Genetic Diversity, Correlations and Path Coefficient Analysis in Popcorn (*Zea mays* L. everta)

By

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“Our deepest fear is not that we are inadequate. Our deepest fear is that we are powerful beyond measure. It is our light, not our darkness that most frightens us. We ask ourselves, who am I to be brilliant, gorgeous, talented, and fabulous? Actually, who are you *not* to be?

We are all meant to shine. And as we let our own light shine, we unconsciously give other people permission to do the same. As we are liberated from our own fear, our presence automatically liberates others.”

Marianne Williamson

ABSTRACT

Popcorn is the most popular snack food in the world. Genetic diversity is of major concern in popcorn breeding. High genetic diversity allows manipulation of different genotypes to breed new varieties. There is very little published work on popcorn production in Sub-Saharan Africa primarily in South Africa. Popcorn production in South Africa could be hampered by the lack of superior and adapted varieties with large genetic base, good popping ability and high yield. Studies relating popping expansion volume and grain yield are of fundamental importance for popcorn improvement, but they are limited. Furthermore, there is limited number of studies regarding popcorn genetic diversity among locally developed popcorn varieties.

The objectives of the study were; (i) to investigate genetic variability among the popcorn inbred lines, (ii) to study the magnitude of genetic diversity among the popcorn inbred lines, (iii) to establish the relationship between popping ability and seed yield, and with secondary traits, and (iv) to evaluate the effect of popping methods on popping ability of different popcorn inbred lines. Two populations designated as Population 1 and Population 2 with 83 and 81 inbred lines, respectively, were used in the study.

On the study of the appraisal of popping methods, the highest popping expansion volume (cm^3) and less number of unpopped kernels were obtained from hot air popping than in the microwave popping method. The study revealed that hot air popping method is more effective and efficient in discriminating popping ability of the inbred lines. The study further revealed that the two methods rank genotypes differently. The presence of genotype × popping method interaction resulted in three different groups. (i) Genotype adaptation across methods, (ii) specific adaptation to microwave popping, and (iii) specific adaptation to hot air popping method. Hence, when breeders evaluate popping ability of different genotypes, they should consider the method, depending on the way consumers will do the popping.

The study of relationship between traits showed that popping expansion volume and seed yield was positively and significantly correlated. Nevertheless, the relationship between seed yield and popping expansion volume was weak. Popping expansion volume was negatively and weakly correlated with most secondary traits except kernel aspect and number of unpopped kernels. The direct effects of kernel aspect score on popping expansion volume were large and negative. Other traits showed small direct and indirect effects on popping expansion volume. Traits including days to anthesis, ear prolificacy and ear aspect exhibited large direct effects on seed yield. Indirect and direct effects of other traits on seed yield were small. Relationship among several secondary traits was small. The results obtained showed that selection for high seed yield will not negatively impact popping expansion volume and vice versa, therefore, popping expansion volume and seed yield can be improved concurrently. Overall, indirect effects of secondary traits on seed yield and popping expansion volume were small; this supported the focus on direct selection of these traits to improve seed yield and popping ability.

Based on the study of genetic diversity and variability, inbred lines showed large genetic variation and high heritability for 18 traits. Phenotypic and genetic coefficient of variation was high in seven and six traits, respectively. A large percentage of genetic advance was recorded in 11 traits. Dendogram derived from phenotypic data grouped the inbred lines into four to seven clusters depending on heritability. Dendogram produced from 22 SSR markers grouped inbred lines into five clusters.

Overall, the study showed that, maximum popping ability of inbred lines is dependent on the method used. Simultaneous improvement of seed yield and popping expansion volume is possible through selection of inbred lines combining both high popping expansion volume and seed yield. Improvement of the two traits should be based on selection for traits with large direct effects. The magnitude of genetic diversity among the inbred lines was large; therefore, distant inbred lines can be selected as parents and crossed to develop new varieties that are locally adapted. Above all, the results have implications for the methods which would be used to process popcorn by consumers especially in developing rural communities.

DECLARATION

I, Siphiwokuhle Shandu declare that:

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

2. This dissertation has not been submitted for any degree or examination at any other University.

3. This dissertation does not contain other personsô data, pictures, graphs, or other information, unless specifically acknowledged as being sourced from other persons.

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5. This dissertation does not contain text, graphics or tables copied and pasted from the internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the references sections.

Signed

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Siphiwokuhle Shandu

As the candidateôs supervisors, we agree to the submission of this dissertation:

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Dr Alfred Odour Odindo

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Prof John Derera

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DEDICATION

This work is dedicated to the memories of my parents who did not live to see this work:

- Mother: I.B Dube, who never gave up, who overcame the tremendous obstacles just for me to grow up and who taught me not to give up and who gave me strength to propel in spite of the lifeôs challenges.
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LIST OF ABBREVIATIONS

QTL	Quantitative Trait Loci
PEV	Popping Expansion Volume
FV	Flake Volume
UPK	Unpopped Kernel
SSR	Simple Sequence Repeats markers
SNP	Single Nucleotide Polymorphism
SCAR	Sequence characterized amplified region
CAPS	Cleaved Amplified Polymorphic Sequence
RAPD	Randomly amplified Polymorphic DNA
RFLP	Restriction Fragment length polymorphism
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
H^2	Broad sense heritability
GV	Genetic variation
PCV	Phenotypic coefficient of variation
GCV	Genetic coefficient of variation
GA	Genetic advance
PH	Plant height
EH	Ear height
PTB	Primary tassel branches
NL	Number of leaves
EA	Ear aspect
FA	Flake aspect
ER	Ear rot
DA	Days to anthesis
DS	Days to silking
NP	Number of plants

SL	Stem Lodging
RL	Root lodging
NE	Number of ears per plant/plot (prolificacy)
GM	Grain moisture percentage at harvest (h) and popping(p)
CC	Chlorophyll concentration
LA	Leaf area
ET	Ear turc
SY	Seed yield
V_G	Genetic variance
V_P	Phenotypic variance
m.a.s.l	Metres above sea level
PC	Positive check
NC	Negative check
PCR	Polymerase chain reactions
DNA	Deoxyribonucleic acid
OPV	Open pollinated varieties
CIMMYT	International Maize and Wheat Improvement Center
REML	Linear Mixed Models
TBE:	Tris-Borate -EDTA

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INTRODUCTION TO DISSERTATION

1 Origin and Unique traits

Popcorn (*Zea mays* L) is a small flint maize that is widely consumed as a snack food worldwide. Popcorn was first discovered by native Americans in Mesoamerica (Hallauer, 1994). This type of maize originated as a mutant of flint corn (Kantey *et al.*, 1995). The early varieties in the area of origin were, White rice, Queens Golden and Japanese Hulless (Hallauer, 1994). Popcorn popularity has been increasing overtime throughout the world (Ahmet and Halil, 2011; Sakin *et al.*, 2005). United States is the largest popcorn producer and has been principally the consuming country of popcorn (Dhliwayo, 2008).

Popcorn is distinguished from other types of maize by its unique feature of popping when heated. Popcorn kernels contain a small amount of water stored in soft starch within the endosperm. When the kernels are subjected to heat, the superheated water expands causing the pressure to build up within the kernel, as the kernel expands the pericap explodes (Hoseney *et al.*, 1983; Lu *et al.*, 2003). Grain moisture of 14% is ideal for popping, and the ideal popping percentage should be approximately 98% (Gokmen, 2004; Tiner, 2008). Popcornsô ability to pop is associated with its composition (Dhliwayo, 2008; Matz, 1991) for example, the presence of a very hard pericap and outer layers of the endosperm allowing the internal temperature and pressure to adequately rise for kernels to pop (Karababa, 2006). Popcorn kernels have three distinct shapes, rice, pearl shape and American type. Rice shaped kernels are white, long, small and sharp at the top, the pearl shaped are yellow, oval shaped and smooth at the top and the American type is large and round (Dhliwayo, 2008).

2 Potential for food security

There is a potential for turning popcorn into a food security crop in developing countries. Popcorn has high nutritive value for example, it contains relatively high amount of vitamins (B1, B2 and niacin), proteins, minerals, and calories. For example, one cup of popcorn contains about 25 calories and the minerals iron, phosphorous and calcium contents that are comparable to that of beef. Popcorn also contains large amount of carbohydrates and low fat content. The presence of calcium and phosphorous in popcorn contributes to strong teeth. Popcorn also supplies bulk and roughage (Amusa *et al.*, 2005; Muhammad, 2005). Its popularity as the worldôs snack food has increased as a result of the flavour enhanced by the addition of salt, butter, margarine and honey on popped kernels (Muhammad, 2005). Because of the high nutritive value of popcorn, it can be used in combating malnutrition problems in rural developing countries.

3 Genetic diversity and variety development with implication for plant breeding

Popcorn has a small gene pool than dent maize. For most productivity traits such as diseases and pests resistance, stalk strength and grain yield; popcorn shows a small genetic variation which has limited its improvement (Matz, 1991). The narrow genetic base of the crop resulted from the use of small popcorn lines developed from flint maize germplasm (Kantey *et al.*, 1995). Trindade *et al.* (2010) reported that a large genetic base is useful in developing superior varieties. In all types of maize, high genetic diversity allows manipulation of different genotypes resulting in genetic improvement of open pollinated varieties (OPV) and heterotic hybrids (Hallauer and Miranda-Filho, 1988; Munhoz *et al.*, 2009).

Dent maize was used as a genetic source of improving the elite gene pool of popcorn. This was achieved through phenotypic backcrossing where popcorn was used as a recurrent parent (Zeigler, 2001). However, improvement using germplasm of dent maize resulted in new popcorn genotypes with improved agronomic performance and other agronomic characteristics. For example large kernel size,

reduced stem and root lodging, improved grain yield but with low popping expansion volume indicating that, popping ability is compromised in backcross programs which include dent maize (Zeigler, 2001).

4 Opportunity for breeding: limited technology options

The importance and utilization potential of popcorn is steadily increasing in South Africa. However, almost all the popcorn consumed in the country is imported. Exceptionally small amount of popcorn is produced in South Africa, where most of production occurs at the private seed industries. Therefore, there are no current yield figures recorded for South African popcorn production. Popcorn production in South Africa was lastly reported in 1953 by Josephson *et al.* (1954). The estimated production from 1947-1954 ranged from 13000 ï 20000 bags of 200 lb. (each which is equivalent to about 6 ï 9 tons) per annum. Production dropped dramatically as a result of the poor product availability. The reason for poor production could be attributed to little or no attention received by popcorn production with regard to the research studies. For example, studies evaluating genetic and phenotypic diversity of different genotypes and consequently genetic improvement of popcorn are not well documented. Genotype × environment interaction (G×E) could also be another reason for poor popcorn production in South Africa. Genotype × environment interaction has also been reported by various researchers as the major constraint in popcorn production (Paula *et al.*, 2010). As a result, there are a limited number of varieties for farmers to choose from.

5 Summing up rationale for the study

Research studies based on genetic and phenotypic diversity in popcorn and overall production improvement are important in breeding programs to establish new varieties adapted to South African growing conditions, and to reduce heavy reliance on popcorn imports. Therefore, this research will lead to enhancement of popcorn germplasm to enable development of new varieties with acceptable popping

expansion volume and grain yield. Furthermore, the study will contribute to an increase in South African popcorn production.

Among the 83 inbred lines which were developed at the University of KwaZulu-Natal (UKZN) breeding programme, it is not known whether the genotypes vary genetically and phenotypically, and whether their variability can contribute to local adaptation. Lack of knowledge of the genetic diversity among different popcorn genotypes has led to the need for investigating the important genetic parameters and genetic diversity among popcorn inbred lines. It is also important to quantify the relationship between popping ability, yield and agronomic performance. The study of the relationship between popping expansion volume and seed yield and with secondary traits is therefore crucial. Genotypes are also likely to display an interaction with the popping method.

6 Research objectives

The major objectives of the study were to quantify the level of genetic diversity and to study correlations and path coefficients of the secondary traits on seed yield and popping ability in two experimental populations designated as Population 1 and Population 2 with 83 and 81 inbred lines, respectively.

The specific objectives were to:

i. Evaluate the effect of microwave oven and hot air popping method in popping expansion volume and number of unpopped kernels of different popcorn inbred lines.

ii. Establish the relationship between seed yield and popping expansion volume, and with secondary traits as well as the relationship among secondary traits.

iii. Determine genetic variation among popcorn inbred lines.

iv. Investigate the magnitude of genetic diversity among popcorn inbred lines, using genetic and molecular data.

7 Research hypotheses

i The ability of popcorn inbred lines to pop differs with the popping method used.

ii There is a positive relationship between seed yield and popping expansion volume and with secondary traits, and there is also a relationship among secondary traits.

iii There is high genetic variation among genotypes under study

iv The magnitude of genetic diversity among the studied genotypes is high suggesting that selection would be effective to breed new varieties.

8 Dissertation Outline

This dissertation comprises the introduction and five main chapters as follows:

Introduction

Chapter 1: Literature Review

Chapter 2: Appraisal of Microwave and Hot Air Popping Methods for Rapid Screening of Popcorn Inbred Lines

Chapter 3: Correlations and Path Coefficient Analysis for Seed Yield, Popping Expansion Volume and Secondary Traits in Popcorn Inbred Lines

Chapter 4: Genetic Parameters and Diversity in a Popcorn Inbred Line Population.

Chapter 5: General overview of the research findings

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CHAPTER 1

Literature review

1.1 Introduction

The chapter reviews genetic and phenotypic diversity, its importance and assessment, popping expansion volume, the effect of different popping methods and other factors on popping ability of different popcorn genotypes. The section also reviews literature on correlations and path coefficients on seed yield, popping expansion volume and secondary traits. The chapter, therefore, provides framework for the study of genetic diversity, correlations and path coefficient analysis in popcorn.

1.2 Types of corn

In addition to popcorn, there are other types of maize which are categorized on the basis of their endosperm, and these include dent and flint maize.

1.2.1 Dent maize (*Zea mays* indenata)

The characteristics of dent maize include a soft central core and a floury endosperm which extends to the crown of the endosperm. This endosperm collapses upon drying and becomes indented. The degree of denting is influenced by the genetic background of a variety. The presence of the horny endosperm at the back and in the sides of the kernel is another characteristic of dent maize (Brown *et al.*, 1985; Logsdon, 2009; Smith, 1999). Unlike popcorn, dent maize does not pop even when the conditions are ideal for popping. Inability of dent maize to pop is associated with the presence of porous hulls that do not trap steam and therefore kernels fail to pop (Davidson, unpublished data).

1.2.2 Flint maize (*Zea mays* indurata)

Flint maize is characterized by vitreous, thick and hard endosperm layer. The flint kernels are small, generally long and skinny and have fewer number of kernels per row (Brown *et al.*, 1985; Smith, 1999). The amount of starch is small and varies with genotypes. When the kernels are dried, they do not shrink which differentiates it from dent maize. Flint maize kernels are not easily digested as dent maize and the endosperm does not become dented at maturity (Logsdon, 2009). Flint maize normally performs better than dent, for example, when grown in temperate regions, it germinate quickly and matures faster (Brown *et al.*, 1985). Flint maize can pop under proper conditions, however the flakes become hard (Smith, 1999).

1.3 Popping expansion volume (PEV)

Popping expansion volume is the fundamental determinant of popcorn quality and therefore, improvement of this trait is the primary objective in popcorn breeding. Popping expansion volume is defined as the volume of popped corn per gram of unpopped corn (Dhliwayo, 2008) Popping expansion volume is a heritable trait and its inheritance is of additive type (Dhliwayo, 2008). Kernel explosion and flake formation are the two primary events that occur during popping expansion. An increased arrangement of cellulose, increased degree of fabrillar packing in the pericap and the greater ratio of hard (translucent) endosperm to soft endosperm (opaque) are associated with kernel explosion and large flake formation (Babu *et al.*, 2006; Zeigler, 2001). In plant breeding, popping quality is the major trait that is emphasized in popcorn improvement while in dent maize, grain yield and other agronomic traits are mostly underlined (Dhliwayo, 2008; Zeigler, 2001).

1.3.1 Factors affecting popping expansion volume

Popping expansion volume is influenced by several factors. These factors include, physical properties of the kernel such as kernel size, kernel composition, kernel

damage, kernel density, shape, pericarp thickness and hardiness (Sweley *et al.*, 2012a; Sweley *et al.*, 2012b). Genotype, type of endosperm, popping method, popping temperature, varietal maturity, effect of fungal diseases, structural damage, moisture content, and kernel composition can also influence popping ability. Unfavorable environmental conditions such as frost also influence popping expansion volume (Ahmet and Halil, 2011; Arnhold *et al.*, 2006; Broccoli and Burak, 2004; Coyle *et al.*, 2000; Hoseney *et al.*, 1983; Santos *et al.*, 2004; Soylu and Tekkanat, 2007; Sweley *et al.*, 2012a; Tian *et al.*, 2001). The effect of moisture content, kernel size, popping method, genotype and popping temperature is explained below.

1.3.1.1 Moisture content

Moisture content is the primary factor affecting popping expansion. According to Hoseney *et al.* (1983), moisture content is associated with the rate and degree of pressure build up within the starch granules. Gokmen (2004) studied the effect of moisture content on popping ability. Gokmen (2004) obtained low popping quality at moisture content below or above the optimum. For example, when moisture content deviated by ±2% from the optimum, expansion volume was reduced by 20%. The highest popping ability (flake size and the lowest percentage of unpopped kernel) was obtained from kernels popped at 14% moisture content. Gokmen (2004), therefore, concluded that, the optimum moisture of 14% was ideal for popping (Figure 1.1). Changes in popping ability at given moisture content are associated with the pericarp. The pericarp allows the kernel to maintain high pressure when the mechanical resistance is high, for example, it serves as the pressure vessel that holds the steam of heated water (Broccoli and Burak, 2004; Soylu and Tekkanat, 2007). When the moisture content of popcorn kernels increases, the temperature at which the pericarp melts decreases. At high moisture content, the pressure within the kernels is low and consequently reduces the popping expansion volume and vice versa (Gokmen, 2004; Hoseney *et al.*, 1983; Shimoni *et al.*, 2002). Kernel moisture content varies with varieties and kernel size. For example, small kernels require a higher moisture content than large kernels (Ahmet and Halil, 2011; Coyle *et al.*, 2000; Ertas *et al.*, 2009; Gokmen, 2004; Tian *et al.*, 2001).

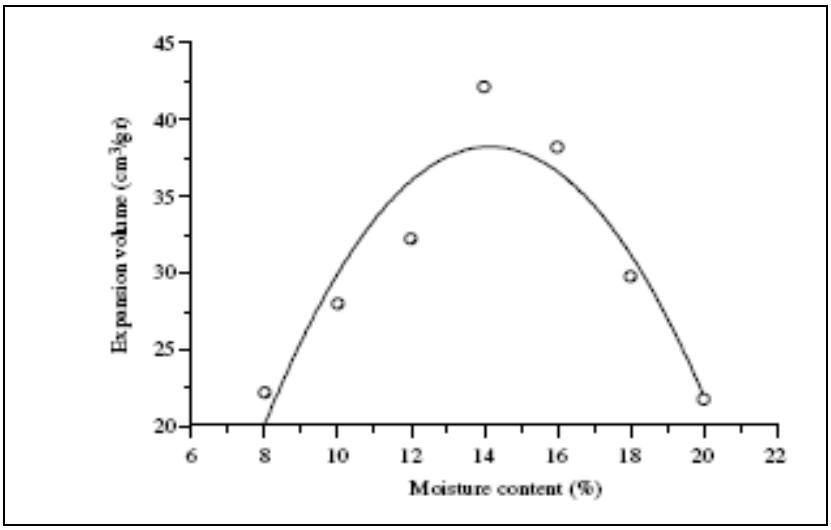

Figure 1.1: Effect of moisture content in popping ability (Gokmen, 2004)

1.3.1.2 Kernel size

There is contradicting information regarding the relationship between kernel size and popping ability. Gokmen (2004) observed that, genotypes with large kernel size had a high expansion volume, flake size and low percentage of unpopped kernels than small to medium kernels. Pajic and Babic (1991) reported that, large kernels are characterized by high percentage of soft endosperm. Soft endosperm contains the highest amount of water and starch which lowers popping ability (Fantini *et al.*, 2006). Tian *et al.* (2001) observed no significant effect of kernel size on popping ability. Further studies are therefore recommended to elucidate the effect of kernel size on popping ability.

1.3.1.4 Popping methods

Performance of popcorn varieties may depend on the method used. Gokmen (2004) studied the effect of popping methods on popping quality. The methods used were microwave popping, and conventional methods (hot-air popper and cooking pan). Hot air popping method, cooking pan without oil and salt gave the highest popping

ability and fewer unpopped kernels. However, the microwave popping method gave the lowest popping ability (flake size and more unpopped kernels). Low popping ability in the presence of oil and salt was associated with differences in heat conductivity and loss factors of both oil and heat (Singh and Singh, 1999). Dofing *et al.* (1990) also observed better popping ability and fewer number of unpopped kernels under hot air popping than in the microwave popping. These findings suggest that, a recommended popping method should be used in order to obtain high flake volumes.

1.3.1.5 Genotype

Popping ability is also influenced by the genetic makeup of individual genotypes. Ahmet and Halil (2011) observed differences in popping ability among genotypes, some genotypes (TCM-05-16, TCM-05-07, TCM-05-14, and TCM-05-08) showed higher popping expansion volume and less unpopped kernel than commercial hybrids. Hybrid varieties generally show high popping ability when compared with open pollinated genotypes, therefore most of the commercial popcorns are three way or single cross hybrids (Dofing *et al.*, 1990). Soylu and Tekkanat (2007) and Sakin *et al.* (2005) also reported that, when hybrids are compared with open pollinated varieties they give better popping ability. Therefore, popping ability is not uniform across genotypes, as a result, special selection for the best genotypes with regard to popping ability is essential.

1.3.1.6 Genotype × popping method interaction

Popping ability of a genotype may be influenced by the popping method used, for example, under different methods, similar genotype may pop differently. Dofing *et al.* (1990) evaluated the presence of genotypes × popping method interaction on expansion volume and the associated components. The presence of genotype × popping method interaction on popping expansion volume, unpopped kernels and flake size was detected. Genotypes ranked differently under two methods, for example popping expansion volume for genotype R20-60 was higher than M8386 and P410 in conventional methods. When the same genotypes were ranked under

microwave popping, popping ability of R20-60 was lower compared to M8386 and P410. Zeigler (2001) also observed a significant genotype popping × method interaction. The observations suggested that, the effect of popping methods on popping ability is not consistent across genotypes. These observations also underlined the importance of evaluating popping ability of different genotypes using distinct popping methods since genotypes may be adapted to a specific method.

1.3.1.7 Popping temperature

Popping temperature is another factor influencing popping ability. The maximum temperature for popping is approximately 177^{o}C, when temperature drops below the critical temperature, the ability of the kernels to pop declines (Hoseney *et al.*, 1983). The temperature, internal pressure and power absorbance required for popping differs for individual kernels (Byrd and Perona, 2005). Sweley *et al.* (2012b) evaluated the microwave popping performance across hybrids. They reported that, during microwave heating, some of the kernels are shielded by others or may be in a position of the bag where they fail to absorb sufficient energy. These kernels therefore, do not reach the optimum temperature required for popping. This effect was verified by re-popping the unpopped kernels, and was able to pop. These findings underline the importance of choosing the most appropriate method for evaluating popcorn genotypes.

1.4 Correlations and path coefficients

Knowledge of the direct and indirect effects of different components on dependent traits such as yield and popping expansion volume, and the interrelationship among different components is essential during the selection process in a breeding program (Qaizar *et al.*, 1991; Vijayabharathi *et al.*, 2009). Path coefficient analysis gives information about the direct and indirect effects of different traits on a complex trait. Path coefficient analysis also suggests the selection criterion, and reduce the time taken by a breeder during the selection process (Qaizar *et al.*, 1991; Vijayabharathi *et al.*, 2009). For example, the breeder focuses only on the traits with a large direct effect on a dependent traits such as popping expansion volume and yield, thus

selection is only restricted to a few essential traits (Qaizar *et al.*, 1991; Vijayabharathi *et al.*, 2009). Separating correlation coefficients into direct and indirect effects using path coefficient analysis facilitates the breeding process (Darvishzadeh *et al.*, 2011; Machikowa and Saetang, 2008; Makanda *et al.*, 2009). The relationship between secondary traits and their direct and indirect effects on seed yield and popping expansion volume will be beneficial in the improvement of both popping expansion volume and seed yield.

1.4.1 Relationship between yield and popping ability

The relationship between popping expansion volume and seed yield is important in order to establish the value for cultivation and use (VCU) of popcorn varieties. A negative correlation between grain yield and popping expansion volume has been reported by several researchers (Arnhold *et al.*, 2006; Broccoli and Burak, 2004; Daros *et al.*, 2002; Sweley *et al.*, 2012b). Camara (2002) observed an approximately zero correlation between grain yield and popping expansion volume. Vijayabharathi *et al.* (2009), Pipolo *et al.* (2003) and Dofing *et al.* (1991) also reported a weak and non-significant correlation between the two traits. Their findings suggested that, concurrent improvement of popping ability and seed yield is difficult.

The good strategy for developing cultivars with high grain yield and high popping ability is to select plants with more than one ear and with good popping expansion volume (Broccoli and Burak, 2004). Arnhold *et al.* (2006), therefore found an opposite (positive) correlation between yield and popping expansion volume. During previous selection cycles, Arnhold *et al.* (2006) considered both grain yield and popping expansion volume simultaneously, and they concluded that, grain yield and popping ability may be positively correlated only when they are considered simultaneously during the selection process. However, information on the effect of concurrently selecting for both grain yield and PEV is limited. A positive and significant correlation between grain yield and popping expansion volume (r= 0.86) was also reported by Sakin *et al.* (2005) in open pollinated genotypes. According to Arnhold *et al.* (2006), Dofing *et al.* (1990), the two traits can be improved

simultaneously if breeding methods use dominance variation and additive genetic variation for grain yield and popping expansion, respectively. The foregoing indicates that, improvement of popcorn for popping ability will not necessarily compromise seed yield and vice versa.

