Quality prediction and improvement in Lesotho commercial wheat (*Triticum aestivum* L.) cultivars

by

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Submitted in fulfilment of the requirements for the degree of
Philosophiae Doctor in Plant Breeding

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2005

Dedication

This thesis is dedicated with affection to my wife, 'Maithabeleng Edith and my three loving daughters, Ithabeleng Janice, Lemohang Violet and Amohelang Bernice whose courage, good humour and patience have been an inspiration to me.

Acknowledgements

Several groups of people and individuals deserve special recognition for the assistance they provided during my research. First, I wish to extend my sincere gratitude to my supervisor, prof. M.T. Labuschagne and to dr. H. Maartens for their wholehearted support and guidance during my research and acknowledge their critical comments and suggestions.

Special thanks go to Mrs. Sadie Geldenhuys for logistics and administrative support and for making my studies very enjoyable.

I am also indebted to the National Research Foundation for awarding me the scholarship that made it possible for me to pursue my studies.

Finally, and by no means least, I wish to thank my parents, my father Moses Neo and my late mother, Malebohang Julia, all my sisters and brothers for their moral support and encouragement during my academic adventure.

Table of contents

List of tables

List of figures

Abbreviations

AACC	American Association of Cereal Chemists
ANOVA	Analysis of variance
ASA	American Society of Agronomy
BPH	Best Parent Heterosis
CSSA	Crop Science Society of America
CV	Coefficient of Variation
DDT	Dithiothreitol
DF	Degree of Freedom
F_1	First fillial generation
F_2	Second fillial generation
FPC	Flour protein content
FLY	Flour yield
GxE	Genotype x Environment Interaction
GCA	General Combining Ability
Gli	Gliadin
Glu	Glutenin
h_b^2	Heritability in the broad sense
h_n^2	Heritability in the narrow sense
HMW-GS	High Molecular Weight Glutenin Subunits
HPH	High Parent Heterosis
Kda	Kilodalton
LMP	Large Monomeric Protein
LMW-GS	Low Molecular Weight-Glutenin Subunits
LPP	Large Polymeric Protein
LSD	Least Significant Difference
MDT	Mixograph development time
MS	Mean square
MSE	Mean square error
NCSS	Number Cruncher Statistical System

P	Probability
PR	Predictability ratio
R^2	Coefficient of determination
SCA	Specific Combining Ability
SDSS	Sodium Dodecyl Sulphate Sedimentation Volume
SKCSD	Single Kernel Characterization Seed Diameter
SKCSH	Single Kernel Characterization Seed Hardness
SKCSW	Single Kernel Characterization Seed weight
SMP	Small Monomeric Protein
SPP	Small Polymeric Protein
SDS-PAGE	Sodium Dodecyl Sulphate- Polyacrylamide Gel Electrophoresis
SE-HLPC	Size exclusion – High Performance Liquid Chromotography
V_a	Additive Variance
V_d	Dominance Variance
δ	Variance
δ_P	Phenotypic variance
δ_g	Genotypic variance

Chapter 1

Introduction

Common wheat (*Triticum aestivum* L. em.Thell) is one of the world's most economically important crop as evidenced by its production and land allocated to it. It ranks second after rice. It is grown mainly for breadmaking, while a small quantity is used for pasta, macaroni and biscuits (Briggle & Curtis, 1987).

In Lesotho, wheat is an economically important crop, ranking third after maize and sorghum (Lesotho Agricultural Sector Investment Programme, 1997). It is grown countrywide for home consumption in the mountain areas and commercially in the lowlnands (Ntokoane, 1992). In the mountain areas, wheat is grown in summer, while in the lowlands, it is grown in winter when high yields are realised. According to Moeletsi (2004), each crop can produce high yield if the environmental conditions are suitable for it.

According to Namane (1992), wheat was introduced by the missionaries upon arrival around 1833 when sorghum, maize and millet were predominating. Its production increased dramatically, to such an extent that Lesotho started exporting to South Africa. In 1891, a total of 8648 ton was exported to South Africa from Lesotho, thereafter a decline in national production was experienced due to depletion of soil fertility, inadequate technical advice and trade restrictions imposed on Lesotho. According to Mokitimi (1990), wheat was exported until 1972.

Many wheat cultivars were and are still imported from other countries into Lesotho where they are tested under different environments for adaptability, yield potential, disease and pest resistance, fertiliser levels, time of planting and seeding rate (Namane, 1992). No breeding programmes have been established in Lesotho. Kena (2003) provided the strategies for small scale farmers to manage plant diseases as an effrt to increase production.

The wheat requirement in Lesotho is over 122 000 metric tons per annum, of which 22% is produced in the country (Ntokoane, 1992). This shows that wheat contributes significantly to the imbalance of payments and a large trade deficit. According to Ntokoane (1992), production of wheat has declined

gradually in terms of total acreage and production per unit area. In 1972/73, area allocated to wheat was 18622 ha, while in 1990/91 it reached a low level of 3000 ha. Productivity from a hectare averaged 1396 kg ha $^{-1}$ in 1976/77 and declined to as low as 625 kg ha $^{-1}$. According to the Lesotho Agricultural Sector Investment Programme (1997) and Mokitimi (1990), many reasons have been put forward to explain the causes of decline in yields, which include, among others; lack of animal power and farm machinery, drought prevalence, low soil fertility, insufficient financial assistance to farmers, lack of knowledge on proper agronomic practices, use of poor yielding cultivars, serious Russian aphid infestation, pre-sprouting of wheat on a standing crop and unattractive wheat prices offered by milling companies. They further indicated that for wheat to once more be an export crop and a foreign currency earner, the above-mentioned problems must be overcome by growing high yielding and Russian aphid resistant cultivars, planting at the right time, applying adequate fertilisers and harvesting at the appropriate time. Similarly, Ranthamane (2001) indicated that maize production in Lesotho is limited by low soil fertility, low temperature, unreliable rainfall, low organic matter content and the use of unimproved seed.

