pure

line varieties show homozygosity, a basic requirement in diallel hybridization (Oduori et al., 2008).

The cereals genomes greatly vary in their size, which is due to the presence of varying amounts of repetitive sequences within their genomes (Heslop-Harrison, 2000). The genome size of finger millet has been estimated by flow cytometry to be 1.8 Picogram (Pg) which is larger than for most cereals (Mysore & Baird, 1997). The fraction of the genome in other cereals increases with genome size from diploid rice being the smallest cereal genome of 0.4 Gigabyte (Gb) ($2n = 24$) (Tarchini et al., 2000), through maize a diploid genome of 2.5 Gb ($2n = 20$) (SanMiguel et al., 1996), barley a diploid genome of 5.1 Gb ($2n=2x=14$) (Komatsuda et al., 1999) to wheat a hexaploid genome of 16 Gb ($2n = 42$) (Gill & Appels, 2004). Development of improved cultivars with enhanced resistance or tolerance to biotic and/or abiotic stresses and higher agronomic performance in self pollinating crops can be greatly accelerated through emasculation and hybridization.

Emasculation and hybridization have been employed in other cereals like rice, in that the wild species have been donors of valuable genes containing resistance to biotic and hybrid vigor traits (Friebe et al., 1996; Tanksley & McCouch, 1997). Systematic bio-diversity within cultivated and wild finger millet varieties should be exploited for desirable traits like resistance to blast (Dida et al., 2006; Dida et al., 2008). Attempts have been made to analyze nutritional profile, blast resistance and early vigor for both cultivated and wild *E.coracana* for breeding (Barbeau & Hilu, 1993; Vadivoo et al.,

1998). Single nucleotide polymorphisms should be in-corporated in future mapping studies due to their abundance in the entire genome (Rafalski, 2002; Koebner & Summers, 2003). Developments of good genetic maps using markers that are simple to generate, highly reproducible, codominant, and specific for particular linkage groups are desirable for application in breeding programs. The outcome from such mapping studies is a greater tool to researchers as estimates for the number of loci, allelic effects, and gene action controlling important traits of interest in plants of interest can be understood (Yu et al., 2008).

The first finger millet genetic map was developed in 1998. This was a lee way for construction of smaller linkage groups (Dida et al., 2007). Restriction fragment length polymorphism (RFLP) (Dida et al., 2007), amplified fragment length polymorphism (AFLP), expressed-sequenced tag (EST) and simple sequence repeat (SSR) (Srinivasachary et al., 2007) markers were used to develop finger millet genetic map.

Studies have established that variability in crops is caused by genotype, environment, and their interaction.There are some simply inherited finger millet traits that could be useful as morphological markers to identify true crosses in an F_1 population. Such traits include purple pigmentation (Takan et al., 2004), brown seed colour, panicle shape, plant height, peduncle and glume length. The purple pigmentation is dominant and can be used as a genetic marker in identifying true crosses at the seedling stage. Brown colour in seeds is controlled by chromosomal duplication, and is dominant over white. Ayyangar (1932); Ayyangar & Rao (1932) reported that panicle shape inheritance is due to a Q gene which

when present results in fingers curving. The tight ‘fisted’ fingers were found to be simple recessive to ‘top curved fingers (Vijayaraghavan & Sarma, 1938). (Babu et al., 2014) reported that E1 and E2 genes are responsible for plant height and peduncle length. Ayyangar & Wariar (1936c) carried out a study on glume shape, and they found that short glumes were dominant over long glumes. This is as a result of three genes G11, G12, and G13 all acting together to produce short glumes. Interaction between any two genes at a time produces medium glumes while one or none gives long glumes.

2.2 Morphology and Cultivation

Millets are referred to as coarse, small edible grasses belonging to grass (*Gramineae/Paniceae*) family beside maize, sorghum, oats (*Avena sativa*), and barley (*Hordeum vulgare*) (Bouis, 2000). On the other hand, they are also referred to as small seeded annual grasses that are cultivated as grain crops on marginal lands in dry, temperate, sub-tropical and tropical regions (Baker, 1996). The spike length of finger millet ranges from 3cm to 13cm and they are of different shapes (Plate 2.1) and plant height ranging between 40cm up to 140cm, with slender stems and shallow fibrous root system hard to pull from the ground by hand (de Wet, 2006; FAO, 2012). The panicle has 4 to 19 fingers (spikes) (de Wet, 2006). Spikes develop 1 to 70 spikelets each carrying 4 to 7 small seeds (Dida et al., 2006). The colour of finger millet grains vary from white through orange-red, deep brown, purple to almost black (Crawford & Lee, 2003). Coloured (brown) finger millet grain (Plate 2.2) varieties contain good levels of phenolics, tannins (Ramachandra et al., 1977; McDonough a, 1986) and do not allow oxidation of other molecules in the body than white grains.

(a) (b) (c)

Plate 2. 1: Finger millet ear shapes: (a) open, (b) fist and (c) incurved

(Source: Author, 2012)

(a) (b) (c)

Plate 2. 2: Finger millet grain colours: (a) white, (b) red and (c) light brown

(Source: Author, 2012)

Finger millet has been in cultivation in East Asia for the last 10,000 years (Lu et al., 2009). In Africa, it is the second most important millet grown and comprises about 8% of the cultivated area and 11% of production of all millets worldwide (Obilana & Manyasa, 2002). In Kenya, finger millet is mainly produced in west of the Rift Valley counties (Oduori, 1993; (Oduori et al., 2008). Finger millet is very important but the yields are low when compared to other cereals like maize, sorghum, wheat, barley, rice etc (National Research Council, 1996), accounting for only 15-16 % of the potential in Kenya (Takan et al., 2002). Farmer grain yields range between 4.5 – 6.75 tonnes/ ha

(Takan et al., 2002) compared to research yields of 3.8 – 4.0 tonnes/ha. Yields in Africa are generally low and breeding for higher yields is needed to elevate production.

2.3 Global Importance

Finger millet is a staple grain for most of the world's population especially in south Asia and East Africa. It is highly nutritious as its grains contain 65-75% carbohydrates, 5-8% protein, 15-20% fibre and 2.5-3.5% minerals (Chetan & Malleshi, 2007) (Table 2.1). Because of its high levels of carbohydrate content it is consumed in terms of porridge and pancake-like flat breads to provide energy to different categories of people (ICRISAT/FAO, 1996; Bhatt et al., 2003). This crop is on high demand for food for infants, invalids, convalescents and the elderly because of its high nutritive properties. It is also important for culture and cash (Holt, 2000; Oduori, 2000). In regions like India, majority of millets produced are used as green forage for livestock (Upadhyaya et al., 2011), and alcohol production (Crawford et al., 2005). Straws left in the fields after harvesting can be grazed by animals because it contains up to 61% total digestible nutrients. The straws can also be used in house thatching (de Wet, 2006).

Potential health benefits derived from finger millet have been reported as follows; prevention of cancer and cardiovascular diseases, reduction of tumor incidence, lowering blood pressure and heart attack disease, reduction of cholesterol and rate of fat absorption, delaying gastric emptying, and supplying of gastrointestinal bulk (Truswell, 2002; Gupta et al., 2012).

Table 2.1: Nutritive content of finger millet as compared with other cereals

Food	Protein (g)	Fat (g)	Ash (g)	Fiber (g)	Carhohydrate (g)	Kcal	Ca (mg)	Fe (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)
Rice (brown)	7.9	2.7	1.3	1	76	362	33	1.8	0.41	0.04	4.3
Wheat	11.6	2	1.6	2	71	348	30	3.5	0.41	0.1	5.1
Maize	9.2	4.6	1.2	2.8	73	358	26	2.7	0.38	0.2	3.6
Sorghum	10.4	3.1	1.6	2	70.7	329	25	5.4	0.38	0.15	4.3
Pearl millet	11.8	4.8	2.2	2.3	67	363	42	11	0.38	0.21	2.8
Finger millet	**7.7**	**1.5**	**2.6**	**3.6**	**72.6**	**336**	**350***	**3.9**	**0.42**	**0.19**	**1.1**
Foxtail millet	11.2	4	3.3	6.7	63.2	351	31	2.8	0.59	0.11	3.2
Common millet	12.5	3.5	3.1	5.2	63.8	364	8	2.9	0.41	0.28	4.5
Little millet	9.7	5.2	5.4	7.6	60.9	329	17	9.3	0.3	0.09	3.2
Barnyard millet	11	3.9	4.5	13.6	55	300	22	18.6	0.33	0.1	4.2
Kodo millet	9.8	3.6	3.3	5.2	66.6	353	35	1.7	0.15	0.09	2

Sources: Singh & Raghuvanshi (2012).

Finger millet has high calcium level which is one of the most important minerals for the the human body. Calcium helps in formation and maintainance of healthy teeth and bones that can help prevent osteoporosis (Bakhru, 1996).

In Africa and other parts of Asia, finger millet is mostly produced for food and in malted form for production of weaning foods, more so, in Zimbabwe it is commercially brewed and used to prepare opaque beer (ICRISAT/FAO, 1996; McDonough et al., 2000). The crop has also received attention from developed countries due to its good potential in the manufacture of bioethanol and biofilms (Li et al., 2008).

Even though finger millet production is declining in areas where 30 years ago it was the major crop, it is still an important cereal that fetches high prices on the market (National Research Council, 1996). Globally finger millet trading is estimated to range between 200,000 – 300,000 tons, which represent only 0.1% of the world trade in cereals or 1.0% of world millet production (ICRISAT/FAO, 1996). Finger millet production in the developing countries is 97%; it grows under different environmental conditions having short maturity period, with resistance to pests and diseases thus very much favoured by farmers compared to other cereals (McDonough et al., 2000; Devi et al., 2011). In Kenya, it sells over double the price of sorghum and maize. A participatory rural appraisal (PRA) conducted in western Kenya showed that finger millet production was most commercialized in Kisii and least in Teso districts (Obilana et al., 2002). Finger millet yields can be improved in Kenya by growing improved varieties and improving management practices (Oduori, 2000). In Uganda,

finger millet still occupies 50% of Uganda's cereal area helping raise farmer's income through selling of surplus in the market.

2.4 Production Constraints

The production of finger millet has been constrained by several biotic and abiotic factors. Under abiotic constraints, the small finger millet seed size leads to higher plant densities that demands high labour input during cultivation (National Research Council, 1996; Consultative Group on International Agricultural Research, 2001). The small grains have large portions of husk and bran around them and require dehusking and debranning before consumption (Hulse et al., 1980). Dry, moistened grains are normally pounded with wooden sticks, which is laborious and tedious although in some cases moistening the grain by adding water enhances quick removal of fibrous husk (Lupien, 1990; Hadimani & Malleshi, 1993). The small seeds, however, are an advantage when it comes to storage, allowing the crop to be stored safely for many years without insect damage. Other abiotic constraints include lodging, acid soils, heat and drought. Biotic constraints include *Striga* pest and wild relatives (*Eleusine coracana* sub-species *africana*) that elude weeding but don't contribute to yield because they appear like finger millet at vegetative stage but panicles are grasslike (Oduori, 2000). Some communities' negative attitude towards the crop is also a major constraint to production (National Research Council, 1996).

Magnaporthe grisea (Hebert) Barr (syn: *Pyricularia grisea* Sacc.) fungus is a serious filamentous ascomycetous causative organism of blast in finger millet (Anon, 2008).

Hyphal fusion, mitotic and parasexual recombination help characterize blast pathogen (Tsujimoto & Shigeyama, 1998). This disease affects finger millet at all growth stages from seedlings (causing lesions and premature drying of young leaves) to affecting the panicle causing neck and/or finger millet blast (Gashaw et al., 2014). Blast (a close relative of rice blast) is the most serious disease of finger millet which significantly lowers yield (National Research Council, 1996; Consultative Group on International Agricultural Research, 2001). Despite finger millet importance, its productivity has been limited by blast disease caused by *Magnaporthe grisea* in the eastern African countries. Gashaw et al. (2014) has shown that *Magnaporthe grisea* is a disease capable of devastating finger millet, and causing significant yield reduction worldwide (Dunbar, 1969).

2.5 Finger Millet Breeding

Traits that need to be bred for in finger millet in Africa include blast resistance, early maturity, large panicle size, increased high-density grain filling, high finger number and branching, resistance to *Striga*, lodging, tolerance to stressful soil and moisture conditions (National Research Council, 1996). Report from a study done in different parts of India suggested that grain yield per plant is significantly influenced by days to emergence of finger, days to 50% flowering, finger length, finger width, and weight of grains of main head (Bendale et al., 2002). Additional effort aimed at breeding for superior genotypes in Africa will likely result in the development of genotypes with novel traits (National Research Council, 1996). A more efficient

emasculation and pollination technique will have to be developed to enable routine hybrid production in finger millet.

2.6 Diallel Analysis

Diallel cross is a mating design used in breeding programmes to determine genetic manifestation in terms of quantitative characters (Crusio et al., 1984). Diallel analysis is performed after making diallel crosses where all possible crosses among a group of parents in a segregating population to check on dominance of traits in genotypes (Hayman, 1954; Singh & Chaudhary, 1977).There are three diallel analysis approaches that are commonly used which include: the Hayman (1954); Jinks (1954) and the Griffing (1956). The approaches of Griffing (1956) (Method 1, Model 1) and Hayman (1954) are similar statistically but differ in genetic assumptions and interpretations (Hayman, 1960). Griffing analysis stresses on general and specific combining ability (GCA and SCA) whereas Hayman deals with genetic interpretation. Jinks diallel crosses involves information on parental heterozygosity.

The full diallel design basically allows testing for maternal and paternal effects leading to heterotic patterns in plant breeding (Crusio, 1987; Carena, 2005). Utilization of this mating design with different parents having different agronomic traits e.g. seed yield, will in turn help determine gene effects, combining abilities (both GCA and SCA), heritability and heterosis in species (Crusio, 1993; Topal et al., 2004). Use of genotypes in this mating design that posse desirable genetic components will result in superior genotypes (Khan & Hassan, 2011). Diallel mating

design and analysis has been broadly used in genetic and breeding programs on various crops including cotton, finger millet (Ashraf & Ahmad, 2000; Oduori, 2008).

2.7 Plant Emasculation Techniques

Emasculation entails removal of stamens from bisexual flowers of the female parents with the aim of avoiding self-pollination, where it is not required (Hussain et al., 2012). Emasculation is done before anthers are mature and therefore prevents undesired self-pollination in the plant of interest (Chapman, et al., 2002). Emasculation allows cross breeding hence combining of elite lines to produce high quality progeny (Knight et al., 2000). There are many plant emasculation techniques that are used depending on plant species, genetics and floral architecture (Kittelson & Maron, 2000). Emasculation is classified into three categories; mechanical, chemical, and genetic (House, 1985).

2.7.1 Mechanical Emasculation

Mechanical pollination control refers to any approach by which pollen transfer is mechanically prevented. An example is in monoecious crops, where male and female flowers are at different positions on the plant (Arias et al., 2005). To achieve cross-pollination in such a plant, the whole male flowers can be manually removed from the plant (Fleming, 2004). However, removal of anthers from the flowers is the most commonly used emasculation technique done in hermaphrodite flowers (Kudo, 2003). Although manual emasculation is very common in breeding, it is time consuming, labour intensive, and expensive (Acquaah, 2007). In maize, hand emasculation is

widely used and is done before pollen is shed or before the silks appear (Hayes, 2007). This approach requires manual labour and often results in foliar damage (Basra, 1999). Considering the microscopic florets and delicate pistils of finger millet, mechanical emasculation remains a very difficult undertaking.

Other methods have been devised to remove the microscopic florets manually. Hot and cold-water emasculation depend on sensitivity of the stamens to both genetic and environmental factors than the pistil (Virmani, & Ilyas-Ahmed, 2001). This property is therefore utilized to kill the pollen grains with hot or cold water (or other agents) without damaging the pistil (Singh et al., 2008). Both hot and cold water techniques have setbacks in small millets (Riley et al., 1989), due to the delicate pistils that are to a larger part protected by glumes. Another mechanical approach is the use of plastic bags reported to work in certain crops, including finger millet (Gupta, 1993). The plastic bags create high humidity that prevents anther dehiscence when florets open resulting in anthers emerging without shedding pollen (House, 1985). Such anthers can then be tapped off the ear and the ear cross pollinated. This method may not work successfully in finger millet where anthers collapse and open before the florets open (Dabholkar, 2006).

2.7.2 Genetic Emasculation

Genetic emasculation technique involves the use of nuclear or cytoplasmic male sterility genes of a targeted population to come up with female male sterile parent plants for the purpose of hybridization ((Edwardson, 1970; Budar & Pelletier, 2001).

In some plant species, certain genes occur naturally that contribute to genetic control for pollination. This occurs mainly by two mechanisms, male sterility and self-incompatibility. Male sterility was first observed when anthers were aborted in corn hybrids (Vinod, 2005). Male sterility (MS) results from non-functional pollen grains in flowering plants due to mutations, diseases, unfavourable environmental and growth conditions (Budar & Pelletier, 2001). Male sterility enhances out-breeding enabling breeders to produce F_1 hybrid cultivars. Male sterility also prevents unwanted gene flow between inbred and native species (Gardner et al., 2009). There are three categories of MS: cytoplasmic, nuclear, and cytoplasmic-genetic. Cytoplasmic male sterility (CMS) is an extra-nuclear genetic control that shows non-Mendelian inheritance (Chase, 2007). It is caused by expression of mitochondrial genes that result in production of non-viable pollen (Pelletier & Budar, 2007). Cytoplasmic male sterility is a maternally inherited trait associated with unusual open reading frames (ORFs) found in mitochondrial genomes (Hanson & Bentolila, 2004; Eckardt, 2006). Cytoplasmic male sterility results from inter-specific or intra-specific cross combination that contain different nucleus with different genes (Mihr et al., 2001). Recessive male sterile genes contained in the cytoplasm can be inherited by offspring's naturally and then expressed phenotypically due to natural mutation (Schnable & Wise, 1998).

This is possible if the seed of interest has CMS mutants associated with high yields and restorer genes, (Perez-Prat & van Lookeren Campagne, 2002). In some cases, CMS hybrids have been reported to be associated with disease susceptibility and

unstable sterility (Rao et al., 1990). In the presence of restorer (T-urf13) genes the amount of T-URF13 polypeptide is highly reduced, an advantage as it controls the fertility of a hybrid progeny (Kotchoni et al., 2010).

Nuclear male sterility is also called genic male sterility (GMS). It is controlled by a single recessive gene “ms” in the nucleus and is inherited following Mendelian fashion (Edwardson, 1970; Hanson, 1991). It results in reduced pollen production, anther size and total pollen abortion (Poehlman & Sleper, 1995). This type of sterility is not useful to breeders because it cannot be used to maintain pure lines of male sterile lines (Acquaah, 2007). Male sterile lines are pure homozygous (msms) so must be crossed with a heterozygous (Msms) source (Acquaah, 2007). Attempts to use NMS in cotton (*Gossypium spp.*), tomato (*Lycopersicon esculentum*), potato (*Solamum tuberosum*), soybean (*Glycine max*), barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*) turned out to be undesirable as the F_1 hybrids were sterile (Chahal & Gosal, 2002).

Mariani et al. (1990) reported the first transgene to confer GMS that facilitate hybrid seed production. Transgenes result in male sterility by disrupting tissue specific gene expression of the protein that is needed for production of functional pollen (Koltunow et al., 1990; Mariani et al., 1990). The introduction of one or more genes that can alter levels of metabolites, like amino acids and sugars that are needed for pollen formation can also be used to cause male sterility (Goetz et al., 2001).

Cytoplasmic-genetic male sterility is caused by nucleic and cytoplasmic genes (Vinod, 2005). It is restored by a fertility restoring (Rf) gene (nuclear gene), which results in the development of normal anthers and pollen (Akagi et al., 2004). This system enables breeders to control the sterility expression by manipulating the gene–cytoplasm combinations in any selected genotype, and becomes highly desirable. It has been used in hybrid seed production in onion (*Allium cepa*), sorghum, safflower (*Carthamus tinctorius*), corn, and sugar beet (Chahal & Gosal, 2002), as it overcomes the problems of GMS and CMS. R-lines (parental lines that have the ability to produce large and viable amounts of pollen for cross pollination-restores fertility) with higher ability to restore fertility can be used in production of hybrids with high heterosis level (Zhang et al., 2010). 'A'- lines (female parental lines that possess homozygous recessive non-restore genes) can be pollinated with 'R'-lines naturally in the field (Poehlman & Sleper, 1995). Cytoplasmic male sterility has been studied extensively in open pollinating crops and least on self pollinating cereals especially in finger millet has not yet been found.

Self incompatibility (SI) is another mechanism for preventing inbreeding depression (Porcher & Lande, 2005). It is found in some cultivated crops like rye (*Secale cereale*), potato (*Solamum tuberosum*), alfalfa (*Medicago sativa*), clover (*Trifolium spp*.), pearl millet (*Pennisetum glaucum*), and sugar beets (*Beta vulgaris*) (Borba et al., 2001). Self-incompatibility is the most widespread strategy employed in plants, because it enables the pistil of a flower to recognize and reject self-pollen or pollen from genetically related individuals, thus preventing inbreeding and promoting out

crossing (McCubbin & Kao, 2000). Self incompatibility feature is genetically controlled by a single polymorphic S locus (Nettancourt, 2001). Self incompatibility promotes heterozygosity and therefore is desirable (Igic et al., 2008). Self incompatibility can be either heteromorphic, where differences in the length of style and stamen pose a barrier in fertilization (Ebert et al., 1989), or homomorphic. Homomorphic is further divided into gametophytic, where the genotype of the pollen determines the fate thus fertilization is prevented when the S allele expressed by the haploid pollen grain matches one of the S alleles expressed in the pistil (Lawrence, 2000). Homomorphic SI can also be sporophytic, where the genotype of the sporophyte that produces the pollen determines the incompatibility and the phenotype of the pollen is determined by the diploid genome of the parent plant (Hiscock & Tabah, 2003). Receptor protein S-receptor kinase covers the stigmatic surface towards anthesis thus acts as a barrier of the stigma germinating pollen grains (Silva & Goring, 2001).

Self incompatibility is overcome under external and physiological conditions like treatment with CO_2 gas, heat treatment, irradiation and pistil grafting (Nettancourt, 2001). Selfing is achieved by using immature flowers in which the S phenotype is not yet expressed or older flowers in which SI is getting old (Mable et al., 2008). Pollination with mixtures of compatible and incompatible flowers can be the option too (Nettancourt, 2001). This mechanism is widely used in *Brassica* and *Raphanus* for production of single-cross hybrid seeds (Chahal & Gosal, 2002).