1.4.2 Relationship between popping ability and secondary traits

Another consideration that breeders make is the relationship between popping and secondary traits with implication for selection strategy to enhance popping ability. Vijayabharathi *et al.* (2009) analyzed correlations and path effect for popping expansion volume. Traits including cob weight, plant height, number of kernels per row, days to maturity, and days to silking showed a strong correlation and a very high direct and positive effects on popping expansion volume. These observations indicated that, these traits can be used to enhance popping ability. Cob length and 100 kernel weight were negative and significantly correlated with popping expansion volume. Therefore, these secondary traits should also not be ignored during popping ability enhancement programme.

Pipolo *et al.* (2003) and Ceylan and Karababa (2004) also observed a significant negative correlation between 100 kernel weight and popping expansion. The observed relationship was attributed to large percentage of soft endosperm in large kernels, hence, low popping expansion volume (Coyle *et al.*, 2000; Pajic and Babic, 1991; Song *et al.*, 1991). These findings contradicted the observations of Dofing *et al.* (1990) and Gokmen (2004) who reported large popping expansion volume in large than in small kernels. Broccoli and Burak (2004) found a positive relationship between kernel thickness, caryopsis roundness index and popping expansion volume, suggesting that, kernel shape is important in popping ability. Further, they reported that, expansion kernel density was strongly related with popping ability suggesting the direct proportion of this trait in popping ability. Significant relationship between secondary traits and popping ability can be exploited to improve popcorn varieties.

1.4.3 Relationship between yield and secondary traits

Literature on secondary traits has been scarcely reported in popcorn but, a lot has been done on maize. Ear prolificacy index correlated positively and significantly with yield (Broccoli and Burak, 2004). Agrama (1996) reported the highest direct effects of number of ears per plant (prolificacy) and grain size on grain yield. Ear prolificacy was suggested to be the best selection criterion in yield improvement. The other traits were suggested to be the potential components for developing lines with superior grain yield. Ear length, ear diameter and kernel length were positively correlated with yield. A negative correlation was found between yield and days to silking. Grain yield was negatively and significantly correlated with anthesis silking interval (Borras *et al.*, 2007). Grain yield was positively correlated with plant and ear height and days to anthesis (Bello *et al.*, 2012). The study of this relationship in popcorn will be exploited to improve popcorn varieties in the breeding programme.

1.4.4 Relationship among secondary traits

Secondary traits are important in plant breeding in selection for a genotype for a specific location. Days to anthesis was positive and significantly correlated with days to silking, however correlation with plant height and anthesis silking interval was positive and non-significant (Bello *et al.*, 2012). The relationship between ear aspect, ear height and plant aspect with days to anthesis was negative but non-significant. Plant height, ears per plant, plant aspect and ear aspect were positively correlated (Bello *et al.*, 2012). However correlation between plant height, plant aspect and ears per plant was not significant (Bello *et al.*, 2012). The relationship among traits themselves is important in improving individual traits associated with yield and popping ability in popcorn genotypes.

1.5 Genetic diversity

Genetic diversity is of fundamental importance in popcorn and other crop improvement. Frankham *et al.* (2010) described genetic diversity as, the variety of alleles and genotypes present in a population or in species. They reported that, genetic diversity present in different populations originated from mutation and by migration. Genetic diversity at a population level is measured by genetic distance between populations, and diversity at a molecular level is measured by the number of alleles per locus (Leal *et al.*, 2010). Generally, low level of genetic diversity in plants decreases the opportunities for improvement through breeding. A very wide range of genetic diversity is important for favorable genes. For example, genes for adaptation, higher yield and resistance to pests and diseases (Engels, 2002). Measurement of genetic diversity also allows breeders to focus only on the promising crosses as the number of crosses made is minimized (Pipolo *et al.*, 2003).

When species are subjected to various environments they encounter different changes such as, diseases, pests, competition and also pollution. High genetic diversity in species, therefore, enables them to evolve and adapt to the adverse environments, enables evolutionary change (Frankham *et al.*, 2010). Large populations that result from natural out breeding usually have a large genetic diversity. While loss of genetic diversity in a population is attributed to high levels of inbreeding thus reducing the individual's ability to adapt to environmental fluctuations (Frankham *et al.*, 2010; Simberloff and Rejmanek, 2011).

1.6 Phenotypic diversity and variation

Phenotypic diversity is important in indicating the genetic diversity of the segregating lines (Zavala, 2008). Favorable germplasm that are related can be grouped using information on phenotypic diversity (Melchinger, 1999; Weir, 1996 as cited by Zavala (2008)). Phenotypic variation in a population is largely dependent on the genetic and environmental effects, and the interaction between genotype and the environment. Hence, phenotypic variation is defined as the sum of genetic variance, environmental

variance and the total variance due to genotype × environmental interaction (G×E) (Leal *et al.*, 2010; Moose and Mumm, 2008; Plomin, 1986). Phenotypic variation gives useful information to be used by plant breeders in improvement of popcorn (Yoshida and Yoshida, 2004). The effect of genetic and environmental factors on phenotypic diversity can be best described by the phenotypic effect model (Zavala, 2008), as follows:

$P_{ij}= \mu+ G_i +E_j+ (GE)_{ij}+d_{ij}$

Where i= 1,2,3,é n genotypes, j= 1,2,3,é .p environments; P_{ij}=phenotypic value of the i^{th} genotype in the i^{th} environment, μ = population mean; G_i = effect of the i^{th} genotype, E_j= effect of the j^{th} environment, $(GE)_{ij}$ = effect of the interaction of the i^{th} genotype with the j^{th} environment and d_{ij} = random error term.

1.7 Assessment of genetic diversity using molecular markers

Assessment of genetic and phenotypic diversity in popcorn may be based on agronomic and morphological data. The use of molecular markers has been increasingly important in plant breeding in assessing genetic diversity and characteristics of the germplasm, estimating the genetic differences between genotypes, breeding material and population, identifying and fingerprinting the genotype and screening of parents by detecting variation in the DNA sequence (Dandolini *et al.*, 2008; Junior *et al.*, 2011; Munhoz *et al.*, 2009). Molecular markers also distinguish between homo and heterozygous individuals in one population without progeny testing (Jain and Brar, 2009). Molecular markers are the indicators of polymorphism. For example, when the DNA sequence varies between individuals under study, the molecular markers are polymorphic (Srivastava, 2004). Markers also identify the useful gene sequence and detects the Quantitative Trait Loci (QTL) (Junior *et al.*, 2011). Molecular markers are stable and can be detected in all plant tissues, for example, at any growth and development stage. Markers are also independent of environmental, pleiotropic and epistatic effects (Agarwal *et al.*, 2008; Collard and Mackill, 2008; Moose and Mumm, 2008).

1.7.1 Quantitative trait loci (QTLs)

When molecular markers are used in the assessment of genetic diversity in popcorn and other crops, the quantitative trait loci (QTL) in association with the traits under study must be identified (Dhliwayo, 2008). Agronomic performance including yield components and quality are mainly influenced by the quantitative traits. Molecular markers allow the identification and genetic localization of the quantitative traits that contribute to the overall agronomic performance of the varieties (Lorz, 2008). Li *et al.* (2007) evaluated six grain yield components in 220 selected families BC2F2, which were derived from a cross between Dan232 and an elite popcorn inbred. The families were evaluated under two environments using 170 SSR markers; 19 QTLs were found and favorable alleles were detected on 18 QTL. The favorable alleles were contributed by the dent parent (Dan232).

Babu *et al.* (2006) mapped the QTL for popping expansion volume (flake volume-FV and unpopped kernels-UPK) using the SSR markers for the three families. Four QTLs were detected on chromosome 1,3,8,10 and these QTLs indicated 62% of the phenotypic variance. Four QTLs for FV were identified on chromosome 1, 5, 9 and 10 (44% of the phenotypic variance), while QTLs for UPK were detected on chromosome 1, 3, 4, 5 and 9 (57% of the phenotypic variance). Lu *et al.* (2003) also identified four QTLs on chromosomes 1S, 3S, 5S and 5L. These four QTLs indicated 45% of the phenotypic variation for PEV in a cross between popcorn and dent maize cross. The QTLs influencing the three kernel composition traits (starch, proteins and oil concentration) were identified in different maize populations ($F_{2:3}$) and BC_2F_2 derived from a cross between dent and an elite popcorn inbred (Yanyang *et al.*, 2008). Four and two QTLs were detected for starch, four and three for protein (5.0-14.3%), and one for oil (5.2-8.5%) in both populations. The detected QTLs contributed to the diversity in different maize populations. The findings indicate that, QTLs with favourable effects can be selected to improve popping ability in new varieties.

1.7.2 Types of Molecular Markers

There are several markers employed in quantifying genetic diversity among crops. Other markers are also called biochemical markers (Allozymes) (Kumar *et al.*, 2009). Among the available markers are; Simple sequence repeats (SSR) and Single Nucleotide Polymorphism (SNP) which are commonly used in automated systems which reduces costs and have high output laboratories. The SSRs and SNPs are discussed below. The features of other markers that have been used to study genetic diversity in crops are summarized in Table 1.1. (Benchimol *et al.*, 2000; Ibitoye and Akin-Idowu, 2010; Kumar *et al.*, 2009; Schierwater and Ender, 1993; Spooner *et al.*, 2005).

1.7.2.1 Single Nucleotide Polymorphism (SNP)

Sequence polymorphism between individuals can occur in various forms, for example it can result from insertion or deletion of multiple bases or due to single nucleotide polymorphism (SNP) (Srivastava, 2004). SNPs are defined as single base differences in a given DNA at which the sequence variation is present between different individuals within species or population (Lorz, 2008). The SNPs normally occur at different frequencies. However, the frequency at which they occur depends on the genome and species under study. The major advantages of SNPs are multiple detection and abundance in a single copy DNA sequence. The DNA segment containing SNP is amplified by the Polymerase chain reaction, a restriction enzyme is used when the product is incubated; if SNP is available, the enzyme breaks down the molecule to create the cognition site (Lorz, 2008; Srivastava, 2004).

1.7.2.2 Simple Sequence Repeats markers (SSR)

The SSR markers, also referred to as microsatellites, identify variation in a number of short repeat sequences. For example, they detect variation in two or three base repeats, at high frequency, the number of repeats changes allowing the detection of multiple alleles (Lorz, 2008). A number of researchers have used the SSR markers

to assess genetic relationship among popcorn lines. SSR markers are highly polymorphic, reproducible, multiallelic and co-dominant (both homozygous and heterozygous alleles present in one individual are expressed) (Hamon *et al.*, 2003; Spooner *et al.*, 2005). Genetic information provided by the SSR markers in situ is also reliable (Balestre *et al.*, 2008; Dandolini *et al.*, 2008; Trindade *et al.*, 2010). SSR markers can be automated, are easy to use, highly informative and large number of SSR primer pairs is available (Spooner *et al.*, 2005). Genetic diversity in eight popcorn S6 lines using 10 SSR markers was evaluated by Trindade *et al.* (2010). The results indicated a few clusters of the lines in a dendogram obtained, they further indicated that hybrids could be developed by crossing lines from different clusters because the level of genetic diversity was not very low. For studying diversity, the SSRs markers are recommended over the SNPs, therefore, SSR were used in the current study.

Table 1.1:Features and comparisons of other different molecular markers

Markers	**Advantages**	**Disadvantages**
Restriction Fragment Length Polymorphism (RFLP)	-Number of loci unlimited -Co-dominant -Highly reproducible -Good genome coverage -Not species specific -High availability	-Large DNA quantity required -Laborious -Difficult to automate -Low polymorphism -Expensive
Amplified Fragment Length Polymorphism (AFLP)	-High genomic abundance -Highly polymorphic -Automated -Small DNA quantities required	-Not easy to use -Cannot get consistent map -Need very good primers
Randomly Amplified Polymorphic DNA (RAPD)	-High genomic abundance -Good genome coverage -Can be automated -Low DNA quantity required -Quick -Easy to use	-No probe information -Dominant -Low reproducibility -Species specific -Not very well-tested
ISOZYMES	-Useful for evolutionary studies -Not species specific -No radioactive labeling -Need no sequence information	-Labor intensive -Limited in polymorphism -Expensive -Not easily automated
Sequence-Tagged Site (STS)	-No radioactive labeling -Fairly good genome coverage -Highly reproducible -Can use filters many times	-Laborious -Cannot detect mutations -Need sequence information - Require cloning and probe characterization
Inter- Simple Sequence Repeat amplification (ISSR)	-robust in usage -can be automated -highly polymorphic	-dominant -species specific
Sequence characterized amplified region / Cleaved Amplified Polymorphic Sequence (SCARS/CAPS)	-highly reproducible -small DNA quantity required -co-dominant -usually single locus -species-specific	-Labour intenstive -Hard to find

Source: (Ibitoye and Akin-Idowu, 2010; Kumar ***et al.***, 2009; Schierwater and Ender, 1993; Spooner ***et al.***, 2005).

1.8 Heritability

Heritability, defined by Sesardic (2005) as the proportion of phenotypic variation that is due to genetic differences is important in breeding programs. Phenotypic variation or phenotypic value contributes to the overall plant breeding value, therefore, the reliability of phenotypic value is expressed by heritability (Anholt and Mackay, 2010). Heritability measures the extent to which the phenotypic and breeding value corresponds. It also measures quantitative traits, for example, it is mostly used to calculate the expected response to selection in a population (Zavala, 2008). Heritability can be expressed in two ways. Firstly as broad sense heritability ($H^2_B=V_G/V_P$) which is defined as the proportion of phenotypic variability that is due to the overall genetic effect in a population (Bernardo, 2002; Rao and Gu, 2008). Secondly, narrow sense heritability ($H^2_N=V_A/V_P$) which is defined as the proportion of the total phenotypic variance that is associated with the additive effects of genes transmitted from parents to progenies (Bernardo, 2002; Rao and Gu, 2008).

High heritability is normally obtained in crops with a diverse genetic background, therefore, contributes to genetic diversity. Low environmental variation also increases heritability, for example, in traits with high heritability, the differences observed in a population will be the result of genetic factors. However, in the traits with low heritability, differences will be attributed to environmental factors (Rao and Gu, 2008). High heritability has been reported for popping expansion volume. For example, heritability of 78% and 83% were reported by Pereira and Junior (2001) in different popcorn populations. Therefore, popping expansion volume contributes to the presence of high genetic variability in popcorn populations. A 57% and 18% heritability was reported for seed yield, respectively, (Pereira and Junior, 2001). These heritability figures implied that, high genetic gain from selection can be expected for popping ability than yield.

1.9 Popcorn yield and improvement limitations

High yielding popcorn genotypes are desired. However, low yield is a major constraint in popcorn production (Effa *et al.*, 2011). Ziegler (2001) reported that, the reason for low yield in popcorn compared with dent maize is the lack of the number of studies focusing on recurrent selection and hence genetic improvement of popcorn varieties. However, several studies focus primarily on genetic and phenotypic improvement of dent maize. Low yield has been reported in popcorn, for example, popcorn yield is approximately half that of dent maize (Duffy and Calvert, 2010). Developing genotypes with high seed yield is, therefore, important in improving popcorn production.

Lack of adapted varieties with desirable agronomic traits primarily popping expansion volume also limit popcorn improvement (Dhliwayo, 2008; Freitas *et al.*, 2009; Miranda *et al.*, 2003; Rinaldi *et al.*, 2007). Therefore, studies focusing on improving popping ability of popcorn genotypes are also important. Other production constraints include, increased susceptibility to a number of insects and vertebrate pests such as birds. Vertebrate pests affect the growing cobs by opening the cobs and thus increasing its susceptibility to a number of pathogens, which consequently affects grain yield and popping quality (Agele *et al.*, 2008; Amusa *et al.*, 2005; Effa *et al.*, 2011). Diseases such as, maize rust, corn leaf blight, ear or stalk rots and downy mildew also limit popcorn production. These factors can cause a serious reduction in yield, primarily when the infection occurs at the early stages of growth (Agele *et al.*, 2008). Striga weed are another major problem in popcorn production, generally. According to Matz (1991), popcorn grows relatively slower than dent maize and as a result of the small size of the plants at maturity, the crop is susceptible to weed infestation.

1.10 Genotype × environment interaction

Genotype × environment interaction influences genetic improvement in maize. Genotype × Environment (G×E) interaction refers to the ability of genotypes to

perform in various environments (Frankham *et al.*, 2010). A G×E interaction occurs when different populations adapt differently in various environments. For example, their reproductive and survival ability differs, they exhibit strong and better performance in their local environments compared to other environments (Frankham *et al.*, 2010). A G × E interaction in populations is more common when the genetic and environmental differences are large (Frankham *et al.*, 2010). One genotype may exhibit an outstanding performance than the other in one environment and poorly in another environment (Frankham *et al.*, 2010; Hartl and Ruvolo, 2012). Genotypes with greater uniformity are more vulnerable to environmental changes, therefore, genotypes with large genetic diversity, high level of stability and adaptability are desired in plant breeding.

Ahmet and Halil (2011) reported that G x E interaction affected grain yield in 18 popcorn genotypes. Sakin *et al.* (2005) observed that, hybrid genotypes were significantly affected by the environment, for example differences were observed in popping ability and yield. Relatively low yield was recorded in hybrids than open pollinated cultivars when the temperature and rainfall was unfavorable for production. Scapim *et al.* (2010) suggested two strategies which could be used to develop cultivars with low levels of G × E interaction. The first strategy involves selecting cultivars that are stable and adapted and productive in a variety of environments. Selection process for these cultivars can be carried through the use of multivariate, parametric and non-parametric statistical analysis. The second strategy is dividing heterogeneous areas into homogeneous and to develop cultivars that are adapted to those small divisions of homogenous areas.

Scapim *et al.* (2010) evaluated correlations between stability and adaptability statistics of popcorn cultivars. They reported a significant effect of genotypes, environments, and G×E interaction for popping expansion and grain yield. Broccoli and Burak (2004) investigated the effect of genotype x environment interactions in popcorn maize yield and grain quality in fourteen popcorn hybrids. The environment was favourable for yield and hence higher yield was observed, however low popping expansion volume was found in the same environment. Daros *et al.* (2002) found a

significant environmental interaction for popping ability, this indicated the effect of GXE on popping ability. The effect of environment on popping ability was a result of the quantitative inheritance (Daros *et al.*, 2002). It is, therefore, crucial that, popcorn varieties are evaluated for yield in multi-locational environments, and G×E interaction should be considered in ranking genotypes according to both popping ability and seed yield.

1.11 Conclusion and implications for breeding

From the review of literature, it is clear that, there is still a huge gap in popcorn improvement in Sub-Saharan Africa. Popcorn production primarily in South Africa is limited by the unavailability of adapted popcorn varieties and hence, variety options are limited for farmers. Therefore, there is a need for conducting a study that focuses on making improvements in this crop. For example, breeding efforts based on the development of adapted local popcorn varieties are limited. Therefore, development of local varieties, and a study of genetic diversity among them is important. Superior popcorn varieties should be characterized by high yield and mainly better popping ability. A study of the effect of popping methods in determining popping ability is important in deciding on the method that is efficient and effective. The study of relationship among secondary traits and their direct and/or indirect effects on primary traits, grain yield and popping expansion volume will also enhance the breeding progress. However, a literature does not give a clear indication of whether selection large kernels would enhance popping ability or not. The association of popcorn seed yield with secondary traits is also not well documented. From the review of literature, it can be concluded that popping methods have an effect on popping ability of different popcorn genotypes. Popping ability and yield are strongly related, a strong correlation is contributed by genotypes with both high yield and popping ability; there is no evidence for any large genetic variation or diversity in popcorn populations.

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CHAPTER 2

Appraisal of Microwave and Hot Air Popping Methods for Rapid Screening of Popcorn Inbred Lines

Abstract

Popcorns are very important food and snacks in Sub-Saharan Africa (SSA). However, adequate production is hampered by limited variety options for farmers. The development of popcorn varieties requires effective and efficient methods for discriminating genotypes according to popping ability. The study was conducted to evaluate the effect of popping methods on popping expansion volume (PEV) of different popcorn inbred lines and genotype × popping method interaction. Two popping methods were employed in the study; these were the microwave oven popping (MWP) and hot air popping method (HAP). Under both methods the kernels were popped for 2 minutes. Popping expansion volume and the number of unpopped kernels differed significantly ($P<0.001$) between the two methods. The highest popping expansion volume and less number of unpopped kernels were obtained from the hot air popping. The average popping expansion volume was 862 cm^3 and 726 cm^3 for hot air and microwave popping method, respectively, while 98 unpopped kernels were obtained under hot air popping and 115 under microwave. However, the popping ability of some of the inbred lines was slightly consistent in both methods. The Spearmanôs rank correlation coefficient ($r=0.54$) and the coefficient of determination (28%) between popping methods indicated that, in general the two methods would rank genotypes differently. Genotype × method interaction was significant for both PEV and number of unpopped kernels. Hot air popping was a more efficient method with a greater discriminating power (CV =15%) than the microwave (CV=34%) popping method. Hence, HAP is highly recommended rather than the MWP for breeders to use when conducting their selections. The presence of genotype × popping method interaction resulted in the formation of three groups; genotypes suitable for both methods, those suitable for microwave popping and those suitable for hot air popping method. These results have implications for both breeding and processing of popcorn at the household level.

Keywords: Popcorn, Popping method, Popping expansion volume, Genotype × method interaction

2.1 Introduction

Popcorn is a nutritious snack food that is used for human consumption and, therefore, requires genetic improvements. The main characteristic of popcorn that distinguishes it from other maize grains is that it expands (pops) when heated at atmospheric pressure (8.1×10^5 pa) (Soylu and Tekkanat, 2007). Its ability to pop is associated with the physical structure of the kernel, starch and endosperm microscopic structure (Matz, 1991; Soylu and Tekkanat, 2007). Popularity of popcorn has been steadily increasing over the years in Sub-Saharan Africa (SSA). However, almost all the popcorn consumed is not from the locally produced varieties. Inadequate production of popcorn in SSA results from the limited number of varieties available to the farmers. The availability of popcorn varieties is required to minimize the amount imported. However, the available varieties must possess high popping expansion volume as the primary determinant of popcorn quality. Hence, effective and efficient methods for rapid screening of varieties for popping ability are required.

Popping expansion volume (PEV) and the number of unpopped kernels (UPKs) are the primary traits that measure quality in popcorn (Borras *et al.*, 2006; Song *et al.*, 1991; Soylu and Tekkanat, 2007; Sweley *et al.*, 2012a). Popping expansion volume is defined as the volume of popped corn per gram of unpopped corn (Dofing *et al.*, 1990). The kernels are sold on weight basis, however, the final product (popped kernels) is sold by volume basis. Therefore, popcorn kernels ability to pop is a fundamental determinant of its quality (Goneli *et al.*, 2007). Popcorn consumers prefer popcorn with high PEV, fluffy and tender kernels. Kernels with high PEV are generally more palatable and have greater commercial value (Allred-Coyle *et al.*, 2000). Therefore, efficient methods that can discriminate genotypes according to PEV are crucial.

The primary objective of popcorn producers is to maximize PEV and to minimize the number of UPKs (Quinn Sr *et al.*, 2005). Unpopped kernels were defined by Song *et al.* (1991) and Singh *et al.* (1997) as kernels that fail to pop when subjected to popping test and can pass through a 7.14 mm square hole.

Popcornsôability to pop can be affected by several factors such as kernel sphericity, hybrid variety, storage time, 1000 kernel weight, endosperm type, pericarp thickness, test weight and physical properties of the kernel including size, density and shape, (Soylu and Tekkanat, 2007). Structural damage, popping temperature, slurry formulation, type of fatty acid inside the kernel, level of zein proteins, harvesting and handling practices and moisture content can also affect popping ability of popcorn (Allred-Coyle *et al.*, 2000; Borras *et al.*, 2006; Gokmen, 2004; Karababa, 2006; Lin and Anantheswaran, 1988; Mohamed *et al.*, 1993; Singh *et al.*, 1997; Soylu and Tekkanat, 2007). The effect of popping methods on popping ability has also been reported (Dofing *et al.*, 1990; Gokmen, 2004).

There are several distinct popping methods used in screening popcorn varieties. The methods used include microwave and conventional popping method. Conventional methods include cooking pan with some oil added, and these methods are commonly used than the microwave popping method (Gokmen, 2004; Quinn Sr *et al.*, 2005). Microwave popping method is associated with a number of constraints limiting maximum popping ability. For example low popping ability, scorching of the popped kernels and a large number of unpoppped kernels (Ceylan and Karababa, 2004; Gokmen, 2004; Singh and Singh, 1999). Different popcorn genotypes may show differences in the ability to pop in different methods (genotype × popping method interaction). For example, one method can give high popping expansion volume and less number of unpopped kernels compared to the other method, even, for the same genotype (Dofing *et al.*, 1990; Gokmen, 2004).

When choosing the popping method, it is important to consider all the factors that could probably interfere with popping during popping test. For example, distribution of heat among kernels (Hoseney *et al.*, 1983). The objectives of the study were, therefore, to evaluate the effect of two different popping methods on popping expansion volume of different popcorn genotypes, and to investigate the existence of genotype × popping method interaction for popping ability.

2.2 Materials and methods

2.2.1 Experimental site

The study was conducted in South Africa at Ukulinga Research Farm of the University of KwaZulu - Natal, Pietermaritzburg (Latitude 29.67ºS; Longitude 30.41ºE; 812 m.a.s.l.) and Cedara (KwaZulu - Natal Department of Agriculture, Latitude 29.54ºS; Longitude 30.26ºE; 1066 m.a.s.l.) during the period of December 2011 – April 2012.

2.2.2 Experimental material

Two populations of popcorn inbred lines were used in the study. The first population, designated "Population 1" was the advanced and fixed population of 83 inbred lines and ten checks (controls). The controls were, positive (popping) controls (P618: commercial hybrid, CHECK1, CHECK 2, CHECK3, CHECK4, CHECK5, CHECK6 and CHECK7: 100% dent), and negative (non-popping) controls (P1* and 8CED6-7). The second population designated "Population 2" of 81 inbred lines (F5 generation) originated from a nursery plot from Makhathini Research station and were derived from F2 segregations of a flint x popcorn population. The bi-parental population was a cross between a flint maize line P1 and an F3 popcorn inbred bulk population.

2.2.3 Experimental design

In Population 1, the experiment was laid out as an augmented alpha lattice design, with 9 blocks × 12 plots, and 3 major controls; P618 from Capstone Seeds, P1 and 8CED6-7 (both from University of KwaZulu-Natal Breeding programme), where each control was replicated 9 times. Population 2 was grown in a nursery observation plot at Ukulinga Farm. The trial was also laid out as an augmented alpha lattice design with 9 blocks and 9 plots and without replicates.