Wheat producers comprise the commercial farmers, applying a high input model, semi-commercial farmers employing a medium input model and subsistence farmers applying low input models (Ntokoane, 1992). According to data from the Food Self Sufficient Food Program (Ntokoane, 1992), commercial farmers must produce 2000 kg ha $^{-1}$ of wheat to meet the break-even point, while a subsistence farmer must produce 500 kg ha $^{-1}$ to break-even.

In an effort to increase yield in Lesotho, many exotic cultivars have been imported into Lesotho, broadening the genetic base of the wheat crop in Lesotho, even though some landraces are extinct due to negligence. These landraces and exotic cultivars have not been characterised by either morphological or biochemical means to distinguish or identify those that could be used in future programmes for valuable traits, that can contribute to both yield and quality improvement of the crop in Lesotho (Lesotho Agricultural Sector Investment Programme,1997). Several efforts have been made to institute a cultivar release committee, but all in vain. The Ministry of Agriculture, through the crops division, documented a proposal which was accepted, but not implemented (Lesotho Agricultural Sector Investment Programme, 1997).

In the past, wide collections of cultivars obtained from outside Lesotho were tested for agronomic characteristics only and no effort was made to evaluate them for breadmaking quality (Spiller Milling Limited, 1984). It was only in the advent of National Milling Companies that wheat quality became important as the price at which wheat was bought, was based on the milling and breadmaking quality (Spiller Milling Limited, 1984). Most of Lesotho's wheat cultivars were poor in quality to such an extent that hard wheat had to be imported from elsewhere to blend and improve them. Some were obtained from South Africa, the Netherlands, Argentina and Australia. This conscientized the Lesotho farmers to such an extent that they have now embarked on improvement of wheat quality by manipulating cultural methods, applying more fertiliser and selecting cultivars with good milling and baking quality. It was therefore imperative to determine the breadmaking quality of the most commonly grown cultivars in Lesotho and to estimate their heritability.

The rich genetic pool of wheat grown in Lesotho could be exploited to produce hybrids that would give good baking quality. This can only be achieved by crossing the prospective wheat cultivars in all possible combinations and identifying progenies that have heterotic effects, after the transgressive segregants would be produced and integrated in the breeding programme. According to Arunachalam (1976) and Baker (1978), combining ability is instrumental in facilitating improvement in plant breeding programmes. Diallel analysis provides an opportunity to evaluate a number of lines in all possible combinations.

The objectives of this study were to

(1) characterize wheat cultivars grown in Lesotho according to their glutenin and gliadin banding patterns, with the use of Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE);

(2) estimate general and specific combining ability, heritability, heterosis and correlation of wheat quality traits by crossing poor, medium and good breadmaking cultivars in a full diallel design to produce F_1 and F_2 progeny;

(3) determine the potential of Size Exclusion-High Performance Liquid Chromatography (SE-HPLC) in predicting wheat quality.

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Chapter 2

Literature review

2.1 Protein

Both eukaryotic and prokaryotic cells contain thousands of different types of proteins. Because each species of life possesses a chemically distinct group of proteins, millions of different proteins exist in the biological world (Armstrong, 1983). The genetic material contained in chromosomes determines the protein composition of an organism which makes a species with a macro-molecular uniqueness (Poehlman, 1987).

According to Datta (1994) and Devlin and Witham (1983), proteins are involved in a number of biological functions which include amongst others enzymatic activities, transport of oxygen and copper, storage of food reserves, form structural components of some cell organelles, contraction, protection, hormonal activity and toxins. Biologically active proteins range in molecular weight from six kilodaltons (kDa) to several million kDa (Watson *et al.*, 1987).

2.1.1 Protein structure

Proteins are made up of amino ($-NH_2$) and carboxyl (-COOH) groups joined together by peptide bonds. A compound consisting of two amino-acids joined together is called a dipeptide, while three amino-acids joined together are called tripeptides. When many amino-acids are joined together, they are referred to as polypeptides. Amino acids differ from one another by the chemical composition of their R-group (side-chains). The amino-acids are divided into two broad categories, namely: those 20 having their proteins as their constituents and those occurring free and are designated as the non-protein amino-acids. This latter category has a limited distribution in plants and form a large group of over 200, representing a unique feature of amino-acid metabolism (Armstrong, 1983; Devlin & Witham, 1983).

2.1.2 Levels of protein structure

The polypeptide bond, number and the definite sequence of amino-acids imparts a primary structure of the protein molecule. Many proteins contain more than one polypeptide chain connected to each other by the sulphide bonds. Polypeptide chains reveal three major types of arrangements or orientations, namely; helical, plated sheet or random. These particular coils or spiral arrangements of the polypeptide chain constitute its secondary structure. A pleated sheet is formed when the segments of the polypeptide chain are side by side and joined by hydrogen bonds in such a manner as to produce a zig-zag appearance of the peptide back-bone. In random arrangement, polypeptide secondary structure shows no geometrical order, which is attributed to the surface resulting from folding of amino-acid side-chains rather than the folding of the peptide back-bone. The helical structure may fold over and bend to form various specific patterns called the tertiary structure of the protein molecules. The tertiary structure is maintained primarily by hydrogen bonding and involves interaction of the R-groups. The structure may be disrupted resulting in the irreversible loss of its function when exposed to high temperature, change in pH and radiation (Watson *et al.*, 1987).