2.7.3 Chemical Emasculation

Chemical hybridizing agents are used in breeding to selectively render pollen non-functional, thus allowing one to make crosses without laborious hand emasculation (Yu et al., 2005). Chemical induction of male sterility provides a means of enforcing hybridization between any populations where it eliminates the long and expensive process of converting promising breeding lines to the cytoplasmic male sterility conditions (Padidam, 2003). Chemical pollen control is advantageous for inducing male sterility because any parent line can be used as a female, and there is no requirement for a fertility restorer gene (Chahal & Gosal, 2002). A variety of chemicals with gametocidal properties are used to produce male sterility temporarily in crops. Dalapon®, Estrone® and Ethrel® (Amiran Kenya Limited, Nairobi, Kenya) are a few examples of chemicals that are used commercially to induce male sterility (Acquaah, 2007). The effects of gametocides moreover, are not inherited and are relatively easier to use (de Milliano, 1983). Ethrel (2-chloroethylphosphonic acid) is one of the chemical hybridizing agents (CHAs), which acts more strongly in retarding anthers (or inactivates the stamens) of the flower to promote male sterility especially in most self-pollinating crops (Rowell & Miller, 1971).

Ethrel is known to advance the formation of pistillate flowers and delay staminate flower formation thus has great potential in being useful in plant hybridization experiments (Lower & Miller, 1969). Gametocidal response happens in a way that, the anthers of the florets remain retarded in approximately 7 days before pollen

release (Masoudi-Nejad et al., 2002). This mechanism requires appropriate timing for application of the chemical to attain desirable response. Male sterility in crops caused by use of ethrel treatment results from the microsporogenesis interference before, during and or in post meiosis stage (Colhoun & Steer, 1983). Ethrel plant regulator hormone is advantageous because it reduces plant height which prevents lodging. Grain yield in cereals have been reported to increase on application of ethrel (Simmons et al., 1988). Yield increases with ethrel application have been attributed to increased spikes per plant (Ramos et al., 1989). Increased grain mass was observed in barley treated with ethrel, which also had reduced lodging (Brown, 1996).

Despite the above mentioned positive effects of ethrel, Rowell & Miller (1971); Stoskopf & Law (1972); de Milliano (1983); Oduori et al. (2008) observed poor ear exertion, reduced plant height, delayed heading, anthesis, reduced spikelets per head, reduced awns, delayed maturity, enhanced tillering and reduced panicle length that seemed to increase with increased ethrel concentration in cereals. Stoskopf & Law (1972) observed similar setbacks of ethrel effects in barley and pearl millet respectively. Germination of F_1 female parent plantof Triticale reduced from 93% in control to 73% when treated with ethrel at a concentration of 500 ppm. Higher Ethrel® concentration of 1000 ppm, 2000 ppm and 4000 ppm further reduced seed germination to 57%, 42% and 30% respectively (Sapra et al., 1971). The application of ethrel at 5,000 ppm in cucumber reduced plant height significantly. Stoskopf & Law (1972) studied the effect of ethrel at 1,500 ppm in *Hordeum vulgare* and noticed a severe reduction in plant height too. The application of ethrel at 100 ppm and 200

ppm in *Beta vulgaris* reduced the plant height drastically (Hecker & Smith, 1975). Application of ethrel at 250 ppm concentration inhibited the production of male flowers in cucumber (Rudich et al., 1969).

Despite all the negative effects, ethrel is easily and cheaply available on the market and it cuts down on labour compared to other emasculation techniques (Verma & Kumar, 1978). Ethrel has the potential of eliminating floral sensitivity which is a major setback experienced in manual emasculation of tef. (Berhe & Miller, 1978). Exploitation of ethrel in finger millet hybridization with high success rate would promote exploitation of mass selection and even manual crossing (Oduori et al., 2008).

2.8 Zadoks Developmental Stages in Cereals

Developmental stages in cereals are important consideration in the application and timing of forage management practices (Shewry & Halford, 2002). They can be grouped as follows: germination to seedling (S); growth stage 1 (GS1) from seedling to tillering; growth stage 2 (GS2) from tillering to booting; growth stage 3 (GS3) booting to inflorescence emergence; growth stage 4 (GS4) inflorescence emergence to anthesis; and growth stage 5 (GS5), which includes the grain filling period that is from anthesis to maturity (Table 2.2) (Meier, 2001). Leaf development in tillers of cereals is useful for determining the timing of management practices such as defoliation, burning, fertilization, and growth regulator and pesticide application (Moore et al., 1991). Physiological maturity is the time when the flag leaf and spikes turn brown (Acevedo et al., 2002).

Table 2.2: Developmental stages in cereals

Days	**Description** (Zadoks et al., 1974)
0-9	Germination
10-19	Seedling/leaf development
20-29	Tillering
30-39	Stem elongation
40-49	Booting
50-59	Inflorescence emergences
60-69	Anthesis/flowering
70-77	Milk development
80-87	Dough development
90-99	Ripening

2.9 Detection and Selection of True F_1 Hybrids

It is important to device methods to enable simple detection of true hybrids in breeding programs. Hybridization entails interbreeding between individuals of different species or genetically divergent individuals from the same species of heritable trait variation, selected and repetitively evaluated morphologically and genetically in order to identify key adaptation features for both local and commercial purpose (Lambertini et al., 2012). Heterosis utilization is a fundamental approach used in breeding to increase crop yield, resistance to pests and diseases in the F_1 plant as some of the good traits considered by breeders (Zhao & Gai, 2006). The differences that distinguish one plant from another are encoded in the plants genetic material, the deoxyribonucleic acid (DNA) which is packed in chromosome pairs. Genes that control plant characteristics are located on specific segments of each chromosome and are carried by a single gamete called a genome (Semagn &

Ndjiondjop, 2006). Genetic marker is a chromosomal mark, a specific piece of DNA with a known position on the genome (Shen et al., 2005) or a gene whose phenotypic expression is easily discerned and used to identify an individual or a cell that carries it (King & Stansfield, 1990).

There are three major categories of markers that are used in plant breeding. These include: morphological markers which are also called classical or visible markers; biochemical markers, which include allelic variants of enzymes called isozymes; and DNA or molecular markers, which reveal sites of variation in DNA (Semagn & Ndjiondjop, 2006). Besides several phenotypic or morphological descriptors, various molecular markers have been used to characterize finger millet germplasm (Table 2.3).

2.9.1 Morphological Markers

Morphological traits are the easiest of all markers to score. They are visually characterized using flower colours, shape of the seed, growth habits or pigmentation in plants (Sumarani et al., 2004). Though morphological markers are easy to score, they have the disadvantage that they may be limited in number and also are influenced by environmental factors or the developmental stage of the plant (Winter & Kahl, 1995). In addition, the expression of such markers can also be altered by epistatic and pleiotropic interactions (Kumar, 1999). The number of morphological markers is very limited; their alleles interact in a dominant-recessive manner, thereby making it impossible to distinguish the heterozygous individuals from homozygous

individuals. However, they are useful to plant breeders because they do not require expertise to score (Eagles et al., 2001).

2.9.2 Biochemical Markers

Biochemical markers are proteins produced as a result of gene expression and reveal polymorphism in germplasm by detecting differences in the gene sequence and function as co-dominant markers (Moore & Durham, 1996). Biochemical markers include allelic variants of enzymes called isozymes and allozymes. Isozymes were first described by Market (2012) who defined them as different variants of the same enzyme having identical functions and present in the same individual and are detected by electrophoresis and specific staining. Uzunov & Weiss (1972) described isozymes as enzymes that are the product of different alleles of the same gene. These markers are limited in number and are influenced by environmental factors or plant developmental stage (Winter & Kahl, 1995). Despite these limitations, biochemical markers have been extremely useful to plant breeders (Eagles et al., 2001). Allozyme markers have provided a valuable tool for population genetic studies in natural populations of woody plants (Adams, 1983). In addition, allozyme analysis is relatively fast and inexpensive (Ledig, 1998).

2.9.3 Molecular Markers

DNA markers reveal polymorphisms at DNA level (Collard et al., 2003). They are widely used due to their abundance in the genome and their ability to detect changes in the DNA sequence (Gupta et al., 1999). There are a good number of molecular

markers and each one of them has advantages and disadvantages (Koebner et al., 2001) as presented in Table 2.3.

Table 2.3: Chronological evolution of molecular makers

Acronym	Nomenclature	Reference
RFLP	Restriction Fragment Length Polymorphisms	Grodzicker et al., 1974
RAPD	Random Amplified Polymorphic DNA	Williams et al., 1990
SSR	Simple Sequence repeats	Akkaya et al., 1992
SNP	Single Nucleotide Polymorphisms	Jordan & Humphries, 1994
ISSR	Inter-Simple Sequence Repeats	Zietkiewicz et al., 1994
AFLP	Amplified Fragment Length Polymorphisms	Vos et al., 1995

2.9.3.1 Microsatellites

The genomes of higher organisms contain three types of multiple copies of simple repetitive DNA sequences that include; satellite DNAs, minisatellites and microsatellites (Armour et al., 1999). Microsatellites (Litt & Luty, 1989), also called simple sequence repeats (SSRs), are the smallest class of simple repetitive DNA sequences. Simple sequence repeats are easy to score, have multiallelic nature, co-dominant inheritance, are more informative because it can detect multiple alleles per locus, reliable and highly reproducible than most of other DNA markers (Agarwal et al., 2008). They have a wide range of applications such as in population genetic studies, determination of paternity, genotyping and genetic mapping, systematic taxonomy, molecular evolution, hybrid selection (Kalia et al., 2011).

Simple Sequence Repeat (SSRs) markers are advantageous and can be highly applicable in detecting hybrids (Schlotterer, 2000). This is because they are highly polymorphic (can show differences between different individuals analyzed), co-dominant (can discriminate between individuals that are heterozygous and homozygous) and multi allelic (produce a good number of different sized PCR products for a single pair of primers across a range of individuals) (Powell et al., 1996). These factors have led to increased attention and attraction by molecular scientists (Zane et al., 2002). These markers are among the most popular markers that have been used previously on crops like rice (Jin et al., 2010), wheat (Emon et al., 2010), maize (Chakraborti et al., 2011), soyabean (Powell et al., 1996), pea (Loridon et al., 2005), for genetic analysis. Because SSRs are highly polymorphic and easy to use, they can be selected and used in any study.

2.9.3.2 SNP Markers

Single nucleotide polymorphisms occur at a single base pair position in the genome of an organism and are numerous (Gupta et al., 2001).These markers will provide greater information about an organism. The genetic code is specified by the four nucleotides A (adenine), C (cytosine), T (thymine), and G (guanine). SNP variation occurs when a single nucleotide, such as an A, replaces one of the other three nucleotide letters—C, G, or T (NCBI, 2005). For example, a SNP might change the DNA sequence as shown in Figure 2.1 below.

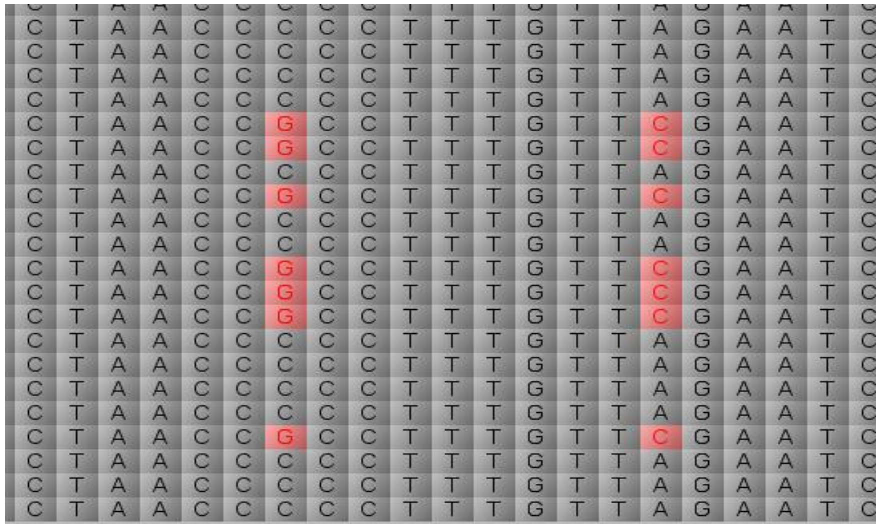

Figure 2. 1: Tablet screen shot showing SNP location

(Source: Musia, G. D., Muchugi, A. & De Villiers S. unpubl)

Single nucleotide polymorphisms are of two types: substitution (transitions/transversions) and or indels (insertion/deletion) of a base. Transition is as a result of pyrimidine to pyrimidine (C/T) or purine to purine (G/A) bases change, while transversions could be pyrimidine to purine (C/G, A/T, C/A or T/G) and vice versa (Batley et al., 2003). Single nucleotide polymorphisms are currently the most popular genetic markers as it contributes directly to phenotype or due to linkage disequilibrium can be associated with a phenotype in organisms (Nasu et al., 2002). These markers are biallelic (there usually only exist two possible alleles at a target site), occuring in coding and non coding regions and tends to be more in repetitive sequences in the genome than microsatellites (Batley et al., 2003) and are present in most animal and plant marker systems (Gupta et al., 2001).

Single nucleotide polymorphism markers are developed either by mining sequence databases and or, sequencing regions of interest in the genome of an organism where SNPs are screened. There are two methods that have been devised currently to develop SNP markers, miraEST (Chevreux et al., 2004) and QualitySNP (Tang et al., 2006). Expressed sequence tags can be clustered with CAP3 and used in SNP mining programs to develop SNPs (Baker, 2003). QualitySNP (Tang et al., 2006) identifies and localizes SNP after clustering them with a condition that every allele is represented by more than two reads (Batley et al., 2003). This helps in identifying a probe with a known sequence and target DNA that contain the SNP site through PCR for polymorphism (Gupta et al., 2001; Rafalski, 2002; Weising et al., 2005). This marker has been developed and used in some cultivated crop species like rice (Monna et al., 2006) and maize (Bi et al., 2006).

When full sequences of most plant genomes will be complete, SNPs are going to gain more popularity for molecular work in the near future (Ganal et al., 2009). Due to their abundance and distribution throughout the genome, molecular markers will help differentiate, create, maintain and improve heterosis among plant cultivars (XU et al., 2003). For this reason, this study investigated the possibility to identify heterozygosity in finger millet F_1 genotypes.

2.10 High resolution melting (HRM) Analysis

High resolution melting analysis (HRMA) is high-throughput post-PCR analysis method used to enable researchers identify genetic variation in nucleic acid sequences

without sequencing or determining genetic differences in a population before sequencing (White et al., 2007). HRM is the most inexpensive, simple and rapid of these technologies to detect SNPs (Gundry et al., 2003). It is usually based on PCR melting curve techniques enabled by measurement of fluorescence change which is then accompanied by double stranded DNA melting using a saturated DNA intercalating dye together with next generation real-time PCR detection system and analysis software (Palais et al., 2005). When melted, each PCR product exhibit characteristic disassociation behaviour (Montgomery et al., 2007).Variation in the DNA sequence leads to detectable changes in the melting curve, and thus the allelic differences among PCR amplicons are distinguished based on their composition, length, GC content or strand complementarity, sequence and heterozygosity of the amplified region, provided that the salt, buffer conditions and the volume of each sample remain constant (Smith et al., 2010). DNA template quality has great impact on HRM results meaning, DNA concentrations should be similar for all samples and controls preferably 10–20 ng of DNA template for better results (Applied Biosystems, Inc. 2009).

Based on previous studies, HRM analysis has proved to be a relatively efficient, accurate and inexpensive method to detect polymorphisms especially in SNPs (Wu et al., 2008). The HRMA being a reliable and cost effective post PCR technology, has been used successfully in various plant species in identifying SNPs, this includes, barley (Lehmensiek et al., 2008), capsicum/pepper (*Capsicum spp.)* (Park et al.,

2009). During HRM analysis, fluorescent-labelled primers are used to distinguish between homozygotes and heterozygotes (Gundry et al., 2003).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

The study was conducted in screen house, field and laboratory. Screen house and field experiments were carried out at Kenya Agricultural and Livestock Research Organization (KALRO) Kakamega (Kakamega County) due to the favourable climatic conditions. Laboratory experiments were conducted at the International Crop Research Institute for the Semi-Arid Tropical (ICRISAT) Nairobi (Nairobi County) due to availability of resources.

3.2 Plant Materials

The finger millet varieties used in this study were obtained from the working germplasm maintained at Kenya Agricultural and Livestock Research Organization (KALRO) Kakamega seed store. They consisted of six finger millet varieties: Gulu-E, IE 2872, IE 4115, KACIMMI 72 (KA-72), U-15 and Okhale-1 (OK-1) with contrasting traits on; blast, drought, grain colour, maturity period and all were *Striga* resistant (Table 3.1).

Table 3.1: Finger millet varieties used in the study

Variety	Code	Grain colour	Maturity Period (Days)	Origin and Source	Blast Resistance
IE 2872	IE 2872	Red	118	ICRISAT	Susceptible
IE 4115	IE 4115	Light brown	127	ICRISAT	Resistant
GULU-E	GE	Brown	130	Uganda	Moderate resistant
KACIMMI 72	KA-72	Brown	128	Kenya	Resistant
OKHALE-1	OK-1	Brown	129	Nepal	Moderate resistant
U-15	U-15	Brown	122	Uganda	Resistant

3.3 Screen House Study

3.3.1. Parent Arrangement and Ethrel Treatment

The six parental varieties were planted in pots in a screen house in a staggered manner based on maturity period to achieve synchrony in flowering to facilitate crossing. IE 4115, GE, OK-1 and KA-72 were planted on the same day; U-15 was planted five days later followed by IE 2872 after another two days. Later the pots were paired to make 6×6 diallel scheme of the elite varieties (Figure 3.1), with each variety pair having eight plant pairs as follows (all parents were crossed excluding selfs):

i. Designated female and male control plants not to be sprayed with ethrel (0 ppm).

ii. Designated female and male plants – female to be sprayed with ethrel at 1,500 ppm GL and its reciprocal.

iii. Designated female and male plants – female to be sprayed with ethrel at 1,750 ppm GL and its reciprocal.

iv. Designated female and male plants – female to be sprayed with ethrel at 2,000 ppm GL and its reciprocal.

The female plants in each pair to be treated with ethrel were sprayed with Ethrel at Zadoks development stage 45, found most effective by (Oduori et al. (2008).

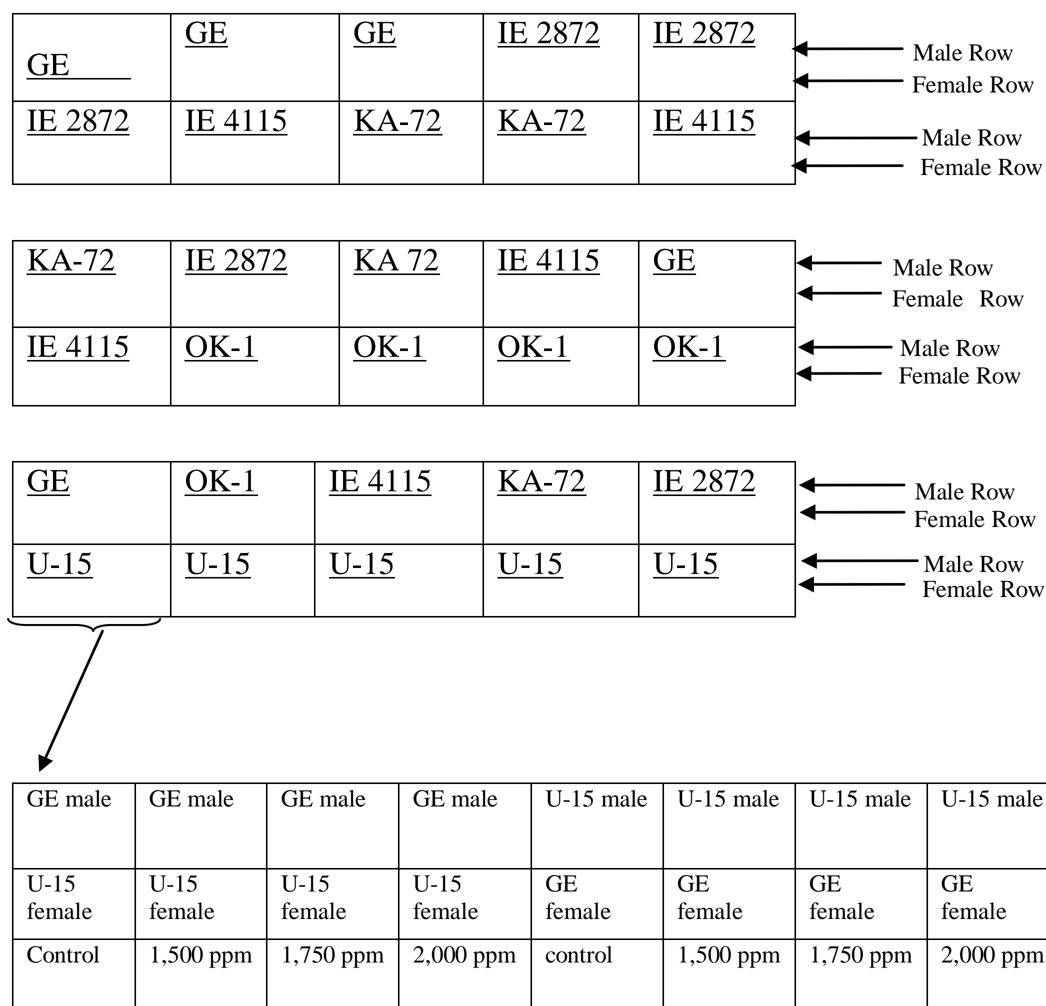

Figure 3.1: Parent arrangement in the screen house for 6×6 diallel mating

KEY: **GE = Gulu E; KA-72 = KACIMMI 72; OK-1 = Okhale 1**

Four gametocide levels (GL) of ethrel sourced from Amiran Kenya Limited, Nairobi, Kenya (1,500, 1,750 and 2,000ppm) including control at zero ppm check (sprayed using double distilled water) was studied on six selected finger millet varieties at Zadoks development stage 45.