2.2.4 Management practices

Inbred lines in Population1 were planted in a 4 m row plot with 0.8 m × 0.3 m spacing. Inbred lines in Population 2 were planted in 3m row plot with 0.9 m × 0.3 m spacing. Both locations were similar with regard to management practices. Sowing was done after land preparation where 2 seeds/ hole were dipped by hand. A 250kg basal fertilizer (NPK, 2:3:4) was applied before planting. The proportion of N, P, and K was 55kg, 83 kg and 111kg, respectively. Limestone Ammonium Nitrate (LAN) containing 28% N was applied as top dressing four weeks after planting. Both locations were rainfed, however, supplementary irrigation was applied at Ukulinga Farm. Weeds were controlled by hand weeding and by the use of herbicides. Herbicides used were Gramoxone, Troopers and Basagran. The plots were harvested manually after physiological maturity.

2.2.5 Sample preparation

A 50 cm^3 kernel sample for each genotype was weighed using a 100 mL measuring cylinder. Two replicates of 50 cm^3 kernel sample were evaluated for popping ability under both methods. Before popping, grain moisture percentage of the inbred lines was measured using a grain moisture metre. Kernel size was also measured for all genotypes before popping. Kernel size was determined by counting the number of kernels per 10 g sample. Kernel size was thereafter estimated by grouping kernels into classes as follows; 76-105, small; 68ï 75, medium and 52ï 67, large (Ziegler *et al.*, 1984).

2.2.6 Popping methods

Popping ability was evaluated using two different popping methods; microwave popping (MWP) and hot air popping (HAP) method. For each genotype and popping method, the experiment was replicated twice in the laboratory.

2.2.6.1 Microwave popping

Two microwave ovens of the same model (DMO: 351 metallic with 900W power) were used in the study. The sample was placed inside a brown paper S.O.8 bag (dimensions = 165 x 102 x 301, product code = 6410534). The bag was folded to allow the steam to be released through the small space left on top of the bag. Popcorn sample was placed at the centre of the microwave and was allowed to pop for two minutes. The optimum popping time for the microwave method was evaluated on commercial popcorn (variety not known) at different time intervals (1-5 minutes). The total popping expansion volume (cm^3) was measured in a 2000 mL plastic graduated cylinder which was inverted once to allow uniform distribution of the kernels inside the cylinder. The unpopped kernels were counted and recorded. Flake quality/flake aspect was determined after popping; the aspect was rated from 1(good) to 5(poor).

2.2.6.2 Hot air popping

Hot air popping test was carried out using a 900W Salton-popcorn maker (White-SPC 900). The base of the hot air popcorn maker was fed with 50 cm^3 popcorn sample. A heat proof bowl was placed under the popping chute to collect the popped kernels and prevent them from flying off. The kernels were allowed to pop for two minutes (standard time for the hot air popping machine as recommended by the manufacturer), after which the machine was switched off. After popping three samples, respectively, the machine was allowed to rest for 15 minutes according to the user guide, to avoid overheating.

2.2.7 Statistical analysis

General analyses of variance were performed for all quantitative data using GenStat 14^{th} edition. Experimental data were subjected to Linear Mixed Models (REML). The model used was:

$P_{ijk}=\mu+E_i+r_j+E_{ijk}$, where μ was the overall population mean, E_i=Entry effect, r_j= replicate and E_{ijk} = random experimental error term.

2.3 Results

2.3.1 Popping time and popping ability

Popping expansion volume differed significantly at different time intervals (P<0.001). Popping expansion volume increased from 1 ï 4 minutes and decreased at 5 minutes. The highest popping expansion volume was obtained at 4 minutes (Figure 2.1).

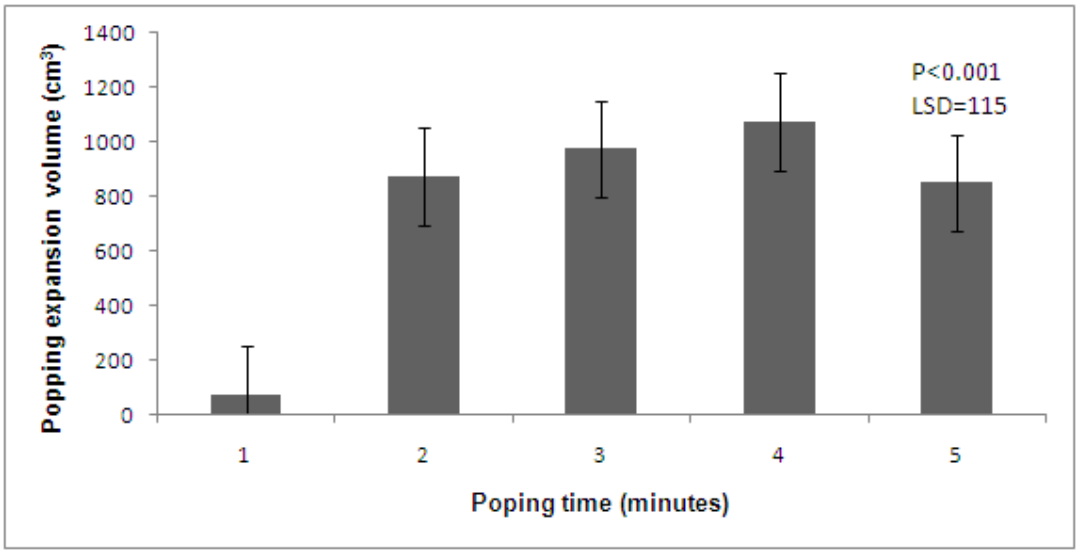

Figure 2.1: Popping expansion volume (cm^3) for commercial varieties at five different time intervals using microwave popping method

The number of unpopped kernels differed significantly (P<0.001) at different time intervals. There was a decrease in the number of unpopped with an increase in popping time from 1-4 minutes; however, a slight increase was observed at five minutes (Figure 2.2).

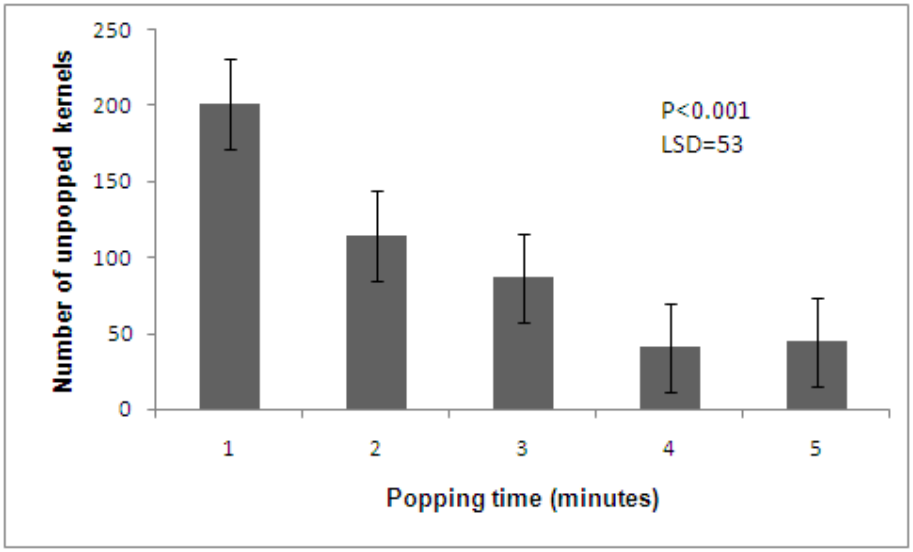

Figure 2.2: Number of unpopped kernels for commercial varieties at five different time intervals using microwave popping method

2.3.2 Popping Methods

Popping methods differed significantly (P<0.001) for popping expansion volume. Large popping expansion volume was obtained from the hot air popping method than the microwave method (Figure 2.3).

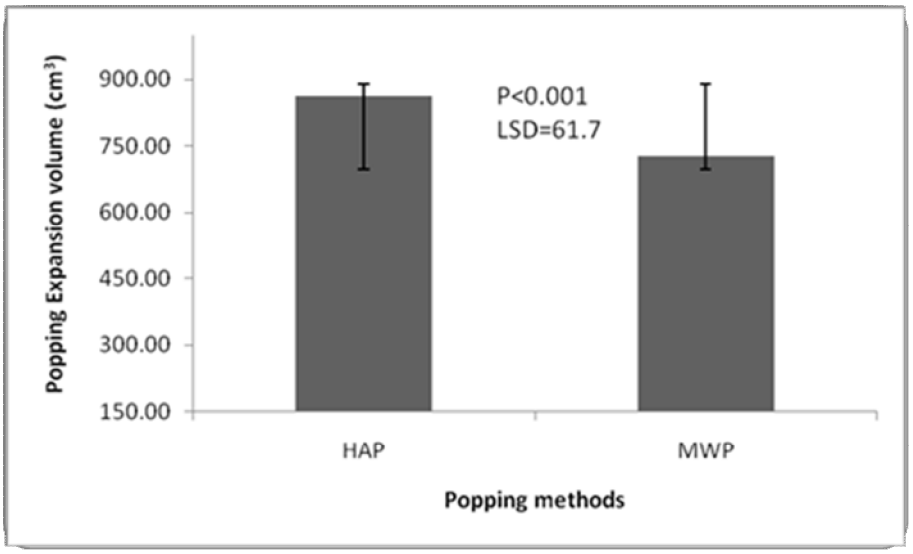

Figure 2.3: Popping expansion volume (cm^3) under hot air and microwave popping method

Popping methods differed significantly (P<0.001) for the number of unpopped kernels. A fewer number of unpopped kernels were obtained from hot air popping method than the microwave popping method (Figure 2.4).

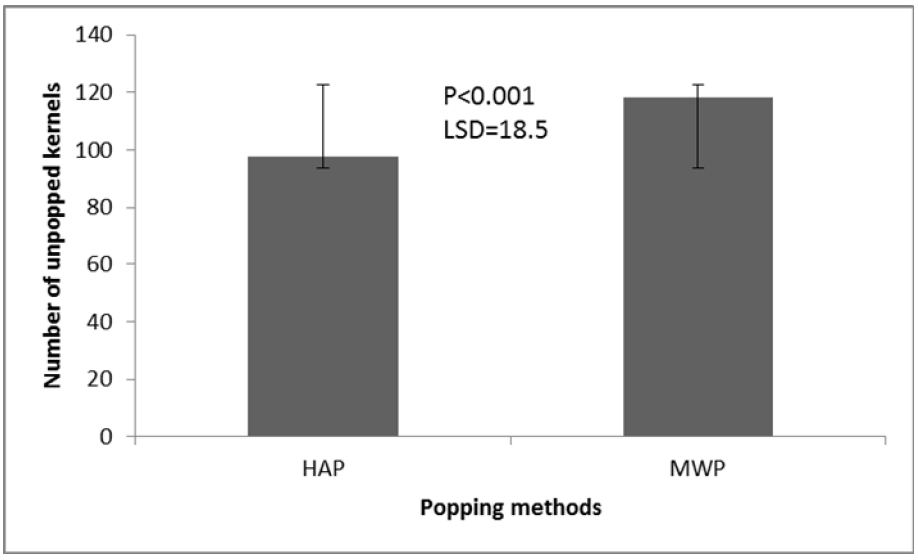

Figure 2.4: Number of unpopped kernels under hot air and microwave popping method

2.3.3 Genotype x popping method interaction

Genotype × popping method interaction was significant for popping expansion volume (P<0.001) (Table 2.1).

Table 2.1: Analysis of variance for popping expansion volume (cm^3)

Source of variation	d.f	(m.v.)	m.s	v.r
REP stratum	2		. 20892.	1.64
Genotype	**92**		**499372.****	**39.18**
Method	**1**		**1437472.****	**112.77**
Genotype. Method	**90**	**(2)**	**70767.****	**5.55**
Residual	443	(17)	12747.	
Total	628	(19)		

*** significant at P< 0.001*

Genotype ×popping method was significant (P< 0.001) for the number of unpopped kernels (Table 2.2).

Table 2.2: Analysis of variance for the number of unpopped kernels

Source of variation	d.f.	(m.v.)	m.s.	v.r. .
REP stratum	2		5157.	4.21
Genotype	**92**		**17091.****	**13.95**
Method	**1**		**28318.****	**23.11**
Genotype. Method	**90**	**(2)**	**7640.****	**6.24**
Residual	443	(17)	1225.	
Total	628	(19)		

*** significant at P< 0.001*

Popping expansion volume and the number of unpopped kernels for the check varieties were different. The negative checks showed poor popping performance than the positive checks (Table 2.3).

Table 2.3: Popping expansion volume (cm^3) and percentage of unpopped kernels for the check genotypes

Controls	Popping expansion volume (cm^3)		Number of unpopped kernels	
	HAP	MWP	HAP	MWP
Positive control				
CHECK1	961.5	1116.7	95	61
CHECK2	1061.5	666.7	72	108
CHECK3	974	333.3	107	215
CHECK4	911.5	716.7	106	144
CHECK5	776.7	733.3	156	157
CHECK6	861.5	316.7	128	238
CHECK7	911.5	800	102	160
P618 (hybrid)	1203	1433.3	51	32
Mean	**957.65**	**764.5875**	**102**	**139**
Negative Control				
P1 (flint)	266.7	233.3	54	75
08CED6-7 (dent)	116.7	100	86	101
Mean	**191.7**	**166.65**	**70**	**88**
CV	**15.98**	**34.29**	**23**	**61**

HAP: Hot air popping method; MWP: Microwave popping method

Some genotypes performed relatively better under both methods with regard to PEV (Figure 2.5).

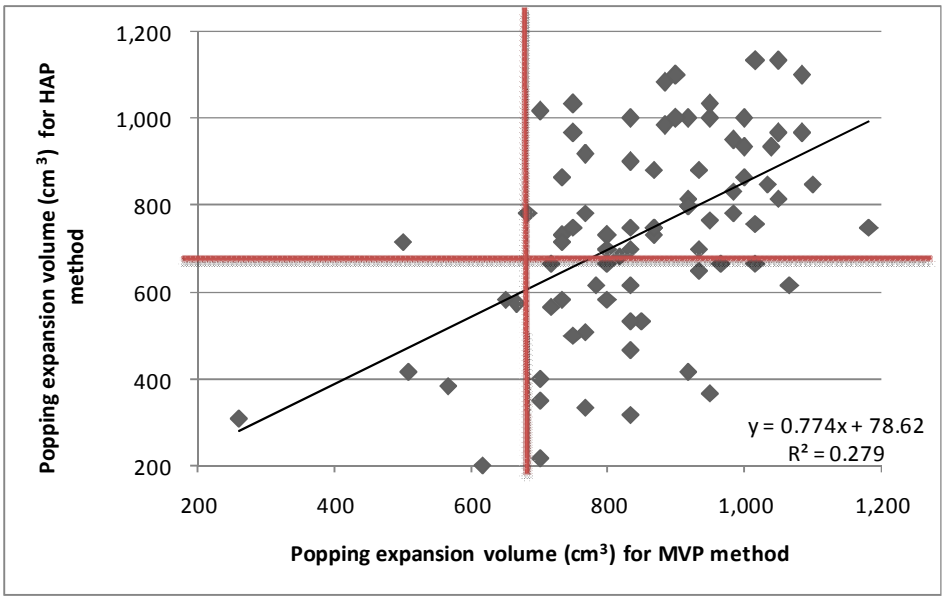

Figure 2.5: Scatter plot of popping expansion volume for different genotypes under Hot air and microwave popping method.

The inbred lines were ranked by popping expansion volume based on hot air popping method. The top 28 inbred lines showed a higher popping ability (Table 2.4) than the middle (Table 2.5), and the bottom inbred lines (Table 2.6). When genotypes were ranked by microwave popping method, they ranked differently (Table 2.7-Table 2.9).

Table 2.4: Top 28 inbred lines ranked by popping expansion volume (cm^3) and their relative means (trial mean, hybrid mean, mean of positive and negative checks and hybrid mean) for hot air popping method

Rank	Entry	HAP	MVP	PEV change	%Trial mean	%Mean Positive Check	%Mean Negative Check	%Mean hybrid
1	83	1186.5	750	436.5	138	129	619	99
2	41	1099	1100	-1	127	119	573	91
3	64	1074	966.7	107.3	125	116	560	89
4	55	1074	850	224	125	116	560	89
5	13	1049	833.3	215.7	122	114	547	87
6	75	1036.5	966.7	69.8	120	112	541	86
7	61	1036.5	816.7	219.8	120	112	541	86
8	51	1036.5	616.7	419.8	120	112	541	86
9	21	1011.5	933.3	78.2	117	110	528	84
10	82	1011.5	866.7	144.8	117	110	528	84
11	49	1011.5	747.8	263.7	117	110	528	84
12	42	999	766.7	232.3	116	108	521	83
13	71	999	666.7	332.3	116	108	521	83
14	53	991.5	933.3	58.2	115	107	517	82
15	62	986.5	1000	-13.5	114	107	515	82
16	20	986.5	850	136.5	114	107	515	82
17	39	986.5	650	336.5	114	107	515	82
18	22	983.3	783.3	200	114	107	513	82
19	72	961.5	1133.3	-171.8	112	104	502	80
20	1	961.5	1100	-138.5	112	104	502	80
21	80	961.5	1033.3	-71.8	112	104	502	80
22	74	961.5	950	11.5	112	104	502	80
23	18	961.5	416.7	544.8	112	104	502	80
24	73	961.5	366.7	594.8	112	104	502	80
25	65	949	883.3	65.7	110	103	495	79
26	23	933.3	700	233.3	108	101	487	78
27	48	924	816.7	107.3	107	100	482	77
28	17	924	733.3	190.7	107	100	482	77
Mean		**862.3**	**726.3**					

Table 2.5: Middle 27 inbred lines ranked by popping expansion volume (cm^3) and their relative means (trial mean, mean of positive and negative checks and hybrid mean) for hot air popping method

Rank	Entry	HAP	MVP	PEV change	%Trial mean	%Mean Positive Checks	%Mean Negative Checks	%Mean hybrid
1	2	916.7	1000	-83.3	106	99	478	76
2	63	911.5	900	11.5	106	99	475	76
3	6	911.5	883.3	28.2	106	99	475	76
4	58	911.5	800	111.5	106	99	475	76
5	77	899	1133.3	-234.3	104	97	469	75
6	15	899	1000	-101	104	97	469	75
7	59	899	1000	-101	104	97	469	75
8	68	899	800	99	104	97	469	75
9	28	899	316.7	582.3	104	97	469	75
10	5	886.5	800	86.5	103	96	462	74
11	79	874	1083.3	-209.3	101	95	456	73
12	70	874	750	124	101	95	456	73
13	81	874	750	124	101	95	456	73
14	67	861.5	1000	-138.5	100	93	449	72
15	47	861.5	983.3	-121.8	100	93	449	72
16	78	861.5	583.3	278.2	100	93	449	72
17	12	849	966.7	-117.7	98	92	443	71
18	38	849	866.7	-17.7	98	92	443	71
19	16	849	750	99	98	92	443	71
20	46	849	533.3	315.7	98	92	443	71
21	27	836.5	666.7	169.8	97	91	436	70
22	66	824	916.7	-92.7	96	89	430	68
23	50	824	533.3	290.7	96	89	430	68
24	52	824	466.7	357.3	96	89	430	68
25	56	824	333.3	490.7	96	89	430	68
26	40	811.5	1016.7	-205.2	94	88	423	67
27	33	811.5	750	61.5	94	88	423	67
Mean		**862.3**	**726.3**					

Table 2. 6: Bottom 28 inbred lines ranked by popping expansion volume (cm^3) and their relative means (trial mean, mean of positive and negative checks and hybrid mean) for hot air popping method

Rank	Entry	HAP	MVP	PEV change	%Trial mean	%Mean Positive Checks	%Mean Negative Checks	%Mean hybrid
1	9	811.5	666.7	144.8	94	88	423	67
2	7	811.5	616.7	194.8	94	88	423	67
3	54	800	700	100	93	87	417	67
4	35	799	750	49	93	87	417	66
5	24	799	616.7	182.3	93	87	417	66
6	34	786.5	1033.3	-246.8	91	85	410	65
7	10	786.5	783.3	3.2	91	85	410	65
8	8	786.5	726.9	59.6	91	85	410	65
9	3	786.5	700	86.5	91	85	410	65
10	44	786.5	583.3	203.2	91	85	410	65
11	25	774	666.7	107.3	90	84	404	64
12	57	761.5	683.3	78.2	88	83	397	63
13	11	761.5	216.7	544.8	88	83	397	63
14	45	749	500	249	87	81	391	62
15	32	749	497.8	251.2	87	81	391	62
16	60	733.3	716.7	16.6	85	79	383	61
17	36	711.5	726.9	-15.4	83	77	371	59
18	29	700	350	350	81	76	365	58
19	26	674	716.7	-42.7	78	73	352	56
20	30	674	583.3	90.7	78	73	352	56
21	31	674	566.7	107.3	78	73	352	56
22	43	674	400	274	78	73	352	56
23	69	674	383.3	290.7	78	73	352	56
24	19	674	200	474	78	73	352	56
25	37	666.7		666.7	77	72	348	55
26	76	636.5	783.3	-146.8	74	69	332	53
27	4	621.2		621.2	72	67	324	52
28	14	260	297.8	-37.8	30	28	136	22
Mean		**862.3**	**726.3**					

Table 2. 7: Top 28 inbred lines ranked by popping expansion volume (cm^3) and their relative means (trial mean, mean of positive and negative checks and hybrid mean) for microwave popping method

Rank	Entry	HAP	MVP	PEV change	%Trial mean	%Mean Positive Checks	%Mean Negative Checks	%Mean Hybrid
1	72	961.5	1133.3	-171.8	156	169	680	79
2	77	899	1133.3	-234.3	156	169	680	79
3	41	1099	1100	-1	151	164	660	77
4	1	961.5	1100	-138.5	151	164	660	77
5	79	874	1083.3	-209.3	149	162	650	76
6	80	961.5	1033.3	-71.8	142	154	620	72
7	34	786.5	1033.3	-246.8	142	154	620	72
8	40	811.5	1016.7	-205.2	140	152	610	71
9	62	986.5	1000	-13.5	138	149	600	70
10	2	916.7	1000	-83.3	138	149	600	70
11	15	899	1000	-101	138	149	600	70
12	59	899	1000	-101	138	149	600	70
13	67	861.5	1000	-138.5	138	149	600	70
14	47	861.5	983.3	-121.8	135	147	590	69
15	64	1074	966.7	107.3	133	144	580	67
16	75	1036.5	966.7	69.8	133	144	580	67
17	12	849	966.7	-117.7	133	144	580	67
18	74	961.5	950	11.5	131	142	570	66
19	21	1011.5	933.3	78.2	129	139	560	65
20	53	991.5	933.3	58.2	129	139	560	65
21	66	824	916.7	-92.7	126	137	550	64
22	63	911.5	900	11.5	124	135	540	63
23	65	949	883.3	65.7	122	132	530	62
24	6	911.5	883.3	28.2	122	132	530	62
25	82	1011.5	866.7	144.8	119	130	520	60
26	38	849	866.7	-17.7	119	130	520	60
27	55	1074	850	224	117	127	510	59
28	20	986.5	850	136.5	117	127	510	59
Mean		**862.3**	**726.3**					

Table 2. 8: Middle 27 inbred lines ranked by popping expansion volume (cm^3) and their relative means (trial mean, mean of positive and negative checks and hybrid mean) for microwave popping method

Rank	Entry	HAP	MVP	PEV change	%Trial mean	%Mean Positive Checks	%Mean Negative Checks	%Mea Hybrid
1	13	1049	833.3	215.7	115	125	500	58
2	61	1036.5	816.7	219.8	112	122	490	57
3	48	924	816.7	107.3	112	122	490	57
4	58	911.5	800	111.5	110	120	480	56
5	68	899	800	99	110	120	480	56
6	5	886.5	800	86.5	110	120	480	56
7	22	983.3	783.3	200	108	117	470	55
8	10	786.5	783.3	3.2	108	117	470	55
9	76	636.5	783.3	-146.8	108	117	470	55
10	42	999	766.7	232.3	106	115	460	53
11	83	1186.5	750	436.5	103	112	450	52
12	70	874	750	124	103	112	450	52
13	81	874	750	124	103	112	450	52
14	16	849	750	99	103	112	450	52
15	33	811.5	750	61.5	103	112	450	52
16	35	799	750	49	103	112	450	52
17	49	1011.5	747.8	263.7	103	112	449	52
18	17	924	733.3	190.7	101	110	440	51
19	8	786.5	726.9	59.6	100	109	436	51
20	36	711.5	726.9	-15.4	100	109	436	51
21	60	733.3	716.7	16.6	99	107	430	50
22	26	674	716.7	-42.7	99	107	430	50
23	23	933.3	700	233.3	96	105	420	49
24	54	800	700	100	96	105	420	49
25	3	786.5	700	86.5	96	105	420	49
26	57	761.5	683.3	78.2	94	102	410	48
27	71	999	666.7	332.3	92	100	400	47
Mean		**862.3**	**726.3**					

Table 2.9: Bottom 28 inbred lines ranked by popping expansion volume (cm^3) and their relative means (trial mean, mean of positive and negative checks and hybrid mean) for microwave popping method

Rank	Entry	HAP	MVP	PEV change	%Trial mean	%Mean Positive Checks	%Mean Negative Checks	%Mean Hybrid
1	27	836.5	666.7	169.8	92	100	400	47
2	9	811.5	666.7	144.8	92	100	400	47
3	25	774	666.7	107.3	92	100	400	47
4	39	986.5	650	336.5	89	97	390	45
5	51	1036.5	616.7	419.8	85	92	370	43
6	7	811.5	616.7	194.8	85	92	370	43
7	24	799	616.7	182.3	85	92	370	43
8	78	861.5	583.3	278.2	80	87	350	41
9	44	786.5	583.3	203.2	80	87	350	41
10	30	674	583.3	90.7	80	87	350	41
11	31	674	566.7	107.3	78	85	340	40
12	46	849	533.3	315.7	73	80	320	37
13	50	824	533.3	290.7	73	80	320	37
14	45	749	500	249	69	75	300	35
15	32	749	497.8	251.2	69	74	299	35
16	52	824	466.7	357.3	64	70	280	33
17	18	961.5	416.7	544.8	57	62	250	29
18	43	674	400	274	55	60	240	28
19	69	674	383.3	290.7	53	57	230	27
20	73	961.5	366.7	594.8	50	55	220	26
21	29	700	350	350	48	52	210	24
22	56	824	333.3	490.7	46	50	200	23
23	28	899	316.7	582.3	44	47	190	22
24	14	260	297.8	-37.8	41	45	179	21
25	11	761.5	216.7	544.8	30	32	130	15
26	19	674	200	474	28	30	120	14
27	37	666.7		666.7	0	0	0	0
28	4	621.2		621.2	0	0	0	0
Mean		**862.3**	**726.3**					

Hot air popping method gave a lower coefficient of variation (CV=15.98%; CV=23%) than microwave popping method (CV=34.29%; CV=61%) for popping expansion volume and number of unpopped kernels, respectively. The Spearman rank correlation coefficient were 0.54 for popping expansion volume and 0.28 for number of unpopped kernels. These coefficients were significant ($P<0.001$) for both popping expansion volume and number of unpopped kernels.