Some proteins have a quaternary structure which occurs when several protein units, each with its own primary, secondary and tertiary structure, combine to form a complex unit. Hydrogen bonds are involved in holding subunits together in the maintenance of a quaternary structure. Another type of interaction that holds the quaternary structure together is the association of hydrophobic groups that combine and exclude water. Many proteins that contain multiple subunits seem to be constructed and held together by hydrophobic side chain interactions among sub-units (Ayala & Kiger, 1984; Gardner & Snustad, 1984).

2.1.3 Protein classification

Proteins are broadly classified into three categories (chemical, morphological and biological proteins) according to (1) solubility in water, salt, alcohol and acid, (2) their functions which include structural, contractile, storage protein, transport, hormones, enzymes, protective or toxic and (3) their shape and dimensions, globular folded into a ball shape (albumin and globulin), fibrous consisting of parallel polypeptides that are coiled and stretched out. Last but not least, they are classified according to their chemical composition, being simple proteins, complex proteins, lipoproteins, glycoproteins and

nucleoproteins (Armstrong, 1983; Maartens, 1997).

2.1.4 Occurrence and uses of proteins

The part of the plant that contains the largest amount of protein is the seed, which has been studied intensively over the past centuries. Seed protein comprises organic reserves for young seedlings and is also the major source of world supply of edible proteins for man and animals (Datta, 1994). Seed proteins are given different names depending on the crop species. Seed proteins in maize, dry beans, barley, peas and soybeans are called zein, phaseolin, hordein, vicilin and glycinin, respectively. The structural proteins are found in the endosperm, aleurone layer and embryo. The proteins performing biological functions are located in the cytoplasm, enzymes, membrane, ribosomes, regulation and storage protein. Chemical proteins contitute simple and complex compound such as lipoproteins, glucoprotein and nucleoproteins (Bewley & Black, 1994).

2.1.5 Seed storage proteins in wheat

In wheat seed, storage proteins account for about 8 - 15% of mature grain compared to 40% in legume seed (Shewry *et al.*, 1987). Storage proteins are stored in the form of protein bodies and aleurone grains in the endosperm and aleurone layer, respectively. Storage proteins in wheat endosperm constitute about 80% of the total protein in the grain (Maartens, 1997; de Swardt, 1998).

According to Osborne (1907), seed proteins are divided into four classes on the basis of their solubility; (1) albumin which is water soluble in dilute buffers at neutral pH, (2) globulin which is soluble in salt solution but insoluble in water, (3) glutenin which is soluble in dilute acid or alkaline solution and (4) gliadin which is soluble in 70 - 90% ethanol. Solubilization of some protein requires harsh extraction procedures (Bewley & Black, 1994).

2.1. 6 Classes of seed proteins on the basis of molecular weight

The tendency has been to define seed protein classes on the basis of their molecular weight. Proteins with molecular weights higher than 100 kDa were considered as glutenin, while gliadin was considered to have a molecular weight ranging between 25 and 100 kDa and those proteins with a molecular weight lower than 25 kDa were classed as albumin. Globulin has a molecular weight of 25

kDa. Classification of seed proteins based on molecular weight resulted in an overlapping between globulin and albumin (Watson *et al.,* 1987).

2.1.7 Description of seed protein

Gliadins

Gliadins are wheat proteins soluble in 70 - 90% ethanol when the extraction procedure of Osborne (1907) is followed. It accounts for about 30 - 35% of the total seed protein. According to Shewry *et al.* (1986), gliadins are monomeric proteins suggesting that all the disulphide bonds present are intra-molecular and that the gliadin conformations are stabilized by hydrogen bonding and hydrophobic interactions.

Using one dimensional polyacrylamide gel electrophoresis, gliadins can be separated into four distinct groups, namely; α, β, γ and ω gliadin (Bushuk & Zillman, 1978). The α- gliadins have the highest relative mobility, while ω-gliadin has the slowest relative mobility (Eliasson & Larsson, 1993; de Swardt, 1998). The ω-gliadins account for about 8 - 13% of the total proteins, while α, β, and γ together contribute about 34 - 38% of the total protein. The ω-gliadins differ from the other gliadins because of the absence of α -helix and β-sheet structure.

Payne *et al.* (1984) indicated that the majority of the gliadin proteins of wheat endosperm are simple polypeptides without subunit structure under normal conditions of extractions. Most gliadins have a molecular weight in the range of 30 – 40 kDa, except for ω-gliadins which have a weight of 60 – 70 kDa (Bietz, 1979). Gliadins are comprised of extremely high levels of proline and glutamic acids. The secondary structure of the gliadin polypeptides is affected by the high level of proline which hinders the formation of α-helices. There is a tendency of gliadins to have larger amounts of cysteine, isoleucine, phenylalanine and amide (Ewart, 1967). Almost all of the glutamic acid content of the gliadins is present as glutamine. All gliadins have relatively low levels of aspargine and aspartic acid. Gliadins contain a very low amount of amino-acids such as lysine, glycine, tryptophan, isoleucine and phenylalanine. Because of the combined effect of the low level of these amino-acids, and that of free carboxyl groups, the gliadins are positioned among some of the least charged proteins (Eliasson & Larsson, 1993).

Gliadins are less mobile than other proteins because of their specific amino-acid sequence containing many neutral amino-acids such as glutamine and proline (Patey & Waldron, 1976). The molecular structure of gliadin components take a globular shape and as a result of the high level of proline in all components, the proportion of the α-helical parts are relatively low. The intramolecular sulphide bonds appear in every case (Eliasson & Larsson, 1993).