Two hundred and forty pots were used in this experiment and were kept under screen house conditions and watered regularly whenever there was no rain, watering was twice in a day (morning and evening) to achieve the required moisture content for optimum growth. Each pot had a maximum of two plants after thinning and top-dressed with CAN fertilizer, according to Nyende et al. (2001). The gametocide levels (GL) were constituted by computing the requisite volumes to dilute from the package label information of 480g/l chemical in 1 litre active ingredient (a.i). A pipette was used in drawing the volumes for mixing of the chemical for each concentration together with double distilled water in a 2 litre hand sprayer that was used for chemical application. The chemical was applied to dripping wetness of the plant according to Oduori et al. (2008). After the plants had been treated with ethrel, each female plant was labelled with indications of the cross, gametocide concentration level and date of application. When heads emerged on the stalks (main stalk), they were covered using a pollination bag.

3.3.2 Plant Hybridization

Female plant heads were monitored daily towards flowering and were pollinated with the designated male parent pollen immediately the stigmas stuck out. Pollination was done in the morning between 8:00 and 11:00 am by shaking the male parent pollen in a pollination bag and shaking the bag over the female plant head. This was done when pollen was evident on the designated male plant head. Pollen transfer was done more than once to ensure maximum transfer of the pollen from the male plant head to the female. In cases where there was disparity in parent pair maturity, tillers of the early

variety were used to provide pollen. The female head remained covered until grain filling was complete. At maturity, the bagged heads were harvested independently, each in its own labelled bag, dried, threshed and seeds packed and stored safely.

3.3.3 Data collection

Data was collected on seven parameters e. g. days to heading, days to flowering, days to maturity, plant height, number of productive tillers per plant, ear exertion and plant grain yield.

3.3.3.1 Days to Heading

Days to heading was taken from the date of sowing to the stage when the ears emerged from flag leaf. This parameter was observed critically for gametocide application.

3.3.3.2 Days to Flowering

The days to flowering was taken from emergence date to the stage when florets opened.

3.3.3.3 Days to Maturity

Days to physiological maturity was taken by calculating days from date of sowing to stage when 50 per cent of main tillers had mature ears, that is, when the grain were hard and could not be crashed when rubbed between the thumb and fore finger.

3.3.3.4 Plant Height (cm)

The height of the main stalk was measured from the soil level in the pot to the tip of the panicle in centimetres.

3.3.3.5 Number of Productive Tillers per Plant

Number of tillers bearing mature ears were counted from each of tagged plants and averaged per plant variety.

3.3.3.6 Ear Exertion Length (mm)

Length of peducle (length from flag leaf ligule to the base of a panicle) was measured in millimetres.

3.3.4.7 Plant Grain Weight (Gms)

Weight of grain from main stalk covered panicle for each plant was recorded using electronic balance and the mean yield recorded in grams.

3.4 Field Screening of F_1s

3.4.1 Site Climatic and Edaphic Conditions

Kakamega County receives a mean annual rainfall of 2,010 mm and mean monthly temperatures of 28°C. The soil types in this region are Dystro-mollic Nitosol with pH of 5.2 (FURP, 1987). Rainfall is heaviest in April and May (“long rains”) with slightly drier June and lighter rain in August to September (“short rains”). January and February are the driest months. Temperature is fairly constant throughout the year, ranging between 20°C - 30°C (Ng’etich, 2013).

3.4.2 Field Lay-out

Screening for F_1 was done in 2013 long rain season (LR). Seeds harvested from single female parent plants were planted head to row in 20m long rows spaced at

50cm. Heads from each parent pair were planted in a block in which the first row was the female parent variety, followed by four rows from progeny plant heads where it was the female parent treated with 0ppm (control), 1,500ppm, 1750ppm and then 2,000ppm GL, then male parent and the reciprocal of the preceding arrangement and closing the block with the initial variety, that had served as female. There was a total of fifteen blocks with each block having eleven rows as presented in Table 3.2 below and Appendix I. The fifteen blocks added to a total of 165 rows. The parental lines were planted to help elucidate true F_1s in the population and also to serve as checks for evaluation of gametocide effect on post treatment generation. Trial management was as per recommendation for finger millet. Plant colour was a major component in F_1 screening. Plants intermediate between male and female parent in terms of morphological features including; plant colour, ear shape, plant height and flowering period were considered as true F_1s. For example in a cross between IE 2872 x IE 4115, the offspring was to have an intermediate colour between green and purple. Plants that looked like the maternal parents were considered to be selfed plants.

Table 3.2: Field arrangement of plants in a block

BLOCK 1
IE 2872 Female parent plant
IE 2872 × U-15 0 PPM
IE 2872 × U-15 1,500 PPM
IE 2872 × U-15 1,750 PPM
IE 2872 × U-15 2,000 PPM
U-15 Male/Female parent
U-15 × IE 2872 0 PPM
U-15 × IE 2872 1,500 PPM
U-15 × IE 2872 1,750 PPM
U-15 × IE 2872 2,000 PPM
IE 2872 Male parent

3.5 Molecular Screening for F_1

3.5.1 Laboratory Screening for True F_1s from Parents

Seeds that were harvested from the female plants in the screen house were planted in pots alongside parents in the screen house at Kakamega. After two weeks the plants were thinned to five per pot. Four weeks after planting, four young leaves were picked from each plant and packed in zip-lock bags that contained silica gel for molecular DNA work.

3.5.2 DNA Extraction Protocol by CTAB

The DNA was extracted from preserved dry leaves according to the protocol described by Mace et al. (2003), where phenol: chloroform step was omitted in the extraction. Silica gel was used for preservation of the leaves to prevent DNA degradation.

One hundred and twenty seedlings from treated female plants (F_1s) and six parent varieties of finger millet were sampled for DNA extraction as presented in Table 3.3. Dry, four weeks old, healthy leaf tissues were cut into small pieces of 1-2 cm that weighed 3 mg and placed in 12 × 8 well strip tubes with strip caps (Green tree Scientific, USA) in a 96 deep well plate containing 4 mm stainless steel grinding balls. A 2000 Geno/Grinder© (SpexCertiPrep Inc., USA) was used to homogenize leaf tissues by grinding at 30 frequencies per second for 5 minutes into powder form. Four hundred and fifty µl of pre-heated (65°C) CTAB extraction buffer containing (100mM Tris-HCI [pH8], 1.4 M NaCI, 20 mM EDTA CTAB [3% w/v], and betamercaptoethanol-alcohol ([3% v/v]) was added to each sample.

The samples were incubated for 45 minutes at 65°C water bath with occasional mixing. Incubation at 65°C was important to inactivate protease enzymes (Müller et al., 1994). Four hundred and fifty µl of chloroform-isoamyl alcohol (24:1) was added to each sample and inverted twice to mix. Chloroform is an organic molecule that removes all organic matter from DNA. The samples were centrifuged at 4000 x g for 10 minutes using an AllegraTM 25R centrifuge (BECKMAN COULTER Inc., USA) at 23°C. Fixed volume (400 µl) of aqueous layer was transferred to fresh strip tubes (Green tree Scientific, USA).

To each sample 0.7 vol of cold isopropanol was added to precipitate the DNA and inverted once to mix. The samples were centrifuged at 4000 x g for 20 minutes to

pellet the DNA. The supernatant was decanted from each sample and pellet air-dried for 30 minutes. The DNA pellets were then resuspended in 200 µl of low salt TE (10 mM Tris, 0.1 mM EDTA [pH 8]) was added to each sample. A total of 10 µl of RNase A (10mg/ml) was added and incubated at 37°C to digest the RNA.

Two hundred µl of chloroform-isoamylalcohol (24:1) was then added to each sample and inverted twice to mix. The samples were then centrifuged at 4000 x g for 5 minutes to separate the aqueous solution and the organic phase. One hundred and fifty µl of aqueous layer was transferred to a fresh 96 deep-well plate (Green tree Scientific, USA).

Three hundred and fifteen µl of ethanol-acetate solutions (30 ml EtOH, 1.5 ml. 3 M NaOAc [pH 5.2]) was added to each sample and placed at -20°c for 1 hour to precipitate the DNA. Samples were then centrifuged at 4000 x g for 20 minutes. The supernatant was decanted from each sample and the DNA pellet was washed twice with 70% ethanol (EtOH). The DNA pellet was then air dried and resuspended in 100 µl of low salt TE (Ven der Beek et al., 1992).

Table 3.3: Total number of samples used in DNA extraction

Parents	P1	P2	P3	P4	P5	P6
P1	-	4	4	4	4	4
P2	4	-	4	4	4	4
P3	4	4	-	4	4	4
P4	4	4	4	-	4	4
P5	4	4	4	4	-	4
P6	4	4	4	4	4	-

3.5.3 Genomic DNA, Quality and Quantity

The concentration of extracted DNA was estimated using a Nanodrop© 1000 (Thermo Scientific, USA) and Qubit™ fluorometer. The quality of DNA checked on 0.8% agarose dissolved in 100 ml 1 × TBE (0.1M Tris base, 0.1M boric acid and 0.02M EDTA; pH 8.0) buffer as shown in Appendix II. The mixture was then heated in a microwave for 3 minutes for agarose to dissolve. The gel was left to cool for five minutes on the bench at 25°C before adding 5 µl gel red (Biotium, USA) which is less mutagenic, then poured in a horizontal gel tray that was fitted with appropriate combs. The gel was left to set for 40 minutes then combs were removed carefully and the tray immersed in an electrophoresis tank that contained 1x TBE buffer. Then 2 µl of extracted DNA was mixed with 1 µl of 3x loading dye that contains bromophenol blue, xylene cyanol FF, a high density glycerol reagent and deionized water. Lambda (λ) DNA IEcoRI +*Hind 111* 500µg/ml, 100µg Promega MADISON, WI U.S.A. was loaded (40 ng and 60 ng) alongside the DNA samples in order to check the integrity of the DNA. The DNA was then subjected to electrophoresis at 80v for 45 minutes. The DNA was visualized under UV light using a UV gel documentation system (Bio Doc-IT™, Ultra-Violet Products, Cambridge, UK).

3.5.4 SNP Primer Design

Mining of the single nucleotide polymorphism (SNP) molecular marker was done using sequences generated from finger millet genotypes KNE 755 and KNE 796. DNA was extracted from the genotypes and specific target of dinucleotide motifs (CT and GT) was considered then sequences (454 GS-FLX Titanium) obtained. Nucleotides of good quality in fna data files were in turn selected into qual data files (Musia, G. D., Muchugi, A. & De Villiers S. unpubl). The rules for primer sequence design followed were adapted from (http://www.premierbiosoft.com/netprimer/index.html). Primers were designed using the NetPrimer a free primer analysis software (web-based tool) (Van et al., 2004) with the following conditions: 57-63°C melting temperature, 30-70% GC content, 18-25 bp primer length (shorter primers tend to be non-specific and longer ones are inefficient and more expensive) (Sambrook & Russel, 2001) and 200-800 bp amplicons size. All secondary structures were removed (Hillier et al., 2003).

3.5.5 Finger Millet SNP Markers

A total of four SNP markers out of the total five ordered from Inqaba Biotechnology Industries (Pty) Ltd (South Africa) were used in this study to determine heterozygosity in the 126 samples (Table 3.4).

Table 3.4: Four finger millet SNP primers

S/No.	Primer	Primer Sequence	Product Length (kb)	Amplified
1	904-1538 L	TGTGTTTGACACCTTAGACAGG	_	NO
	904-1538 R	CATGAAATGGCCCTAGATGG		
2	1458-1992 L	TTCAAGGTCAGATATGATTTAGC	285	YES
	1458-1992 R	AGCCCACGAACACCTTAGAC		
3	1925-2474 L	GCAGTATTCATGTGCTAGACTCC	170	YES
	1925-2474 R	GGAAATTGGCTCGAATGAAG		
4	2837-3585 L	TCTTCATTCGAGCCAGTGTC	235	YES
	2837-3585 R	AGCCCACGAACACCTTAGAC		
5	3502-4102 L	AGACTCCTCCTCTTTCCGATG	175	YES
	3502-4102 R	GCAACTGGCTCGAATGAAG		

3.5.6 Polymerase Chain Reaction (PCR)

Polymerase chain reaction was performed on parental lines using a thermal cycler (GeneAmp PCR system 9700®, Applied Biosystems, USA) to determine the temperature range before running all samples using real time PCR machine. PCR machine.The concentrations and volumes for each PCR reaction are shown in Table 3.5 below. The master mix contained 1x PCR buffer (20mM Tris-HCl (pH7.6); 100mM KCl; 0.1mM EDTA; 1mM DTT; 0.5% (v/v) Triton X-100; 50% (v/v) glycerol), 2mM $MgCl_2$, 0.16mM dNTPs, 0.04µM M13-forward primer, 0.2µM reverse primer, 0.2 units of *Taq* DNA polymerase (SibEnzyme Ltd, Russia) and 40ng of template DNA (Table 3.5).

Table 3.5: Concentrations and volumes for each PCR reaction for SNP primer

Master mix M13		
Components	Final concentration	×1 volume
PCR buffer without $MgCl_2$	1×	1µl
$MgCl_2$	2 Mm	0.4 µl
dNTPs	0.16 mM	0.8 µl
Forward primer	0.04 µM	0.5 µl
Reverse primer	0.2 µM	0.5 µl
Taq polymerase	0.2 U	0.04 µl
Double distilled water H_2O		4.76 µl
DNA template (total 40 ng)		2 µl

The total volume for each PCR reaction was 10 µl (Table 3.5). A master Mix was prepared for each marker and loaded in plates then DNA template was added. Amplification was performed using a thermal cycler (GeneAmp PCR system 9700®, Applied Biosystems, USA). Touchdown (TD) PCR (Don et al., 1991) program was run over 63°C - 58°C and 65°C-60°C temperature range for 35 cycles and optimal annealing temperatures of 57°C and 62°C respectively. The initial denaturation step was performed at 94°C for 3 min followed by 10 cycles at 94°C for 30 s, at appropriate annealing temperatures and 1 min at 72°C (extension). In the initial annealing step, the annealing temperature was decreased by 1°C/cycle until the lowest temperature within each range was reached. The products were then amplified for 25 cycles at an appropriate optimum annealing temperature with a final extension at 72°C for 7 min.

A 2% agarose gel (Sigma A9539) was prepared using 1x TBE buffer was then heated in the microwave for 4 minutes. The gel was cooled for 5 minutes on the bench at 25°C then 5 µl of GelRed® (Biotium, USA) was added for staining. Before

immersing in the electrophoresis tank that contained electrophoresis buffer (1x TBE), it was left to set on the bench for 15 minutes. A total of 2 µl of the PCR DNA product and 1 µl of the 3x loading dye was picked and loaded in the separate wells submerged in the buffer. O'GeneRuler (Thermo Scientific, USA) 100 bp plus DNA ladder of 0.1 µg/µl, 50 µg was also loaded alongside the samples to estimate the fragment sizes. Electrophoresis was performed at a constant voltage of 100 for 1 hour visualization of the bands was done under a UV transilluminator and photographed using Alpha digi doc system (Alpha Innotech).

3.5.7 PCR Run on High Resolution Melting Analysis for Screening F_1s

To detect SNPs using high resolution melting analysis, finger millet DNA was amplified in a total volume of 20 µl (Table 3.6). Master Mix for each primer was prepared in an eppendorf tube and then loaded in a 24 well plate then centrifuged at 2000 x g for 1 minute using an AllegraTM 25R centrifuge (BECKMAN COULTER Inc., USA) at 4°C PCR run.

Table 3.6: Master Mix preparation (1x volume)

Nuclease free water (-)	8.4 µl
DNA Template (20 ng - gDNA)	1 µl
Forward primer (0.2 µM)	0.3 µl
Reverse primer (0.2 µM)	0.3 µl
Master mix SYBR Green (1×)	10 µl
Total volume	20 µl

The PCR temperatures were programmed as follows: initial denaturation for enzyme activation at 95°C for 3 minutes, 35 cycles followed by final denaturation 95°C for 1

minute, annealing at 60°C for 30 seconds; data acquisition- elongation at 60°C for 30 seconds and melting curve 60°C, 95°C (60-95°C) at infinite hold. The samples were run in Piko-Real equipment (Thermo Scientific, USA). After the run, the melting curves were displayed on the computer screen having PikoReal™ software 2.1 connected to the Piko-Real equipment (Thermo Scientific, USA) for analysis.

3.6 Data Analysis

The data collected from observations in the screenhouse and field were subjected to analysis of variance (ANOVA) using general linear models (GLM) procedure and means separated by Fischer's protected least significant differences ($p \leq 0.05$) resident in SAS 8.2 ed statistical package(SAS Institute, 2001).The screen house data were analysed as 6 varieties × 3 gametocide levels factorial treatment arrangement in a completely randomized design (CRD). Field data were analysed as Randomised Complete Block Design (RCBD) with each crossing block serving as a replication. The high resolution melting analysis was performed using PikoReal™ software 2.1. The results were scored in an alternating manner of the parent (female and male) melt curves and the progenies in order to determine heterozygosity.

CHAPTER FOUR

RESULTS

4.1 Screen House Ethrel Application and Agronomic Traits Study.

4.1.1 Production of Controlled Crosses

Plate 4.1 shows a treated and pollinated head between two parental genotypes OK and GE.

Resolution = 160 x 120

Plate 4. 1: Ethrel emasculated and pollinated finger millet head

(Source: Author, 2012)

4.1.2 Preclusive study on Ethrel gametocide

The screening for F_1, across the 15 crossing blocks resulted in 118 plants which represented an average of 4.31% and ranging between 0.85-16.35% female cross success rate per each female head in all the four treatments (Table 4.1). True F_1 successful crosses were in the range of 1-17 per treated female head. Higher success

rates were recorded in crosses made between GE x KA-72 having the highest at 16.5%, IE 4115 x U-15 (11.92%) and IE 4115 X GE (7.87%).

Table 4.1: Percent pair success rate of true F_1 crosses

Block No.	Cross	Mean % Success Rate per Head
1	IE 2872 x U-15	1.65
2	GE x IE 2872	1.65
3	IE 2872 x KA-72	3.45
4	IE 4115 x GE	7.87
5	OK-1 x U-15	2.94
6	GE x KA-72	16.35
7	OK-1 x GE	0.85
8	IE 4115 x IE 2872	4.76
9	IE 4115 x U-15	11.92
10	KA-72 x IE 4115	0.89
11	OK-1 x IE 2872	5.13
12	IE 4115 x OK-1	1.90
13	U-15 x KA-72	1.65
14	OK-1 x KA-72	1.90
15	U-15 x GE	1.79
Mean		**4.31**

4.1.3 Effect of Ethrel on Finger Millet Agronomic Traits

Table 4.2 shows significant GL and genotype and GL x Genotype interaction differences for plant height. There was no significant GL and genotype and no significant GL x Genotype interaction for productive tillers. The ANOVA representation did not show significant GL and GL x Genotype interaction but showed significant differences in genotype for ear exertion length per plant. There was no significant GL x Genotype interaction and significant GL and genotype differences for seed weight per plant.

Table 4.2: Analysis of variance mean squares for finger millet agronomic traits as influenced by gametocide level

		Mean Squares			
Source	df	Plant Height	Productive Tillers	Ear Exertion Length	Grain Weight
GL	3	7084.01**	1.3270 ^{ns}	99253.9**	46.712**
Genotype	5	1326.10**	1.9133 ^{ns}	647.2 ^{ns}	20.527**
GL x Genotype	15	207.74**	0.6756 ^{ns}	538.6 ^{ns}	5.580 ^{ns}
Error	213	65.04	0.8508	432.3	2.721
Total	236				

ns = not significant, * = significant at $p \leq 0.05$ and **= significant at $p \leq 0.01$

The means showing effect of ethrel on days to heading, days to anthesis, days to maturity, productive tillers, plant height, ear exertion and grain weight across the four gametocide levels is presented in Table 4.3.

Table 4.3: Screen house agronomic trait means under different gametocide levels (GL) across six finger millet varieties per plant

GL (ppm)	DH (days)	DA (days)	DM (days)	EE (mm)	PH (cm)	GW (gms)	NPT
0 ppm	64	76	119	102.7	95.95	9.91	1.946
1,500 ppm	64	76	119	24.0	76.50	11.85	2.083
1,750 ppm	64	76	119	20.5	74.08	11.65	2.083
2,000 ppm	64	76	119	19.9	72.75	10.97	2.306
Grand Mean	64	76	119	41.8	79.82	11.10	2.105
L.S.D (0.05)	-	-	-	18.33**	7.109**	1.454**	0.8131^{ns}
CV%	0.0	0.0	0.0	49.8	10.1	14.9	43.8

DH = days to heading; DA = days to anthesis; DM = days to maturity; GL = gametocide level; EE = ear exertion; PH = plant height; GW = grain weight; PT = productive tillers; ns = not significant, * = significant at $p \leq 0.05$ and **= significant at $p \leq 0.01$

Ethrel did not have any significant effect on days to heading, days to anthesis of parental plants and days to maturity. The effect of ethrel increased with increased gametocide concentration for ear exertion and plant height. Table 4.3 above shows that concentrations of ethrel significantly affected ear exertion and plant height. The average grain weight was more at 1,500ppm (11.85 g) and zero ppm (9.91 g) having the least. There was significant difference in ear exertion, plant height and grain weight caused by GL and no significant difference in number of productive tillers. The cross between GE x KA 72 gave highest weight of 12.063 grams compared to others (Table 4.4). Through interaction of GL x Genotype, OK x GE recorded the

highest grain weight grand mean of 13.125 grams with lowest of 8.750 grams IE 4115 x OK (Appendix III).

Table 4.4: Grain weight means for the 15 crosses generated from six finger millet genotypes

Serial No.	Cross	Grain Weight (gms)
1	IE 2872 x U-15	11.813
2	GE x IE 2872	10.943
3	IE 2872 x KA-72	10.500
4	IE 4115 x GE	11.188
5	OK-1 x U-15	10.755
6	GE x KA-72	12.063
7	OK-1 x GE	11.938
8	IE 4115 x IE 2872	10.500
9	IE 4115 x U-15	11.360
10	KA-72 x IE 4115	10.750
11	OK-1 x IE 2872	11.750
12	IE 4115 x OK	9.375
13	U-15 x KA-72	10.313
14	OK-1 x KA-72	12.000
15	U-15 x GE	11.375

Screen house data from each parent single plant are presented in Appendix IV. There was slight variation in days to 50% heading, flowering and maturity across the six varieties planted.