2.4 Discussion

2.4.1 Popping time and popping ability

Differences were observed in popping expansion volume and number of unpopped kernels at different time intervals. When microwave popping method was used, popping expansion volume increased with an increase in time while, the number of unpopped kernels decreased. The highest popping expansion volume was obtained at 4 minutes, this time also recorded the least number of unpopped kernels. However, with regard to flake quality (FA), most of the kernels were burnt and hence the burnt kernels were not acceptable. The popping capacity of the kernel depends on the microwave power used during microwave popping test and the kernelsô ability to absorb power (Hoseney *et al.*, 1983; Singh and Singh, 1999). Popping time is estimated based on the microwave power used (unpublished data), for example at 800 W kernels are popped from 2-3 minutes, and at 600 W they are popped from 3-4 minutes. Therefore, the best quality of the popped kernels was estimated at two minutes because the power of the microwave oven used was 900 W. Low expansion volume at one minute interval was likely to be associated with low microwave temperature. Sweley *et al.* (2012b) suggested that popping capacity is affected by microwave temperature; if the required temperature is not attained, kernels may fail to pop. This suggests that, the power of the microwave used is crucial when determining popping ability of the genotypes.

2.4.2 Popping methods and popping ability

Popping expansion volume and the number of unpopped kernels were significantly affected by popping methods ($P<0.001$). Hot air popping gave a significantly higher popping expansion volume and less number of unpopped kernels than microwave popping method. Similar observations have been reported by several authors who also observed high popping expansion volume and low number of unpopped kernels under conventional methods than in the microwave popping method (Ceylan and

Karababa, 2004; Gokmen, 2004). Dofing *et al.* (1990) reported 10 times lower unpopped kernels under conventional methods than in microwave popping method.

According to the popping mechanism described by Hoseney *et al.* (1983), the pericarp and the outer layers of the kernel act as a pressure vessel which encloses the contents of the kernels such as starch and water. During heating; moisture inside the kernel converts to superheated steam that builds temperature and pressure until it can overcome the combined force of the pericarp and atmosphere and the kernels begin to pop. Individual kernels have a distinct ability to pop, for example each kernel has its different interior critical vapor pressure and power absorbance. Low microwave power is likely to result in low popping expansion volume and more number of unpopped kernels. The effect can be accelerated by variation in the critical vapor pressure of individual kernels. Therefore, the popping ability depends on the microwave power used and the kernels ability to absorb power during the microwave popping test (Hoseney *et al.*, 1983; Singh and Singh, 1999).

Popping temperature is another critical factor affecting popping ability during microwave popping test. Sweley *et al.* (2012b) collected the unpopped kernels from microwave oven and re-popped them; the kernels were able to pop when heated for the second time and the total percentage of unpopped kernels was reduced. This explains that, kernels from the same sample do not all pop at once when subjected to microwave heating (Byrd and Perona, 2005). Sweley *et al.* (2012b) further explained that; failure of the kernels to pop is not always an inherited factor, however, kernels do not all reach the minimum thermodynamic required for popping. Each individual kernel may have its different threshold popping temperature even though the kernels may be subjected to the same temperature.

Gokmen (2004) reported that a large number of unpopped kernels in the microwave method could be attributed to the fact that, popped kernels reduce the intensity of the electromagnetic waves to reach unpopped kernels at the bottom of the microwave.

Sweley *et al.* (2012b) further explained that, the number of unpopped kernels within the popping bag during microwave popping are those kernels that are positioned along the side of the bag. These kernels are shielded by popped kernels and hence they fail to absorb reflective energy and, therefore, sufficient temperature required for maximum popping is not attained. The temperature within the microwave during may also exceed the optimum level above which kernels fail to pop, hence low popping expansion volume and high unpopped kernels results.

Low popping expansion volume observed from the microwave popping method could be attributed to the bag capacity, for example, the amount of space available for kernels distribution within the bags interfere with popping, small space significantly result in low PEV. Allred-Coyle *et al.* (2001) explained that popping bags with a small capacity allow more steam to escape as they hastily open and hence low PEV results. Therefore, there are several factors that are likely to interfere with popping ability which must be considered during microwave test.

2.4.3 Genotype × popping method interaction

Differences in popping expansion volume under different popping methods were observed in different genotypes. However the popping expansion volume of some genotypes was consistent in both methods. For example, the popping expansion volume of 11MAK2-72, 11MAK2-41, 11MAK2-77, 11MAK2-1 and 11MAK2-62 and some of the genotypes was slightly affected by popping method. The average performance of some varieties was superior to the positive checks. For example, 11MAK-41, 11MAK-72, and 11MAK-77 performed above all the popping checks under both methods with regard to popping ability suggesting that, the performance of other genotypes is reliable regardless of the method used. However, when genotypes were compared with the hybrid check variety, none of them showed a relative percentage of hybrid check greater or equal to 100. Therefore, performance of the hybrid check variety was above that of the tested inbred lines under both methods. This was expected and is explained by hybrid vigor.

Genotype × method interaction was highly significant for both popping expansion volume and the number of unpopped kernels (P<0.001). Inbred line 11MAK2-83, 55, 64, 13 and other inbred lines showed a high popping ability under hot air popping method than microwave. However, some inbred lines including 11MAK2-41, 80, 2 and 6 performed better under MWP than HAP. Similar findings were reported by Gokmen (2004) and Ziegler *et al.* (1984) who observed variation in popping ability of different genotypes at different popping methods, suggesting that the effect of popping method on popping ability is also influenced by the genotypesôability to pop at a given popping method. Dofing *et al.* (1990) investigated genotype × popping method interaction for expansion volume in popcorn, and reported that, genotype × popping method interaction was significant for popping expansion volume and unpopped kernels.

The presence of genotype × popping method interaction was further explained by cross over interaction, for example, when genotypes were ranked by hot air popping method, genotype 83, 41, 55, 64 and 13 were the top five genotypes respectively. When ranking was based on the microwave popping method, genotypes ranked differently, the top 5 genotypes were 72, 77, 41, 1 and 79. A similar trend was observed for unpopped kernels. Dofing *et al.* (1990) also observed a switch in rank of genotypes. Popping expansion volume for genotype R20-60 was higher than M8386 and P410 in conventional methods, but popping ability of R20-60 was lower compared to M8386 and P410 when ranked under microwave method. These observations suggested that some genotypes are specifically adapted to microwave oven popping method, some are specific to hot air popping method and other genotypes are adapted to both methods. Therefore, when breeders evaluate genotypes for popping ability they should evaluate popping performance under both conventional and microwave popping methods. At the end, when new varieties are released, the information regarding the best method for popping should be disseminated to the end users.

2.5 Conclusion and implications

Hot air popping method is an effective and efficient method for determining popping ability. Therefore, industrial hot air poppers should be developed by the manufacturers to be used by breeders, as they work with large samples and require a quick method for screening purposes. Microwave popping is not an efficient and effective method for determining popping expansion volume as a result of greater variation for popping expansion volume and the number of unpopped kernels. The ability of the kernels to yield high popping expansion volume and fewer numbers of unpopped kernels depends on the genotype and the method used. Three categories of genotypes were identified; specific genotype suitable for microwave oven method, specific genotype suitable for hot air popping method and genotype suitable for both methods. This kind of information would be crucial for both popcorn breeders as well as popcorn end users. Importantly, the popping methods which are used by consumers are recommended for use in rapid screening of popcorns.

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CHAPTER 3

Correlations and Path Coefficient Analysis for Seed Yield, Popping Expansion Volume and Secondary Traits in Popcorn Inbred Lines

Abstract

Correlation and path analysis are important for studying the relationship between secondary traits with popping expansion volume (PEV) and seed yield (SY), and partitioning direct and indirect effects of each secondary trait on SY and PEV. Unfortunately, not much work has been done in South Africa in this regard. The objectives of the study were to establish the relationship between PEV and SY, and also with secondary traits. Two popcorn populations comprising 83 and 81 inbred lines were evaluated at two sites, following the standard cultural practices for maize. The traits were subjected to correlations and path coefficient analysis in SAS. Seed yield and PEV were positive and significantly correlated. However, the association between SY and PEV was weak (R^2=18%). The PEV was weakly associated with several traits but negative and significantly correlated with flake aspect (FA) and number of unpopped kernels (UPKs). The association among many secondary traits was small. The model for path analysis was significant in both populations for the correlation of secondary traits with PEV (R^2 = 41% and 49%; P<0.01) and SY (R^2 = 81%; P<0.01). The FA score showed large negative direct effects on PEV, while other agronomic and plant traits did not show any large effects on PEV. Days to anthesis (DA), ear prolificacy and ear aspect (EA) exhibited large direct effects on SY. The results obtained showed that, PEV and SY can be selected concurrently during the selection cycle with selection based on genotypes that possess high SY and PEV. In general, the weak relationship between PEV and SY implies that selection for high PEV will not negatively impact on yield and the vice versa. Weak association and small direct effects of secondary traits on PEV suggested that PEV cannot be improved through selection of most secondary traits. Overall, the indirect effects of secondary traits were small on both yield and PEV supporting the strategy of direct selection of these traits to enhance yield and popping ability.

Keywords: Correlations, Path analysis, Popping expansion volume, Seed yield, Secondary traits

3.1 Introduction

Popping expansion volume (PEV) and seed yield (SY) are the major quality and agronomic traits in popcorn, respectively. Therefore, popcorn varieties that provide value for cultivation and use (VCU) would be released. Popcorn yield is below that of dent maize. Low yield could be associated with yield components and unfavorable environmental factors (Ahmet and Halil, 2011). Genotype is a major factor affecting yield in popcorn; as a result, yield can be improved by breeding (Pajic and Babic, 1991). Developing high yielding genotypes in popcorn depends primarily on the selection of superior cultivars and use of high quality seeds (Sakin *et al.*, 2005). However, popcorn yield in South Africa (SA) and in general Sub-Saharan Africa (SSA) has not been quantified.

Popcorn genotypes that are stable in various environments should be characterized by high yield and popping ability. High SY and PEV allow plant breeders to concurrently select for high yield and high PEV (Zorica *et al.*, 2008). According to Zorica *et al.* (2008), SY and PEV are heritable traits and could be influenced by certain heredity factors. Genotypes with high SY and PEV can be developed, however, developing cultivars with these two traits can be difficult (Zorica *et al.*, 2008). The use of breeding methods that utilize additive genetic variation for PEV and dominance variation for SY can result in improvement of PEV alongside with SY (Dofing *et al.*, 1990). Popping expansion volume is normally negatively correlated with several secondary traits and it is, therefore, more prone to diseases and many unfavorable conditions (Pipolo *et al.*, 2003). However, the association between PEV and secondary traits has not been studied in SA.

Phenotypic traits are important in plant breeding in improving primary traits such as yield and popping ability and in selection of a genotype for a specific location. Association between secondary traits, SY and PEV is important in improvement of popcorn genotypes with regard to PEV and SY (Ahmet and Halil, 2011). These two traits may be singly or jointly influenced by several factors (Grafius, 1959). Factors influencing PEV and SY require investigation under cultivation conditions in SA. Estimating correlation among different traits is also a useful tool during the selection

process. For example, it provides information that selection for one trait can be used in the improvement of another trait if correlation is significantly large. Correlation among agronomic traits can also make selection process more efficient if heritability of the selected correlated traits is high (Manggoel *et al.*, 2012). The development of superior popcorn genotypes in relation to yield and popping ability requires the understanding of the relationship between traits influencing yield and popping ability (Yoshida and Yoshida, 2004). This therefore, underlines the call to study the complex relationship between popping ability with secondary traits, and also between yield and its components.

Path coefficient analysis is a useful tool for determining the direct and indirect effects of interrelated agronomic traits on a complex trait such as SY and PEV. It also measures the direct effects of one trait to another and simplifies the relationship with the dependent character such as yield and PEV (Rajper et.al. (1986) as cited by Qaizar *et al.* (1991)). Path analyses also enable plant breeders to decide between direct and indirect selection and to give the proportion contributed by individual traits (Coimbra *et al.*, 2002; Darvishzadeh *et al.*, 2011; Wende *et al.*, 2012). Seed yield and PEV results from the combination of various polygenic traits and, therefore, are quantitatively inherited (Babu *et al.*, 2006; Darvishzadeh *et al.*, 2011). Hence, PEV is a complex trait with implication for breeding strategy. However, the direct selection of traits influencing PEV and SY may be difficult but indirect selection through the associated traits may be useful in improving these two complex traits (Darvishzadeh *et al.*, 2011). Large direct effects of one secondary trait on a complex trait indicates a good selection criterion that will not negatively impact the complex trait (Qaizar *et al.*, 1991). Path coefficients also reduces the timeline for the selection process by restricting selection to the major few traits rather than looking at several traits with little or no impact on yield and popping ability (Qaizar *et al.*, 1991).

The studies evaluating correlation coefficients among secondary traits, popping ability and yield, and the direct and indirect effects of secondary traits on popping ability and yield in popcorn are limited. Therefore, there is a need of understanding this relationship for successful development of adapted local popcorn varieties with

high value for cultivation and use. Understanding direct and indirect effects of agronomic traits on a complex trait can be attained if correlations among secondary traits are determined (Manggoel *et al.*, 2012). Knowledge of the association between popping ability and yield and among secondary traits may also be beneficial in developing an effective and efficient breeding programme. The objective of the study was to estimate the relationship between SY and PEV, and the relationship between SY and secondary traits, PEV and secondary traits, the association among secondary traits themselves, and their direct and indirect effects on SY and PEV. This information would be crucial in devising an appropriate popcorn breeding strategy.

3.2 Materials and methods

3.2.1 Experimental site

The study was conducted in South Africa at Ukulinga Research Farm of the University of KwaZulu - Natal, Pietermaritzburg (Latitude 29.67ºS; Longitude; 30.41ºE; Altitude 812 m.a.s.l) and Cedara (Latitude 29.54ºS; Longitude 30.26ºE; Altitude1066 m.a.s.l.) during the period of December 2011 – April 2012.

3.2.2 Experimental material

Two populations of popcorn inbred lines were used in the study. The first population designated "Population 1" was the advanced and fixed population of 83 inbred lines and ten checks (controls). The controls were, positive (popping) controls (P618: commercial hybrid, CHECK1, CHECK 2, CHECK3, CHECK4, CHECK5, CHECK6 and CHECK7, 100% dent), and negative (non-popping) controls (P1* and 8CED6-7). The second population, designated "Population 2", of 81 inbred lines (F5 generation) originated from a nursery plot from Makhathini Research Station and were derived from F2 segregations of a flint x popcorn population. The bi-parental population was a cross between a flint maize line P1 and an F3 popcorn inbred bulk population.

3.2.3 Experimental design

In Population 1, the experiment was laid out as an augmented alpha lattice design, with 9 blocks × 12 plots, and 3 major controls; P618 (from Capstone Seeds), P1 and 8CED6-7 (both from University of KwaZulu-Natal Breeding programme), where each control was replicated 9 times. Population 2 was grown in a nursery observation plot at Ukulinga Farm. The trial was also laid out as an augmented alpha lattice design with 9 blocks and 9 plots and without replicates.

3.2.4 Management practices

Inbred lines from Population1 were planted in a 4 m row plot with 0.8 m × 0.3 m spacing. Inbred lines of Population 2 were planted in 3m row plot with 0.9 m × 0.3 m spacing. Both locations were similar with regard to management practices. Sowing was done after land preparation where 2 seeds/ hole were dipped by hand. A 250kg basal fertilizer (NPK, 2:3:4) was applied before planting. The proportion of N, P, and K was 55kg, 83 kg and 111kg, respectively. Limestone Ammonium Nitrate (LAN) containing 28% N was applied as top dressing four weeks after planting. Both locations were rainfed, however, supplementary irrigation was applied at Ukulinga Farm. Weeds were controlled by hand weeding and by the use of herbicides. Herbicides used were Gramoxone, Troopers, and Basagran. The plots were harvested manually after physiological maturity.

3.2.5 Data collection

The following traits were measured:

- Plant height (PH): Measured from the ground level to the point of insertion of the flag leaf.
- Ear height (EH): Measured from the ground level to the insertion of the highest ear in the stem.
- Number of primary tassel branches (PTB): Total number of primary tassel branches counted per plant/plot.

- Number of leaves (NL): Total number of leaves counted per plant.
- Ear aspect (EA): Was rated on scale from 1 to 5, where 1= good and 5 = bad.
- Flake aspect (FA): Was rated on scale from 1 to 5, where 1=good and 5= bad.
- Ear rot (ER): Determined by counting the number of rotten ears per genotype/plot.
- Flowering date: Number of days to 50% tasseling (days to anthesis, DA) and 50% silking (days to silking).
- Number of plants (NP): Number of plants counted per genotype/plot.
- Stem lodging (SL): Determined by counting the number of plants broken below the upper ear at harvest.
- Root lodging (RL): Determined by counting the number of plants broken by more than 45^{o} from the bottom of the root.
- Number of ears (NE/ ear prolificacy: Total number of ears per genotype/plot.
- Grain moisture (GM%): Measured using grain moisture metre.
- Chlorophyll concentration (CC index): Measured using the chlorophyll content metre (CCM-200 plus).
- Leaf Area (LA) (m^2): Determined using the leaf area metre.
- Popping expansion volume (PEV): Determined using hot air popping machine.
- Number of unpopped kernels (UPKs): Number of unpopped kernels counted after popping.
- Ear turc (ET): Disease was determined at Cedara using 1 to 5 rating scale, where 1=resistant and 5=susceptible.
- Seed yield (SY) in tons/ha was determined using the following equation and moisture content was adjusted to 14% (ideal grain moisture for popping).
- Grain yield (tons/ha) = (Field weight x10/plot area)* (100-GM) x shelling percent.

  Where GM = Grain moisture percentage, shelling percentage (weight shelled/ weight unshelled)*100.

3.2.6 Statistical Analysis

Correlation coefficients among and between traits were performed using GenStat 14th edition. The path coefficient analysis was performed using SAS Software (Cramer *et al.*, 1990).

3.3 Results

3.3.1 Correlations among traits

The scatter plot for popping expansion volume and seed yield is shown in Figure 3.1. The relationship between popping expansion volume and seed yield was weak (R^2 =0.18).

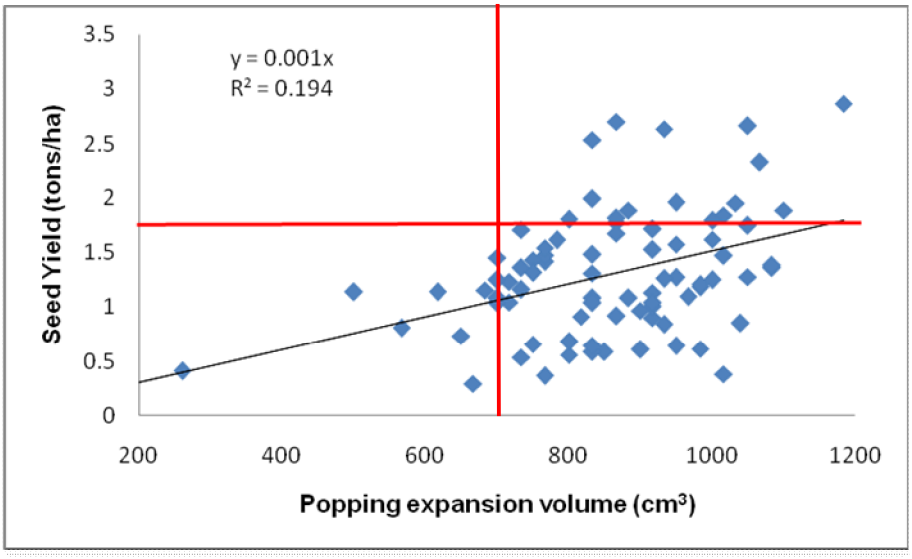

Figure 3.1: Scatter plot of seed yield (tons/ha) and popping expansion volume (cm^3) (n=83)

The correlation coefficient data are represented in Table 3.1 and Table 3.2 for Population 1 and 2, respectively.

Table 3.1: Correlation Coefficient (r) between popping expansion volume, seed yield and secondary traits in Population 1

Traits	Popping expansion volume (cm^3)	Seed yield (cm^3)
Seed yield (tons/ha)	**0.507****	
Anthesis silking interval (days)	-0.270*	-0.364**
Chlorophyll concentration	0.211	0.477**
Days to anthesis	-0.260*	-0.135
Days to silking	-0.313*	-0.236*
Ear aspect (score 1-5)	-0.340*	-0.668**
Ear height (cm)	0.042	0.189
Ear length (cm)	0.266*	0.414**
Ear Rot	0.06	-0.204
Ear Turc (score 1-5)	-0.117	-0.424
Grain moisture (%harvest)	0.351*	0.697**
Grain moisture (%popping)	0.207	0.520**
Flake aspect (score 1-5)	-0.609**	-0.419**
Kernel size (kernels /10g)	-0.266*	-0.431**
Leaf area (m^2)	0.247*	0.256*
Number of ears	0.298*	0.616**
Number of leaves	-0.094	0.254*
Number of plants	0.091	0.207
Plant height (cm)	0.154	0.247*
Number primary tassel branches	-0.112	0.143
Root Lodging	-0.070	-0.208
Stem lodging	-0.180	-0.215
Shelling percentage	0.130	0.185
Number of unpopped kernels	-0.528**	-0.381**

*,**significant at P Ò0.05 and PÒ0.01, respectively.

Table 3.2: Correlation coefficient (r) between popping expansion volume, seed yield and secondary traits in Population 2

Traits	Popping expansion volume (cm^3)	Seed yield (tons/ha)
Seed yield (tons/ha)	-0.055	-
Chlorophyll concentration	0.189	0.165
Ear height (cm)	-0.182	0.151
Flake aspect	-0.517**	0.109
Number of leaves N	-0.122	0.253*
Grain moisture (%popping)	0.006	0.559**
Plant height (cm)	-0.176	0.172
Primary tassel branches	-0.171	0.207
Number of unpopped kernels	-0.685**	-0.094
Kernel size	0.347*	-0.369*

*,**significant at P Ò0.05 and PÒ0.01, respectively.

The correlation coefficients among secondary traits associated with popping expansion volume and seed yield in Population 1 and 2 is presented in Table 3.3 and Table 3.4, respectively.

Table 3.3: Correlation coefficients (r) among popping expansion volume associated traits of population1

Traits	1	2	3	4	5	6
Chlorophyll concentration	-					
Ear turc	-0.370**	-				
Grain moisture (%)	0.313*	-0.189	-			
Kernel size	-0.150	0.271*	-0.194	-		
Flake aspect	-0.285*	0.489**	-0.161	0.447**	-	
Unpopped kernels	-0.280*	0.307*	0.003	0.372**	0.684**	-

*,**significant at P Ò0.05 and PÒ0.01, respectively.

[1]Chlorophyll concentration, [2]=Ear turcum, [3]= Grain moisture (%), [4]=Kernel size, [5]= Flake aspect, [6]= Unpopped kernels

Table 3.4: Correlation coefficients (r) among seed yield related components in Population 1

Traits	1	2	3	4	5	6	7	8	9	10	11	12
LA	-											
NE	-0.056	-										
NL	0.222*	0.217*	-									
PH	0.282*	0.201	0.591**	-								
PTB	0.079	0.146	0.444**	0.255*	-							
ASI	0.009	-0.233*	-0.044	0.058	0.097	-						
CC	0.396**	0.135	0.378**	0.400	0.265*	-0.218*	-					
DA	0.136	-0.081	0.544**	0.396**	0.285*	0.053	0.329*	-				
DS	0.131	-0.146	0.502**	0.390	0.292*	0.334*	0.255*	0.956**	-			
EA	-0.285*	-0.182	-0.405**	-0.314*	-0.159	0.241*	-0.455**	-0.248*	-0.168	-		
EL	0.186	0.228*	-0.053	0.111	-0.170	-0.259*	0.181	-0.103	-0.171	-0.353*	-	
ET	-0.190	-0.270*	-0.394**	-0.165	-0.218*	0.079	-0.371	-0.302*	-0.261*	0.423**	-0.032	-

*,**significant at P Ò0.05 and PÒ0.01, respectively.

[1]LA= leaf area (cm[3]), [2]NE=No. of ears, [3]NL= number .of leaves, [4]PH=plant height (cm), [5]PTB=No. of primary tassel branches [6]ASI= anthesis silking interval, [7]CC=chlorophyll content, [8]DA= days to anthesis,[9]DS=days to silking, [10]EA= ear aspect, [11]EL= ear length (cm), [12]ET=ear turc.

Correlation coefficients data for secondary traits in Population 2 is represented in Table 3.5.

Table 3.5: Correlation coefficient (r) among secondary traits in Population 2

Traits	1 CC	2 EH	4 LN	5 GM	6 PH	7 PTB	8 UPK	9 KS
CC	-							
EH	0.038	-						
FA	-0.131	0.341*	-					
LN	0.089	0.215	-0.008	-				
GM	0.108	0.117	0.165	0.230	-			
PH	0.091	0.651**	**0**.282*	0.198	0.016	-		
PTB	0.152	0.346*	0.295*	0.196	0.261	0.291*	-	
UPK	-0.257*	0.082	0.259*	0.122	-0.064	0.166	0.119	-
KS	-0.134	-0.034	-0.051	0.011	-0.123	-0.088	-0.140	0.034

*,**significant at P Ò0.05 and PÒ0.01, respectively.