The level of lysine in gliadin is low, which renders it less valuable. Analysis of gliadin physical properties reveals it to be cohesive, low in elasticity and composed of proteins of relatively low molecular weight (Crow & Rothfus, 1968). According to Popineau and Pineau (1987), the surface hydrophobicities of α, β and γ -gliadins depend on both aromatic and aliphatic amino-acids side chains, whereas those of the ω-gliadins depend mainly on aromatic side-chains.

Further analysis of gliadins revealed that endosperm specific prolamines are most abundant in α-gliadin (Smith, 1984), while ω-gliadins constitute the S-poor prolamins of wheat. Conversely, γ-gliadins are the S-rich prolamin (Tatham *et al.*, 1990). Regardless of the importance of gliadins in breadmaking, they can be toxic to a person with coeliac disease, particularly α-gliadin. Conversely, β-gliadin and other gluten confer immunological properties associated with coeliac disease (Bietz *et al.*, 1977).

Glutenin

Based on the solubility classification of Osborne (1907), glutenins are described as proteins that remain after albumins, globulins and gliadins have been extracted. Glutenin accounts for 40 - 50% of the total protein of wheat flour. Glutenins are soluble or rather dispersible in diluted alkali or acid, in denaturants such as urea and in surfactants. Solubility of glutenins is low, which is attributed to their high molecular weight and to the fact that some of the individual subunits are only sparingly soluble in aqueous alcohols (Shewry *et al.*, 1987). High molecular weight glutenin subunits (HMW-GS) of up to 20 million dalton have been reported (Eliasson & Larsson, 1993).

When glutenin subunits are reduced and fractionated by electrophoretic methods, two groups are revealed on the basis of their apparent molecular mass, namely; HMW-GS ranging from 80 – 150 kDa and low molecular weight subunits (LMW-GS) ranging from 30 – 50 kDa. Where reversed-phase high performance liquid chromatography (RP-HPLC) is employed to analyse glutenin subunits, three groups of glutenin subunits are found differing in hydrophobic groups containing the low molecular weight glutenin, intermediate hydrophobicity containing the high molecular weight glutenin and the lowest hydrophobicity group containing medium molecular weight (Maartens, 1997).

According to Gupta *et al.* (1995), the average molecular weight of the LMW-GS (M_r. 40 kDa) is about one half the average of the HMW-GS (M_r. 85 kDa). The ratio of the LMW-GS to HMW-GS is approximately 1:6. Because of the formation of large polymers and dough strength, high molecular weight glutenins have higher perceptible influence than LMW-GS and it has been inferred from calculations that the effect of one high molecular weight subunit is four times that of one low molecular weight subunit.

The differences between HMW-GS and gliadins are that the former has a high level of glycine and low level of proline (Shewry *et al.,* 1986). Furthermore, glutenin consists of polymers with molecular weights extending into millions which is the product of polymerization of polypeptides through intermolecular disulphide linkage (Hammanzu *et al.,* 1972).

2.1.8 Genes encoding for gliadins and glutenins

Genetic studies revealed that genes encoding most of the wheat α and β gliadins are located on the short arms of homoelogous group 6A and 6B chromosomes, while most ω and γ gliadin genes are on the short arms of chromosomes 1A, 1B and 1D. Genes encoding gliadins are inherited as blocks of components, suggesting that gliadin gemnes are clustered in discrete loci, each of which may vary in composition (Josephides *et al.,* 1987; Lorenzo & Kronstad, 1987). Group 1 and 6 chromosome loci are inherited independently, causing variation that allows cultivars to be distinguished (Mansur *et al.,* 1990: Dong *et al.,* 1991). According to Metakovsky (1990), there is an occurrence of multiple allelism on these loci.

While gliadin is coded by genes located on the short arms of group 1 and 6 chromosomes, HMW-GS are coded by genes on the long arms of chromosome 1A, 1B and 1D. Genes responsible for synthesis of HMW-GS are designated *Glu*-A1, *Glu*-B1 and *Glu*-D1 (Lawrence & Shepherd, 1980). These genes are inherited as separate units, although some are linked. These loci are adjacent to the centromeres (Shewry *et al.,* 1986). HMW-GS range in number from three to five. Due to this small number, cultivars often have the same subunits which make it ineffective for cultivar identification (de Swardt, 1998).

Crosses between cultivars having different HMW-GS composition have resulted in at least 21 allelic variants at glutenin subunits loci *Glu*-A1, *Glu*-B1 and *Glu*-D1 (Payne *et al.,* 1984). HMW-GS are inherited co-dominantly (Shewry *et al.,* 1986). According to Mansur *et al.* (1990) and McIntosh *et al.* (1994), LMW-GS are coded by genes on the short arms of chromosomes 1A, 1B and 1D and the genes are designated *Glu*-A3, *Glu*-B3 and *Glu*-D3, respectively. Gliadin bands 42 and 45 are coded by two co-dominant alleles of a single gene on chromosome 1B which were used as markers for good breadmaking quality in wheat cultivars (Bushuk & Zillman, 1978).

Recently, two new groups of LMW-GS have been discovered, namely; minor D and minor C. Minor D is controlled by *Gli*-B3 and *Gli*-D3 while minor C is controlled by genes on either group 1 or group 6 chromosomes (Pogna *et al.,*1982). According to Shewry *et al.* (1986), the basic LMW-GS are encoded by genes of the *Gli*-1 loci, while the acidic sub-units are encoded by loci *Glu*-B2 and *Glu*-D2 which are found between the *Gli*-1 and the centromeres on chromosomes 1B and 1D.