Figure 4.1 below is a histogram representation of days to heading, days to flowering, days to maturity, plant height, ear exertion, grain weight and number of productive tillers comparison of treated and crossed genotypes across GLs. Days to 50% heading was varied, variety GE, OK-1, KA-72 and IE 4115 had (67 days), variety U-15 (61

days) and variety IE 2872 (56 days) in the four treatments. The highest days to 50 % flowering was recorded in variety IE 4115, OK-1, KA-72 and GE (79 days) followed by U-15 (73 days) and IE 2872 (68 days) being the least. Among the six varieties, variety IE 2872 matured early (110 days), followed by U-15 (115 days), KA-72 and IE 4115 at (121 days) then OK-1 and GE matured late (122 days). Looking at gametocide concentration effects on the varieties, there was slight variation on the plant height across the varieties though IE 2872 seemed to record the least thus it was sensitive. Means across the treatments for each cross is presented in Appendix V. Variety U-15 recorded the highest ear exertion length compared to variety IE 2872 that was much affected by the treatment. Crosses under the check recorded very high ear exertion while emasculated genotypes under ethrel treatment were mostly affected having the least mean length. Means across the treatments for each cross are presented in Appendix VI. Gametocide concentration levels had significant influence on grain weight per plant across the varieties. The means for grain weight per treatment in the 15 crosses generated plus the reciprocals is presented in Appendix III. Gametocide levels did not have any significant influence the number of productive tillers which is clearly seen across the varieties. Mean numbers of productive tillers per plant across the four treatments are presented in Appendix VII.

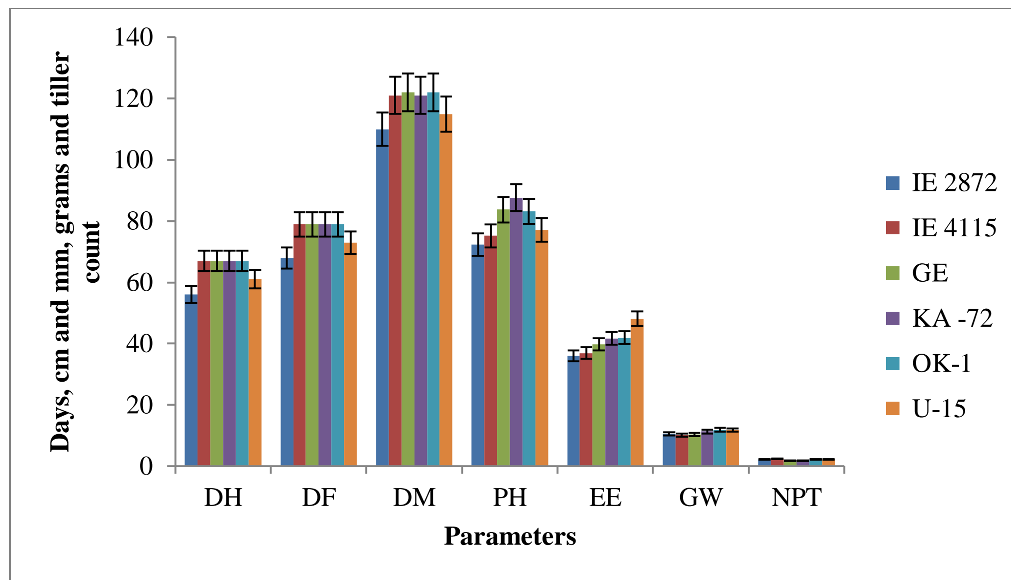

Figure 4. 1: Mean gametocide effect frequency across seven parameters for the six genotypes crossed

4.2 Field Study on Post Emasculation Generation Plants

4.2.1 Field F_1 Screening from Selfs

The histogram in Figure 4.2 below compares percent success rate per each treatment. Ethrel application at 2,000ppm was the highest in the production of true F_1 plants compared to 1,750ppm, 1,500ppm and 0ppm. This figure represents GL success rates where 2 crosses were from 0ppm GL treatment (1.65%), 11 from 1,500ppm GL treatment (9.67%), 34 from 1,750ppm GL treatment (29.79%) and 71 from 2,000ppm GL treatment (63.98%).

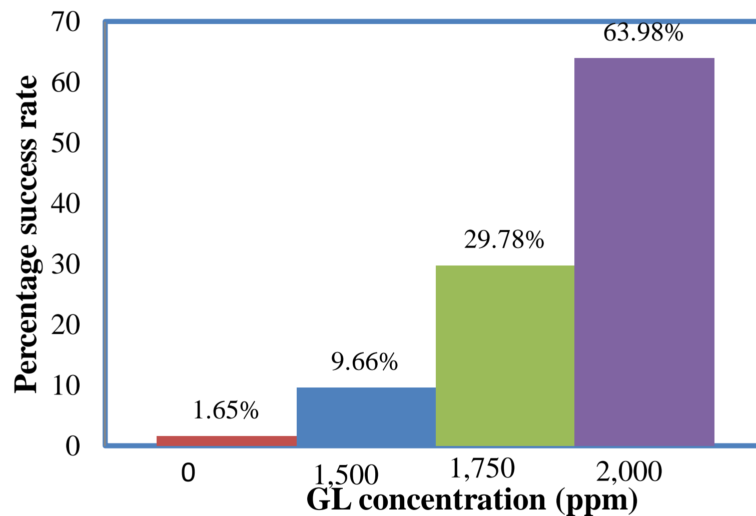

Figure 4.2: Gametocide level percent proportion on Post ethrel treated generation showing number of recorded F_1s

Mean variations for the quantitative traits is presented in Table 4.5 below. There was no significant GL difference for plant colour, ear shape, plant height and days to flowering traits indicating that ethrel did not have effect on post on post treated generation. The means for 15 crosses and success rate of the counted true F_1s were significantly different. Highest plant stand count was recorded at 1,500ppm with 116.933 followed by 1,750ppm with 114.167. Gametocide levels 0ppm and 2,000ppm had 112.520 and 111.900 respectively and was statistically different attributed with the number of seeds harvested per treated female head.

Table 4.5: Fischer's protected least significant differences for comparing means of finger millet post ethrel treated generation

GL	Traits						
	Plant colour	Ear shape	Plant height (cm)	Plant stand count	Days to 50% flowering	Crosses	Success rate
0ppm	1.560a	1.720a	73.987a	112.520ab	79.467a	0.027c	0.022c
1,500ppm	1.533a	1.667a	75.667a	116.933a	79.033a	0.367bc	0.322bc
1,750ppm	1.533a	1.700a	75.767a	114.167ab	78.500a	1.133b	0.993b
2,000ppm	1.533a	1.733a	74.067a	111.900b	78.800a	2.367a	2.132a
LSD	0.181^{ns}	0.193^{ns}	2.743^{ns}	4.550**	2.122^{ns}	0.934**	0.847**

Means with the same letter in a row are not significantly different at P<0.05 level of significance. *- significant at P < 0.05, **- significant at P < 0.01, ns- not significant

LSD = Least Significant Difference

4.3 Laboratory Screening for True F_1s from Parents.

4.3.1 Genomic DNA Quality and Quantity.

The DNA extracted from the six parental varieties and 120 progenies was of good quality with high molecular weight. The Nanodrop© 1000 (Thermo Scientific, USA) spectrophotometer measured absorbance (A) from 220 to 350 nm wavelengths and displayed the DNA concentration. For pure DNA, the A260/280 should be between 1.8 to 2.0 and the A260/230 between 1.8 to 2.3 where lower values indicate the presence of protein contamination.The concentration of DNA ranged from 62.8 ng/µl to 551.6 ng/µl (Appendix VIII).

4.3.2 Polymerase Chain Reaction.

Four SNPs out of the total five primer pairs synthesized (Table 3.4) successfully amplified consistent bands (Figure 4.3, 4.4, 4.5). Samples 1 and 2 in Figure 4.3, samples 1,2,3,4,5 and 6 in Figure 4.4 and samples 1,2,3,5, and 6 in Figure 4.5 the right primer had double bands, lane 1 being the correct PCR fragment size. Because the samples were not to be taken for sequencing, this was not necessary and could not affect analysis using PikoReal™ software 2.1 in determining hybrids.

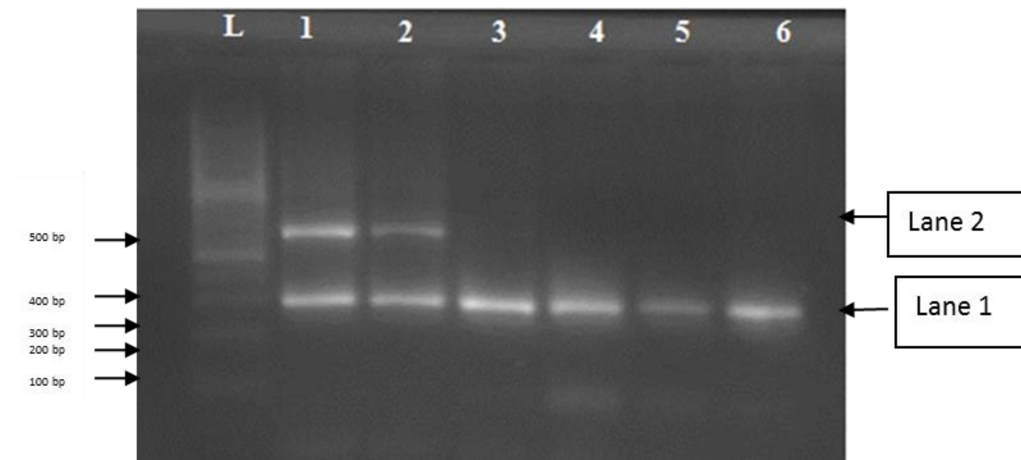

Figure 4.3: Agarose gel (2%) zymogram of parental DNA PCR amplification products for SNP 2(1458-1992 L and 1458-1992 R) run under a PCR touch-down program with annealing temperature ranging from 60°C - 55°C

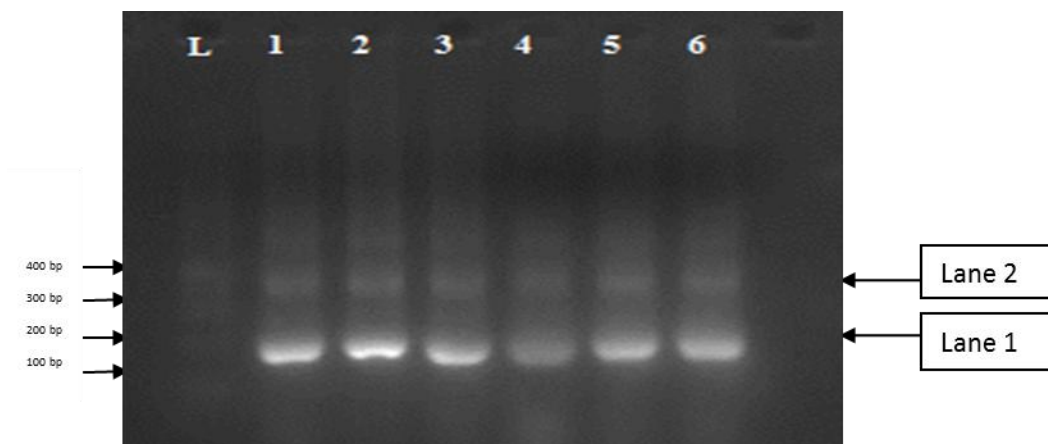

Figure 4.4: Agarose gel (2%) zymogram of parental DNA PCR amplification products for SNP 3(1925-2474 L and 1925-2474 R) run under a PCR touch-down program with annealing temperature ranging from 65°C - 60°C

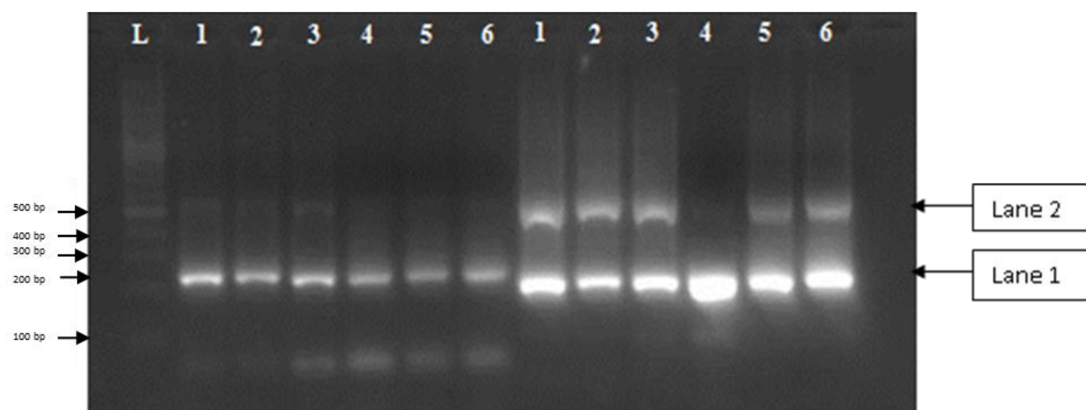

Figure 4.5: Agarose gel (2%) zymogram of parental DNA PCR amplification products for SNP 4(2837-3585 L and 2837-3585 R) on the left and SNP 5(3502-4102 L and 3502-4102 R) on the right run under a PCR touch-down program with annealing temperature ranging from 63°C

The two primers were run on the same gel because they had the same annealing temperature range (Figure 4.5).

4.3.3 High Resolution Melting Analysis (HRMA).

Differences in melting curve shape that correlated to genotype were revealed by HRMA. SNP markers were able to distinguish the multiple alleles in the progenies from those of the parents as shown in Table 4.6 and Figure 4.6.

Table 4.6: Genetic analysis of the 120 progenies alongside six finger millet varieties across GLs and their reciprocals

Sample ID		8 PROGENIES PER EACH CROSS ACROSS THE 4 SNP MARKERS							
♀	♂	SNP 2		SNP 3		SNP 4		SNP 5	
			Rec		Rec		Rec		Rec
P1 (A-IE 4115)	P2 (B-IE 2872)	AAAH	BBBH	HHHH	HHHH	AHHH	AHHH	HHHH	HHHH
P1 (A-IE 4115)	P3 (B-GE)	HHHH	HBHH	HHHH	HHHH	HHHH	HHHH	HHHH	HBBH
P1 (A-IE 4115)	P4 (B-KA-72)	AHAH	AHHH	HHAH	HHHH	HHHH	HHHH	AHAA	HHHB
P1 (A-IE 4115)	P5 (B-OK-1)	AHHH	BHHH	AAHH	BBBH	HHHH	HBBH	HAHA	BHHH
P1 (A-IE 4115)	P6 (B-U-15)	HHHH	BBBH	HHHH	HHHH	AHHH	BHHH	HAAH	HHBB
P2 (A-IE 2872)	P3 (B-GE)	AAHH	BHHH	AHHH	HBBB	HHHH	HHHB	AAHH	HBBH
P2 (A-IE 2872)	P4 (B-KA-72)	HHHH	HHHH	HHAA	HHHH	HHHH	BHHH	HHAA	HBBH
P2 (A-IE 2872)	P5 (B-OK-1)	HAAA	HHBH	HHHH	BBHH	HAHA	BBBB	AAAA	HHBB
P2 (A-IE 2872)	P6 (B-U-15)	HHAA	HHHH	AHHH	HHHB	HHHH	HBHH	HAHH	HHHH
P3 (A-GE)	P4 (B-KA-72)	HHHH	HHHH	HHHH	HHHH	HHHH	HHHH	HHHH	BHHH
P3 (A-GE)	P5 (B-OK-1)	AHHH	HHHH	HHAH	HHHH	HHHH	BHHH	HHAH	HBHH
P3 (A-GE)	P6 (B-U-15)	AHHH	HBBH	HAHH	HHHH	AHAA	HHHB	HHHH	HHHH
P4 (A-KA-72)	P5 (B-OK-1)	HHHH	HBHH	HAAH	HHHH	AAHA	BBBH	HHHH	BHHH
P4 (A-KA-72)	P6 (B-U-15)	HHHH	HHHB	HHAH	HHHH	HAHH	HHHB	AHAA	HHBH
P5 (A-OK-1)	P6 (B-U-15)	HHHH	HHHH	AAHA	HHHH	AAHH	HHHH	AHHH	HHHH

P – Parent; A - Female parent; B - Male parent; A or B - Homozygote progenies at the locus screened;

H - Heterozygote progeny at the locus screened; Rec - Reciprocals

Melt profile observations showing fluorescence range of parents and progenies is shown in Figure 4.6.The data obtained after PCR run was transformed to derivative of fluorescence to temperature dissociation (dF/dT) to give melting profiles of the parental lines and heterozygotes using SNP4 (2837-3585) (Figure 4.7). Comparison in melting curves from PCR products of parents and F_1s were overlaid at high temperature (Figure 4.7). Heterozygotes could be easily identified by a change in melting curve shape from selfs. After analysis progenies were clearly distinguished from their respective parental lines (Table 4.7). The table shows successful crosses obtained across the four GLs and their recipricals using four SNP markers. Heterozygote success rates were high at 2,000ppm (5.05%), 1,750ppm and 1,500ppm (4.58%) and 0ppm having the least (4.27%).

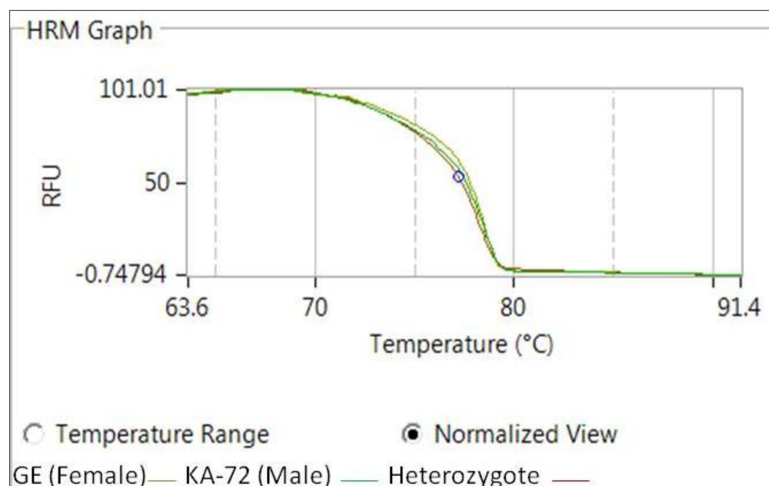

Figure 4.6: Fluorescence change over increasing temperature range for GE, KA-72 and heterozygote PCR amplification products

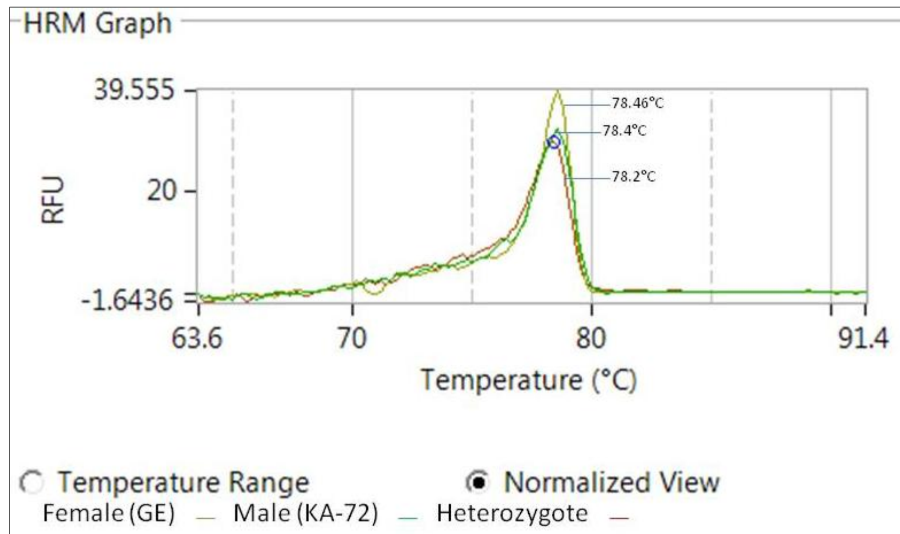

Figure 4.7: Melt profile for GE, KA-72 and heterozygote PCR amplification products from HRM analysis

High Resolution Melting Analysis gave best results in differentiating between hybrids and selfs. The difference in the melting curves between the parental lines and progenies was 0.23°C for GE and KA-72 but varied across the parents.

CHAPTER FIVE

DISCUSSION

5.1 Screen house and Field Study

The screen house success rate range of 0.85-16.35% on true F_1 identification is indeed a potential aspect to be considered in breeding. Ethrel treatment to induce male sterility depends on the concentration and the stage of the plant (Rowell & Miller, 1971). When the florets are not sterile, this hinders emasculation. Finger millet treated heads were sterile due to the gametocidal reaction which was ideal to ensure cross pollination for hybrid development, however, unfertilized heads under went self fertilization. Accuracy in calculations during ethrel preparation and application during appropriate developmental stage as reported by (Fairey & Stoskopf, 1975; Chakraborty et al., 2000) hinders 100% hybrid production. Rowell & Miller (1971) reported that, ethrel spray induced male sterility in wheat, and that the percentage of sterility depended on the concentration and the stage of the plant.

More hybrids were generated at 2,000 ppm. Rowell & Miller (1971) reported that ethrel, applied at rates of 2,000 and 3,000 ppm at the early, midboot, and late boot stages of wheat development produced up to 100% sterility. Chemical emasculation proves to be an effective technique to be adopted in creation of hybrids especially in 100% self pollinating crops. Conventional techniques are laborious, cumbersome and need highly skilled personnel. Breeders need to employ more of chemical

emasculation using ethrel particularly in finger millet to generate hybrids with desirable traits in breeding programs.

Ethrel applied at 1,500 ppm, 1,750 ppm and 2,000 ppm having no significant physiological effect on days to anthesis and maturity for the screen house study is promising. Looking at maturity period, it is an important aspect most farmers consider when selecting a variety for planting, as they prefer early maturing ones. These results were in agreement with the findings of Oduori et al. (2008). Application of ethrel on barley did not have any effect on flowering period as reported by (Moes and Stobbe (1991a). In these findings, it indicates that ethrel could be used at any point without interfering with flowering or maturity period in finger millet.

Besides, Earley & Slife (1969) reported delayed flowering and maturity in maize, Stoskopf & Law (1972) and De Milliano (1983) observed delayed heading on wheat, and Law & Stoskopf (1973) observed delayed heading in barley, this did not agree with the current study findings on finger millet. Days to 50% flowering on emasculated plants in the screen house trial was not significantly affected as compared to the post emasculated plants in the field because during planting the plants were staggered. The results obtained were in consonance with green house findings of Oduori et al. (2008) which facilitated cross pollination in achieving success true F_1s.

Application of the four gametocide levels in the screen house experimental study caused significant reduction in plant height. This could be due to slow cell division and reduction in expansion (Moore, 1950) where the cells expand laterally producing thick shoots (Alberts et al., 1989) after ethrel application. According to Karchi (1969), ethrel application was shown to reduce plant height in certain legumes such as soybeans, corn and pea which in turn results in resistance to lodging. The decrease in finger millet plant height by ethrel in this study was similar to the observations made by Oduori et al. (2008). Plant height reduction across the four GL was in agreement with the outcomes observed in barley by Earley & Slife (1969) and in maize by Rowell & Miller (1971); Stoskopf & Law (1972); Law & Stoskopf (1973) and De Milliano (1983).