[1]CC= chlorophyll content, [2]EH =ear height (cm), [3]FA=flake aspect, [4]LN= leaf number, [5]GM= grain moisture, [6]PH=plant height, [7]PTB=primary tassel branches, [8]UPK=No. of unpopped kernels, [9]KS= kernel size

3.3.2 Path analysis

Path analysis results for popping expansion volume with associated traits in Population 1 and 2 are presented in Table 3.6 and 3.7 respectively. Flake aspect score had a large negative direct effect (p=-0.63, p= -0.57) in both Populations.

Table 3. 6: Path analysis for popping expansion volume direct (diagonal) and indirect effect in the (**R^2=0.41,** n=83) in Population 1

Traits	Seed yield	Ear turc	Chlorophyll concentration	Grain moisture	Flake aspect	Kernel size	**Total correlations to PEV**
SY	**0.16**	-0.01	-0.02	0.01	0.16	-0.11	**0.18**
ET	-0.06	**0.04**	0.02	0	-0.01	0.12	**0.11**
CC	0.07	-0.02	**-0.06**	0.01	-0.02	-0.09	**-0.1**
GMp	0.08	-0.01	-0.02	**0.02**	0.12	-0.04	**0.15**
FA	-0.04	0	0	0	**-0.63**	0.04	**-0.63**
KS	-0.08	0.02	0.02	0	-0.11	**0.22**	**0.07**

SY= seed yield, ET=ear turc, CC= chlorophyll content, GM_P=grain moisture at popping, FA=flake aspect, KS= kernel size (No. of kernels per 10g sample), PEV=popping expansion volume.

Table 3.7: Path analysis for popping expansion volume direct (diagonal) and indirect effect (**R^2=0.49,** n=81) in Population 2

Traits	Ear turc	Chlorophyll concentration	Moisture content	Kernel size	Flake aspect	Seed yield	**Total correlations to PEV**
ET	**-0.25**	-0.02	0.00	0.06	0.01	-0.01	**-0.20**
CC	0.05	**0.11**	0.01	-0.06	0.07	0.01	**0.20**
GM	0	0.01	**0.12**	-0.05	-0.09	0.04	**0.02**
KS	-0.04	-0.01	-0.01	**0.44**	0.03	-0.03	**0.37**
FA	0.01	-0.01	0.02	-0.02	**-0.55**	0.01	**-0.55**
SY	0.04	0.02	0.06	-0.17	-0.09	**0.07**	**-0.07**

SY= seed yield, ET=ear turc, CC= chlorophyll content, GM=grain moisture at popping, FA=flake aspect, KS= kernel size (No. of kernels per 10g sample), PEV=popping expansion volume.

Path analysis results for seed yield with associated traits are presented in Table 3.8.The model for path coefficient was significant in Population 1 (R^2=81%; P<0.01) but non-significant in Population 2. Therefore the results for Population 2 have not been presented.

Table 3.8: Direct (diagonal) and indirect effect of agronomic traits on seed yield in Population 1. (R^2=0.81; n=83)

Name	DA	DS	ASI	PH	NE	EL	EA	ET	NL	PTB	CC	LA	**Total correlation to seed yield**
DA	**0.8**	-1.14	0.03	0.0	-0.08	-0.01	0.06	0.04	0.02	0.0	0.06	-0.01	**-0.23**
DS	0.77	**-1.18**	0.09	0.0	-0.1	-0.01	0.02	0.04	0.02	0.0	0.05	-0.01	**-0.32**
ASI	0.08	-0.41	**0.26**	0.0	-0.09	-0.02	-0.13	-0.01	0.0	0.0	-0.04	-0.01	**-0.37**
PH	0.12	-0.15	-0.01	**-0.01**	0.08	0.01	0.2	0.01	0.03	-0.01	0.08	0.04	**0.39**
NE	-0.15	0.28	-0.06	0.0	**0.41**	0.02	0.05	0.02	0.01	0.0	0.01	0.0	**0.58**
EL	-0.09	0.18	-0.05	0.0	0.11	**0.09**	0.11	0.0	0.0	0.01	0.01	0.02	**0.38**
EA	-0.1	0.05	0.07	0.0	-0.04	-0.02	**-0.46**	-0.03	-0.02	0.01	-0.09	-0.04	**-0.67**
ET	-0.32	0.43	0.02	0.0	-0.07	0	-0.16	**-0.1**	-0.02	0.0	-0.08	-0.02	**-0.32**
NL	0.42	-0.56	-0.02	0.0	0.05	-0.01	0.2	0.04	**0.05**	-0.01	0.08	0.02	**0.27**
PTB	0.1	-0.14	0.01	0.0	0.04	-0.02	0.11	0.01	0.02	**-0.03**	0.04	0.01	**0.15**
CC	0.26	-0.3	-0.06	0.0	0.03	0.01	0.22	0.04	0.02	-0.01	**0.19**	0.04	**0.44**
LA	-0.04	0.09	-0.03	0.0	0.01	0.02	0.2	0.02	0.01	0.0	0.08	**0.1**	**0.44**

DA= days to anthesis, DS=days to silking, ASI= anthesis silking interval, PH=plant height (cm), NE=No. of ears, EL= ear length(cm), EA= ear aspect, ET=ear turc, NL= number of leaves, PTB=No. of primary tassel branches, CC=chlorophyll content, LA= leaf area(cm^3),SY=seed yield.

3.4 Discussion

3.4.1 Relationship between popping expansion volume and seed yield

There was a positive relationship between SY and PEV. A positive significant correlation was observed between PEV and SY in Population 1 (r=0.507**). This relationship suggests that, PEV and SY can be improved simultaneously during the breeding progress. The study is in accordance with the findings by Sakin *et al.* (2005) who found a positive relationship (r=0.86**) between yield and PEV. Arnhold *et al.* (2006) also observed a positive correlation between these traits after having considered both SY and PEV during previous selections. Therefore, a positive correlation between PEV and SY is only possible when these two traits are considered concurrently during the selection cycle. Popping expansion volume and SY can, therefore, be improved jointly.

Genotypes combining high SY and PEV would thus be beneficial in concurrent improvement of SY and PEV. For example, some inbred lines showed high yield and high PEV. Those included inbred lines (entries) 83, 55, 72 and 77 and other inbred lines. Inbred line 83 was the more superior line which exhibited the highest PEV and SY amongst others. Selecting cultivars that combine both high SY and PEV can therefore be an effective way for simultaneous improvement of PEV and SY. Ahmet and Halil (2011) investigated yield and PEV in 18 popcorn hybrids and could select for genotypes with both high yield and PEV. Broccoli and Burak (2004) suggested that, these two traits can be improved at once when plants with more than one ear (prolific) and high PEV are selected. Daros *et al.* (2002) also reported that, selecting genotypes with both high SY and PEV is possible, however, SY improvement is generally easier than PEV. Selecting inbred lines with both high SY and PEV can lead to superior genotypes possessing high yield and popping ability and therefore, development of superior and locally adapted cultivars.

However, other researchers have reported negative significant correlation between SY and PEV (Arnhold *et al.*, 2006; Arnhold *et al.*, 2009; Broccoli and Burak, 2004; Li *et al.*, 2008). They reported that, a negative correlation between these two traits interfered with simultaneous selection of the two traits, suggesting that genes controlling the two traits were distinct. Zorica *et al.* (2008) reported low PEV in genotypes with high yield and high PEV in genotypes with low to moderate SY. This indicated that seed yield improvement lowered PEV and vice versa. Li *et al.* (2008) explained that traditional plant breeding may not be efficient in improving SY and maintaining high PEV due to a possible negative association between these two traits. Contrasting results could result from genotypes with different genetic backgrounds and maybe environmental conditions used. For example, when high yielding genotypes but with a generally poor popping ability are continuously used in the breeding cycle, a negative relationship may be obtained. Nevertheless, simultaneous selection of these traits during the selection progress will not hamper the other trait and, hence, a positive relationship is obtained. Some genotypes may also fail to express both high yield and popping ability in certain environments, therefore, evaluation of different genotypes for PEV and SY in various environments may be crucial.

3.4.2 Correlation and path coefficient analysis for popping expansion volume

There was a negative association between PEV and flake aspect score and number of unpopped kernels with implications for breeding. Popping expansion volume was significant and negatively correlated with the number of unpopped kernels. The negative correlation between these two traits could be explained by the fact that in general, large number of unpopped kernels present in a sample contributes to a low PEV. Therefore, as PEV increases the number of unpopped kernels decreases and vice versa. The flake aspect and PEV also showed a large negative and significant correlation. According to the present study, flake aspect refers to the physical appearance of the popped kernels. An increase in the value of flake aspect for example, flake aspect =5 implies poor popped kernels, and this was observed during the study. Inbred lines which showed

high PEV were characterized by a flake aspect between 1 and 2, while inbred lines with low PEV had a high flake aspect (>3). The relationship between PEV and most secondary traits was small and insignificant, suggesting no association between PEV and most of the secondary traits. Therefore, PEV can only be improved through the selection of fewer traits. Reviews regarding the observed relationship are not well documented. Nevertheless, results obtained from the current study suggest that, when resources are limited (time and budget), breeders can measure only PEV because the number of UPKs gives similar results regarding genotypesôpopping ability.

The path coefficient analysis model was significant in both populations for the correlation of secondary traits with PEV. Therefore, information obtained from the current study would be considered in developing a breeding strategy. Path analysis showed large negative direct effects of FA on PEV (p= -0.63 and p= -0.57) in Population 1 and 2, respectively. The direct effects of FA on PEV was not influenced by other traits for example, moisture content, implying that indirect selection of these traits would not be necessary in PEV improvement. Large direct effects of FA on PEV suggests that breeding for FA will not interfere negatively with the objective of obtaining high PEV. The direct effects of kernel size on PEV were large and positive, suggesting that an increase in kernel size is likely to increase popping expansion volume. This relationship has been reported by Dofing *et al.* (1990) and Song *et al.* (1991). Selection for large kernel size would therefore be emphasized in the breeding program.

Other traits did not show any large direct effect on PEV, this indicated that, direct selection of these traits, for example chlorophyll, would not be effective in improving PEV. Large direct effect of secondary traits including days to silking, days to maturity and plant height was reported by Vijayabharathi *et al.* (2009). This suggests that associations between traits are influenced in part by the environment and population under study. Babic (2001) also observed a weak relationship between PEV and most of the secondary traits, and concluded that most secondary traits do not determine popping expansion volume at a great extent. This is consistent with findings from the

current study. Thus, secondary traits generally play a limited role in influencing popping ability; as a result, they will not be emphasized in the breeding programme.

3.4.3 Correlations and path coefficient analysis for seed yield

Unlike PEV, for seed yield the secondary traits play an influential role. Correlation between seed yield and most secondary traits was significant, for example, there was as strong positive and significant correlation between seed yield and NE (r=.0.616**), GM (r= 0.520** and 0.559**) in Population 1 and 2, respectively. The large positive correlation between seed yield and ear prolificacy is attributed to the fact that in general, more ears per plant contribute to high seed yield. This suggests that selection for ear prolificacy should be emphasized to improve seed yield. Similar observations have been reported by Broccoli and Burak (2004). Prolificacy can, therefore, be selected during seed yield improvement. Other traits showed a positive significant but small correlation with seed yield, for example, chlorophyll concentration, ear length, plant height and number of leaves. Therefore, these traits should not be ignored when selecting for yield enhancement, direct selection for these traits should be applied.

Relationship between seed yield and other traits was small but significant. For example, the relationship between seed yield and days to anthesis was small. Similar findings were reported by Makanda (2009) in sorghum where the shorter the period to flowering the higher was the yield. Genotypes with early flowering period generally mature faster and are higher yielding (Makanda, 2009). High yield in these genotypes was also associated with high vigor and increased ability to escape adverse conditions that may subsequently lower yield (Ahmet and Halil, 2011; Broccoli and Burak, 2004). A negative significant correlation was found between anthesis silking interval and yield. This relationship has been reported by several authors, but in dent maize (Moss and Stinson, 1961; Woolley *et al*., 1962; Edmeades and Daynard, 1979 and Hall *et al*., 1982 as cited by Borras *et al.* (2007). Further investigations are, therefore, required in popcorn breeding to confirm this trend.

The path coefficient analysis model was highly significant for seed yield (R^2=81%; P<0.01), indicating that this information would be crucial in developing breeding strategy. The direct effects of the number of ears (ear prolificacy) on seed yield were large and positive (Table 3.8). Days to anthesis also showed large direct effects on seed yield indicating that direct selection for these traits would be effective to improve seed yield in popcorns. The direct effect of days to silking on seed yield was large and negative suggesting that direct selection for this trait might compromise seed yield. Therefore, genotypes with early silk emergence are generally low yielding. Emphasis on ear prolificacy can be effective in improving seed yield as there was also a considerable significant correlation between number of ears and seed yield. Indirect effect of other traits, such as plant height, number of leaves, chlorophyll concentration, and other traits was small. Therefore, indirect selection of these traits may not be considered during seed yield improvement.

Darvishzadeh *et al.* (2011) reported that traits to be considered for indirect selection on seed yield should have a positive significant correlation and a positive direct effect on seed yield. Other previous researchers have also indicated that considering the indirect effect of a trait on seed yield without accounting for the magnitude and nature of correlation between that trait and seed yield can be unreliable (Das and Taliaferro, 2009; Dewey and Lu, 1959). Therefore, selection based on secondary traits with positive and significant correlation, large direct effects on seed yield may be effective in seed yield improvement. These secondary traits would be emphasized in the breeding programme.

3.4.4 Correlation among secondary traits

Investigating the association among secondary traits themselves and how they can impact popping ability is also important. There was a large positive and significant correlation between the number of unpopped kernels and kernel size (r=0.684**). An increase in the number of unpopped kernels was associated with an increase in the

number of kernels/ 10g (small kernel size), which agrees with the findings of Song *et al.* (1991). They reported that, smaller kernels are more likely to be unpopped than the larger kernels. Dofing *et al.* (1990) also observed a large number of UPKs in genotypes with small kernels than those with medium to large kernels. However, the findings contrast the results obtained by Soylu and Tekkanat (2007) who reported low PEV and large number of UPKs in genotypes with large kernel size than small to medium sized varieties. Soylu and Tekkanat (2007) explained that large kernels contained a high percentage of soft endosperm which interfered with the kernels ability to pop. However, our observations suggest that popping ability could be improved by selecting for large kernels. Nonetheless, small correlation among several traits that are likely to contribute to high PEV was observed suggesting that the expression of high PEV is not influenced by a large number of traits as reported by Babic (2001) and Vijayabharathi *et al.* (2009). These contrasting observations may require further investigations.

Correlation among many secondary traits associated with yield was weak. However, some traits showed a large positive correlation with other traits such as days to silking and days to anthesis (r=0.956**), plant height and number of leaves (r=0.591**) and other traits. This showed that, indirect selection can be applied to improve these traits in popcorn, depending on what is easy to measure, heritability of these and other economic factors. For example, to save resources breeders can measure only days to anthesis and estimate days to silking because the two traits are strongly correlated. Ear aspect was negative, significant, but weakly correlated with most secondary traits, suggesting that these traits cannot be a good selection criterion for improving ear aspect

3.5 Conclusion and implications

Popping ability in popcorn is as significant as yield because new varieties will be released on the basis of value for cultivation and use (VCU). Therefore genotypes with optimum seed yield and popping expansion volume are required. This study has shown

that such varieties could be found because popping ability and seed yield were positively correlated, especially in Population 1. Therefore, selecting genotypes with high seed yield may be the efficient procedure for improving popping ability. Seed yield was positively correlated with several secondary traits. Therefore, improvement of seed yield can be made through selection for some secondary traits for example ear prolificacy. Since popping expansion volume was poorly correlated with most secondary traits, fewer traits can be selected to significantly increase or to maintain high popping expansion volume.

Path analysis further revealed that, direct selection for popping expansion volume and seed yield would be important than indirect selection. Ear prolificacy and days to anthesis showed large positive direct effects on seed yield and therefore, they are qualified as the key secondary traits which must be emphasized to enhance yield in popcorns. The direct effects of ear aspect on seed yield were large and negative, indicating that these traits should not be ignored in breeding programs that emphasize seed yield improvement. Flake aspect showed a large and positive direct effect on popping expansion volume, implying that direct selection for superior flake aspect in popcorn would result in improved popping ability. Overall, direct selection for ear prolificacy, ear aspect, and flake aspect would be emphasized to improve both seed yield and popping expansion volume.

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CHAPTER 4

Genetic Variation and Diversity in a Popcorn Inbred Line Population

Abstract

Production of popcorn (*Zea mays* L. everta) in South Africa is limited by the lack of adapted local varieties. The number of research studies focusing on genetic variation and diversity in popcorn are also scanty. High genetic diversity among popcorn inbred lines is essential for selection and development of varieties. The objective of the study was, therefore, to evaluate the level of genetic diversity and variation among 83 popcorn inbred lines (Population 1) and 81 lines (Population 2) using phenotypic traits measured at two sites. Genetic diversity was investigated in 20 randomly selected inbred lines using hierarchical cluster analysis based on morphological data, and using 22 Simple Sequence Repeats (SSR) markers. Results indicated a significant genetic variation among the popcorn inbred lines. The dendogram based on seed yield and popping expansion volume formed six and four clusters, respectively, while the dendogram based on the 17 phenotypic traits with high heritability (>50%) grouped inbred lines into 7 distinguishable clusters. Moreover, the dendogram performed using 22 SSR markers allocated 5 clusters to the inbred lines. Diversity was also observed from the distribution of phenotypic traits. The presence of a large genetic diversity was also detected by the distribution of phenotypic traits in Population 2. The results indicated overwhelming evidence in support of genetic diversity in the evaluated popcorn population. Hence, the distant inbred lines can be conserved and used in subsequent selection for popcorn improvement and development of locally adapted inbred lines and hybrids.

Keywords: Genetic diversity, Genetic distance, Popcorn inbred lines, SSR markers, Phenotypic diversity

4.1 Introduction

Genetic diversity in popcorn and most crop species is important in crop improvement. Plants with high genetic diversity are more desirable (Leal *et al.*, 2010). High genetic variation is associated with several advantages. For example, increased production and adaptation to various environmental conditions. These include adaptation to abiotic and biotic stresses. However, a narrow genetic diversity may limit crop improvement as a result of limited variety options. Plants exhibiting a narrow genetic base for selection are more prone to stress because their ability to withstand stress is low (Khodadadi *et al.*, 2011). Large and more diverse gene pool in popcorn allows the exploitation of different genotypes (Bispol *et al.*, 2009). Breeding progress of popcorn is depended on the diversity and the number of original germplasm used during the breeding process (Joshi *et al.*, 2004). However the level of diversity in popcorn has been scarcely reported in the literature. Estimation of genetic components in popcorn populations could lead to the best breeding procedure and maximize genetic gain (Pereira and Junior, 2001).

In any breeding program, parental selection is generally the first step (Joshi *et al.*, 2004). According to Joshi *et al.* (2004) and Leal *et al.* (2010), measuring genetic distance between parents is important for the benefits of transgressive segregation and for parental selections and therefore, overall development of potential varieties. High genetic distance between parents is associated with high heterosis (Joshi *et al.*, 2004; Leal *et al.*, 2010). Genetic diversity among popcorn genotypes can be estimated based on molecular, morphological, biochemical and agronomic information (Goncalves *et al.*, 2009; Mohammadi and Prasanna, 2003; Sudre *et al.*, 2007). The methods including factor analysis, cluster analysis, principal components analysis (PCA) are also employed for measuring genetic diversity. The presence of distinct groups among inbred lines is associated with high allelic diversity and furthermore, large genetic diversity (Wende *et al.*, 2012). Grouping popcorn genotypes based on their genetic background also minimizes the number of crosses to be made and evaluated (Terron *et al.*, 1997). Superior individuals are selected based on their genetic diversity. However, selecting individuals based only on their genetic diversity without accounting for the

behavior of the individuals may not be useful in genetic improvement of popcorn. Selection should also account for the superior agronomic traits to make genetic improvement more efficient (Pipolo *et al.*, 2003).

There are several molecular markers employed in the study of genetic diversity. Among them are randomly amplified polymorphic DNA (RAPD), inter simple sequence repeats (ISSR), simple sequence repeats (SSR) markers, amplified fragment length polymorphism (AFLP), single nucleotide polymorphisms (SNPs) and other markers (Chen and Sullivan, 2003; Tautz, 1989; Vos *et al.*, 1995; Welsh and McClelland, 1990; Zietkiewicz *et al.*, 1994). Molecular markers are useful in characterizing inbred lines and grouping them based on their genetic diversity (Reif *et al.*, 2003). For example, molecular markers can distinguish between homo and heterozygous individuals in one population without progeny testing (Jain and Brar, 2009). The major advantage of molecular markers is early screening of parents (Balestre *et al.*, 2008; Chen and Sullivan, 2003; Dandolini *et al.*, 2008; Mohammadi and Prasanna, 2003; Munhoz *et al.*, 2009).

Several authors have employed molecular markers in investigating the magnitude of genetic diversity in popcorn and other maize populations (Babu *et al.*, 2006; Lorz, 2008; Lu *et al.*, 2003; Qi-Lun *et al.*, 2008; Yoshida and Yoshida, 2004). Measurement of genetic and phenotypic diversity in field trials may not be adequate in investigating variation among genotypes as a result of the large environmental variations which may interfere with genetic strength of genotypes (Yoshida and Yoshida, 2004). Therefore, combining molecular data analysis and phenotypic data may be more effective and efficient in discriminating genotypes and developing superior varieties within a short timeline. The objective of the current study was to investigate genetic variation and diversity among popcorn inbred lines.

4.2 Materials and methods

4.2.1 Experimental site

The study was conducted in South Africa at Ukulinga Research Farm of the University of KwaZulu - Natal, Pietermaritzburg (Latitude 29.67ºS; Longitude; 30.41ºE; Altitude 812 m.a.s.l) and Cedara (Latitude 29.54ºS; Longitude 30.26ºE; Altitude1066 m.a.s.l.) during the period of December 2011 – April 2012.

4.2.2 Experimental material

Two populations of popcorn inbred lines were used in the study. The first population, designated "Population 1", was the advanced and fixed population of 83 inbred lines and ten checks (controls). The controls were, positive (popping) controls (P618: commercial hybrid, CHECK1, CHECK 2, CHECK3, CHECK4, CHECK5, CHECK6 and CHECK7, 100% dent), and negative (non-popping) controls (P1* and 8CED6-7). The second population, designated "Population 2", of 81 inbred lines (F5 generation) originated from a nursery plot from Makhathini Research Station and were derived from F2 segregations of a flint x popcorn population. The bi-parental population was a cross between a flint maize line P1 and an F3 popcorn inbred bulk population.

4.2.3 Experimental design

In Population 1, the experiment was laid out as an augmented alpha lattice design, with 9 blocks × 12 plots, and 3 major controls; P618 from Capstone Seeds, P1 and 8CED6-7 (both from University of KwaZulu-Natal Breeding programme), where each control was replicated 9 times. Population 2 was grown in a nursery observation plot at Ukulinga Farm. The trial was also laid out as an augmented alpha lattice design with 9 blocks and 9 plots and without replicates.

4.2.4 Management practices

Inbred lines from Population1 were planted in a 4 m row plot with 0.8 m × 0.3 m spacing. Inbred lines of Population 2 were planted in 3m row plot with 0.9 m × 0.3 m spacing. Both locations were similar with regard to management practices. Sowing was done after land preparation where 2 seeds/ hole were dipped by hand. A 250kg basal fertilizer (NPK, 2:3:4) was applied before planting. The proportion of N, P, and K was 55kg, 83 kg and 111kg, respectively. Limestone Ammonium Nitrate (LAN) containing 28% N was applied as top dressing four weeks after planting. Both locations were rainfed, however, supplementary irrigation was applied at Ukulinga Farm. Weeds were controlled by hand weeding and by the use of herbicides. Herbicides used were Gramoxone, Troopers, and Basagran. The plots were harvested manually after physiological maturity.

4.2.5 Data collection: phenotyping

The following traits were measured:

- Plant height (PH): Measured from the ground level to the point of insertion of the flag leaf.
- Ear height (EH): Measured from the ground level to the insertion of the highest ear in the stem.
- Number of primary tassel branches (PTB): Total number of primary tassel branches was counted per genotype/plot.
- Number of leaves (NL): Total number of leaves counted per genotype/plot.
- Ear aspect (EA):Rated on scale from 1 to 5, where 1= good and 5 = bad.
- Flake aspect (FA) : Rated on scale from 1 to 5, where 1=good and 5= bad
- Ear rot (ER): Determined by counting the number of rotten ears per genotype/plot.
- Flowering date: Number of days to 50% tasseling (days to anthesis, DA) and 50% silking (days to silking).

- Number of plants (NP): Number of plants counted per genotype/plot.
- Stem lodging (SL): Determined by counting the number of plants broken below the upper ear at harvest.
- Root lodging (RL): Determined by counting the number of plants broken by more than 45o from the bottom of the root.
- Number of ears (NE/ ear prolificacy: Total number of ears per genotype/plot.
- Grain moisture (GM%): Measured using grain moisture metre.
- Chlorophyll content (CC index): Measured using the chlorophyll metre (CCM-200 plus).
- Leaf Area (LA) (m^2): Determined using the leaf area metre.
- Popping expansion volume (PEV): Determined using hot air popping machine.
- Number of unpopped kernels (UPK): Number of unpopped kernels counted after popping.
- Ear turc (ET) was determined at Cedara using 1 to 5 rating scale, where 1=resistant and 5=susceptible.
- Seed yield (SY) in tons/ha was determined using the following equation and moisture content was adjusted to 14% (ideal grain moisture for popping).
- Grain yield (tons/ha) = (Field weight x10/plot area)* (100-GM) x Shelling percent

  Where GM = Grain moisture percentage, shelling percentage (weight shelled/ weight unshelled)*100.

4.2.6. Sample for cluster analysis

Twenty randomly selected popcorn inbred lines from the 83 inbred lines used in genetic diversity analysis are described in Table 4.1.