2.1.9 Cultivar identification by storage proteins

Cultivar identification is of paramount importance in commerce where quality and purity have to be assured. Duplication, fraudulence and violation of breeder's rights have to be detected. In breeding programmes, genotypes with desirable traits have to be identified. Several methods are used to identify cultivars such as morphological markers, Storage proteins, Random Amplified Polymorphism DNA, Simple Sequence Repeats and Amplified Fragment length Polymophism.
In wheat, gliadin, glutenin, albumin, globulin and total protein extracts have long been used with great success in distinguishing cultivars. Nonetheless, gliadin supersedes the other three classes of

protein when identifying cultivars because of their heterogeneity, monomeric nature, ease of extraction, stability and near constant expression under varying conditions. Glutenin is less commonly used in identifying wheat genotypes than gliadin because it has to be converted to its sub-units before electrophoresis, through reduction of disulphide bonds in the presence of a detergent or denaturant. Total protein extracts can be used to distinguish wheat genotypes qualitatively and quantitatively (Cooke,1992).

Hika (2000) conducted a study on the identification of 20 Ethiopian bread and durum wheat cultivars using gliadin, HMW-GS and LMW-GS. Gliadin banding patterns uniquely distinguished all cultivars. HMW-GS showed similarities in three breadmaking cultivars at bands 2*,17+18, 5+10 using Payne and Lawrence nomenclature system (ref.3.2.1). Five durum wheat cultivars shared bands at 0, 14+15, two other durum wheat cultivars at 0, 6+8. Results indicated that gliadins had more discriminatory power than HMW-GS. Maartens (2000) tried to distinguish 147 cultivars of durum and bread wheat using HMW-GS and was unable to distinguish all the cultivars. LMW-GS distinguished all cultivars. De Villiers and Bosman (1993) identified wheat cultivars by electrophoretic analysis of gliadin patterns. The maximum number of bands found in all cultivars at different zones were 23, 12, 19, 14 and 9 in E, D, C, B, and A, respectively using Bushuk and Zillman (1978) nomenclature (ref.2.1.13). The bands on the B to E were used to identify cultivars and all cultivars were distinguished from each other. The cultivar with the highest number of bands had 36 and the lowest had 19.

2.1.10 Principles of electrophoresis

The term electrophoresis was first coined by Michaelis (1909) as cited by Mumba (1994). It is the method used to separate charged molecules, chiefly proteins and nucleic acids, on the basis of size and net charge. Electrophoresis is usually carried out on a supporting medium such as agar, agarose, paper, cellulose acetate and polyacrylamide gel in an electrical field, causing molecules to migrate towards the anode or cathode depending on the net charge, size and shape of the molecules, ionic strength, viscosity and temperature of the medium in which the molecules are moving (Hayward *et al.,* 1993; Cooke,1992).

2.1.11 Protein solubilization

Proteins are not all soluble in aqueous solutions and need a powerful negatively charged detergent such as sodium dodecyl sulphate that causes the proteins to unfold into extended polypeptide chains, resulting in individual protein molecules being freed from their association with other proteins or lipid molecules, and becoming soluble in the extraction solution. In addition, a reducing agent, mercapto-ethanol is added to the extraction solution in order to break any S-S linkages present in the protein so that the constituent polypeptides in multi-disciplinary molecules can be analysed. When a solution of sodium dodecyl sulphate solubilized protein is electrophoresed through a slab gel, each protein migrates towards the positive electrode because of its association with many negatively charged detergent molecules. Small proteins move much more readily through holes in the gel meshwork than large ones, and as a result, a series of discrete protein bands is produced from a complex mixture, arranged in order of molecular weight.

2.1.12 The rationale for the use of electrophoresis

The success of electrophoresis in identifying and distinguishing cultivars within a crop species relies on the fact that proteins are 'markers' for the structural genes that encode them. As genes are connected into genetic systems, protein markers can be used to label these systems which might be a set of genes, part or all of the chromosome or genome as a whole. By considering sufficient markers, a large portion of the genome could be covered. Crop cultivars consist of collections of germplasm differing in genetic expression. A comparison of the composition of particular proteins within these collections becomes useful for typing or characterizing the material. For comparison to be successful, it was found necessary to consider proteins which are polymorphic. In almost all crop species, seed storage proteins exhibit considerable polymorphism with regard to charge, size or both parameters. Furthermore, they are encoded at several loci and are present in comparatively large amounts and are readily extracted (Cooke, 1992).

2.1.13 Nomenclature system

Nomenclature is the language that is used by scientists in communicating research work. It tends to combine concepts and methods, i.e. it specifies the protein being studied and distinguishes it from the others in terms of its functions. Nomenclature must be consistent in order to avoid misunderstanding

and improve interaction amongst researchers (Wrigley *et al.*, 1996.)

Protein banding patterns formed on the electrophoregram are used to distinguish the cultivars of the same crop species and this is achieved by scoring the bands generating the matrix of 0 for absence and 1 for presence of a band. The resolution of the bands provide sufficient proof of the distinction among cultivars. Bushuk and Zillman (1978) showed that the easiest way to establish difference among cultivars is to examine the presence and absence of a specific band by the patterns in a vertical plane starting from the origin. They further indicated that the banding patterns of most cultivars are distinct.

Gliadins

Jones *et al.* (1959) were the first to classify gliadin into four zones designated as α, β, γ and ω on the basis of four distinct peaks in free or moving boundary electrophoresis in aluminium lactate buffer at pH 3.1. In 1961, Woychik *et al.* indicated that the four zones could be distinguished as four groups of bands by starch gel electrophoresis using aluminium lactate buffer pH 3.1 containing 3M urea. They furthermore showed that within a group, resolved bands could be identified by Arabic letters and the band having the highest mobility being assigned 1.