However, significant reduction in plant height would be a great setback in the use of ethrel as a tool in developing hybrids in cereals as it would make cross pollination and harvesting tedious because the plants are too short (Crook & Ennos, 1994). All these depend on the variety; developmental stage of application, concentration, and environmental conditions which could promote or inhibit ethrel effects on internode length as observed by (Krishnamoorthy, 1993). There was a significant genotype and gametocide difference with ethrel application. Because looking at gametocide concentration effects on the varieties, there were significant differences in height though IE 2872 seemed to record the least thus was sensitive to ethrel. Study report by Grabowska et al. (2005) indicates that, male flowers in monoecious hemp plant were achieved successfully by appliying ethrel at 1,000ppm to 2,000ppm.

Lack of significant Genotype x Gametocide interaction and no significant differences between genotype and GL in finger millet tillering was not in agreement with the findings of Woodward & Marshall (1987) in barley. Number of productive tillers and fingers per head has positive impact on grain yield in crops as reported by Ravindran et al. (1996). Farmers of finger millet, in most cases will go for a variety that has high tillering trait (Lenne et al., 2006).

Application of ethrel at varied concentrations from the results caused significant difference in genotypes on ear exertion where treated plants with 2,000 ppm had much effect on ear length compared to the control. The observation was that ethrel effect increased with the concentration irrespective of the genotype. Although ear length is not a crucial characteristic, it is important to breeders because poor ear exertion renders cross pollination difficult (Fairey & Stoskopf, 1975; Oduori et al., 2008). This will not permit production of large numbers of hybrids in breeding programs as reported on barley and wheat (Rowell & Miller, 1971; Stoskopf & Law, 1972; Law & Stoskopf, 1973).

The lowest grain weight was recorded on the untreated control plants, this implies that, ethrel application increases yield and weight due to increased spikes. Ethrel application at 1,500ppm is the best as it resulted in increased grain yield as supported by reports of Early & Slife (1969), and Oduori et al. (2008) in maize and finger millet, showing increased concentration reduced yield. Ethrel delays staminate flower growth and thus effectively causing male sterility (Lower & Miller, 1969).

Low significant negative correlation of grain yield with productive tillers and plant height were observed by Pedersen & Toy (2001) in sorghum. Significant GL and Genotype differences on grain weight indicate that ethrel effect is irrespective of the genotype of which is in agreement with the findings of Beek (1988).

Genotypes with open ear shape exposes much surface area to wind compared to incurved headed genotypes. The results obtained were in accordance with those of Oduori et al. (2008). Determination of true F_1 plants from selves relied on the use of morphological markers. Purple pigmentation being dominant over green was a determinant factor in identifying successful crosses as reported by (Takan et al., 2004). Kearsey & Pooni (1996) reported that different genes that have dominance on the character may control the character in different aspects.

Though morphological markers are easy to score, the disadvantage is that they may be limited in number and influenced by environmental factors or the developmental stage of the plant (Winter and Kahl, 1995). During identification of successful crosses using plant colour, it was abit tedious because some hybrids had slight colour difference from selfs. Some parameters are to be collected at a specific developmental period, for example plant stand count and height were recorded at almost maturity time and ear shape at ripening stage. Oduori et al. (2008) reported that, accuracy of morphological markers in hybrid determination is abit low especially in finger millet where pure homozygotes mask desirable heterozygotes. This engineered the use of molecular markers (SNPs) which is specific and gives quality results.

5.2 Molecular Analysis

The F_1 hybrids were initially selected using morphological means but heterozygosity further confirmed using SNP markers. Single nucleotide polymorphism marker detected difference in parental and F_1 genotypes in this study; this is because they are many and highly distributed throughout the genome of the plant as supported by (Batley et al., 2003). By detecting polymorphism that comes as a result of changes in the DNA sequences, molecular markers have opened an avenue in identifying lines with traits of interest for breeding programmes.

Homozygous parental and heterozygous lines amplified PCR fragments of less than 300 bp at an annealing temperature of 55°C with the four designed finger millet SNP marker. This is because shorter PCR products show larger melting temperature differences especially between homozygous parental lines that have some similarity between the base sequences. However, the shorter PCR products for the parents did not show significantly larger temperature shifts.

The parental lines were unmistakably distinguished from each other in the melt profiles because they differed slightly or no difference in the melt temperature differnce. The heterozygote profile was more clearly distinguishable in the difference plots and this was used to define the heterozygous genotypes though some looked like the parents thus treated as selfs.

Despite the fact that HRMA accuracy is high, there are factors reported that might affect the accuracy, these include; sample source and preparation, amplicon length, GC content, dye, equipments (Montgomery et al., 2010), to mention but a few. High resolution melt analysis is very much sensitive to DNA PCR products having smaller fragment sizes though they might be up to 600 bp as reported by Wittwer (2009). In this study, the size (bp) of the HRMA fragments was less than 300 bp from the parental and F_1 lines was clearly differentiated with the HRM analysis. Due to this, it resulted in 100% sensitivity and specificity in identifying heterozygote and homozygote genotypes. However, high fragment sizes ranging between 400 and 1000 bp reduce sensitivity to 96.1% and 99.4% specificity (Montgomery et al., 2010). When the PCR product length increases it makes the difference between the melting curves to decrease thus causing errors in detecting a particular allele.

Errors can occur during detection of the particular allele when PCR product length is larger, leading to decreased difference between the melting curves suggesting a dependence on product length. Higher sensitivity is achieved specifically using fresh samples; this is because the DNA might have degenerated during sampling, preservation and or poor handling during extraction (Takano et al., 2007). Degradation of the DNA is mostly caused by endonucleases (Sahu et al., 2012).

The differences between the homozygous parental PCR products can be more clearly visualized and quantified basing on the difference in the difference plots of normalized curves than the melting curve analysis. This is due to the difference

between the melting temperatures of the parental line PCR products being very small or there being only one or a few bases that differ between the amplified products. This indicates that they can be used to differentiate between the homozygous and heterozygous genotypes more clearly than the melting curves, especially in cases where the melting differences are very small. The distinctive shape of the heterozygote is used to differentiate heterozygous from homozygous genotypes.

The PCR products of the heterozygote F_1's in this study was differentiated from pure homozygous parental line PCR products due to their distinct characteristic melting curves in the difference plots generated after high resolution melting. The shape of the PCR amplicon for the heterozygotes is identified by a unique "wave" shape in the difference plots, confirmed by the difference between genotypes visible in difference plots due to unique trace patterns (Wittwer et al., 2003). The obtained results were in accordance with those of Park et al. (2009) who successfully distinguished between homozygotes and heterozygotes in F_2 populations in pepper (*Capsicum spp)* using Pp201 SNP marker.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. Optimal ethrel concentration that resulted in high hybrids was 2,000ppm compared to 1,750 ppm, 1,500 ppm and zero ppm. There was an average of 4.31% female cross success rate ranging between 0.85-16.35%.

2. Ethrel had much effect on ear exertion length, plant height and grain weight though there was no effect for days to heading, days to flowering days maturity and number of productive tillers. Ethrel does not have any effect on post emasculated and residual finger millet generation.

3. Both morphological and molecular markers were able to identify of true F_1s. This is because both techniques were able to detected successful crosses at 2,000ppm. Though molecular markers detected high success rate at 1,500 and 1,750ppm compared to morphological markers. Single nucleotide polymorphism marker was able to successfully differentiate between homozygous and heterozygous lines by the differences in melt profiles using HRM analysis.

6.2 Recommendations

I would like to recommend that:

1. Further studies should be done especially application of ethrel beyond 2,000 ppm in finger millet to to see the difference in success rate.

2. The F_2 and subsequent generations should be planted in order to check how the progenies segregate using based on morphological and molecular markers.

3. Diallel mating design in conjunction with chemical emasculation technique using ethrel should be emphasised in breeding programmes to allow exchange of valuable traits between varieties through crossing.

REFERENCES

Acevedo, E., Silva, P., & Silva, H. (2002). Wheat growth and physiology. CURTIS, BC et al. Bread wheat. *Rome, Italy: FAO Plant Production and Protection Series (FAO)*, *30*.

Acquaah, G. (2007). *Principles of plant genetics and breeding*. John Wiley & Sons.

Adams, W. T. (1983). Application of isozymes in tree breeding. In T. J. [eds. . In Tanksley, S. D. and Orton (Ed.), *Isozymes in plant genetics and breeding, part A* (pp. 381–400).

Agarwal, M., Shrivastava, N. & Padh, H. (2008). Advances in molecular marker techniques and their applications in plant sciences. *Plant Cell Reports*, *4*(27), 617–631.

Akagi, H., Nakamura, A., Yokozeki-Misono, Y., Inagaki, A., Takahashi, H., Mori, K. & Fujimura, T. (2004). Positional cloning of the rice Rf-1 gene, a restorer of BT-type cytoplasmic male sterility that encodes a mitochondria-targeting PPR protein. *Theoretical and Applied Genetics*, *8*(108), 1449–1457.

Akkaya, M. S., Bhagwat, A. A. & Cregan, P. B. (1992). Length polymorphisms of simple sequence repeat DNA in soybean. *Genetics*, *4*(132), 1131–1139.

Alberts, B., Bray, D., Lewis, J., Raff, M., Robertsand, K. & Watson, J. D. (1989). , Inc. In *Molecular Biology of the Cell.* (2nd Ed.). New York and London.: Garland Pub.

Anon, L. (2008). Finger millet blast in East Africa: *Pathogen Diversity and Disease Management Strategies.*

Applied Biosystems, Inc. (2009). A Guide to High Resolution Melting (HRM). Analysis. Available for download at www.appliedbiosystems.com/hrm (Accessed on July, 2013).

Arias, R. S., Netherland, M. D., Scheffler, B. E., Puri, A. & Dayan, F. E. (2005). Molecular evolution of herbicide resistance to phytoene desaturase inhibitors in Hydrilla verticillata and its potential use to generate herbicide resistant crops. *Pest Management Science*, *61*(3), 258–268.

Armour, J. A. L., Alegre, S. A., Miles, S., Williams, L. J. & Badge, R. M. (1999). Minisatellites and mutation processes in tandemly repetitive DNA. Microsatellites: evolution and applications. (pp. 24–33). Oxford: Oxford University Press.

Ashraf, M. & Ahmad, S. (2000). Genetic effects for yield components and fibre characteristics in upland cotton (*Gossypium hirsutum* L.) cultivated under salinized (NaCl) conditions. *Agronomy*, *8*(20), 917–926.

Ayyangar, G. N. R. (1932). The inheritance of characters in ragi, Eleusine coracana Gaertn. *Madras Agr*, *20*(7), 1–9.

Ayyangar, G. N. R. & Rao, U. A. (1932). Inheritance of characters in ragi, Eleusine coracana (acrtn., Part VI. Earlicad shapes. *Indian*, *12*(7), 254–65.

Ayyangar, G. N. R. & Wariar, U. A. (1936). Inheritance of glume length in ragi, *Eleusine coracana* Gaertn., the finger millet. *Madras Agr.Y*, (24), 132–34.

Babu, B. K., Agrawal, P. K., Pandey, D., Jaiswal, J. P., & Kumar, A. (2014). Association mapping of agro-morphological characters among the global collection of finger millet genotypes using genomic SSR markers. *Molecular Biology Reports*, *8*(41), 5287–5297.

Baker, M. C. (1996). The polysynthesis parameter.

Baker, R. D. (2003). Millet Production, Guide A-414. New Mexico State University, College of Agriculture and Home Economics, Las Cruces, NM.

Bakhru, H. K. (1996). The Complete Handbook of Nature Cure. Jaico publishing house.

Barbeau, W. E. & Hilu, K. W. (1993). Protein, calcium, iron, and amino acid content of selected wild and domesticated cultivars of finger millet. *Plant Foods for Human Nutrition*, *2*(43), 97–104.

Basra, A. S. (1999). *Heterosis and hybrid seed production in agronomic crops*. CRC.

Batley, J., Barker, G., O" Sullivan, H., Edwards, K.J. & Edwards, D. (2003). Mining for single nucleotide polymorphisms and insertions/deletions in maize expressed sequence tag data. *Plant Physiol*, (132), 84–91.

Beek, M. A. (1988). *Selection procedures for durable resistance in wheat.* Wangeningen Agricultural University (WAU) dissertation No. 1239. Abstract.

Bendale, V. W., Bhave, S. G. & Pethe, U. B. (2002). Genetic variability, correlation and path analysis in finger millet (*Eleusine coracana* Gaertn.). *Soils and Crops*, *12*, 187–191.

Bennetzen, J. L., Dida, M. M., Manyera, N. W. M. & Devos, K. M. (2003). *Characterization of genetic diversity in finger millet (Eleusine coracana). [online]Available:http://www.cerealsgenomics.org/documents/BennetzenProp.doc*. (Accessed on November 2012).

Berhe, T. & Miller, D. G. (1978). Studies of Ethephon as a possible selective male gametocide on tef. *Crop Science*, *18*, 35–38.

Bhatt, A., Singh, V., Shrotria, P. K. & Baskheti, D. C. (2003). Coarse Grains of Uttaranchal: Ensuring sustainable Food and Nutritional Security. *Indian Farmer's Digest*, 34–38.

Bi, I. V., McMullen, M. D., Sanchez-Villeda, H., Schroeder, S., Gardiner, J., Polacco, M., Soderlund, C., Wing, R., Fang, Z. & Coe, E. H. (2006). Single nucleotide polymorphisms and insertion-deletions for genetic markers and anchoring the maize fingerprint contig physical map. *Crop Sci*, *46*, 12–21.

Borba, E. L., Semir, J. & Shepherd, G. J. (2001). Self-incompatibility, Inbreeding Depression and Crossing Potential in Five BrazilianPleurothallis (Orchidaceae) Species. *Annals of Botany*, *1*(88), 89–99.

Bouis, H. E. (2000). Enrichment of food staples through plant breeding: A new strategy for fighting micronutrient malnutrition. *Nutrition*, (16), 701–4.

Brown, B. (1996). Lodging.The Cereal Sentinal, University of Idaho. *Cooperative Extension System*, *10*, 2–4.

Brown, C. M., & Earley, E. B. (1973). Response on One Winter Wheat and Two Spring Oat Varieties to Foliar Applications of 2-Chloroethyl Phosphonic Acid (Ethrel). *Agronomy Journal*, *5*(65), 829–832.

Budar, F. & Pelletier, G. (2001). Male sterility in plants: occurrence, determinism, significance and use. *Comptes Rendus de l'Académie Des Sciences-Series III-Sciences de La Vie*, *324*(6), 543–550.

CAB International. (2005).*Crop protection Compendium*. 2005 ed. Wallingford. UK.

Carena, M. J. (2005). Maize commercial hybrids compared to improved population hybrids for grain yield and agronomic performance. *Euphytica*, *3*(141), 201–208.

Chahal, G. S. & Gosal, S. S. (2002). *Principles and procedures of plant breeding: Biotechnological and conventional approaches*. Alpha Science Int'l Ltd.

Chakraborti, M., Prasanna, B. M., Hossain, F., Mazumdar, S., Singh, A. M., Guleria, S. & Gupta, H. S. (2011). Identification of kernel iron-and zinc-rich maize inbreds and analysis of genetic diversity using microsatellite markers. *Journal of Plant Biochemistry and Biotechnology*, *2*(20), 224–233.

Chakraborty, K., Devakumar, C. & Tomar, S. M. S. (2000). Synthesis and screening of anilates as chemical hybridising agents for wheat (*Triticum aestivum* L.). *Tropical Agricultural Research*, (12), 398–407.

Chapman, B., Mondragon, J. C., Bunch, R. A. & Paterson, A. H. (2002). Breeding and biotechnology. *Cacti: Biology and Uses*, 255–271.

Chase, C. D. (2007). Cytoplasmic male sterility: a window to the world of plant mitochondrial–nuclear interactions. *TRENDS in Genetics*, *23*(2), 81–90.

Chetan, S. & Malleshi, N. G. (2007). Finger millet polyphenols: Characterization and there nutraceutical potential. *American. J. Food Technology*, *7*(2), 582 –592.

Chevreux, B., Pfisterer, Drescher, T. B., Driesel, A. J., Muller, W. E. G., T. & Suhai, S. (2004). Using the miraEST assembler for reliable and automated mRNA transcript assembly and SNP detection in sequenced ESTs. *Genome Res*, *14*, 1147–1159.

Clayton, W. D. & Renvoize, S. A. (1986). Genera graminum.Grasses of the world. *Kew Bulletin Additional Series*, 13.

Colhoun, L.W. & Steer, M. V. (1983). The cytological effects of the gametocides ethrel and RH 531 on microsprogenesis in barley (*Hardeum vulgare* L.). Pl. *Cen Environ*, *1*(196), 21–29.

Collard, B. C. Y., Pang, E. C. K. & Taylor, P. W. J. (2003). Selection of wild Cicer accessions for the generation of mapping populations segregating for resistance to ascochyta blight. *Euphytica*, *130*(1), 1–9.

Consultative Group on International Agricultural Research (2001). CGIAR Research: Areas of Research –Millet.

Crawford, G. & Lee, G. (2003). Agricultural origins in the Korean Peninsula. *Antiquity*, *295*(77), 87–95.

Crawford, G., Underhill, A., Zhao, Z., Lee, G., Feinman, G., Nicholas, L. & Cai, F. (2005). Late Neolithic Plant Remains from Northern China: Preliminary Results from Liangchengzhen, Shandong1. *Current Anthropology*, *2*(46), 309–317.

Crook, M. J. & Ennos, A. R. (1994). Stem and root characteristics associated with lodging resistance in four winter wheat cultivars. *Agricultural Science*, *123*(02), 167–174.

Crusio, W. E. (1987). A note on the analysis of reciprocal effects in diallel crosses. *Journal of Genetics*, *3*(66), 177–185.

Crusio, W. E. (1993). Bi-and multivariate analyses of diallel crosses: A tool for the genetic dissection of neurobehavioral phenotypes. *Behavior Genetics*, *1*(23), 59–67.

Crusio, W. E., Kerbusch, J. M. L. & Van Abeelen, J. H. F. (1984). The replicated diallel cross: A generalized method of analysis. *Behavior Genetics*, *1*(14), 81–104.

Dabholkar, A. R. (2006). *General Plant Breeding*. Amsterdam, The Netherlands: Concept Publishing Company Elsevier Science.

de Milliano, W. A. J. (1983). Improvement of wheat in Zambia using incomplete resistance against rusts.

De Wet, J. M. J. (2006). *Eleusine coracana* (L.) Gaertn. PROTA (Plant Resources of Tropical Africa), Wageningen, Netherlands. URL: http://database. prota. org/search. htm (Acessed July 2013).

Devi, P. B., Vijayabharathi, R., Sathyabama, S., Malleshi, N. G. & Priyadarisini, V. B. (2011). Health benefits of finger millet (*Eleusine coracana* L.) polyphenols and dietary fiber. *Food Science and Technology*, 1–20.

Dida, M. M., & Devos, K. M. (2006). Finger millet. In Cereals and Millets. *Springer Berlin Heidelberg*, 333–343.

Dida, M. M., Ramakrishnan, S., Bennetzen, J. L., Gale, M. D. & Devos, K. M. (2007). The genetic map of finger millet (*Eleusine coracana*). *Theoretical and Applied Genetics*, *2*(114), 321–332.

Dida, M. M., Wanyera, S., Subramanian, J. L., Bennetzen, J. L. & Devos, K. M. (2006). Poster at the Fourteenth Plant and Animal Genomes Conference, January 14-18, 2006, Town andCountry Convention Center. In Genetic diversity in finger millet (*Eleusine coracana*) and related wild species. (pp. 148). San Diego, CA, USA.

Dida, M.M., Srinivasachary, Ramakrishnan, S., Bennetzen, J.L., Gale, M.D. and Devos, K. (2008). Population structure and diversity in finger millet (*Eleusine coracana*) germplasm. *Tropical Plant Biol*, *1*(2), 131–141.

Dunbar, A. R. (1969). The annual crops of Uganda. East African Literature Bureau, Dares Salaam, Nairobi, Kampala.

Duvick, D. N. (2001). Biotechnology in the 1930s: the development of hybrid maize. *Nature Reviews Genetics*, *1*(2), 69–74.

Eagles, H. A., Bariana, H. S., Ogbonnaya, F. C., Rebetzke, G. J., Hollamby, G. J. Henry, R. J. & Carter, M. (2001). Implementation of markers in Australian wheat breeding. *Crop and Pasture Science*, *12*(52), 1349–1356.

Earley, E. B. & Slife, F. W. (1969). Effect of ethrel on growth and yield of corn. *Agronomy*, *61*(5), 821–823.

Ebert, P. R., Anderson, M. A., Bernatzky, R., Altschuler, M. & Clarke, A. E. (1989). Genetic polymorphism of self-incompatibility in flowering plants. *Cell*, *2*(56), 255–262.

Edwardson, J. R. (1970). Cytoplasmic male sterility. *Bot. Rev*, *36*, 341–420.

Emon, R. M., Gustafson, J. P., Nguyen, H., Musket, T., Jahiruddin, M., Islam, M. A. & Hassan, M. M. (2010). Molecular marker-based characterization and genetic diversity of wheat genotypes in relation to Boron use efficiency. *The Indian Journal of Genetics and Plant Breeding*, *4*(70), 339–348.

Evenson, R. E. & Gollin, D. (2003). Assessing the impact of the Green Revolution, 1960 to 2000. *Science*, *5620*(300), 758–762.

Fairey, D. T. & Stoskopf, N. C. (1975). Effects of granular ethephon on male sterility in wheat. *Crop Science*, *15*(1), 29–32.

Falcon, W. P., & Naylor, R. L. (2005). Rethinking food security for the twenty-first century. *American Journal of Agricultural Economics*, *5*(87), 1113–1127.

FAO. (2012). Hunger Statistics – Frequently Asked Questions. Accessed April 2012:

Feltus, F. A., Wan, J., Schulze, S. R., Estill, J. C., Jiang, N. & Paterson, A. H. (2004). An SNP resource for rice genetics and breeding based on subspecies indica and japonica genome alignments. *Genome Research*, *9*(14), 1812–1819.

Fendal, R. K. (1967). *Artificial induction of male sterility in wheat.Research Progess. Agronomy Section*. Wash. State Univ. Cir.