Table 4.1: Description of 20 popcorn inbred lines used in genetic diversity study

Inbred line	Code	Entry	Name	Pedigree	Origin	Population of derivation
1	DL01	4	11MAK2-4	LpopF3-5-B-1	CERU-11CR1-5-1	Pop-F_2
2	DL02	13	11MAK2-13	LpopF3-18-B-1	CERU-11CR1-16-1	Pop-F_2
3	DL03	17	11MAK2-17	BRAZ-SE015-6-1-5-B-1	CERU-11CR1-24-1	LR
4	DL04	28	11MAK2-28	LOCALF3-14-B-1	CERU-11CR1-51-1	Pop-F_2
5	DL05	20	11MAK2-20	BRAZ-SE015-6-2-2-B-1	CERU-11CR1-28-1	LR
6	DL06	23	11MAK2-23	BRAZ-SE015-14-3-1-B-1	CERU-11CR1-39-1	LR
7	DL07	41	11MAK2-41	LOCALF3-70-B-1	CERU-11CR1-75-1	Pop-F_2
8	DL08	12	11MAK2-12	LpopF3-17-B-1	CERU-11CR1-15-1	Pop-F_2
9	DL09	6	11MAK2-6	LpopF3-7-B-1	CERU-11CR1-7-1	Pop-F_2
10	DL10	26	11MAK2-26	LOCALF3-1-B-1	CERU-11CR1-48-1	Pop-F_2
11	DL11	36	11MAK2-36	LOCALF3-43-B-1	CERU-11CR1-61-1	Pop-F_2
12	DL12	38	11MAK2-38	LOCALF3-51-B-1	CERU-11CR1-64-1	Pop-F_2
13	DL13	18	11MAK2-18	BRAZ-SE015-6-1-6-B-1	CERU-11CR1-25-1	LR
14	DL14	50	11MAK2-50	09MAK4-122/09MAK20-1-1-1	CERU-11CR1-91-1	BC_1-F_2
15	DL15	55	11MAK2-55	09MAK4-182/09MAK20-3-2-1	CERU-11CR1-97-1	BC_1-F_2
16	DL16	42	11MAK2-42	LOCALF3-72-B-1	CERU-11CR1-77-1	Pop-F_2
17	DL17	9	11MAK2-9	LpopF3-10-B-1	CERU-11CR1-10-1	Pop-F_2
18	DL18	33	11MAK2-33	LOCALF3-40-B-1	CERU-11CR1-58-1	Pop-F_2
19	DL19	75	11MAK2-75	09MAK4-182/09MAK20-15-2-1	CERU-11CR1-127-1	BC-F_2
20	DL20	24	11MAK2-24	BRAZ-SE015-16-1-1-B-1	CERU-11CR1-41-1	LR

LR= land race pop; PopF2= biparental segregating population; BC=backcross F_1 and F_2 population.

4.2.7 Genotyping

Twenty popcorn inbred lines described in Table 4.1 were genotyped.

4.2.7.1 DNA sampling

The random sample of 20 inbred lines was grown in the tunnel and leaf tissues were sampled at three weeks after planting. The tissues were sampled from 8 plants for each inbred line and were bulked. The leaves were cut into 10-15 cm sections. The samples were then placed in a plastic (screen mesh) bag and were identified with tags. The samples were kept cool in an ice box. The DNA was extracted following the CTAB (mixed alkyltrimethyl-ammonium Bromide protocol: DNA extraction buffer) as described by CIMMYT (2005). The concentration of the extracted DNA was determined using 0.7% Tris-Borate-EDTA (TBE) agarose. A working concentration of 10 ng εl-1 was standardized for all extracted DNA (Erasmus, 2008). The samples were bulked and used in SSR amplification.

4.2.7.2 Genotyping – Polymerase Chain Reaction (PCR)

The PCRs were performed using 12 εl of reaction mixture containing 1 x PCR buffer, 2.5 mM Mg++, 0.2 εl each of dNTPs (Bioline), 1 unit of Taq polymerase (Bioline) and 5-10 ng of genomic DNA. Primers were labeled with a 104 fluorescent dye. Two primers were provided for the amplification of each SSR locus: one tailed forward primer (0.05 εmol) and one normal reverse primer (0.25 εmol). The initial denaturation step was performed at 94ºC for 2 minutes, followed by 33 cycles at 94ºC for 30 seconds, Annealing of primer at primer specific 3ºC for 30 seconds and 72ºC for 45 seconds with a final extension for 20 m minutes (Erasmus, 2008).

4.2.7.3 SSR amplification

The SSR amplification was carried out at the INCOTEC PROTEIOS laboratory (South Africa Pty (Ltd). A total of 29 markers were screened for genotyping. However 7 markers did not amplify in PCR. The 22 markers which amplified were used for genotyping and are listed in Table 4.2. The PCR products were labeled fluorescently and were thereafter separated using capillary electrophoresis on an ABI 3130 automatic sequencer (Applied Biosystems, Johannesburg, SA) and were subjected to analysis.

Table 4.2: List of 22 SSR markers used for the study of genetic diversity in popcorn inbred line population

Number	SSR Markers
1	1 Phi079
2	2 Phi062
3	Phi065
4	Phi072
5	nc130
6	nc133
7	Phi029
8	Phi031
9	Phi075
10	Phi084
11	Phi02228
12	Phi112
13	Phi114
14	Phi123
15	Phi299852
16	Phi308707
17	Phi331888
18	Phi374118
19	umc1304
20	umc1545
21	umc2250
22	Phi076

4.2.8 Statistical Analysis

Data were analyzed using REML procedure in GenStat following a random effects model. The model used was $Y_{ijk}= \mu+ E_i+B_j+ E_{ijk}$, where Y_{ijk}= observed response of inbred lines; μ= overall population mean; E_i =entry effect; B_j = effect of the block in the jth replication; E_{ijk}= random error term. Hierarchical cluster analysis was performed on the matrix using Genstat 14th edition. The matrices of genetic distances were used to perform dendograms based on morphological data. For the dendogram based on SSR markers, the program GGT 2.0 was used to calculate the Euclidian distances between popcorn inbred, and the matrix of the genetic distances was used to create UPGMA dendogram. The SSR analysis was performed using GeneMapper® Software Version 4.1 (van Berloo, 2008). Frequency distribution histograms were created using GenStat 14th edition.

Analysis of variance, cluster analysis was performed using GenStat 14th edition. Genetic parameters were calculated by the following equations:

Heritability (broad sense) (H^2) = (V_G/V_P)*100, where V_G is the genetic variance and V_P is the phenotypic variance (Allard, 1960; Burton and DeVane, 1953).

V_P=MSg/r ; V_G=(MSg-MSe)/r and V_E=MSe

Where V_P, V_G, and V_E are the variance components, phenotypic, genetic and environmental variance. MSe is a mean square error and r is the number of replications (Johnson *et al.*, 1955; Uguru, 1995).

PCV =($\sqrt{VP}$/ X) ×100; GCV = ($\sqrt{VG}$/X)×100

Where PCV = phenotypic coefficient of variation; GCV= phenotypic coefficient of variation; X = grand mean for each trait (Allard, 1960; Burton, 1952).

GA = H^2*SD*i, where GA=Genetic advance; H^2 = heritability in a broad sense, SD= standard deviation and i = selection intensity (i=2.01) and was expressed as the percentage of mean GA = (GA/X) *100 where X = mean of each trait.

4.3 Results

4.3.1 Genetic parameters

The genetic parameters are presented in Table 4.3.

Eighteen phenotypic traits showed the highest heritability. Genetic and phenotypic coefficient of variation was high for 6 and 7 traits, respectively. Genetic advance (%) was large for 11 traits, while genetic variation was high for six traits.

Table 4.3: Estimates of variance components, genetic advance and broad sense heritability for 23 traits in 83 popcorn inbred lines

Traits	MEAN	VG	H^2	PCV	GCV	GA	GA(%)
Anthesis silking interval	-0.53	0.36	26.10	-211.59	-108.87	0.61	-131.71
Chlorophyll concentration	9.12	29.09	38.81	96.21	59.05	7.42	82.46
Days to anthesis	72.50	10.36	89.02	4.71	4.44	7.63	10.53
Days to silking	72.02	11.32	86.67	5.02	4.67	7.75	10.77
Ear aspect	3.06	0.97	70.88	37.73	31.87	1.77	57.89
Ear height(cm)	90.52	198.20	61.71	19.45	15.33	22.58	24.91
Ear length (cm)	16.40	2.80	49.78	15.00	10.22	2.25	13.82
Ear rot	2.41	5.53	89.30	103.42	97.73	4.53	188.39
Ear turc	2.95	0.17	81.76	14.84	13.41	0.87	29.31
Grain moisture content(%harvest)	12.67	0.24	28.59	7.71	3.80	0.59	4.62
Grain moisture content(%popping)	12.14	0.74	93.59	6.28	6.19	1.49	12.48
Seed yield (tons/ha)	1.11	0.25	61.64	58.26	45.22	0.97	87.29
Kernel aspect	1.96	0.70	58.71	46.76	35.94	1.55	74.39
Leaf area (m^2)	429.55	3594.50	38.71	22.20	13.85	78.35	18.38
Number of ears	12.20	10.24	65.73	34.33	27.57	5.64	49.05
Number of leaves	11.16	1.10	57.33	12.81	9.47	1.74	15.64
Plant height (cm)	199.50	357.95	58.67	12.24	9.22	30.83	15.32
Number of primary tassel branches	15.48	9.99	44.44	30.81	20.34	4.44	28.79
Root Lodging	1.36	1.98	48.52	144.11	92.38	2.06	138.96
Stem lodging	5.03	13.54	85.37	79.19	73.17	7.22	143.58
Kernel size	81.63	410.69	96.95	25.21	24.83	46.59	57.08
Popping expansion volume (cm^3)	862.30	28957.00	75.73	22.68	19.73	419.19	48.61
Number of unpopped kernels	98.00	3511.00	77.56	68.66	60.46	69.39	70.8

VG=genetic variation; H^2=heritability; PCV=phenotypic coefficient of variation; GCV=genetic coefficient of variation; GA=genetic advance (%)

4.3.2 Genotyping

Results from SSR analysis are presented in Table 4.4. The total number of alleles detected was 76, the alleles ranged from 2-8 with an average of 3.5. A minimum of two

alleles was detected at several loci and maximum number 8 of alleles were detected at Phi114.

Table 4.4: Size range and number of alleles of 22 SSR markers used in the study of genetic diversity among 20 popcorn inbred lines.

SSR Marker	Size Range	Number of Alleles
Phi079	190-215	3
Phi062	170-186	2
Phi065	149-195	3
Phi072	166-186	3
nc130	155-165	5
nc133	116-138	2
Phi029	167-178	2
Phi031	198-210	2
Phi075	241-265	6
Phi084	170-190	2
Phi02228	135-146	2
Phi112	155-180	2
Phi114	152-191	8
Phi123	159-169	7
Phi299852	122-144	4
Phi308707	135-158	3
Phi331888	142-160	4
Phi374118	225-250	6
umc1304	136-166	3
umc1545	90-108	3
umc2250	163-173	2
Phi076	183-197	2
Total		**76**
Average		**3.5**
Maximum		**8**
Minimum		**2**

4.3.3 Cluster analysis

(a) Cluster analysis based on yield.

Cluster analysis based on seed yield (tons/ ha) is shown in Figure 4.1. Grouping of inbred lines is denoted by A-F. The inbred lines at 0.995 cut off point and 3 clusters at 0.970 cut-off point as denoted by alphabets (A-F). The distance among inbred lines ranged from 0-100 (Table 4.5).

(b) Cluster analysis based on popping expansion volume

A dendogram based on popping expansion volume is shown in Figure 4.2. Inbred lines formed four clusters at 0.993 cut-off point. The Euclidean distance among inbred lines ranged from 0-100 (Table 4.6).

(c) Cluster analysis based on traits with high heritability (>50%)

Dendogram based on traits with high heritability (>50%) is presented in Figure 4.3. Popcorn inbred lines were grouped into 7 major clusters. The distance between inbred lines ranged from 1-100 (Table 4.7).

(d) Cluster analysis based on molecular data

The dendogram constructed based on SSR data matrices grouped the inbred lines into five major clusters (Figure 4.4). The distance among inbred lines ranged from 0.14-0.77 (Table 4.8).

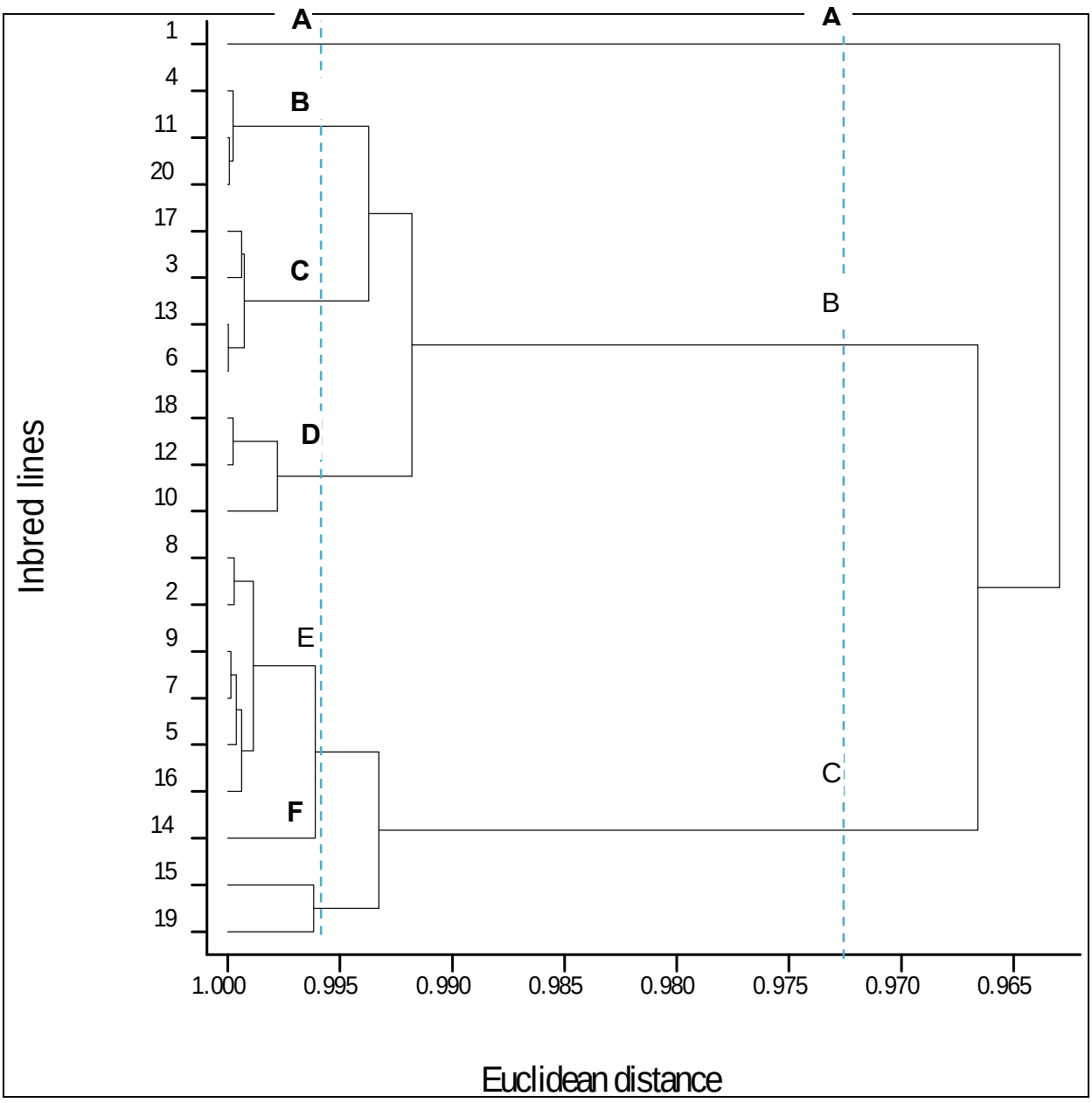

Figure 4.1: Dendogram based on seed yield (tons/ha) for 20 popcorn inbred lines in Population 1.

Table 4. 5: Matrix table of Euclidean genetic distance among 20 popcorn inbred lines based on their seed yield (tons/ ha)

1	----																			
2	51	----																		
3	90	85	----																	
4	96	74	98	----																
5	41	100	80	67	----															
6	88	88	100	98	83	----														
7	44	100	82	69	100	84	----													
8	53	100	87	76	99	89	100	----												
9	46	100	83	70	100	85	100	100	----											
10	75	96	97	90	93	98	94	97	95	----										
11	96	75	99	100	69	98	71	77	72	91	----									
12	79	94	98	93	90	99	92	95	92	100	94	----								
13	88	87	100	98	82	100	84	89	85	98	98	99	----							
14	27	98	71	56	99	75	99	97	99	88	58	84	74	----						
15	12	95	62	44	97	66	96	94	96	81	47	77	65	99	----					
16	37	99	78	64	100	80	100	99	100	92	66	89	80	100	98	----				
17	91	83	100	99	78	100	79	85	81	96	99	97	100	69	59	75	----			
18	81	93	99	94	89	99	91	94	91	100	95	100	99	83	75	88	98	----		
19	0	91	54	35	95	58	94	90	93	75	37	71	57	98	100	96	50	69	----	
20	95	76	99	100	70	98	72	78	73	92	100	94	98	59	48	67	99	95	39	----
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20

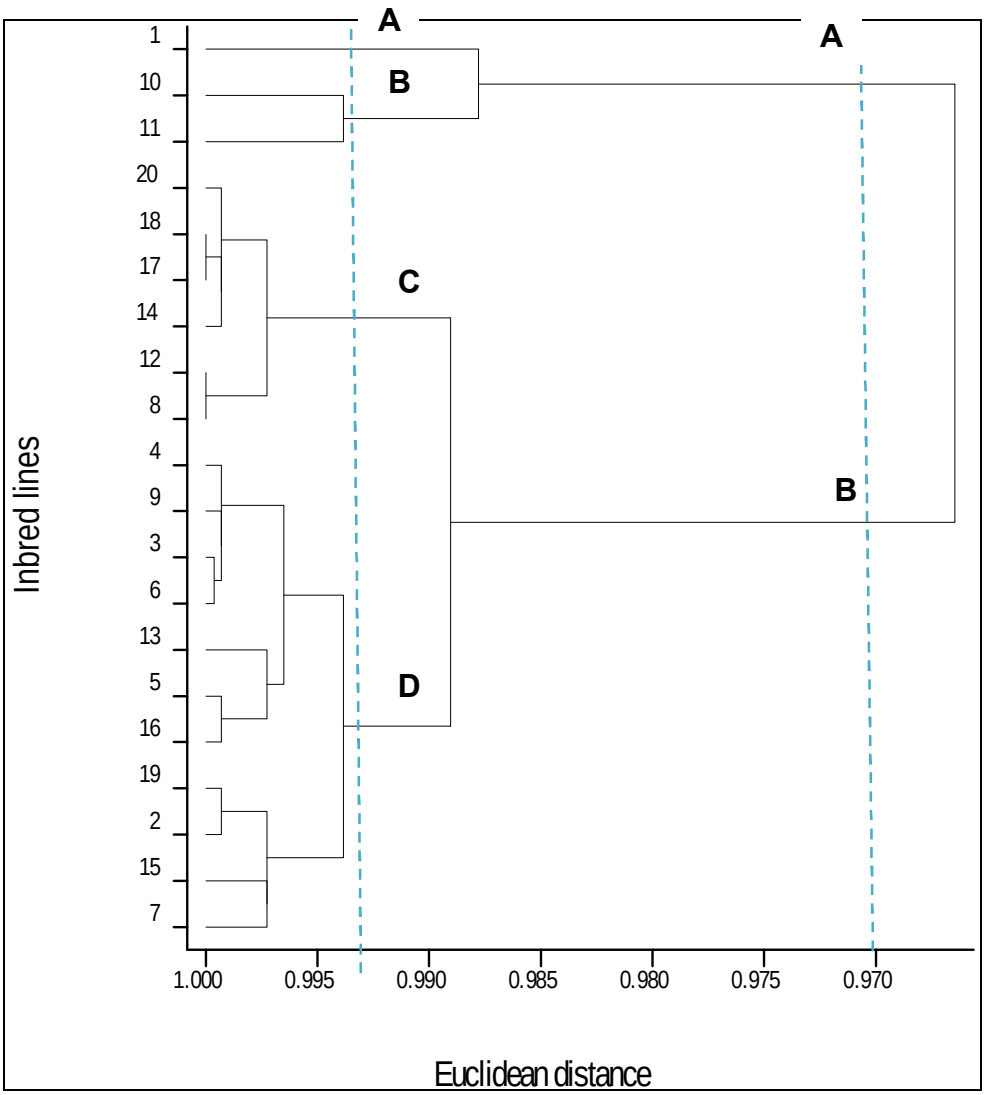

Figure 4.2: Dendogram based on popping expansion volume (cm^3) for 20 popcorn inbred lines in Population 1

Table 4.6: Matrix table of Euclidean genetic distance among 20 popcorn inbred lines based on their popping expansion volume (cm^3)

1	----																		
2	20	----																	
3	60	93	----																
4	66	90	100	----															
5	42	98	98	97	----														
6	57	94	100	100	99	----													
7	0	99	87	83	95	88	----												
8	77	83	98	99	92	97	73	----											
9	63	92	100	100	98	100	85	98	----										
10	99	38	73	78	57	71	21	87	75	----									
11	96	50	80	85	67	79	34	92	83	99	----								
12	77	83	98	99	92	97	73	100	98	87	92	----							
13	49	97	99	98	100	100	92	95	99	64	73	95							
14	82	78	96	98	88	95	67	100	97	90	95	100	92	----					
15	10	100	90	87	97	91	100	78	88	30	42	78	95	73	----				
16	38	99	98	96	100	98	96	90	97	54	64	90	99	87	98	----			
17	84	75	95	97	87	94	64	99	96	92	96	99	90	100	70	85	----		
18	84	75	95	97	87	94	64	99	96	92	96	99	90	100	70	85	100	----	
19	25	100	95	92	99	95	98	85	93	42	54	85	98	80	99	99	78	78	----
20	86	73	93	96	85	92	61	99	95	93	97	99	88	100	67	83	100	100	75	----
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20

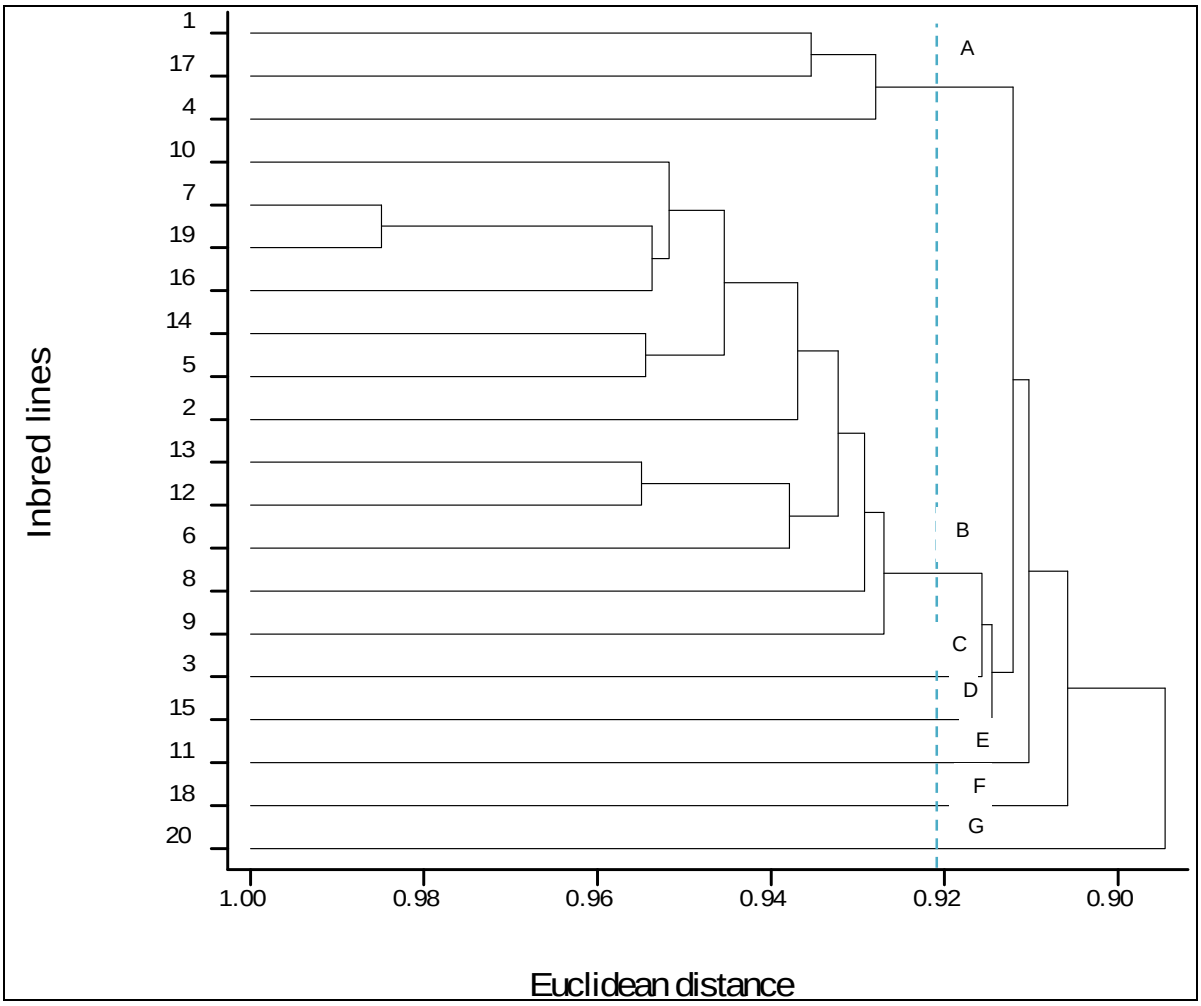

Figure 4.3: Dendogram based on traits with H^2>50 for the twenty randomly selected inbred lines of population 2

Table 4. 7: Matrix table of Euclidean genetic distance among 20 inbred lines based on traits with high heritability (>50%)

1	----																			
2	89	----																		
3	80	83	----																	
4	85	83	67	----																
5	86	89	87	77	----															
6	88	84	89	79	89	----														
7	83	94	71	82	88	80	----													
8	81	89	66	90	83	76	92	----												
9	85	91	84	82	89	92	89	87	----											
10	91	92	81	87	94	90	95	91	93	----										
11	89	78	84	75	80	91	71	66	79	81	----									
12	86	88	84	89	85	90	88	85	93	92	81	----								
13	89	89	92	80	90	94	86	81	91	93	86	96	----							
14	85	91	85	83	95	89	90	91	92	95	78	87	89	----						
15	75	92	84	63	90	76	82	71	79	81	72	71	78	86	----					
16	83	93	79	82	87	87	95	93	92	93	76	89	89	94	81	----				
17	94	90	85	93	87	89	82	86	88	91	91	91	90	88	78	84	----			
18	81	89	91	83	85	87	83	83	87	84	82	88	87	89	84	91	89	----		
19	77	90	70	78	87	80	99	89	89	94	65	86	85	88	80	95	77	81	----	
20	89	83	82	77	84	80	73	79	81	85	80	80	86	88	73	78	90	75	66	----
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20