Wrigley *et al.* (1996) adopted the same greek letter Arabic numeric system in identifying cultivars. A composite formula was developed for AABBDD hexaploid wheat on the basis of number of gliadin bands present in the electrophoregram of the putative progenitors of A (*Triticum aestivum*), B (*Aegilops speltoides*) and D (*Ae. Squarrosa*) genomes. The gliadin electrophoregram produced was divided into four zones as follows; α: 1 - 7, β: 1 - 5, γ: 1 - 5 and ω: 1 - 12. The bands were grouped into high intensity (band number underlined), low intensity (band number put in parenthesis) and doublets represented by two dots above the number. A slight deviation in mobility of β or a band was indicated by a subscript 1 if the displacement was in the direction of the faster neighbouring band and by a superscript 2 if the deviation was in the direction of the slower neighbouring band. Wrigley *et al.* (1996) numbered 130 cultivars, both hard and soft, winter and spring wheat with this nomenclature.

Autran and Bourdet (1975) published the second nomenclature for identifying gliadin bands in the electrophoregram for cultivar identification purposes. They used 73 (60 winter and 13 spring) *Triticum aestivum* cultivars grown in France and obtained 43 bands (maximum being 25 for any one cultivar), each identified by mobility figures relative to 65 for a γ-gliadin band common to all the electrophoregrams analyzed. The mobility of the gliadin ranged between 21 and 100 kDa. They further used statistical analysis to establish the degree of dissimilarity of cultivar electrophoregrams.

Bushuk and Zillman (1978) proposed a nomenclature derived from the modification of Autran and Bourdet (1975). They substituted a γ-gliadin band with one of the major bands of Marquis and assigned this band 0.50 and all other bands were identified relative to this band. This nomenclature necessitated the use of Marquis as a standard cultivar.

Konarev (1982) adopted Woychik *et al.* (1961) s' nomenclature and sub-divided the zones into the sequential digits as follows; α: 1 - 7, β: 1 - 5 , γ: 11 - 5 and ω:1 - 10. This system is the one that is going to be used for wheat cultivars of Lesotho. For LMW-GS, nomenclature system adopted was developed by Gupta and Shepherd (1988), while for HMW-GS, Payne and Lawrence (1983) nomenclature system was employed.

2.2 Wheat quality for breadmaking purposes

2.2.1 Introduction

Wheat cultivars with good breadmaking quality contain high levels of gluten which is responsible for the visco-elasticity of dough. Gluten is a substance possessing the physical characteristics of elasticity and extensibility. When dough ferments under the influence of yeast, carbon dioxide gas is produced, and it is this gas retained within the elastic extensible gluten-starch complex which causes dough to rise. The structure of the risen dough is fixed by heat of the baking oven to give the finished loaf of bread (de Villiers & Laubscher, 1995.

According to Bosman and Agenbag (1998), the low ionic strength of glutenins and gliadins, as well as the disulphide and hydrophobic bonds are responsible for the visco-elasticity aggregates that give

wheat its breadmaking ability. The amount and quality of glutenin forming proteins to a large extent determines the mixing response of flour (Spies, 1990).

Breadmaking quality of wheat is determined by both protein quality and content in the grain. The protein content of the grain is, in turn, determined by environmental conditions, while the protein quality is genetically determined (de Villiers & Laubscher, 1995). The farmer could increase protein content by manipulating cultural methods, while breeders improve the quality by breeding methods (Mailhot & Patton, 1988).

2.2.2 The influence of environment on protein quantity

The environment, cultivar and their interactions affect milling and baking quality of wheat (Baenziger *et al.*, 1985). Protein quantity is affected by crop management, soil fertility, amount and distribution of precipitation, temperature, light intensity, photo-periodism and the length of the grain-filling period (Payne, 1986). Residual soil nitrogen and moisture content influence response of wheat to nitrogen fertilization, particularly pre-anthesis and post-anthesis. Residual nitrogen increases grain protein percentage (Olson *et al.*, 1976). Karathanasis *et al.* (1980) in their study of the effects of different nutrients on protein content, observed that soil potassium had a negative effect on protein content of wheat cultivars, while soil sulpur had a positive influence on grain protein.

Water stress increases protein content only when yield is reduced by water stress. A short period of very high temperature stress can have a marked effect on yield and quality of wheat grain (Blumenthal *et al.*, 1992). Temperatures above 32^0 C during flowering to physiological maturity, tends to increase protein content by decreasing yield, as does moisture stress. According to Austenson (1983), crop management practices such as crop rotation with alfalfa, summer fallow, herbicide use and growth regulators under some production conditions have a positive effect on protein content.

Favourable environmental conditions result in long grain-filling periods that lead to high yield of plump grain with low protein content, while less favourable environmental conditions result in a shorter grain-filling period and lower yields of grain with higher protein content. Although there are many environmental factors affecting grain yield and protein, temperature and rainfall exert more

influence than the others. Campbell and Davidson (1979) found that the effect of water stress on yield components is related to time and intensity of stress.

2.2.3 Correlation of characters

Two or more characters may be related negatively or positively such that a change in one character causes a change in another character (Mayo, 1980). Characters behaving in this manner are of interest for the following reasons; firstly, in relation to the genetic causes of correlations through the pleotropic action of genes. Secondly, in relation to the changes brought about by selection, where an improvement on one character may cause simultaneous change in another. Thirdly, in relation to natural selection, the relationship between quantitative characters and fitness is the primary agent that determines the genetic properties of that character in a population (Falconer & Mackay, 1996).

Genetic correlation is due to pleotropy, even though linkage is a cause of transient correlation particularly in populations obtained from crosses between different strains. According to Falconer and Mackay (1996), the degree of correlation arising from pleotropy expresses the extent to which two characters are influenced by the same gene. They further indicated that correlation brought about by pleotropy is the overall or net effect of all segregating genes that affect particular characters. Some genes may improve characters concerned, while others may improve some characters and reduce others. These are called positive and negative correlation, respectively. The association among the characters that can be directly observed is called phenotypic correlation. When phenotypic values of the individual, genotypic values and their environmental deviations for both characters are known, correlations can be determined between the genotypic values of the two characters as well as between the environmental deviations. Correlation also occurs between dominance deviations and between the various interactions deviations (Falconer & Mackay, 1996: Mayo, 1980).