Fleming, L. (2004). Perfecting cross-pollination. *Harvard Business Review*, *82*(9), 1–2.

Friebe, A., Schultz, G. & Koesling, D. (1996). Sensitizing soluble guanylyl cyclase to become a highly CO-sensitive enzyme. *The EMBO Journal*, *24*(15), 6863.

Ganal, M. W., Altmann, T.& Röder, M. S. (2009). SNP identification in crop plants. *Current Opinion in Plant Biology*, *2*(12), 211–217.

Gardner, N., Felsheim, R. & Smith, A. G. (2009). Production of male-and female-sterile plants through reproductive tissue ablation. *Journal of Plant Physiology*, *166*(8), 871–881.

Gashaw, G., Alemu, T., & Tesfaye, K. (2014). Morphological, physiological and biochemical studies on Pyricularia grisea isolates causing blast disease on finger millet in Ethiopia. *Journal of Applied Biosciences*, *1*(74), 6059–6071.

Gill, B. S. & Appels, R. (2004). NSF/USDA/IGROW workshop on wheat genome sequencing.*Genetics;* in press.

Goetz, M., Godt, D. E., Guivarc'h, A., Kahmann, U., Chriqui, D. & Roitsch, T. (2001). Induction of male sterility in plants by metabolic engineering of the carbohydrate supply. *Proceedings of the National Academy of Sciences*, *11*(98), 6522–6527.

Gomez, M. I. (1993). Preliminary studies on grain quality evaluation for finger millet for food and beverage use in the southern African region.Advances in small millets (Riley, K. W., Gupta, S. C., Seetharam, A. & Mushonga, J. N. eds.). New Delhi, India: Oxford and IBH Publishing, 289-. *The Magic of Heterosis: New Tools and Complexities. Nature and Science*, *9*(11), 42–53.

Grabowska, L., Mankowska, G., Orlov, N. N. & Orlova, L. G. (2005). Application of 2-Chloroethylphosphonic acid in Breeding of Monoecious Hemp. *Natural Fibers*, (1), 15–22.

Graner, A., Ludwig, W. F. & Melchinger, A. E. (1994). Relationships among European barley germplasm: II. Comparison of RFLP and pedigree data. *Crop Science*, *5*(34), 1199–1205.

Griffing, B. (1956). Concept of general and specific combining ability in relation to diallel crossing systems. *Australian Journal of Biological Sciences*, *4*(9), 463–493.

Grodzicker, T., Williams, J., Sharp, P. & Sambrook, J. (1974). Physical mapping of temperature-sensitive mutations of adenoviruses.In Cold Spring Harbor symposia on quantitative biology (Vol. 39, pp. 439–446). Cold Spring Harbor Laboratory Press.

Gundry, C. N., Vandersteen, J. G., Reed, G. H., Pryor, R. J., Chen, J. & Wittwer, C. T. (2003). Amplicon melting analysis with labeled primers: a closed-tube method for differentiating homozygotes and heterozygotes. *Clinical Chemistry*, *49*(3), 396–406.

Gupta, S. C. (1993). Southern African Regional Finger Millet Improvement Programme. In J. N. (ed. . Riley, K.W., Gupta, S. C., Seetharam, A. and Mushonga (Ed.), (pp. 523–535). New Delhi, India: Oxford and IBH.

Gupta, N., Srivastava, A. K. & Pandey, V. N. (2012). Biodiversity and nutraceutical quality of some Indian millets. Proceedings of the National Academy of Sciences, India Section B: *Biological Sciences*, *2*(82), 265–273.

Gupta, P. K., Roy, J. K. & Prasad, M. (2001). Single nucleotide polymorphisms (SNPs): A new paradigm in molecular marker technology and DNA polymorphism detection with emphasis on their use in s3plants. *Current Science*, *80*(4), 524–535.

Gupta, P. K., Varshney, R. K., Sharma, P. C. & Ramesh, B. (1999). Molecular markers and their applications in wheat breeding. *Plant Breeding*, *118*(5), 369–390.

Hadimani, N. A. & Malleshi, N. G. (1993). Studies on milling, physico-chemical properties, nutrient composition and dietary fibre content of millets. *Journal of Food Science and Technology (India)*, *1*(30), 17–20.

Hanson, M. R. (1991). Plant mitochondrial mutations and male sterility. *Annual Review of Genetics*, *25*(1), 461–486.

Hanson, M. R. & Bentolila, S. (2004). Interactions of mitochondrial and nuclear genes that affect male gametophyte development. *The Plant Cell Online*, *16*(suppl 1), S154–S169.

Hayes, H. K. (2007). *Methods of plant breeding* (5th ed.). New York: McGraw-Hill.

Hayman, B. I. (1954). The theory and analysis of diallel crosses. *Genetics*, *6*(39), 789.

Hayman, B. I. (1960). The theory and analysis of diallel crosses. III. *Genetics*, *2*(45), 155.

Hecker, R. J. & Smith, G. A. (1975). TESTS OF GRANULAR ETHEPHON AS A MALE GAMETOCIDE ON SUGAR BEET. *Canadian Journal of Plant Science*, *2*(55), 655–656.

Heslop-Harrison, J. S. (2000). Comparative genome organization in plants: from sequence and markers to chromatin and chromosomes. *The Plant Cell Online*, *5*(12), 617–635.

Hillier, L. W., Fulton, R. S., Fulton, L. A., Graves, T. A., Pepin, K. H., Wagner-McPherson, C., & Minx, P. (2003). The DNA sequence of human chromosome 7. *Nature*, *6945*(424), 157–164.

Hilu, K. W. & De Wet, J. M. J. (1980). Effect of artificial selection on grain dormancy in Eleusine (Gramineae). *Systematic Botany*, 54–60.

Hiscock, S. J. & Tabah, D. A. (2003). The different mechanisms of sporophytic self–incompatibility.Philosophical Transactions of the Royal Society of London. Series B: *Biological Sciences*, *1434*(358), 1037–1045.

Holt, J. (2000). *Investigation into the biology, epidemiology and management of finger millet blast in low-input farming systems in E. Africa.*

House L. R. (1985). *A guide to sorghum breeding* (2nd ed.). Patancheru, AP 502 324 India: International Crops Research Institute for the Semi-Arid Tropics.

Hulse, J. H., Laing, E. M. & Pearson, O. E. (1980). Sorghum and the millets: their composition and nutritive value. *Academic Press.*

Hussain, M., Niaz, M., Iqbal, M., Iftikhar, T., & Ahmad, J. (2012). Emasculation techniques and detached tiller culture in wheat x maize crosses. *J Agric Res*, (50), 1–19.

http://www.premierbiosoft.com/netprimer/index.html (Accessed on May, 2013).

ICRISAT/FAO. (1996). The world Sorghum and Millet. In *The world Sorghum and Millet. Economies: Facts, Trends and Outlook.*

Igic, B., Lande, R. & Kohn, J. R. (2008). Loss of self-incompatibility and its evolutionary consequences. Sciences. *International Journal of Plant*, *1*(169), 93–104.

Jansen, P. C. M. & Ong, H. C. (1996). *Eleusine coracana* (L.) *Gaertner cv. group Finger Millet.* In: Grubben, G. J. H. & Partohardjono, S. (Editors). Plant Resources of South-East Asia No 10. Cereals.Backhuys Publishers, Leiden, Netherlands. pp. 90–95.

Jin, L., Lu, Y., Xiao, P., Sun, M., Corke, H. & Bao, J. (2010). Genetic diversity and population structure of a diverse set of rice germplasm for association mapping. *Theoretical and Applied Genetics*, *3*(121), 475–487.

Jinks, J. L. (1954). The analysis of continuous variation in a diallel cross of Nicotiana rustica varieties. *Genetics*, *6*(39), 767.

Jordan, S. A. & Humphries, P. (1994). Single nucleotide polymorphism in exon 2 of the BCP gene on 7q31-q35. *Human Molecular Genetics*, *10*(3), 1915–1915.

Kalia, R. K., Rai, M. K., Kalia, S., Singh, R. & Dhawan, A. K. (2011). Microsatellite markers: an overview of the recent progress in plants. *Euphytica*, *3*(177), 309–334.

Karchi, Z. (1969). Effect of Ethrel (2-chloroethane phosphonic acid) as Compared to That of CCC on Height and Grain Yield of Spring Wheat. *Israel J Agr Res*, *1*(4), 45–50.

Kearsey, M. J. & Pooni, H. S. (1996). *The genetical analysis of quantitative traits.* Chapman and Hall, London, Weinhein, New York, pp: 381.

Khan, N. U. & Hassan, G. (2011). Genetic effects on morphological and yield traits in cotton (G. hirsutum L.).Span. J. *Agric. Res*, *2*(9), 460–472.

King, R. C. & Stansfield, W. D. (1990). *A dictionary of genetics. 4th ed.* (pp. 188). Oxford University Press, New York-Oxford.

Klichowska, M. (1984). Plants of the Neolithic Kadera (Central Sudan): a paleobotanical study of the plant impressions on pottery. *Origins and Early Development of Food Producing Cultures in North-Eastern Africa. Polish Academy of Sciences, Warsaw.*

Knight, T. W., Cosgrove, G. P., Death, A. F. & Anderson, C. B. (2000). Effect of age of pre-and post-pubertal castration of bulls on growth rates and carcass quality. *New Zealand Journal of Agricultural Research*, *43*(4), 585–588.

Koebner, R. & Summers, R. W. (2003). 21st century wheat breeding: plot selection or plate detection?. *Trends in Biotechnology*, *2*(21), 59–63.

Koltunow, A. M., Truettner, J., Cox, K. H., Wallroth, M. & Goldberg, R. B. (1990). Different temporal and spatial gene expression patterns occur during anther development. *The Plant Cell*, *2*(12), 1201.

Komatsuda, T., Tanno, K. I., Salomon, B., Bryngelsson, T. & Bothmer, R. V. (1999). Phylogeny in the genus Hordeum based on nucleotide sequences closely linked to the vrs1 locus (row number of spikelets). *Genome*, *5*(42), 973–981.

Kotchoni, S. O., Jimenez-Lopez, J. C., Gachomo, E. W. & Seufferheld, M. J. (2010). A new and unified nomenclature for male fertility restorer (RF) proteins in higher plants. *PloS One*, *5*(12), e15906.

Krishnamoorthy, H. N. (1993). Atma Ra and Sons, Delhi. In *Physiology of plant growth and development.* Atma Ra and Sons, Delhi.

Kudo, G. (2003). Anther arrangement influences pollen deposition and removal in hermaphrodite flowers. *Functional Ecology*, *17*(3), 349–355.

Kumar, L. S. (1999). DNA markers in plant improvement: an overview. *Biotechnology Advances*, *2*(17), 143–182.

Lambertini, C., Mendelssohn, I. A., Gustafsson, M. H., Olesen, B., Tenna, R. I. I. S., Sorrell, B. K., & Brix, H. (2012). Tracing the origin of Gulf Coast Phragmites (Poaceae): a story of long-distance dispersal and hybridization. *American Journal of Botany*, *3*(99), 538–551.

Law, J. & Stoskopf, N. C. (1973). FURTHER OBSERVATIONS ON ETIIEPHON (ETHREL) AS A TOOL FOR DEVELOPING HYBRID CEREALS. *Canadian Journal of Plant Science*, *53*(4), 765–766.

Lawrence, M. J. (2000). Population genetics of the homomorphic self-incompatibility polymorphisms in flowering plants. *Annals of Botany*, *suppl 1*(85), 221–226.

Ledig, F. T. (1998). Genetic variation in Pinus. *Ecology and Biogeography of Pinus*, 251, 280.

Lehmensiek, A., Sutherland, M. W. & McNamara, R. B. (2008). The use of high resolution melting (HRM) to map single nucleotide polymorphism markers linked to a covered smut resistance gene in barley. *Theoretical and Applied Genetics*, *5*(117), 721–728.

Lenné, J. M., Takan, J. P., Mgonja, M. A., Manyasa, E. O., Kaloki, P., Wanyera, N. & Sreenivasaprasad, S. (2006). Finger millet blast disease management: a key entry point for fighting malnutrition and poverty in East Africa. *Outlook on AGRICULTURE*, *36*(2), 101–108.

Li, J., Chen, Z., Guan, X., Liu, J., Zhang, M. & Xu, B. (2008). Optimization of germination conditions to enhance hydroxyl radical inhibition by water-soluble protein from stress millet. *Journal of Cereal Science*, *3*(48), 619–624.

Litt, M. & Luty, J. A. (1989). A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. *American Journal of Human Genetics*, *3*(44), 397.

Loridon, K., McPhee, K., Morin, J., Dubreuil, P., Pilet-Nayel, M. L., Aubert, G. & Burstin, J. (2005). Microsatellite marker polymorphism and mapping in pea (*Pisum sativum* L.). *Theoretical and Applied Genetics*, *6*(111), 1022–1031.

Lower, R. L. & Miller, C. H. (1969). Ethrel (2-chloroethanephosphonic acid) a tool for plant hybridizers. *Nature*, (222), 1072–1073.

Lu, H., Zhang, J., Liu, K. B., Wu, N., Li, Y., Zhou, K., & Li, Q. (2009). Earliest domestication of common millet (Panicum miliaceum) in East Asia extended to 10,000 years ago. *Proceedings of the National Academy of Sciences*, *18*(106), 7367–7372.

Lupien, J. R. (1990). Sorghum and millets in human nutrition. *FAO, ICRISAT. At: Ao.org*, 86.

Mable, B. K., Dart, A. V. R., Berardo, C. D. & Witham, L. (2008). Breakdown of self-incompatibility in the perennial Arabidopsis lyrata (Brassicaceae) and its genetic consequences. *Evolution*, *7*(59), 1437–1448.

Mace, E. S., Buhariwalla, K. K., Buhariwalla, H. K. & Crouch, J. H. (). A. high-throughput D. (2003). NA extraction protocol for tropical molecular breeding programs. *Plant Molecular Biology Reporter*, *21*(4), 459–460.

Mariani, C., Beuckeleer, M. D., Truettner, J., Leemans, J. & Goldberg, R. B. (1990). Induction of male sterility in plants by a chimaeric ribonuclease gene. *Nature*, *347*, 737–741.

Markert, C. L. (2012). FORMATION OF ISOZYMES. *Isozymes:Molecular Structure*, *1*.

Masoudi-Nejad, A., Nasuda, S., McIntosh, R. A. & Endo, T. R. (2002). Transfer of rye chromosome segments to wheat by a gametocidal system. *Chromosome Research*, *10*(5), 349–357.

McCubbin, A. G. & Kao, T. H. (2000). Molecular recognition and response in pollen and pistil interactions. *Annual Review of Cell and Developmental Biology*, *1*(16), 333–364.

McDonough, C. M., Rooney, L. W. & Earp, C. F. (1986). Structral characteristics of *Eluesine coracana* (finger millet) using scanning electron and fluorescence microscopy. *Food Microstrucure*, (5), 247–256.

McDonough, C. M., Rooney, L. W. & Serna-Saldivar, S. O. (2000). *The millets. FOOD SCIENCE AND TECHNOLOGY* (pp. 177–202). NEW YORK-MARCEL DEKKER.

Meier, U. (2001). Growth stages of mono-and dicotyledonous plants. BBCH monograph. *German Federal Biological Research Centre for Agriculture and Forestry, Berlin.*

Mihr, C., Baumgärtner, M., Dieterich, J. H., Schmitz, U. K. & Braun, H. P. (2001). Proteomic approach for investigation of cytoplasmic male sterility (CMS) in Brassica. *Journal of Plant Physiology*, *158*(6), 787–794.

Mitaru, B. N., Karugia, J. T., & Munene, C. (1993). Finger millet production and utilization in Kenya.

Moes, J. & Stobbe, E. H. (1991a). Barley treated with ethephon: I. Yield components and net grain yield. *Agronomy*, *83*(1), 86–90.

Mohan, R. A. M. & Saiswal, V. (1972). Ethephon and Plant Development: An appraisal. *Pl. Morph*, 304–314.

Monna, L., Ohta, R., Masuda, H., Koike, A. & Minobe, Y. (2006). Genome-wide searching of single-nucleotide polymorphisms among eight distantly and closely related rice cultivars (Oryza sativa L.) and a wild accession (Oryza rufipogon Griff.). *DNA Research*, *13*(2), 43–51.

Montgomery, J. L., Sanford, L. N. & Wittwer, C. T. (2010). High-resolution DNA melting analysis in clinical research and diagnostics. *Expert Review of Molecular Diagnostics*, *10*(2), 219–240.

Montgomery, J., Wittwer, C. T., Palais, R. & Zhou, L. (2007). Simultaneous mutation scanning and genotyping by high-resolution DNA melting analysis. *Nature Protocols*, *1*(2), 59–66.

Moore, R. H. (1950). Several effects of maleic hydrazide on crop plants. *Crop Sci*, *112*, 52–53.

Moore, G. A. & Durham, R. E. (1996). *Molecular markers. In:Hammerschlag FA and Litz RE (eds) Biotechnology of Perennial Fruit Crops. Cambridge:* (pp. 105–140).

Moore, K. J., Moser, L. E., Vogel, K. P., Waller, S. S., Johnson, B. E. & Pedersen, J. F. (1991). Describing and quantifying growth stages of perennial forage grasses. *Agronomy Journal*, *6*(83), 1073–1077.

Müller, A., Hinrichs, W., Wolf, W. M. & Saenger, W. (1994). Crystal structure of calcium-free proteinase K at 1.5-A resolution. *Journal of Biological Chemistry*, *37*(269), 23108–23111.

Mushonga, J. N., Muza, F. R. & Dhliwayo, H. H. (1993). *Development, current and future research strategies on finger millet in Zimbabwe.* New Delhi, India: Oxford and IBH Pub.

Musia, G. D., Muchugi, A. & Santie, D. (2010). *Identification of Microsatellite Markers forFinger Millet (Eleusine coracana) by Analysis of ROCHE 454 GS-FLX Titanium Sequence Data*. Masters Thesis Kenyatta University, pp 26.

Mysore, K. S. & Baird, V. (1997). Nuclear DNA content in species ofEleusine (Gramineae): A critical re-evaluation using laser flow cytometry. *Plant Systematics and Evolution*, *1-2*(207), 1–11.

Nasu, S., Suzuki, J., Ohta, R., Hasegawa, K., Yui, R., Kitazawa, N. & Minobe, Y. (2002). Search for and analysis of single nucleotide polymorphisms (SNPs) in rice (Oryza sativa, Oryza rufipogon) and establishment of SNP markers. *DNA Research*, *9*(5), 163–171.

National Center for Biotechnology Information (2005). SNPs: Variation on a theme. Retrieved May 17, 2013, from: http://www.ncbi.nlm.nih.gov/About/primer/snps.html (Accessed on May, 2013).

National Research Council. (1996). *Lost Crops of Africa vol. I: Grains. Board on Science and technology for International Development.* (p. National Academy Press Washington, D.C.).

Nettancourt, D. (2001). Incompatibility and incongruity in wild and cultivated plants. *Springer*, *Vol. 3*.

Neves, S. S., Swire-Clark, G., Hilu, K. W. & Baird, W. V. (2005). Phylogeny of Eleusine (Poaceae: Chloridoideae) based on nuclear ITS and plastid trnT and trnF sequences. *Molecular Phylogenetics and Evolution*, *2*(35), 395–419.

Ng'etich, J. K. (2013). Planning and Development of Kakamega County in Kenya: Challenges and Opportunities. *Research Journal in Organizational Psychology and Educational Studies (RJOPES)*, *3*(2), 111.

Nickell, L. G. (1978). Plant Growth Regulator. *Chemical and Engineering News*, pp. 18–34.

Nyende, P., Tenywa, J. S., Oryokot, J. & Kidoido, M. M. (2001). Weed profiles and management assessment for increased finger millet production in Uganda.

Obilana, A. B. & Manyasa, E. (2002). Grain properties and utilization potential. *Springer-Verlag*, 177–217.

Obilana, A. B., Manyasa, E. O., Kibuka, J. G. & Ajanga, S. (2002). Finger millet blast (fmb) samples collection in Kenya: Passport data, analyses of disease incidence and report of activities. ICRISAT, Nairobi, Kenya.

Oduori, C. O. A. (1993). *Small millets production and research in Kenya.Advances in smal*. New Delhi, India: Oxford and IBH Publishing.

Oduori, C. O. A. (1998). Finger millet better varieties–Better crop care-More Food. DFID and GON produced by DEVCOM & AIC.

Oduori C. O. A. (2000). Finger Millet.Kenya Agricultural Research Institute. Available:http://www.kari.org/Farmers%20Guide/Information%20brochures/Finger%20Millet.html. (Accessed on March, 2012).

Oduori, C. O. A. (2008). *Breeding investigations of finger millet characteristics including blast disease andstriga resistance in Western Kenya*. University of KwaZulu-Natal, South Africa.

Oduori C. O. A., Togoona, J., Derera, O. & Odongo, M. (2008). *Finger millet hybridization using ethrel chemical hybridizing agent,* pp 103-128. In C. O. A. Oduori. Breeding investigations of finger millet characteristics including blast disease and *Striga* resistance in Western Kenya. Africa Centre for crop Improvement of the University of KwaZulu-Natal, South Africa Ph.D in Plant Breeding.

Padidam, M. (2003). Chemically regulated gene expression in plants. *Current Opinion in Plant Biology*, *2*(6), 169–177.

Palais, R. A., Liew, M. A. & Wittwer, C. T. (2005). Quantitative heteroduplex analysis for single nucleotide polymorphism genotyping. *Analytical Biochemistry*, *1*(346), 167–175.

Park, S. W., Ann, S. J., Yang, H. B., Kwon, J. K. & Kang, B. C. (2009). Optimization of high resolution melting analysis and discovery of single nucleotide polymorphism in Capsicum. *Hort Environ Biotechnol*, *50*, 31–39.

Pawar, P. A. & Dhanvijay, V. P. (2007). Weaning foods.An overview. *Beverage Food World*, *11*(34), 27–33.

Pedersen, J. F. & Toy, J. J. (2001). Germination, emergence, and yield of 20 plant-colors, seed–color near-isogenic lines of grain sorghum. *Crop Science*, (41), 107–110.

Pelletier, G., & Budar, F. (2007). The molecular biology of cytoplasmically inherited male sterility and prospects for its engineering. *Current Opinion in Biotechnology*, *2*(18), 121–125.

Perez-Prat, E. & van Lookeren Campagne, M. M. (2002). Hybrid seed production and the challenge of propagating male-sterile plants. *Tren Plant Sci*, *7*, 199–203.