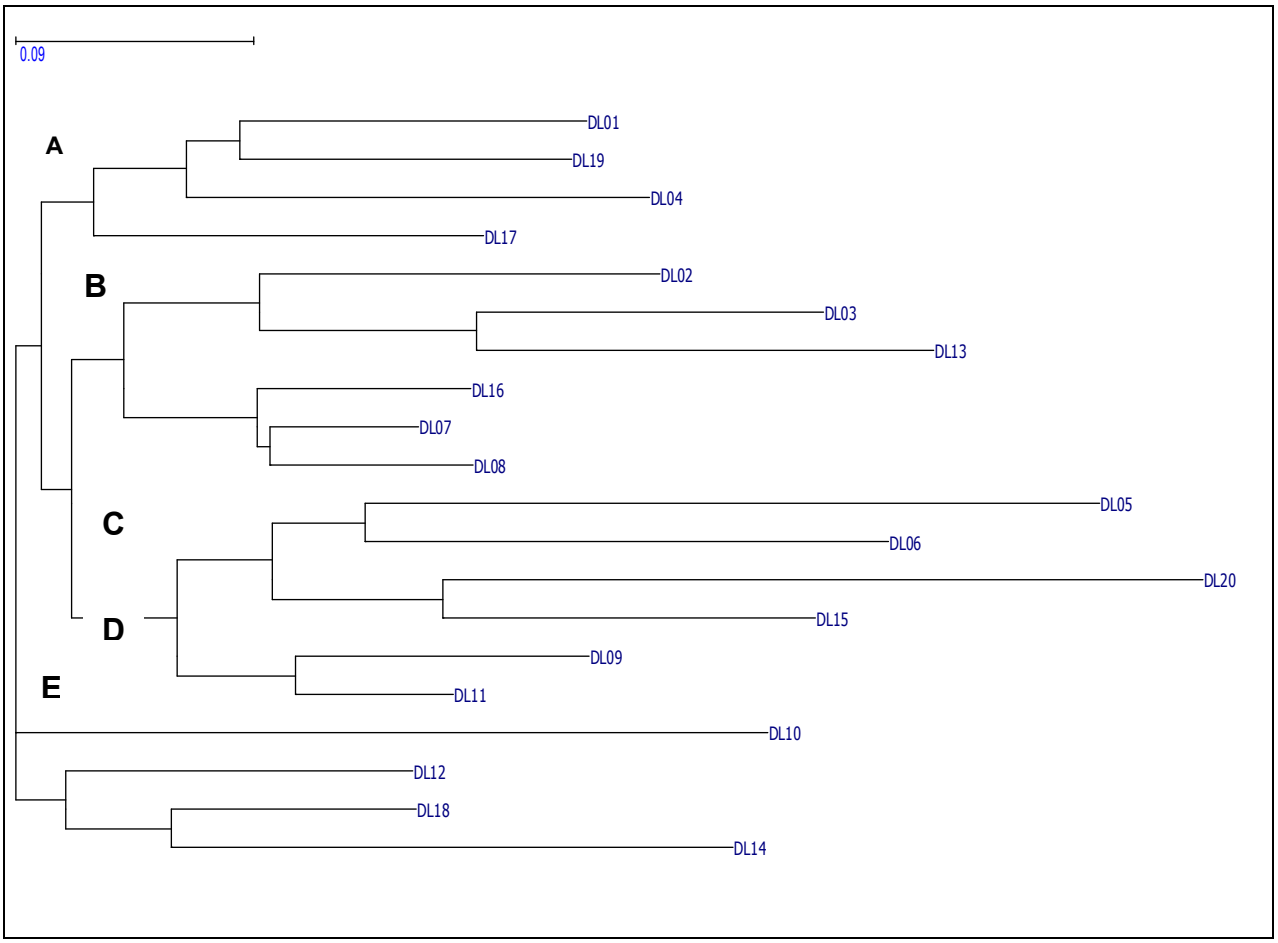

Figure 4.4: Dendogram of 20 popcorn inbred lines achieved by UPGMA grouping based on SSR markers

Table 4.8: Dissimilarity matrix table of Jaccard genetic distance among 20 inbred lines analyzed by 22 SSR markers

	DL01	DL02	DL03	DL04	DL05	DL06	DL07	DL08	DL09	DL10	DL11	DL12	DL13	DL14	DL15	DL16	DL17	DL18	DL19	DL20
DL01																				
DL02	0.41																			
DL03	0.64	0.36																		
DL04	0.36	0.36	0.59																	
DL05	0.68	0.64	0.59	0.59																
DL06	0.64	0.59	0.45	0.50	0.50															
DL07	0.45	0.36	0.36	0.45	0.55	0.45														
DL08	0.45	0.36	0.36	0.50	0.64	0.55	0.14													
DL09	0.50	0.41	0.50	0.36	0.59	0.36	0.27	0.27												
DL10	0.50	0.45	0.68	0.50	0.64	0.64	0.50	0.55	0.50											
DL11	0.45	0.45	0.45	0.45	0.55	0.45	0.18	0.14	0.18	0.55										
DL12	0.27	0.50	0.50	0.45	0.59	0.45	0.27	0.36	0.41	0.45	0.32									
DL13	0.59	0.45	0.32	0.64	0.59	0.59	0.50	0.41	0.59	0.68	0.50	0.50								
DL14	0.59	0.59	0.64	0.59	0.73	0.59	0.50	0.50	0.45	0.55	0.41	0.45	0.64							
DL15	0.59	0.64	0.64	0.55	0.50	0.50	0.36	0.41	0.36	0.64	0.32	0.41	0.68	0.50						
DL16	0.36	0.36	0.36	0.55	0.59	0.55	0.14	0.18	0.41	0.50	0.27	0.27	0.50	0.50	0.50					
DL17	0.27	0.41	0.55	0.41	0.64	0.64	0.32	0.27	0.45	0.50	0.32	0.36	0.50	0.41	0.50	0.27				
DL18	0.32	0.36	0.45	0.41	0.73	0.50	0.36	0.36	0.41	0.50	0.36	0.23	0.50	0.32	0.55	0.27	0.32			
DL19	0.27	0.45	0.55	0.32	0.55	0.55	0.32	0.41	0.50	0.59	0.32	0.36	0.59	0.64	0.55	0.36	0.36	0.41		
DL20	0.64	0.73	0.77	0.55	0.68	0.64	0.64	0.68	0.50	0.73	0.59	0.68	0.77	0.64	0.45	0.77	0.59	0.73	0.59	

4.3.4 Phenotypic traits

The variation among the popcorn inbred lines is reflected by characteristics of the random sample of 20 inbred lines. Economic and secondary traits are presented in Table 4.9 and 4.10, respectively.

Table 4. 9: Economic traits of the 20 inbred lines used in diversity analysis

Inbred line	Name	Popping expansion(cm^3)	Unpopped kernels	Kernel aspect	Grain moisture	Kernel size (kernels/ 10g)
1	11MAK2-4	621.2	171	3.4	11.6	99
2	11MAK2-13	1049	29	1.2	12.9	71
3	11MAK2-17	924	149	1.3	10.7	130
4	11MAK2-28	899	60	2.5	12.0	96
5	11MAK2-20	986.5	25	2.5	12.1	92
6	11MAK2-23	933.3	17	2.0	10.8	78
7	11MAK2-41	1099	67	1.5	12.8	81
8	11MAK2-12	849	154	2.0	12.5	98
9	11MAK2-6	911.5	118	1.5	12.1	88
10	11MAK2-26	674	243	2.2	12.6	92
11	11MAK2-36	711.5	170	3.5	11.8	92
12	11MAK2-38	849	121	1.7	11.8	86
13	11MAK2-18	961.5	91	1.5	11.5	93
14	11MAK2-50	824	52	2.2	12.2	92
15	11MAK2-55	1074	39	1.3	12.5	73
16	11MAK2-42	999	107	1.3	12.5	82
17	11MAK2-9	811.5	160	2.8	10.6	102
18	11MAK2-33	811.5	64	1.3	12.1	83
19	11MAK2-75	1036.5	44	0.9	12.3	64
20	11MAK2-24	799	115	3.0	12.7	98

Table 4. 10: Secondary traits for the 20 inbred lined used in diversity analysis

Inbred lines	Entry code	SY(t/ha)	DA	DS	ASI	PH (cm)	EH (cm)	RL	SL	NP	NE	ER	EL (cm)	EA	ET	NL	PTB	CC	LA(m^3)	GM(%)
1	**E4**	0.23	72	72	0	199.8	86.5	1	3	13	5	4	16.3	4	3.48	11	20	1.61	330.8	11.4
2	**13**	1.03	71	70	-1	201.2	90	0	2	15	11	5	17.4	2.75	3.48	11	13	11.18	451.1	12.9
3	**17**	0.59	76	79	2	202.4	94.5	1	0	14	13	1	14.3	4.5	3	12	24	6.06	416.7	12.0
4	**28**	0.45	69	70	0	178.9	78.5	3	7	15	12	10	16.7	4.5	3	10	11	6.26	342	11.8
5	**20**	1.11	75	75	-1	196.5	87	0	2	14	13	1	16.8	2.5	2.75	10	10	15.77	403	13.0
6	**23**	0.63	74	74	-1	183.4	88	4	1	15	8	1	14.4	3.5	2.77	11	15	5.63	453	11.0
7	**41**	1.09	69	68	-1	191.5	75.5	0	2	12	11	1	17.9	2.75	3	10	9	8.88	460	13.6
8	**12**	1.01	70	68	-1	180.6	69	0	8	15	14	4	16.1	2.5	3.28	11	15	1.91	372.2	12.8
9	**6**	1.07	73	72	-1	180.3	75	3	0	13	13	2	15.6	3	3.53	10	20	3.82	395.5	12.8
10	**26**	0.8	71	72	2	186.1	79	1	4	14	10	1	17.0	3.25	2.99	10	13	7.58	338.4	13.0
11	**36**	0.46	73	72	0	212.3	96	5	2	13	9	0	14.9	4.75	2.95	12	11	10.71	386.3	12.5
12	**38**	0.75	70	71	1	181.6	81.5	1	1	15	13	4	13.8	4.5	3.03	10	12	3.61	469.3	12.9
13	**18**	0.62	73	73	-1	193.2	88	1	2	14	9	0	13.6	4.25	3.01	10	20	5.11	393.8	12.1
14	**50**	1.21	74	73	-1	174	88	0	4	14	14	0	16.7	2.75	3.04	11	15	7.28	329.4	12.7
15	**55**	1.3	74	75	1	218	101	0	1	14	15	2	18.9	2	3	12	20	9.42	522.4	12.8
16	**42**	1.14	70	69	-1	179.1	77.5	1	3	15	12	0	15.9	2.75	3.04	12	14	5.94	425.2	13.2
17	**9**	0.57	72	72	0	202.2	89.5	3	5	14	12	6	15.4	4.25	3.28	11	15	2.59	390.8	11.9
18	**33**	0.73	73	73	-1	193.3	80	1	2	13	15	6	15.5	4	2.77	13	14	7.2	422.4	12.2
19	**75**	1.37	69	69	0	184.8	71.5	0	1	15	11	0	18.3	2.5	2.77	10	15	15.17	448.4	13.6
20	**24**	0.47	75	74	-1	189.1	99.5	0	7	14	11	1	15.1	4.25	3.99	11	25	10.92	307.1	12.7

SY= seed yield; DA= days to anthesis, DS= daus to silking, ASI= anthesis silking interval, EH = ear height (cm), PH= plant height(cm), RL= root lodging, SL=stem lodging, NP=number of plants, NE=number of ears, ER= ear rot, EL=ear length (cm), EA = ear aspect, ET= ear aspect, NL= number of leaves, PTB= primary tassel branches, CC= chlorophyll concentration, LA= leaf area (m^2), GM= grain moisture (%).

4.3.5 Frequency distribution of phenotypic traits

Frequency distributions of phenotypic traits of 83 and 81 popcorn inbred lines in Population 1 and Population 2, respectively, are presented in Figure 4.5 ï Figure 4.20, where a and b denotes traits for Population 1 and 2, respectively. Histograms below present the distribution of phenotypic traits and hence, diversity of the population was detected from the distribution of phenotypic traits.