2.2.4 Wheat quality parameters

2.2.4.1 Flour protein

Flour protein is an economically and nutritionally important property of grain. The price of wheat is based on it, so a drop in protein content results in a drop in price. Nutritionally, protein ranks first followed by carbohydrates and vitamins. It is therefore very important for the breeder to be cognisant

of the genetic control of this trait so that he could design appropriate breeding procedures and efficient selection strategies that could increase protein content of wheat flour (Poehlman, 1987).

Flour protein is described in terms of quality and quantity when measuring its potential in relation to end use. Quality tests relate to physico-chemical characteristics of the glutenin and gliadin components, while quantitative measurements relate to total organic nitrogen in the flour (Mailhot & Patton, 1988). According to Bietz (1988), variation existing in flour quality could be ascribed to variation caused by protein content and composition. Andrews and Skeritt (1996) revealed that flour protein content and total gluten are highly correlated with extensibility.

Several group of researchers found that flour protein is highly correlated with mixing requirements, mixing tolerance, dough handling characteristics, kernel weight, SDS sedimentation volume, Kernel hardnes and loaf volume (Johnson & Swason, 1942; Finney and Shogren, 1972; Bhatt & Derera, 1975).

2.2.4.2 Sodium dodecyl sulphate sedimentation (SDS sedimentation)

The SDS sedimentation test is a quick, simple and precise method of identifying wheat cultivars with good breadmaking quality. This method was introduced by McDermott and Redman (De Villiers & Laubscher, 1995). According to Greenaway *et al.* (1966), evidence has been provided to indicate that SDS sedimentation is the best predictor of breadmaking potential and strength for hard wheat. De Villiers and Laubscher (1995) conducted a study to determine relationship between the SDS sedimentation volume, protein content and loaf volume. The results showed a significant positive correlation between all three parameters. Groger *et al.* (1997) reported SDS sedimentation to be significantly correlated to protein content (r=0.73), extensograph dough strength (r=0.59), extensibility (r=0.6) and all farinograph and alveograph parameters.

2.2.4.3 Mixograph

It is an instrument that records dough parameters during the mixing action. This popular instrument was invented by Swanson in 1933 (Finney and Shogren, 1972) when conducting physical dough testing. The parameters obtained from a mixogram are used to distinguish wheat cultivars and predict

the properties in the finished products (Wilkstrom & Bohlin, 1966). Variation in mixing time among samples are caused by the protein fractions, and were found to be related to total protein, glutenin, residue protein and lastly gliadin/glutenin ratio (Bietz, 1988).

According to Finney and Shogren (1972), mixing requirements of flour containing 7.5% protein is much longer and mixing tolerance greater than those values of flours containing 11 – 13 % protein. The indicated that there is an inverse relationship between mixing time and protein content such that as protein content increases to about 12%, the mixing time decreases. Above 12% protein, the mixing time remains constant. Mixing time obtained from the mixogram is a reliable index of loaf volume potential and protein quality.

Both phenotypic and genotypic correlations indicated that decreased mixing time was related to increased protein levels and kernel weight. The negative genetic correlation between protein and mixing time was a consequence of the typical shorter mixing time characteristics of high protein genotypes.

2.2.2.4 Kernel characteristics

It is of utmost importance to consider the kernel characteristics since they are related to milling and flour quality. The most desirable kernel characteristics are large and uniform kernel size, plumpness, spherical shape, high density, well-filled kernels, smooth surface devoid of corrugations, small to medium sized protruding embryo and semi-translucence (Fowler & Priestley, 1991).

Kernel diameter

Gaines *et al.* (1996) indicated that the size of the kernel influenced flour yield such that an increase in kernel size resulting in increase in flour yield. Fang *et al.* (1998) and Ohm *et al.* (1998) emphasized the significant positive correlations between single kernel weight and diameter. Posner and Hibbs (1997) observed that the large sized kernel has low ash content while small sized kernel has higher ash content. They also observed that the large sized kernel has lower protein content than the small sized ones. Gaines *et al.* (1996) indicated that the small kernels are usually softer than the large kernels and this can be attributed to the fact that small kernels develop later and have little time to produce full plump kernels. Fang *et al.* (1998) reported that large kernel size needs more energy to break during milling than smaller kernels.

Kernel hardness

Wheat is classified according to kernel hardness which is related to milling and flour quality (Meppelink, 1974). The classification is divided into hard and soft wheat. Hard wheat is characterized by high energy requirement to break the kernel, maintenance of large particle size, easy passage through sieves, high protein content, vitreousness and more damaged starch in the flour. The endosperm texture of the kernel is influenced perceptibly by the environmental factors, hence it is variable (Anjum & Walker, 1991). Bran also affects hardness. A vitreous appearance is generally associated with hardness and high protein content, while opaqueness is related to softness and low protein content (Hoseney, 1986). Hardness in bread wheat is caused by protein starch bond strength and protein matrix continuity between granules of the starch. Air-spaces are formed during grain-filling and make opaque grain less dense. The protein shrinks, ruptures and leaves air-spaces upon drying. High protein soft wheat is vitreous, while low protein soft wheat is opaque or mealy. significantly. Low protein kernels have larger air-spaces than high protein kernels. In low protein soft wheat, there is inadequate protoplasmic material to fill the interstices between starch granules in the cells (Meppelink, 1974).