Philips, S. M. (1972). A survey of the genus *Eleusine* Gaertn.(Gramineae) in Africa. *Kew Bulletin*, (27), 251–270.

Poehlman, J. M. & Sleper, D.A (1995). *Breeding field crops.4th ed.* Iowa State University PressAmes.

Porcher, E. & Lande, R. (2005). LOSS OF GAMETOPHYTIC SELF-INCOMPATIBILITY WITH EVOLUTION OF INBREEDING DEPRESSION. *Evolution*, *1*(59), 46–60.

Powell, W., Machray, G. C. & Provan, J. (1996). Polymorphism revealed by simple sequence repeats. *Trends in Plant Science*, *1*, 215–222.

Purseglove, J. W. (1972). *Tropical Crops: Monocotyledons.* London: Longman Group Limited.

Rafalski, A. (2002). Applications of single nucleotide polymorphisms in crop genetics. *Curr.Opin. Plant Biol*, (5), 94–100.

Ramachandra, G., Virupaksha, T. K. & Shadaksharaswamy, M. (1977). Relation between tannin levels and in vitro protein digestibility in finger millet (*Eleusine coracana* Gaertn.). *Journal of Agricultural and Food Chemistry*, *5*(25), 1101–1104.

Ramos, J. M. L. F., Garcia del Moral, J. C., Molina Gano, F., Salamanca, P. & Roca de Tagores, F. (1989). Effect of an early application of sulphur or Ethephone as foliar spray on the growth and yield of spring barley in a Mediterranean environment.J. *Agron. Crop Sci*, (163), 129–137.

Rao, M.K., Devi, K.U. & Arundhati, A. (1990). Applications of gene male sterility in plant breeding. *Plant Breeding*, *105*, 1–25.

Ravindran, G., Rajagopalan, R., Krishnamoorthy, V. S. & Vijayan, K. P. (1996). Correlation and path coefficient analysis in ragi (Eleusine coracana G.). *CROP RESEARCH-HISAR*, *12*, 359–362.

Riley, K. W., Setharam, A. & Harinarayana, G. (1989). Recommendations on breeding and varietal improvement. Small millets in global agriculture. In *First*

International Small Millets Workshop (p. 29 Oct – 2 Nov 1986). Bangalore, India: Oxford and IBH Publishing, New Delhi, IN.

Riley, K. W., Shakya, P. B. & Upreti, R. P. V. (1992). Finger Millet in Nepal: Importance, farming systems and utilization in a socio-economic context. *In:Recent Advances in Small Millets*, 209–226.

Rosenow, D. T., Dahlberg, J. A., Peterson, G. C., Clark, L. E., Hamburger, A. J., Madera-Torres, P. & Woodfin, C. A. (1998). Release of 30 partially converted sorghum lines. *International Sorghum and Millets Newsletter*, (39), 82–84.

Rowell, P. L. & Miller, D. G. (1971). Induction of male sterility in wheat with 2-chloroethylphosphonic acid (Ethrel). *Crop Science*, *11*(5), 629–631.

Rudich, J., Halevy, A. H. & Kedar, N. (1969). Increase in femaleness of three cucurbits by treatment with ethrel, an ethylene releasing compound. *Planta*, *1*(86), 69–76.

Sachidanandam, R., Weissman, D., Schmidt, S. C., Kakol, J. M., Stein, L. D., Marth, G. & Kakol, J. M. (2001). A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. *Nature*, *409*(6822), 928–933.

Sahu, S. K., Thangaraj, M. & Kathiresan, K. (2012). DNA extraction protocol for plants with high levels of secondary metabolites and polysaccharides without using liquid nitrogen and phenol.*International Scholarly Research Notices*, *2012*.

Sambrook, J. & Russel, D. W. (2001). Molecular Cloning: A Laboratory Manual 3rd Ed. Cold Spring Harbor Laboratory Press. *Cold Spring Harbor, NY.*

SanMiguel, P., Tikhonov, A., Jin, Y. K., Motchoulskaia, N., Zakharov, D., Melake-Berhan, A. & Bennetzen, J. L. (1996). Nested retrotransposons in the intergenic regions of the maize genome. *Science*, *5288*(274), 765–768.

Sapra, V. T., Heyne, E. G. & Wilkins, H. D. (1971). Triticale, a man-made species of a crop plant. *Transactions of the Kansas Academy of Science*, *1903*, 52–58.

SAS Institute (2001). *SAS/STAT user's guide*. Release 8.2 ed. SAS Institute Inc., NC, USA.

Saturni, L., Ferretti, G., & Bacchetti, T. (2010). The gluten-free diet: safety and nutritional quality. *Nutrients*, *1*(2), 16–34.

Schlotterer, C. (2000). Evolutionary dynamics of microsatellite DNA. *Chromosome*, *109*, 365–371.

Schnable, P. S. & Wise, R. P. (1998). The molecular basis of cytoplasmic male sterility. *Trends in Plant Science*, *3*(5), 175–180.

Schulman, A. H., Flavell, A. J. & Ellis, T. H. N. (2004). The application of LTR retrotransposons as molecular markers in plants. *Methods Mol Biol*, (260), 145–173.

Semagn, K., Bjørnstad, A., & Ndjiondjop, M. N. (2006). An overview of molecular marker methods for plants. *African Journal of Biotechnology*, *25*(5).

Shen, J. X., Fu, T. D., Yang, G. S., Ma, C. Z. & Tu, J. X. (2005). Genetic analysis of rapeseed self-incompatibility lines reveals significant heterosis of different patterns for yield and oil content traits. *Plant Breeding*, *124*, 111–116.

Shewry, P. R. & Halford, N. G. (2002). Cereal seed storage proteins: structures, properties and role in grain utilization. *Journal of Experimental Botany*, *370*(53), 947–958.

Siedler, H., Messmer, M. M., Schachermayr, G. M., Winzeler, H., Winzeler, M. & Keller, B. (1994). Genetic diversity in European wheat and spelt breeding material based on RFLP data. *Theoretical and Applied Genetics*, *8*(88), 994–1003.

Silva, N. F. & Goring, D. R. (2001). Mechanisms of self-incompatibility in flowering plants. *Cellular and Molecular Life Sciences CMLS*, *14*(58), 1988–2007.

Simmons, S. R., Oelke, E. A., Wiersma, J. V., Lueschen, W. E. & Warnes, D. D. (1988). Spring wheat and barley responses to ethephon. *Agronomy*, *80*(5), 829–834.

Singh, P., & Raghuvanshi, R. S. (2012). Finger millet for food and nutritional security. *African Journal of Food Science*, *4*(6), 77–84.

Singh, R. K. & Chaudhary, B. D. (1977). *Biometrical methods in quantitative genetic analysis.* (Revised ed.). New Delhi, India.: Kalyani Publishers,.

Singh, S. K., Kakani, V. G., Brand, D., Baldwin, B. & Reddy, K. R. (2008). Assessment of Cold and Heat Tolerance of Winter-grown Canola (Brassica napus L.) Cultivars by Pollen-based Parameters. *Journal of Agronomy and Crop Science*, *194*(3), 225–236.

Smith, B. L., LU, C. P., & Alvarado Bremer, J. R. (2010). High-resolution melting analysis (HRMA): a highly sensitive inexpensive genotyping alternative for population studies. *Molecular Ecology Resources*, *1*(10), 193–196.

Srinivasachary, Dida M. M., Gale, M. D. & Devos, K. M. (2007). Comparative analyses reveal high levels of conserved colinearity between the finger millet and rice genomes. *Theor Appl Genet*, (115), 489–499.

Stoskopf, N. C. & Law, J. (1972). Some observations on ethrel as a tool for developing hybrid cereals. *Canadian Journal of Plant Science*, *52*(4), 680–683.

Sumarani, G. O., Pillai, S. V., Harisankar, P. & Sundaresan, S. (2004). Isozyme analysis of indigenous cassava germplasm for identification of duplicates. *Genetic Resources and Crop Evolution*, *51*, 205–209.

Wittwer C. T. (2009). High-resolution DNA melting analysis: advancements and limitations. *Hum Mutat.*, *30*, 857–859.

Takan, J. P., Muthumeenakshi, S., Sreenivasaprasad, S., Akello, B., Bandyopadhyay, R., Coll, R., Brown, A. E. & Talbot, N. J. (2002). Characterization of finger millet blast pathogen populations in East Africa and strategies for disease management.

Takan, J. P., Akello, B., Esele, J. P., Manyasa, E. O., Obilana, B. A., Audi, O. P., Kibuka, J., Odendo, M., Oduori, C. A., Ajanga, S., Bandyopadhyay, R., Muthumeenakshi, S., Coll, R., Brown, A. E., Talbot, N. J and Sreenivasaprasad, S. (2004). Finger millet blast pathogen diversity and management in East Africa: A summary of project activities and outputs. *International Sorghum and Millets Newsletter*, pp. 66–69.

Takano, T., Ohe, Y., Tsuta, K., Fukui, T., Sakamoto, H., Yoshida, T., Tateishi, U., Nokihara, H., Yamamoto, N., Sekine, I., Kunitoh, H., Matsuno, Y., Furuta, K. & Tamura, T. (2007). Epidermal Growth Factor Receptor Mutation Detection

Using High-Resolution Melting Analysis PredictsOutcomes in Patients with Advanced Non–Small Cell Lung Cancer Treated with Gefitinib. *Clin Cancer Res*, *13*, 5385–5390.

Tang, J., Vosman, B., Voorrips, R. E., van der Linden, C. G. & Leunissen, J. A. (2006). QualitySNP: a pipeline for detecting single nucleotide polymorphisms and insertions/deletions in EST data from diploid and polyploid species. *BMC Bioinformatics*, *1*(7), 438.

Tanksley, S. D. & McCouch, S. R. (1997). Seed banks and molecular maps: unlocking genetic potential from the wild. *Science*, *5329*(277), 1063–1066.

Tarchini, R., Biddle, P., Wineland, R., Tingey, S. & Rafalski, A. (2000). The completesequence of 340 kb of DNA around the rice Adh1-adh2 region reveals interrupted colinearity with maize chromosome 4. *Plant Cell*, (12), 381–391.

Taylor, C. F. (2009). Mutation scanning using high-resolution melting.*Biochem Soc Trans,* 37:433-437.

Topal, A., Ayidin, C., Akgun, N. & Babaoglu, M. (2004). Diallel cross analysis in durum wheat (*Triticum durum* Desf.): identification of best parents for some kernel physical features. *Field Crops Research*, (87), 1–12.

Trick, M., Long, Y., Meng, J. & Bancroft, I. (2009). Single nucleotide polymorphism (SNP) discovery in the polyploid Brassicanapus using Solexa transcriptome sequencing. *Plant Biotech J*, (7), 334–346.

Truswell, A. S. (2002). Cereal grain and coronary heart disease. *Eur J Clin Nutr*, *1*(56), 1–4.

Tsujimoto, T. & Shigeyama, T. (1998). New insights into the early stage of the galactic chemical evolution. *The Astrophysical Journal Letters*, *2*(508), 151.

Upadhyaya, H. D., Yadav, D., Reddy, K. N., Gowda, C. L. L., & Singh, S. (2011). Development of pearl millet minicore collection for enhanced utilization of germplasm. *Crop Science*, *1*(51), 217–223.

Uzunov, P. & Weiss, B. (1972). "Separation of multiple molecular forms of cyclic adenosine 3’, 5'-monophosphate phosphodiesterase in rat cerebellum by polyacrylamide gel electrophoresis. *"Biochim. Biophys. Acta*, *284*, 220–226.

Vadivoo, A. S., Joseph, R. & Ganesan, N. M. (1998). Genetic variability and diversity for protein and calcium contents in finger millet (Eleusine coracana (L.)Gaern) in relation to grain colour. *Plant Foods Hum Nutr*, (52), 352–364.

Van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M. & Shipley, P. (2004). Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, (4), 535–538.

Verma, M. M. & Kumar, J. (1978). Ethrel - a male gametocide that can replace the male sterility genes in barley. *Euphytica*, *27*, 865–868.

Vijayaraghavan, C. & Sarma, P. S. (1938). R. 42 an economic recessive mutant from E.C. 593 ragi (Eleusine coracana Gaertn.). *Cur. Sci*, *12*(6), 611–12.

Vinod, K. K. (2005). Cytoplasmic genetic male sterility in plants. A molecular perspective. In *Proceedings of the training programme on advances and accomplishments in heteron breeding*. Tamil Nadu Agricultural University, Coimbotore.

Virmani, S. S. & Ilyas-Ahmed, M. (2001). Environment-sensitive genic male sterility (EGMS) in crops. *Advances in Agronomy*, *72*, 139–195.

Vos, P., Hogers, R., Reijans, M., van de Lee, T., Hornes, M., Friters, A., Pot, J., Peleman, J., Kupier, M. & Zabeau, M. (1995). AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Research*, *23*, 4404–4414.

Waines, J. G. & Hegde, S. G. (2003). Intraspecific gene flow in bread wheat as affected by reproductive biology and pollination ecology of wheat flowers. *Crop Science*, *2*(43), 451–463.

Weising, K., Nybom, Hilde, Wolff, K. & Kahl, G. (2005). DNA Finger printing in Plants -. Principles, Methods, and Applications. - Boca Raton (CRC Press).

Werth, C. R., Hilu, K. W. & Langner, C. A. (1994). Isozymes of *Eleusine* (Gramineae) and the origin of finger millet. *American Journal of Botany*, (81), 1186–1197.

White, H. E., Hall, V. J. & Cross, N. C. P. (2007). Methylation-sensitive high resolution melting-curve analysis of the SNRPN gene as a diagnostic screen for Prader-Willi and Angelman syndromes. *Clin Chem*, *53*, 1960–1962.

Williams, J., Kubelik, A., Livak, K., Rafalski, J. & Tingey, S. (1990). DNA Polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res*, *18*, 6531–6535.

Winter, P. & Kahl, G. (1995). Molecular marker technologies for plant improvement. *World Journal of Microbiology and Biotechnology*, (11), 438–448.

Wittwer, C. T., Reed, G. H., Gundry, C. N., Vandersteen, J. G. & Pryor, R. J. (2003). High resolution genotyping by amplicon melting analysis using LC Green. *Clin Chem*, *49*, 853–860.

Woodward, E. J. & Marshall, C. (1987). Effects of seed treatment with a plant growth regulator on tillering in spring barley (*Hordeum distichum*) cv. *Triumph Ann. Appl. Biol*, (110), 629–638.

Wu, S. B., Wirthensohn, M. G., Hunt, P., Gibson, J. P. & Sedgley, M. (2008). High resolution melting analysis of almond SNPs derived from ESTs. *Theor Appl Genet*, *118*, 1–14.

XU, Y. B., Ishii, T. & Mccouch, S. R. (2003). In B. (Eds.). - In: Mew, T. W., Brar, D. S., Peng, S., Dawe.D. and Hardy (Ed.), *Marker-Assisted Evaluation of Germplasm Resources for Plant Breeding*. (pp. 213–229). Rice Science: Innovations and Impact for Livelihood. Manila (IRRI),.

Yu, C., Hu, S., Zhang, C., YU, Y. & H.E, P. R. (2005). The effect of chemical hybridizing agent EXP on oilseed rape. *Acta Agronomica Sinica*, *11*(31), 1455.

Yu, J., Holland, J. B., McMullen, M. D. & Buckler, E. S. (2008). Genetic design and statistical power of nested association mapping in maize. *Genetics*, (178), 539–551.

Zadoks, J. C., Chang, T. T. & Konzak, C. F. (1974). “A Decimal Code for the Growth Stages of Cereals”. *Weed Research*, *14*, 415–421.

Zane, L., Bargelloni, L. & Patarnello, T. (2002). Strategies for microsatellite isolation:a review. *Molecular Ecology*, *11*, 1–16.

Zhang, X., Wang, X. & Jiang, P. Z. (2010). Inheritance of Fertility Restoration for Cytoplasmic Male Sterility in a New Gossypium barbadense Restorer. *Agri. Sci. China*, *4*(9), 472–474.

Zhao, T. J. & Gai, J. Y. (2006). Discovery of new male-sterile cytoplasm sources and development of a new cytoplasmic nuclear male-sterile line NJCMS 3A in soybean. *Euphytica*, *152*, 387–396.

Zietkiewicz, E., Rafalski, A. & Labuda, D. (1994). Genome finger printing by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics*, *20*, 176–183.

APPENDICES

Appendix I: Field planting layout

BLOCK 1

IE 2872 Female parent	
IE 2872 × U-15	0 PPM
IE 2872 × U-15	1500 PPM
IE 2872 × U-15	1750 PPM
IE 2872 × U-15	2000 PPM
U-15 Male parent	
U-15 × IE 2872	0 PPM
U-15 × IE 2872	1500 PPM
U-15 × IE 2872	1750 PPM
U-15 × IE 2872	2000 PPM
IE 2872 Female parent	

BLOCK 2

GE Female parent	
GE × IE 2872	0 PPM
GE × IE 2872	1500 PPM
GE × IE 2872	1750 PPM
GE × IE 2872	2000 PPM
IE 2872 Male parent	
IE 2872 × GE	0 PPM
IE 2872 × GE	1500 PPM
IE 2872 × GE	1750 PPM
IE 2872 × GE	2000 PPM
GE Female parent	

BLOCK 3

IE2872 Female parent	
IE 2872 × KA 72 0 PPM	0 PPM
IE 2872 × KA 72	1500 PPM
IE 2872 × KA 72	1750 PPM
IE 2872 × KA 72	2000 PPM
KA72 Male parent	
KA 72 × IE 2872	0 PPM
KA 72 × IE 2872	1500 PPM
KA 72 × IE 2872	1750 PPM
KA 72 × IE 2872	2000 PPM
IE 2872 Female parent	

BLOCK 4

IE 4115 Female parent	
IE 4115 × GE	0 PPM
IE 4115 × GE	1500 PPM
IE 4115 × GE	1750 PPM
IE 4115 × GE	2000 PPM
GE Male parent	
GE × IE 4115	0 PPM
GE × IE 4115	1500 PPM
GE × IE 4115	1750 PPM
GE × IE 4115	2000 PPM
IE 4115 Female parent	

BLOCK 5

OK Female parent	
OK × U-15	0 PPM
OK × U-15	1500 PPM
OK × U-15	1750 PPM
OK × U-15	2000 PPM
U-15 Male parent	
U-15 × OK	0 PPM
U-15 × OK	1500 PPM
U-15 × OK	1750 PPM
U-15 × OK	2000 PPM
OK Female parent	

BLOCK 6

GE Female parent	
GE × KA 72 0 PPM	0 PPM
GE × KA 72	1500 PPM
GE × KA 72	1750 PPM
GE × KA 72	2000 PPM
KA 72 Male parent	
KA 72 × GE	0 PPM
KA 72 × GE	1500 PPM
KA 72 × GE	1750 PPM
KA 72 × GE	2000 PPM
GE Female parent	

BLOCK 7

OK Female parent	
OK × GE	0 PPM
OK × GE	1500 PPM
OK × GE	1750 PPM
OK × GE	2000 PPM
GEMale parent	
GE × OK	0 PPM
GE × OK	1500 PPM
GE × OK	1750 PPM
GE × OK	2000 PPM
OKFemale parent	

BLOCK 8

IE 4115 Female parent	
IE 4115 × IE 2872	0 PPM
IE 4115 × IE 2872	1500 PPM
IE 4115 × IE 2872	1750 PPM
IE 4115 × IE 2872	2000 PPM
IE 2872 Male parent	
IE 2872 × IE 4115	0 PPM
IE 2872 × IE 4115	1500 PPM
IE 2872 × IE 4115	1750 PPM
IE 2872 × IE 4115	2000 PPM
IE 4115 Female parent	

BLOCK 9

IE 4115 Female parent	
IE 4115 × U-15	0 PPM
IE 4115 × U-15	1500 PPM
IE 4115 × U-15	1750 PPM
IE 4115 × U-15	2000 PPM
U-15 Male parent	
U-15 × IE 4115	0 PPM
U-15 × IE 4115	1500 PPM
U-15 × IE 4115	1750 PPM
U-15 × IE 4115	2000 PPM
IE 4115 Female parent	

BLOCK 10

KA 72 Female parent	
KA 72 × IE 4115	0 PPM
KA 72 × IE 4115	1500 PPM
KA 72 × IE 4115	1750 PPM
KA 72 × IE 4115	2000 PPM
IE 4115Male parent	
IE 4115 × KA 72	0 PPM
IE 4115 × KA 72	1500 PPM
IE 4115 × KA 72	1750 PPM
IE 4115 × KA 72	2000 PPM
KA 72Female parent	

BLOCK 11

OK Female parent	
OK × IE 2872	0 PPM
OK × IE 2872	1500 PPM
OK × IE 2872	1750 PPM
OK × IE 2872	2000 PPM
IE 2872 Male parent	
IE 2872 × OK	0 PPM
IE 2872 × OK	1500 PPM
IE 2872 × OK	1750 PPM
IE 2872 × OK	2000 PPM
OK Female parent	

BLOCK 12

IE 4115Female parent	
IE 4115 × OK	0 PPM
IE 4115 × OK	1500 PPM
IE 4115 × OK	1750 PPM
IE 4115 × OK	2000 PPM
OKMale parent	
OK × IE 4115	0 PPM
OK × IE 4115	1500 PPM
OK × IE 4115	1750 PPM
OK × IE 4115	2000 PPM
IE 4115Female parent	

BLOCK 13

U-15 Female parent	
U-15 × KA 72	0 PPM
U-15 × KA 72	1500 PPM
U-15 × KA 72	1750 PPM
U-15 × KA 72	2000 PPM
KA 72 Male parent	
KA 72 × U-15	0 PPM
KA 72 × U-15	1500 PPM
KA 72 × U-15	1750 PPM
KA 72 × U-15	2000 PPM
U-15 Female parent	

BLOCK 14

OK Female parent	
OK X KA 72	0 PPM
OK × KA 72	1500 PPM
OK × KA 72	1750 PPM
OK × KA 72	2000 PPM
KA 72 Male parent	
KA 72 × OK	0 PPM
KA 72 × OK	1500 PPM
KA 72 × OK	1750 PPM
KA 72 × OK	2000 PPM
OK Female parent	

BLOCK 15

U-15 Female parent	
U-15× GE	0 PPM
U-15× GE	1500 PPM
U-15× GE	1750 PPM
U-15× GE	2000 PPM
GE Male parent	
GE × U-15	0 PPM
GE × U-15	1500 PPM
GE × U-15	1750 PPM
GE × U-15	2000 PPM
U-15 Female parent	

Appendix II: Preparation of TBE buffer

Component	Molecular Weight	Amount	10x Concentration	Final 1x Concentration
Tris Base	121.2 grams	108 grams	890 mM	89 mM
Boric Acid	61.8 grams	55 grams	890 mM	89 mM
EDTA (pH 8.0)	0.5 M	40 ml	20 mM	2 mM

mM – millimolar

TBE - Tris/Borate/EDTA

EDTA - Ethylenediaminetetraacetic acid

To prepare a 1x working solution from 10x stock buffer, the stock buffer has to be mixed with DNAse (double distilled water) free deionized water in the ratios of 1: 9.