(a) Flake aspect

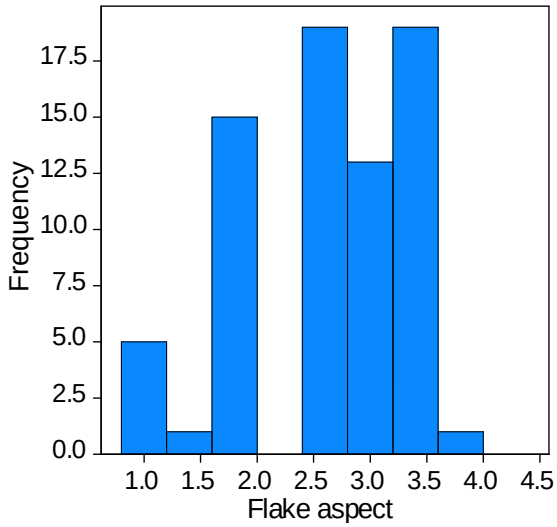

Figure 4.5a: Flake aspect (1-5) for Population 1

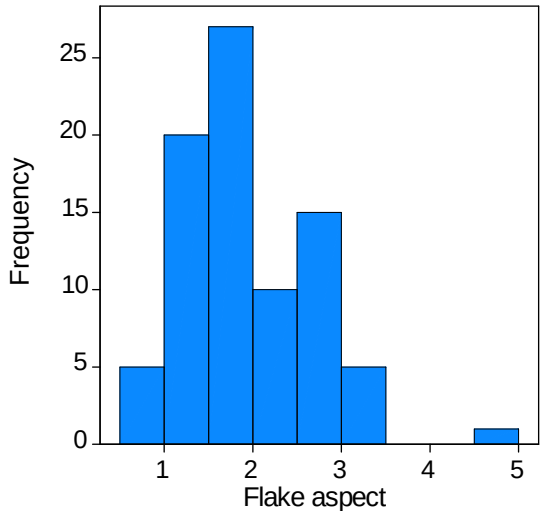

Figure 4.5b: Flake aspect (1-5) for Population 2

(b) Number of unpopped kernels

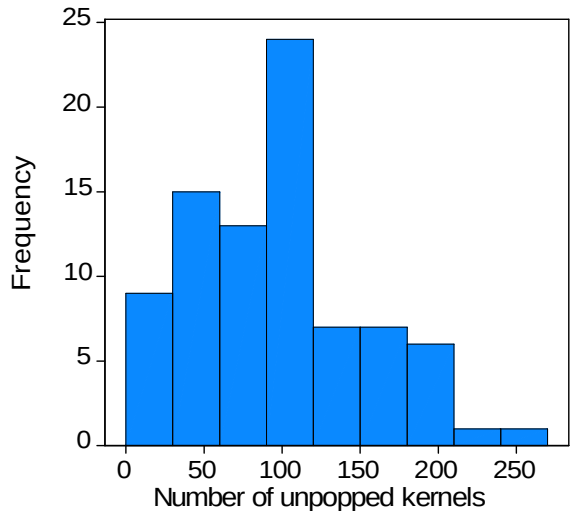

Figure 4.6a: Number of unpopped kernels in Population 1

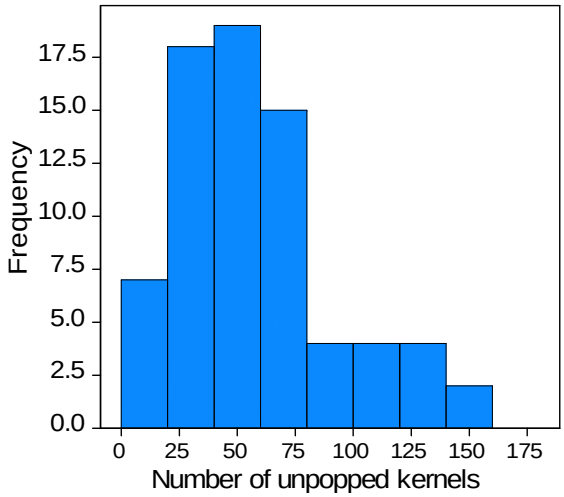

Figure 4.6b: Number of unpopped kernels in Population 2

(c) Popping expansion volume

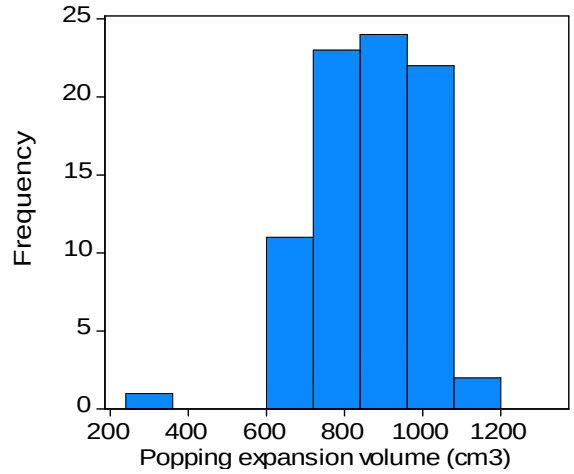

Figure 4.7a: Popping expansion volume (cm^3) in Population 1

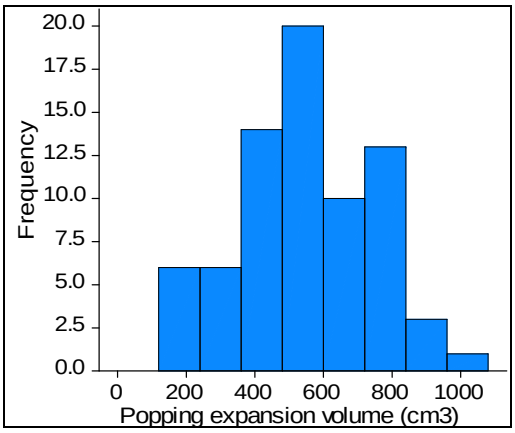

Figure 4.7b: Popping expansion volume (cm^3) in Population 2

(d) Seed yield

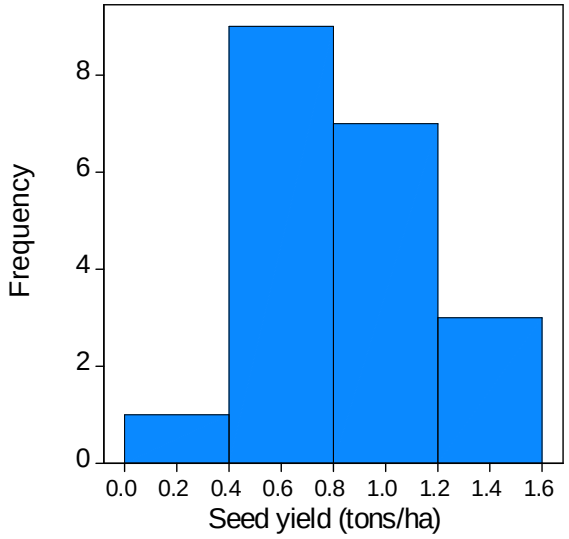

Figure 4.8a: Seed yield (tons/ha) in Population 1

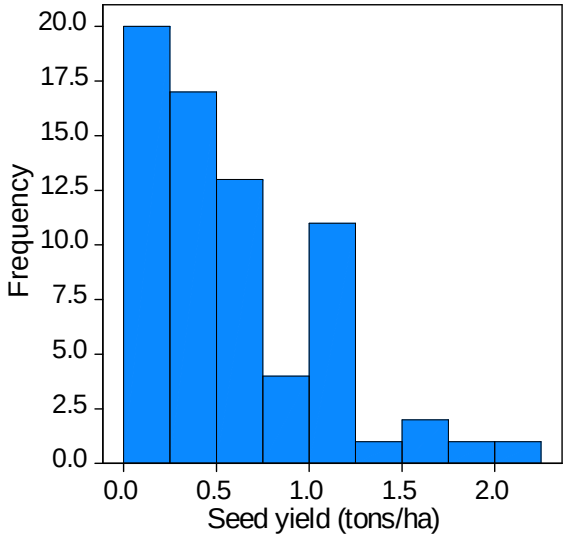

Figure 4.8b: Seed yield (tons/ha) in Population 2

(e) Kernel size

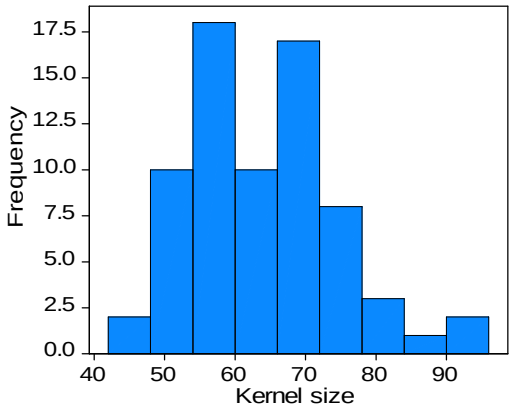

Figure 4.9a: Kernel size in Population

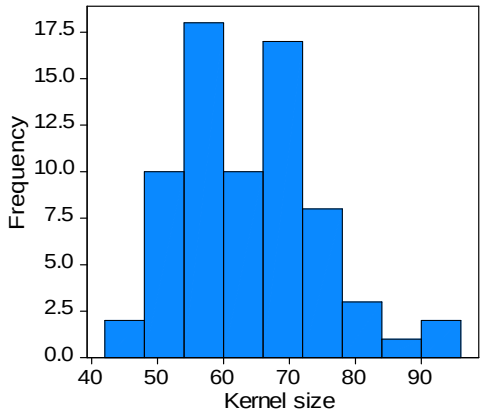

Figure 4.9b: Kernel size in Population 2

(f) Plat height

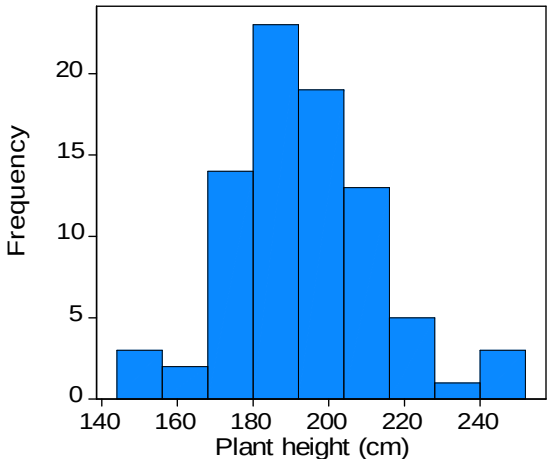

Figure 4.10a: Plant height (cm) in Population 1

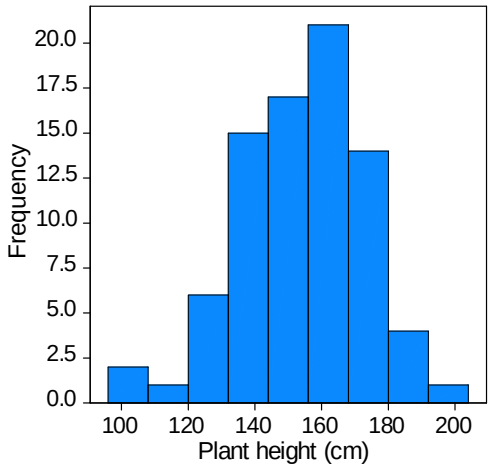

Figure 4.10b: Plant height (cm) in Population 2

(g) Days to anthesis

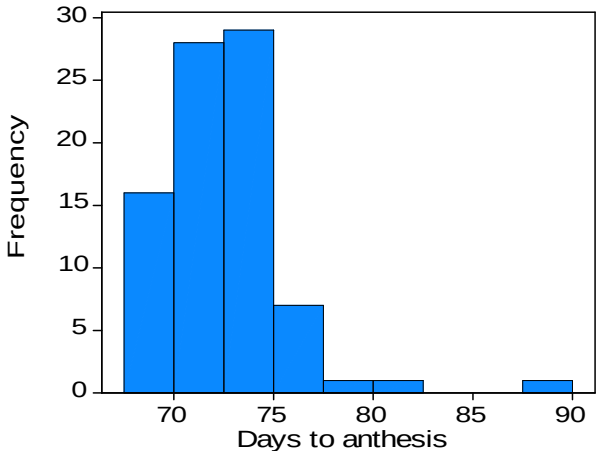

Figure 4.11a: Days to anthesis in Population 1

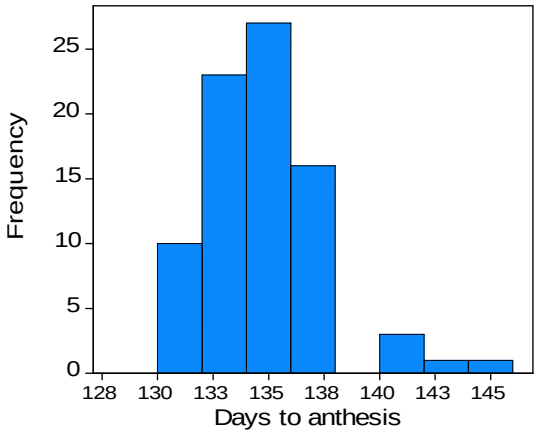

Figure 4.11b: Days to anthesis in Population 2

(h) Days to silking

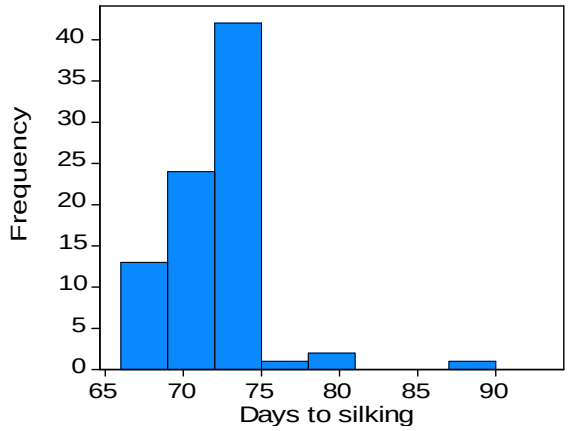

Figure 4.12a: Days to silking in Population 1

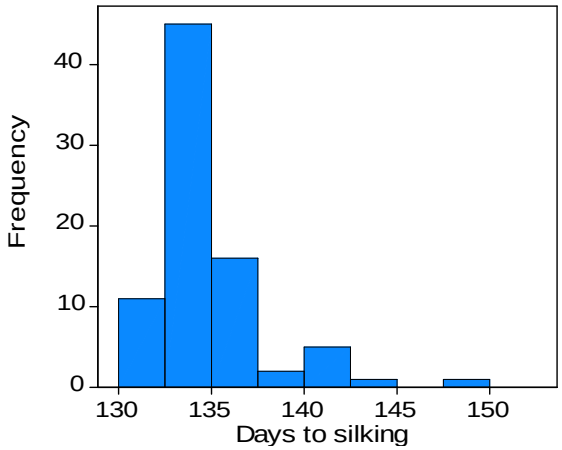

Figure 4.12b: Days to silking in Population 2

(i) Anthesis silking interval

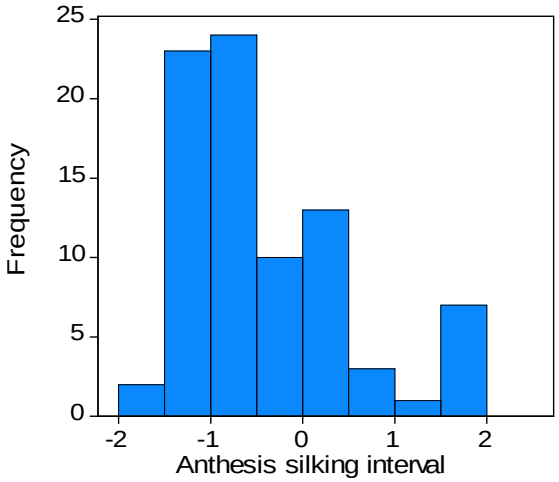

Figure 4.13a: Anthesis silking interval in Population 1

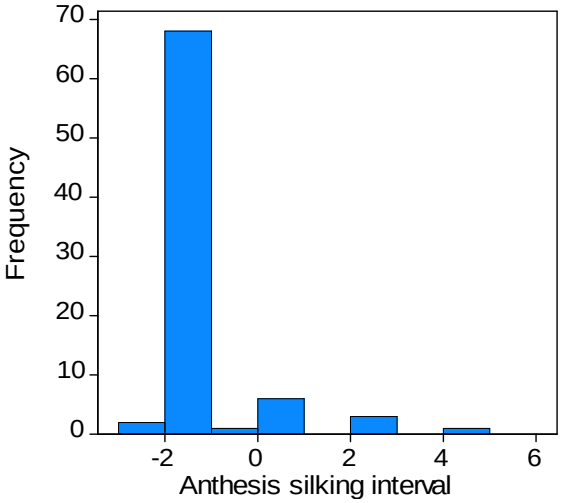

Figure 4.13b: Anthesis silking interval in Population 1

(j) Number of leaves

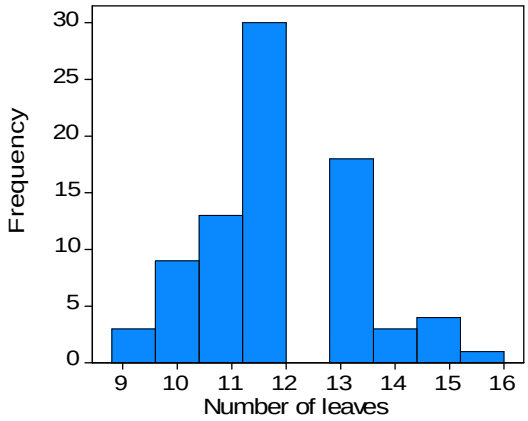

Figure 4.14a: Number of leaves in Population 1

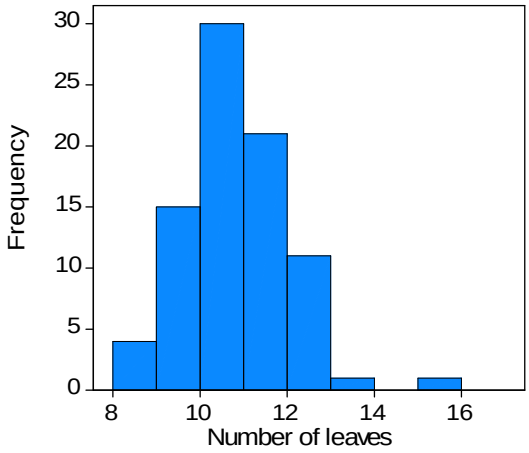

Figure 4.14b: Number of leaves in Population 2

(k) Chlorophyll concentration

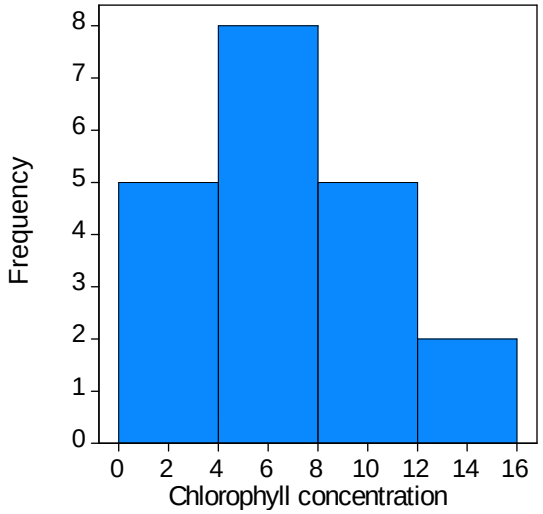

Figure 4.15a: Chlorophyll concentration in Population 1

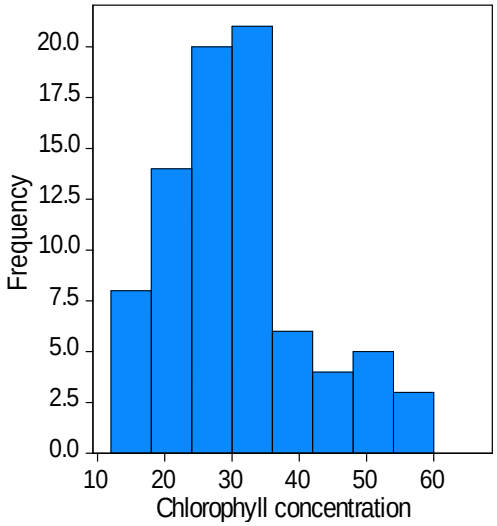

Figure 4.15b: Chlorophyll content in Population 2

(l) Number of primary tassel branches

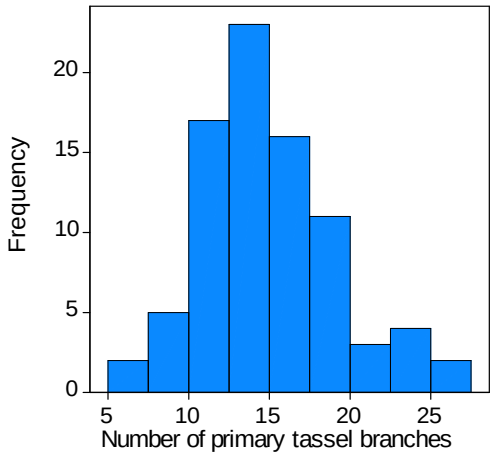

Figure 4.16a: Number of primary tassel branches in Population 1

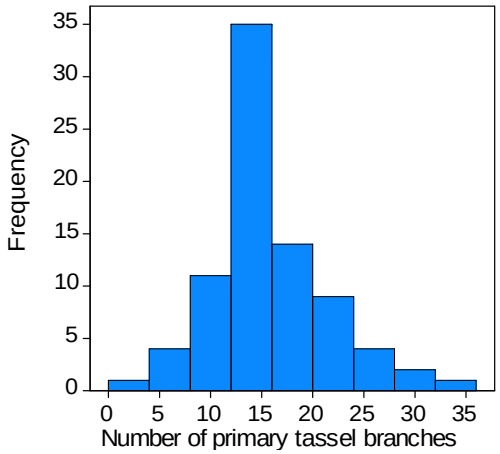

Figure 4.16b: Number of primary tassel branches in Population 2

(m) Ear aspect

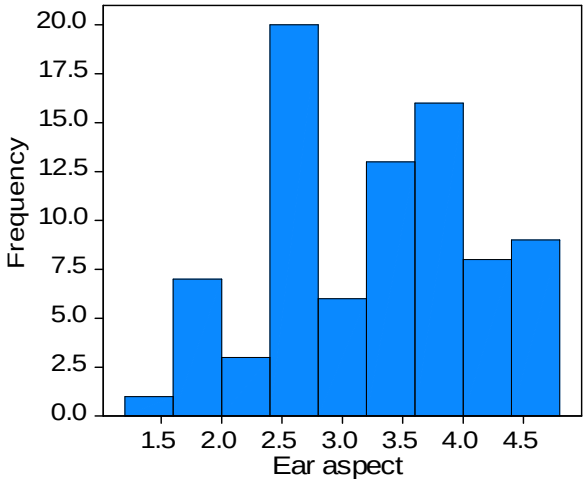

Figure 4.17a: Ear aspect in Population 1

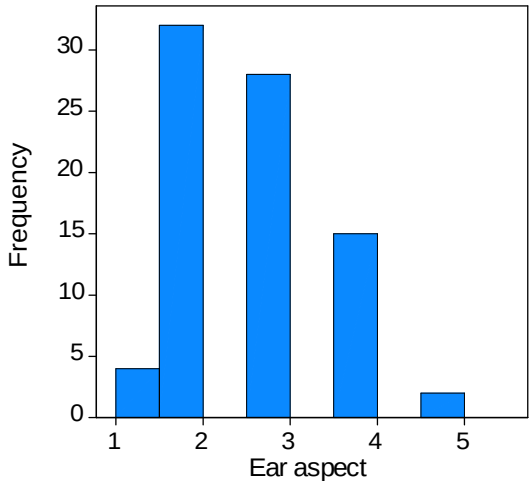

Figure 4.17b: Ear aspect in Population 2

(n) Grain moisture content

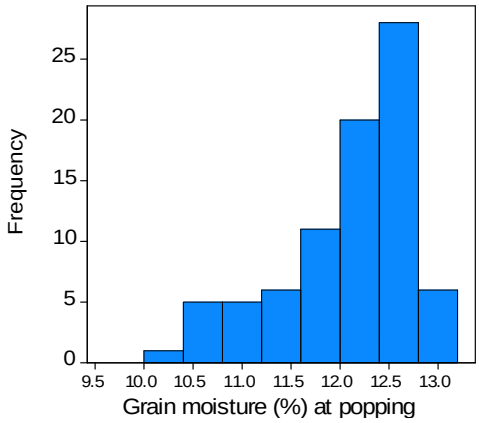

Figure 4.18a: Grain moisture content in Population 1

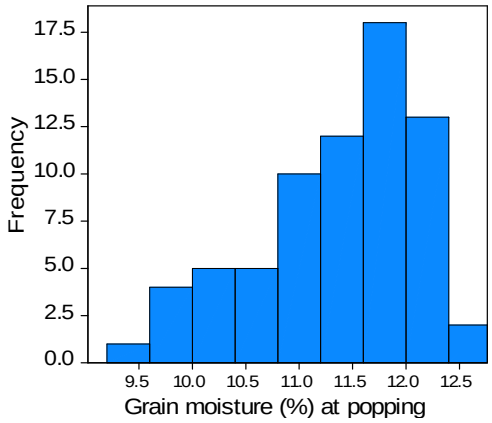

Figure 4.18b: Grain moisture content in Population 2

(o) Ear turcum

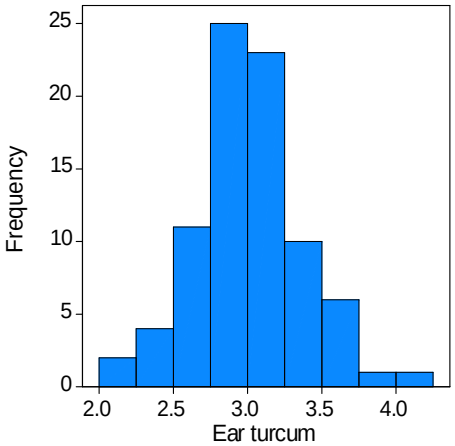

Figure 4.19a: Ear turcum in Population 1

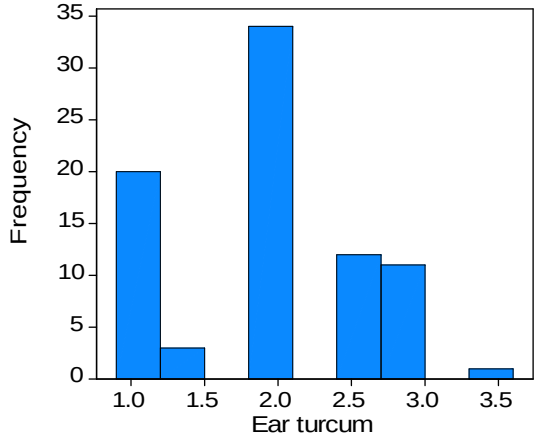

Figure 4.19b: Ear turcum in Population 2

(p) Ear height

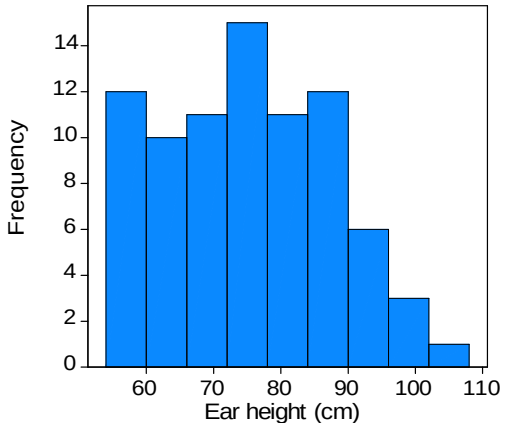

Figure 4.20a: Ear height (cm) in Population 1

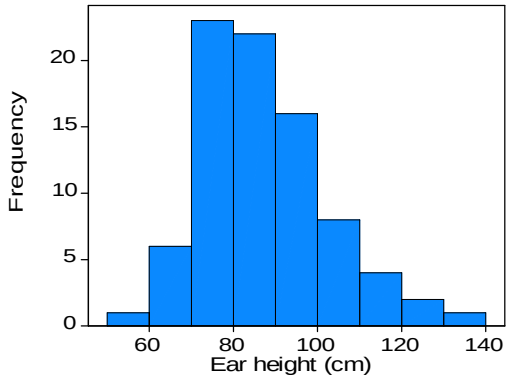

Figure 4.20b: Ear height (cm) in Population 2

4.4 Discussion

4.4.1 Genetic Variation

The results of heritability were high for most of the phenotypic traits. For example the heritability values obtained in the study of two complex traits, popping expansion volume (75.73%) and seed yield (61.64%) are in agreement with other popcorn investigations. Pereira and Junior (2001) found heritability of 77.75 % and 57.48 % for popping expansion volume and seed yield, respectively. In another study, Pereira and Junior (2001) reported a very high heritability for PEV (82.72%) and therefore, expected an expressive genetic gain for popping ability than seed yield during the selection process. Heritability of plant height (58.71%) was closer to the value of 60.47% and 63% obtained by Coimbra *et al.* (2002) and Pereira and Junior (2001), respectively. High heritability obtained for the studied traits is an indication of greater genetic gain from selection during the breeding process. High broad sense heritability is an indication of the presence of large additive gene action of the studied traits. These traits can, therefore, be improved through individual plant selection, which can also contribute to large genetic variation exploited by plant breeders during the breeding process (Pereira and Junior, 2001).

High genetic variance in other traits is the evidence of the presence of genetic diversity in the popcorn population. The results obtained for phenotypic and genetic coefficient of variation suggested that most of the phenotypic traits are governed by genetic factors and, hence, genetic improvement can be achieved through selection. Selection based on the genetic value can be more effective as there is a minor role of the environment in the expression of these traits. Greater genetic advance obtained for ear aspect, seed yield, popping expansion volume and number of unpopped kernels and other traits is an indication of greater chances of selection and therefore, greater genetic gain from selection. Furthermore, it is an evidence of large genetic diversity within the population.

4.4.2 Genetic polymorphisms

Large genetic diversity was found in the inbred line popcorn population. Twenty two SSR markers used detected a total of 76 alleles among 20 inbred lines studied. The number of alleles ranged from 2 - 8 with an average of 3.5. The greater number of alleles detected suggested that SSR markers were spread all over the genome. This further indicated allelic richness , hence, an indication of high genetic diversity at a molecular level among the studied inbred lines (Li *et al.*, 2004). Results further suggested that the germplasm sources of the inbred lines used was broad and, therefore, greater chances of improving popcorn inbred line population. Leal *et al.* (2010) detected a total of 47 alleles for 14 SSR loci, with an average 2 ï 5 alleles per locus. Bracco *et al.* (2009) evaluating 131 popcorn landraces using 9 SSR loci, found a total of 65 alleles. Silva *et al.* (2009) evaluating 25 popcorn genotypes observed a total of 100 alleles from 23 SSR markers. Li *et al.* (2004) detected a total of 306 alleles across 113 loci, with an average of 2.7 per locus. Fifty-seven alleles with an average of 3.7 alleles per locus were detected by Eloi *et al.* (2012) also in a popcorn population. Therefore, results obtained in the current study are generally consistent with previous investigations and findings.

4.4.3 Cluster analysis

Genetic diversity of the 20 randomly selected inbred lines was performed based on morphological data; seed yield (tons/ha) and popping expansion volume (cm^3). The similarity distance ranged from 0 - 100 for both seed yield and popping expansion volume, and many clusters were formed indicating a high genetic diversity among the inbred lines and different heterotic groups. The detected heterotic groups can therefore be used in further improvement of germplasm and development of more superior inbred lines. When the dendogram was performed based on traits with high heritability (>50%), 7 major distinguishable groups were formed. For example inbred lines from cluster A had a similar genetic background based on the source of origin. However, other inbred

lines were not grouped according to their source of origin, this suggested the distant relationship between inbred lines and, hence, the presence of large genetic diversity. Large genetic diversity among the inbred lines could be associated with a large number of ancestors that were used during the development of these lines. The results of the evaluated inbred lines suggest that they have different genetic backgrounds and hence the range of genetic diversity is high in relation to various traits. Therefore, these lines can be useful in the long term improvement of popcorn genotypes and subsequently local and adapted popcorn varieties with a wide range of genetic diversity can be developed by crossing the distant lines.

The SSR markers further revealed a large genetic diversity in the popcorn inbred line population. Based on the dendogram constructed on SSR data, popcorn inbred lines were grouped into five major clusters. The heterotic groups formed indicated great differences among the studied inbred lines. The closest distance was found between inbred line 7 and 8. Cluster C consisted mostly of the lines derived from the Brazilian landraces (5, 6 and 20). These results corresponded with the pedigree relationship and indicated that these inbred lines had similar genetic background. However other lines which were derived from the Brazilian land races did not fall in the same group. Most of the inbred lines were not grouped according to their source of origin. For example, in Cluster A, inbred lines 1(LpopF3-5-B-1) and 17 (LpopF3-10-B-1) were related, but, inbred lines in the same group did not reflect their pedigree. This could be explained by the fact that inbred lines might have similar genetic background contributed by the genes incorporated during the development of these lines and therefore, they are related at a distant level which further contributes to genetic diversity. (Wende *et al.*, 2012).

The study contrasts the findings of other researchers. Li *et al.* (2004), Paula *et al.* (2010), and Dandolini *et al.* (2008) observed that the division and subdivision of all the studied inbred lines reflected their pedigree. Inbred lines from similar sources were grouped together and lines from different germplasm formed separate groups. These

results indicated that the relationship of most of the inbred lines they used was at closer distance and, therefore, narrower genetic diversity. Classification of inbred lines from the same source into different heterotic groups can be essential for broadening popcorn germplasm. Therefore, the results from the current study indicate greater chances of developing popcorn inbred lines with a wide range of genetic diversity and greater value for cultivation and use.

There was no clear relationship between dendograms and distant matrices obtained by SSR markers and phenotypic data. The observations could be attributed to that when SSR markers are compared with morphological data, they cover a large proportion of the genome, which include coding and non-coding regions. The regions covered by molecular markers, therefore, have no relationship with the studied morphological traits (Goncalves *et al.*, 2009; Paula *et al.*, 2010).

4.4.4 Frequency distribution of phenotypic traits

The distribution of phenotypic traits also revealed the presence of genetic diversity among the popcorn inbred lines. The presence of continuous distribution, normal distribution, negative and positive skewness on different traits showed genetic variation for both populations. The observation of continuous distribution for most traits, such as chlorophyll concentration (Figure 4.15a and 4.15b), indicates the role of many genes or quantitative trait loci for the control of the traits. The distribution of genotypes for the number of leaves revealed two classes of genotypes with 9-12 leaves and 13-16 (Figure 4.14a), and 8-14 and 16 leaves (Figure 4.14b). This indicates that there could be major QTLs that confer the number of leaves in this set of popcorn lines. A similar trend was observed for the flake aspect (Figure 4.5a and 4.5b). Overall, most of the secondary traits indicated the presence of large genetic variation among the popcorn inbred line population and, therefore, good chances of popcorn improvement through selection.

4.5 Conclusion and Implications

The SSR markers and phenotypic data grouped inbred lines differently, but, they were equally consistent in displaying diversity among the lines, with 4-7 clusters observed. Further, evidence for genetic variation was revealed by the frequency distribution histograms. The investigated inbred lines showed a wide range of genetic variation and diversity. These inbred lines can, therefore, be used in subsequent selection. Selecting and crossing distant and best inbred lines from different heterotic groups will maximize the level of genetic diversity and minimize genetic vulnerability in the population. The studied inbred lines can be further used for conservation strategies in popcorn breeding.

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CHAPTER 5

General Overview of the Research Findings

5.1 Introduction and objectives of the study

The narrow genetic base of popcorn has been of major concern to the breeders, hence, investigation on genetic diversity of popcorn is extremely important especially within the available popcorn population. The major objectives of popcorn breeders are to develop popcorn varieties with high popping expansion volume and high grain yield. This chapter outlines the findings of the study conducted. The objectives of the study, summary of the research findings, breeding implications of the findings and its challenges, future directions in popcorn breeding and closing remarks are highlighted.

The specific objectives of the study were to:

v. Determine genetic variation among popcorn inbred lines.

vi. Investigate the magnitude of genetic diversity among popcorn inbred lines.

vii. Establish the relationship between seed yield and popping expansion volume, and with secondary traits aswell as the relationship among secondary traits.

viii. Evaluate the effect of microwave oven and hot air popping method in popping expansion volume and number of unpopped kernels of different popcorn inbred lines.

5.2 Summary of research findings

The study on popping methods demonstrated that hot air popping is a more efficient and effective method with regard to discriminating power of different genotypes popping expansion volume and unpopped kernels. The study further showed that genotype x popping method interaction is significant for popping expansion volume

and number of unpopped kernels, therefore different inbred lines are specific to different popping methods.

Positive relationship was found between popping expansion volume and seed yield suggesting the possibility of simultaneously improving yield and popping performance. The results also showed that grain yield could be improved by selecting traits such as, prolificacy, days to anthesis and ear aspect. Popping expansion volume could be increased through selection based on a good kernel aspect. Direct selection for traits contributing to yield and popping expansion volume was more important than indirect selection of these traits, therefore direct selection strategy is supported.

The study indicated the presence of large genetic variation and diversity among the inbred lines from the studied population, indicating the possibility of developing superior and adapted popcorn varieties in South Africa.

5.3 Implications of the research findings

The following implications could be drawn from the study:

- As a result of large genetic diversity identified among popcorn lines, there is room for selection among the distant inbred lines, thereby, allowing popcorn improvement for enhanced seed yield and popping expansion volume. Furthermore, locally adapted popcorn varieties could be developed from the available germplasm.

- The results on the appraisal of popping methods for rapid screening of popcorn inbred lines revealed that most of the inbred lines are adapted to different popping methods. Therefore, when popping performance of different inbred lines is evaluated, the use of different methods will better discriminate popping ability of genotypes. Popcorn varieties will be recommended based on the method that may yield the highest flake volume.

- Results on the relationship between popping expansion, seed yield and secondary traits implied that, seed yield and popping expansion volume can

be improved concurrently with selection based on genotypes combining both high popping expansion volume and high yield. Improvement of seed yield can be made through selection for prolific plants, good ear aspect, and plants with less number of days to anthesis. Popping performance improvement is possible via selection for good kernel aspect and not necessarily other traits.

5.4 Challenges in popcorn breeding

Popcorn production is Sub-saharan Africa (SSA) is hampered by non- availability of superior variety options with high seed yield and high popping ability. This is associated with limitations of information on genetic structure and diversity of popcorn populations as well as varieties with desirable agronomic traits. Efficient popping methods that discriminates popping ability of different genotypes are not well established especially in SSA where consumers use different methods to process popcorn. These methods are required for selecting and developing new improved superior and varieties with high popping ability.

5.5 Recommendations

The following recommendations evolved from the study:

i. Inbred lines which showed high level of genetic diversity should be crossed further to develop new popcorn varieties that are locally adapted in South African conditions. This will lead to the availability of superior varieties with better popping ability and seed yield, hence will increase output and reduce importation.

ii. The performance of the best genotypes in terms of popping expansion volume and seed yield should be further tested in different locations within the country, this will confirm the superiority of the selected genotypes.

iii. Development of industrial hot air popping machines is recommended. This will allow breeders to easily test popping performance on the large samples they work with.

iv. Popcorns can be improved and transformed into a food security crop to benefit children from malnutrition - vulnerable communities. For example, popcorn requires biofortification with vitamin A. Therefore there is a room for popcorn improvement in this regard.

5.6 Conclusion

The major objective of the study was to quantify the levels of genetic diversity, correlations and path coefficients of the secondary traits on seed yield and popping ability in an experimental inbred line population. The study was successful at quantifying the level of genetic variation and diversity among the popcorn inbred lines. Further relationships among traits especially between secondary traits with seed yield and popping expansion volume were ascertained with implications for breeding strategy. Above all, the study confirms that genotype × popping method interaction is crucial as it will impact both breeding progress and dissemination of the variety technology to communities who may use different methods for popping.