Appendix III: Grain weight across the 15 cross replicates generated from the six genotypes

GL (ppm)	Genotype	GE×IE 2872	GE×IE 4115	GE×KA-72
0		10.500	10.500	9.500
1,500		13.000	11.000	13.000
1,750		12.500	10.500	12.000
2,000		10.000	12.000	9.000
Grand Mean		**11.500**	**11.000**	**10.875**
		GE×OK-1	GE×U-15	IE 2872×GE
0		9.500	8.000	10.000
1,500		12.500	10.500	10.500
1,750		11.000	13.500	10.500
2,000		10.000	11.000	10.500
Grand Mean		**10.750**	**10.750**	**10.375**
		IE 2872×IE 4115	IE 2872×KA-72	IE 2872×OK-1
0		11.000	10.000	11.500
1,500		10.000	11.000	12.000
1,750		11.000	11.500	13.000
2,000		10.500	6.500	8.500
Grand Mean		**10.625**	**9.750**	**11.250**
		IE 2872×U-15	IE 4115×GE	IE 4115×IE 2872
0		10.000	10.000	9.500
1,500		12.000	9.500	11.000
1,750		11.000	12.000	11.000
2,000		9.000	10.000	10.000
Grand Mean		**11.500**	**10.375**	**10.375**
		IE 4115×KA-72	IE 4115×OK-1	IE 4115×U-15
0		8.500	8.000	10.000
1,500		11.500	7.500	11.000
1,750		10.500	9.000	11.500
2,000		9.500	10.500	11.500
Grand Mean		**10.000**	**8.750**	**11.000**
		KA-72×GE	KA-72×IE 2872	KA-72×IE 4115
0		11.000	11.000	8.500
1,500		13.500	11.000	13.500
1,750		12.000	12.000	11.500
2,000		12.500	11.000	12.500
Grand Mean		**12.250**	**11.250**	**11.500**
		KA-72×OK-1	KA-72×U-15	OK-1×GE
0		9.000	9.000	11.500
1,500		13.000	10.000	14.000
1,750		12.000	11.000	12.500
2,000		11.500	11.000	14.500
Grand Mean		**11.375**	**10.250**	**13.125**
		OK-1×IE 2872	OK-1×IE 4115	OK×KA-72
0		10.000	7.000	12.500
1,500		13.000	12.500	12.500
1,750		13.000	10.000	11.500
2,000		13.000	10.500	14.000
Grand Mean		**12.250**	**10.000**	**12.625**

	OK-1×U-15	U-15×GE	U-15×IE 2872
0	9.500	10.107	10.500
1,500	13.000	14.500	12.500
1,750	10.500	13.500	12.500
2,000	13.000	9.893	13.000
Grand Mean	**11.500**	**12.000**	**12.125**
	U-15×IE 4115	U-15×KA-72	U-15×OK-1
0	9.500	14.500	7.000
1,500	12.500	13.000	11.000
1,750	13.500	11.500	10.534
2,000	11.500	10.500	11.500
Grand Mean	**11.750**	**12.375**	**10.009**

Appendix IV: Observed morphological traits for the six genotypes used in the screen house study

Variety	Seed colour	Days to 50% Heading	Days to 50% Flowering	Days to 50% Maturity
IE 2872	Red	56 days	68 days	110 days
IE 4115	Light brown	67 days	79 days	121 days
GULU-E	Brown	67 days	73 days	122 days
KA-72	Brown	67 days	79 days	121 days
OK-1	Brown	67 days	79 days	122 days
U-15	Brown	61 days	79 days	115 days

Appendix V: Plant height across the 15 cross replicates generated from the six genotypes

GL (ppm)	Genotype	GE×IE 2872	GE×IE 4115	GE×KA-72
0		107.50	86.00	74.00
1,500		86.00	86.00	83.50
1,750		84.00	73.00	82.50
2,000		83.95	89.50	73.00
Grand Mean		**90.36**	**83.62**	**78.25**
		GE×OK-1	GE×U-15	IE 2872×GE
0		98.50	86.00	86.00
1,500		83.00	90.00	67.50
1,750		76.50	86.00	69.50
2,000		79.50	82.00	57.00
Grand Mean		**84.38**	**86.00**	**70.00**
		IE 2872×IE 4115	IE 2872×KA-72	IE 2872×OK-1
0		93.00	77.50	87.50
1,500		74.00	67.00	72.50
1,750		63.00	65.00	72.50
2,000		68.00	56.50	57.50
Grand Mean		**74.50**	**66.50**	**72.50**
		IE 2872×U-15	IE 4115×GE	IE 4115×IE 2872
0		101.50	99.81	91.50
1,500		66.00	70.00	64.50
1,750		73.50	68.50	63.00
2,000		70.50	83.00	65.00
Grand Mean		**77.88**	**80.33**	**71.00**
		IE 4115×KA-72	IE 4115×OK-1	IE 4115×U-15
0		90.50	94.50	97.50
1,500		64.50	71.00	71.50
1,750		66.00	72.00	67.00
2,000		69.45	67.50	66.50
Grand Mean		**72.61**	**76.25**	**75.62**
		KA-72×GE	KA-72×IE 2872	KA-72×IE 4115
0		103.50	98.00	115.00
1,500		84.50	82.50	79.50
1,750		87.00	87.50	83.00
2,000		76.00	74.50	91.00
Grand Mean		**87.75**	**85.62**	**92.12**
		KA 72×OK-1	KA-72×U-15	OK-1×GE
0		95.81	103.00	114.00
1,500		86.50	84.50	83.50
1,750		83.50	75.00	83.00
2,000		73.50	65.00	78.00
Grand Mean		**84.83**	**81.88**	**89.62**
		OK-1×IE 2872	OK-1×IE 4115	OK-1×KA-72
0		102.50	102.50	107.00
1,500		78.00	78.50	76.50

1,750	79.50	79.50	65.00
2,000	74.50	79.50	71.50
Grand Mean	**83.62**	**85.00**	**80.00**
	OK-1×U-15	U-15×GE	U-15×IE 2872
0	108.50	83.00	93.00
1,500	79.50	80.50	71.50
1,750	56.50	80.50	70.00
2,000	65.00	61.81	67.50
Grand Mean	**77.38**	**76.45**	**75.50**
	U-15×IE 4115	U-15×KA-72	U-15×OK-1
0	97.50	89.00	93.50
1,500	65.50	80.00	67.00
1,750	68.50	62.50	79.50
2,000	76.50	75.00	78.50
Grand Mean	**77.00**	**76.62**	**79.62**

Appendix VI: Ear exertion length across the 15 cross replicates generated from the six genotypes

GL (ppm)	Genotype	GE×IE 2872	GE×IE 4115	GE×KA-72
0		137.50	75.00	41.00
1,500		27.00	43.00	21.00
1,750		29.00	23.00	25.50
2,000		25.00	17.00	5.00
Grand Mean		**55.60**	**39.50**	**23.10**
		GE×OK-1	GE×U-15	IE 2872×GE
0		117.50	86.50	122.50
1,500		10.00	23.00	19.50
1,750		7.00	16.00	36.00
2,000		31.50	36.50	0.00
Grand Mean		**40.50**	**40.50**	**44.50**
		IE 2872×IE 4115	IE 2872×KA-72	IE 2872×OK-1
0		92.50	54.00	123.50
1,500		6.00	23.50	22.50
1,750		20.50	17.00	18.50
2,000		15.00	7.50	9.00
Grand Mean		**33.50**	**25.50**	**43.40**
		IE 2872×U-15	IE 4115×GE	IE 4115×IE 2872
0		112.50	90.20	123.00
1,500		39.00	39.00	17.50
C2		31.00	12.00	10.50
2,000		12.50	20.50	19.50
Grand Mean		**48.80**	**40.40**	**42.60**
		IE 4115×KA-72	IE 4115×OK-1	IE 4115×U-15
0		100.00	95.00	85.00
1,500		6.50	13.50	20.00
1,750		3.00	18.00	23.50
2,000		26.50	32.50	8.00
Grand Mean		**34.00**	**39.80**	**34.10**
		KA-72×GE	KA-72×IE 2872	KA-72×IE 4115
0		106.00	116.00	98.00
1,500		15.00	7.50	11.00
1,750		0.00	12.50	30.00
2,000		17.00	23.50	19.00
Grand Mean		**34.50**	**39.90**	**39.50**
		KA-72×OK	KA-72×U-15	OK-1×GE
0		150.20	120.00	130.50
1,500		8.00	29.50	18.00
1,750		26.00	12.50	13.50
2,000		18.50	18.00	8.50
Grand Mean		**50.70**	**45.00**	**42.60**
		OK-1×IE 2872	OK-1×IE 4115	OK-1×KA-72
0		100.00	100.00	125.00
1,500		16.00	34.50	34.50

1,750	23.00	23.00	17.50
2,000	10.00	5.00	38.00
Grand Mean	**37.30**	**40.60**	**53.80**
	OK-1×U-15	U-15×GE	U-15×IE 2872
0	100.00	19.00	135.00
1,500	22.50	45.00	25.50
1,750	20.00	27.50	21.50
2,000	18.00	3.20	36.50
Grand Mean	**40.10**	**23.70**	**54.60**
	U-15×IE 4115	U-15×KA-72	U-15×OK-1
0	122.00	97.50	120.00
1,500	43.00	58.00	20.00
1,750	18.50	32.50	45.50
2,000	21.00	41.50	40.00
Grand Mean	**51.10**	**57.40**	**56.40**

Appendix VII: Number of productive tillers across the 15 cross replicates generated from the six genotypes

GL (ppm)	Genotype	GE×IE 2872	GE×IE 4115	GE×KA-72
0		2.000	2.000	2.000
1,500		2.000	2.000	2.000
1,750		2.000	1.000	1.500
2,000		2.000	2.000	2.500
Grand Mean		**2.000**	**1.750**	**2.000**
		GE×OK-1	GE×U-15	IE 2872×GE
0		2.000	2.000	0.500
1,500		1.000	2.000	2.000
1,750		2.500	1.500	2.500
2,000		2.000	0.500	3.000
Grand Mean		**1.875**	**1.500**	**2.000**
		IE 2872×IE 4115	IE 2872×KA 72	IE 2872×OK-1
0		1.000	1.500	1.500
1,500		3.000	3.000	2.000
1,750		1.500	3.000	2.000
2,000		2.500	2.500	1.500
Grand Mean		**2.000**	**2.500**	**1.750**
		IE 2872×U-15	IE 4115×GE	IE 4115×IE 2872
0		4.000	2.021	2.500
1,500		1.500	2.000	2.000
1,750		2.000	1.500	3.000
2,000		2.000	3.500	3.500
Grand Mean		**2.375**	**2.255**	**2.750**
		IE 4115×KA-72	IE 4115×OK-1	IE 4115×U-15
0		3.500	1.500	2.000
1,500		3.500	2.000	2.000
1,750		3.000	1.500	2.500
2,000		3.000	1.500	1.000
Grand Mean		**3.250**	**1.625**	**1.875**
		KA-72×GE	KA 72×IE 2872	KA 72×IE 4115
0		2.000	1.500	2.000
1,500		1.000	1.500	1.500
1,750		1.000	2.000	1.000
2,000		2.500	3.000	2.000
Grand Mean		**1.625**	**2.000**	**1.625**
		KA-72×OK-1	KA-72×U-15	OK-1×GE
0		1.021	0.500	1.500
1,500		2.000	2.500	2.000
1,750		1.500	3.000	2.000
2,000		3.000	2.500	2.000
Grand Mean		**1.880**	**2.125**	**1.875**
		OK-1×IE 2872	OK-1×IE 4115	OK-1×KA 72
0		2.000	1.500	2.500
1,500		2.000	1.500	2.500
1,750		2.500	2.500	2.500
2,000		2.000	2.500	2.500
Grand Mean		**2.125**	**2.000**	**2.500**

	OK-1×U-15	U-15×GE	U-15×IE 2872
0	1.500	4.000	1.500
1,500	2.500	2.000	2.500
1,750	3.000	2.000	2.500
2,000	2.500	3.021	2.000
Grand Mean	**2.375**	**2.755**	**2.125**
	U-15×IE 4115	U-15×KA-72	U-15×OK-1
0	1.500	2.500	2.500
1,500	3.000	2.000	2.000
1,750	1.500	2.500	2.000
2,000	3.000	2.000	2.000
Grand Mean	**2.250**	**2.250**	**2.125**

Appendix VIII: Nanodrop© readings for all the samples

Serial No.	Sample ID	Concentration ng/µl	260/280	260/230
1	IE 2872 X U-15 (0PPM)	147.3	1.91	1.65
2	IE 2872 X U-15 (1500PPM)	111.4	1.95	1.46
3	IE 2872 X U-15 (1750PPM)	121.8	1.36	1.09
4	IE 2872 X U-15 (2000PPM)	138.8	1.38	1.13
5	U-15 X IE 2872 (0PPM)	303.5	1.95	1.83
6	U-15 X IE 2872 (1500PPM)	68.7	1.9	1.35
7	U-15 X IE 2872 (1750PPM)	169.5	1.53	1.28
8	U-15 X IE 2872 (2000PPM)	149.1	1.37	1.09
9	GE X 1E 2872 (0PPM)	121.9	1.31	0.94
10	GE X 1E 2872 (1500 PPM)	126.8	1.6	1.1
11	GE X 1E 2872 (1750 PPM)	112.6	1.14	0.84
12	GE X 1E 2872 (2000PPM)	159.7	1.3	0.96
13	1E 2872 X GE (0PPM)	100.2	1.65	1.05
14	1E 2872 X GE (1500PPM)	110	1.75	1.19
15	1E 2872 X GE (1750PPM)	90.4	1.4	1.15
16	1E 2872 X GE (2000PPM)	155.2	1.23	0.89
17	1E 2872 X KA-72 (0PPM)	211.4	1.18	0.84
18	1E 2872 X KA-72 (1500PPM)	278.2	1.96	1.78
19	1E 2872 X KA-72 (1750PPM)	181.9	1.44	1.11
20	1E 2872 X KA-72 (2000PPM)	113.3	1.38	0.93
21	KA-72 X 1E 2872 (0PPM)	116.7	1.75	1.3
22	KA-72 X 1E 2872 (1500PPM)	87.3	2.01	1.27
23	KA-72 X 1E 2872 (1750PPM)	137.6	1.44	1.09
24	KA-72 X 1E 2872 (2000PPM)	190.9	1.15	0.89
25	GE X IE 4115 (0PPM)	272.2	1.22	0.87
26	GE X IE 4115 (1500PPM)	167.2	1.9	1.53
27	GE X IE 4115 (1750PPM)	249.2	1.35	1.04
28	GE X IE 4115 (2000PPM)	120.5	1.33	0.9
29	IE 4115 XGE (0PPM)	163.5	1.91	1.45
30	IE 4115 XGE (1500PPM)	64.9	2.22	1.2
31	IE 4115 X GE (1750PPM)	136	2.09	1.61
32	IE 4115 X GE (2000PPM)	132.1	1.23	0.89
33	U-15 X OK-1 (0PPM)	154.1	1.88	1.4
34	U-15 X OK-1 (1500PPM)	283.2	1.53	1.29
35	U-15 X OK-1 (1750PPM)	167.7	1.64	1.32

36	U-15 X OK-1 (2000PPM)	211.5	1.99	1.64
37	OK-1 X U-15 (0PPM)	211.1	1.99	1.67
38	OK-1 X U-15 (1500PPM)	247.3	1.39	1.15
39	OK-1 X U-15 (1750PPM)	112.1	1.48	1
40	OK-1 X U-15 (2000PPM)	109.1	1.19	0.99
41	KA-72 X GE (0PPM)	303.2	2	1.82
42	KA-72 X GE (1500PPM)	390.2	2.02	1.96
43	KA-72 X GE (1750PPM)	106.1	1.57	1.43
44	KA-72 X GE (2000PPM)	235.2	1.39	1.16
45	GE X KA-72 (0PPM)	142	1.94	1.38
46	GE X KA-72 (1500PPM)	203	1.99	1.68
47	GE X KA-72 (1750PPM)	148.6	1.23	1.06
48	GE X KA-72 (2000PPM)	138.1	1.23	0.96
49	OK-1 X GE (0PPM)	143.2	1.77	1.46
50	OK-1 X GE (1500PPM)	355.5	2.01	1.92
51	OK-1 X GE (1750PPM)	179.8	1.96	1.59
52	OK-1 X GE (2000PPM)	155.3	1.96	1.46
53	GE X OK-1 (0PPM)	474.5	1.49	1.36
54	GE X OK-1 (1500PPM)	86.9	1.72	1.2
55	GE X OK-1 (1750PPM)	128.4	1.77	1.38
56	GE X OK-1 (2000PPM)	93.9	1.43	1.08
57	IE 4115 X IE 2872 (0PPM)	142.7	1.87	1.53
58	IE 4115 X IE 2872 (1500PPM)	170.5	2	1.55
59	IE 4115 X IE 2872 (1750PPM)	161.5	1.99	1.54
60	IE 4115 X IE 2872 (2000PPM)	290	1.22	1.17
61	IE 2872 X IE 4115 (0PPM)	322.9	1.29	1.02
62	IE 2872 X IE 4115 (1500PPM)	216.7	1.39	1.14
63	IE 2872 X IE 4115 (1750PPM)	279.7	1.3	1.03
64	IE 2872 X IE 4115 (2000PPM)	74.3	2.29	1.41
65	IE 4115 X U-15 (0PP	291.1	1.3	0.96
66	IE 4115 X U-15 (1500PPM)	229.7	1.96	1.63
67	IE 4115 X U-15 (1750PPM)	139.2	1.5	1.19
68	IE 4115 X U-15 (2000PPM)	70.5	1.86	1
69	U-15 X IE 4115 (0PPM)	150.1	1.47	1.25
70	U-15 X IE 4115 (1500PPM)	122.5	1.98	1.38
71	U-15 X IE 4115 (1750PPM)	116.8	1.45	1.15
72	U-15 X IE 4115 (2000PPM)	80.7	1.53	1.06
73	IE 4115 X KA-72 (0PPM)	96.5	1.71	1.12

74	IE 4115 X KA-72 (1500PPM)	221.5	2.03	1.87
75	IE 4115 X KA-72 (1750PPM)	62.8	1.91	1
76	IE 4115 X KA-72 (2000PPM)	146.5	2.02	1.62
77	KA-72 X IE 4115 (0PPM)	254.6	1.98	1.8
78	KA-72 X IE 4115 (1500PPM)	185.8	2.03	1.72
79	KA-72 X IE 4115 (1750PPM)	297.1	2.01	1.87
80	KA-72 X IE 4115 (2000PPM)	144.4	1.96	1.55
81	IE 2872 X OK-1 (0PPM)	327.3	1.48	1.23
82	IE 2872 X OK-1 (1500PPM)	72.3	1.99	0.98
83	IE 2872 X OK-1 (1750PPM)	198.6	1.43	1.25
84	IE 2872 X OK (2000PPM)	263.4	1.51	1.28
85	OK-1 X IE 2872 (0PPM)	319.4	1.28	0.96
86	OK-1 X IE 2872 (1500PPM)	111.9	1.96	1.32
87	OK-1 X IE 2872 (1750PPM)	391.8	1.31	1.18
88	OK-1 X IE 2872 (2000PPM)	336.2	1.22	0.88
89	IE 4115 X OK-1 (0PPM)	82.2	1.86	1.04
90	IE 4115 X OK-1 (1500PPM)	120.1	1.92	1.3
91	IE 4115 X OK-1 (1750PPM)	369.5	1.36	1.19
92	IE 4115 X OK-1 (2000PPM)	101.9	2	1.34
93	OK-1 X IE 4115 (0PPM)	243.6	1.95	1.71
94	OK-1 X IE 4115 (1500PPM)	295.7	1.78	1.58
95	OK-1 X IE 4115 (1750PPM)	419.3	1.5	1.32
96	OK-1 X IE 4115 (2000PPM)	195.2	1.96	1.62
97	KA-72 X U-15 (0PPM)	266.2	1.99	1.81
98	KA-72 X U-15 (1500PPM)	350.6	2.05	2.04
99	KA-72 X U-15 (1750PPM)	260.1	1.4	1.09
100	KA-72 X U-15 (2000PPM)	274.5	1.54	1.29
101	U-15 X KA-72 (0PPM)	994.5	2.03	2.33
102	U-15 X KA-72 (1500PPM)	212.6	1.76	1.51
103	U-15 X KA-72 (1750PPM)	238.7	1.54	1.31
104	U-15 X KA-72 (2000PPM)	172.6	1.41	1.08
105	KA-72 X OK-1 (0PPM)	322.2	1.49	1.29
106	KA-72 X OK-1 (1500PPM)	149.1	1.95	1.47
107	KA-72 X OK-1 (1750PPM)	122.4	1.97	1.37
108	KA-72 X OK (2000PPM)	93.2	1.88	1.17
109	OK-1 X KA-72 (0PPM)	102.3	1.91	1.19
110	OK-1X KA-72 (1500PPM)	332.7	2	1.92
111	OK-1 X KA-72 (1750PPM)	155.7	2.01	1.59
112	OK-1X KA-72 (2000PPM)	216	1.95	1.58
113	GE X U-15 (0PPM)	263.3	1.96	1.63
114	GE X U-15 (1500PPM)	551.6	2	2.07
115	GE X U-15 (1750PPM)	297.5	1.65	1.38

116	GE X U-15 (2000PPM)	155.5	1.97	1.39
117	U-15 X GE (0PPM)	204.3	1.59	1.23
118	U-15 X GE (1500PPM)	237.8	1.96	1.71
119	U-15 X GE (1750PPM)	205.8	1.95	1.66
120	U-15 X GE (2000PPM)	67.3	1.56	1.11
121	IE4115-Parent	392.2	1.9	1.83
122	IE 2872-Parent	279.6	1.86	1.72
123	GE-Parent	389.6	1.9	1.98
124	KA-72-Parent	526.5	1.87	1.97
125	OK-1-Parent	354	1.91	1.9
126	U-15-Parent	283.1	1.84	1.91