2. 3 Combining ability

2.3.1 Introduction

The idea of combining ability started in the late 1920's and was vigorously investigated in the early 1930's. Researchers were most interested in crossing maize inbred lines to evaluate combining ability. As many inbred lines as possible were crossed with one another to produce combinations of crosses, which were given a term 'top-cross'. Plant breeders focussed on inbred lines of maize which were showing a superior performance for yield. High performing top-crosses were further crossed in subsequent generations. Those crosses with low performance were discarded ensuring that valuable materials were retained (Falconer &Mckay, 1996). Sprague and Tatum (1942) refined the procedure for assessing combining and partitioned combining ability into general and specific combining ability. They came up with a systematic set of crosses between a number of parents and investigated the extent to which variations among crosses could be interpreted as due to statistically additive features of the parents and what must be attributed to the residual interactions (Simmonds, 1979).

General combining ability (GCA) is defined as an average performance of a strain in a series of crosses, while specific combing ability (SCA) is a deviation from the performance predicted on the basis of GCA (Simmonds, 1979; Mayo, 1980; Falconer, 1981). In statistics, GCA and SCA are main effects and interaction, respectively (Falconer &Mckay, 1996).

Sources of combining ability are partitioned into the variance of GCA and SCA, and are estimated using analysis of variance. According to Falconer (1981), differences in GCA are caused by the additive genetic variance and interactions in the base population, while the differences of SCA are caused by the non-additive genetic variance. Baker (1978) indicated that because of the difficulties caused by correlation of characteristics in the parent, the estimation of GCA and SCA mean squares and effects are of importance to the breeder. Such information is useful for measuring hybrid performance in assessing the potential of a hybrid breeding programme.

When combining ability of grain protein content was subjected to analysis of variance, both GCA and SCA variances were highly significant, indicating that grain protein content is determined by additive and non-allelic gene interaction with additive gene action being predominant (Mihaljev & Kovacev-Djolai, 1978). GCA effects contributed significantly to thousand kernel mass, while grain yield was determined by additive genetic effects (Singh & Chaudhary, 1977). Comparison of GCA x environment interaction and SCA x environment interaction showed higher sensitivity of GCA to environments than that of SCA. Contrarily, Paroda and Joshi (1970) reported GCA variance to be significant for the components of yield, while SCA variance was significant for kernel mass.

Jian and Singh (1978) found estimates of GCA variance to be significant for grain number per ear and thousand kernel mass in two environments and for grain yield under irrigated conditions. Estimates for SCA components were highly significant in both environments for grain yield and ear number, and in one environment for grain number per ear and thousand kernel mass. GCA and SCA variance components were significant for thousand kernel mass under irrigation, while GCA variance components alone were significant for thousand kernel mass under non-irrigated conditions.

GCA to SCA ratio

General combining ability is important as a detection and measure of additive gene action, while specific combining ability is used to estimate the effects of non-additive gene action. The ratio of GCA: SCA can be employed to examine the nature of genetic variability. The presence of additive genes exists when the GCA: SCA ratio is larger (Sayed, 1979). Bitzer *et al.* (1971) reported over-dominance to be associated with a low GCA : SCA ratio.

2.3.2 Mating design

The most common design in combining ability is the diallel cross, which consists of all possible crosses between a number of varieties. If there are n varieties, there will be n^2 combinations consisting of n selfings and n(n-1) crosses. Other designs such as North Carolina 1, 2, and 3, MxN, biparental progeny and triple test cross are also used depending on the information researchers is interested in.

In the diallel cross, as the number of varieties increases, the number of combinations also increases, making the experiment too large. As a result different methods of diallel crosses have been developed which excludes either selfing, parents or reciprocals. These are as follows;

(a) method 1 - crosses include parents, selfings and reciprocals.

(b) method 2 - crosses with parents but no reciprocals.

(c) method 3 - only reciprocals are involved and no parents.

(d) method 4 - no parent, no reciprocals.

2.3.3 Breeding population

According to Griffing (1956), in an analysis based on a fixed model, one is concerned with comparisons of the combining abilities of the actual parents used in the experiment and with the identification of superior combinations, while in an analysis based on the random model, inferences are to be made about the population from which the parents were sampled and these inferences are made from estimates of components of variance. Griffing (1956) analysed his data using both models and concluded that the choice of the correct model was dependent on the nature of the parental material. Eberhart and Gardner (1966) recommended that plant breeders and geneticists interested in genetic information about a particular set of parents should use a fixed model in most cases.

2.3.4 Heritability

Heritability is expressed as the ratio of total variance to the average effects of genes (Mayo, 1980; Falconer, 1981). Heritability determines the degree of resemblance between relatives. The major function of heritability in the genetic study of quantitative traits is a predictive role, showing the reliability of the phenotypic value as a guide to the breeding value. According to Falconer (1981), the phenotypic values of individuals could be measured directly, but it is the breeding value that determines their influence on the next generation. He further indicated that when the breeder chooses individuals on the basis of their phenotype values to be the parents of the next generation, his success in manipulating the characteristics of the population could be predicted only from knowledge of the degree of correspondence between phenotypic and breeding value. Heritability is used as a measure of the degree of correspondence between parents and their progeny.

The relative importance of heredity in determining phenotypic value is called the heritability of the character (Falconer,1981). Heritability confers two meanings depending on whether it refers to genotypic values or breeding values. It could mean the extent to which an individual is determined by genotype which is termed heritability in the broad sense (V_g/V_p) or the extent to which an individual is determined by the genes transmitted from the parents to the off-spring (V_a/V_p). The one that is of utmost importance in breeding programmes is heritability in the narrow sense because it determines the degree of resemblance between relatives (Falconer,1981; Poehlman,1987). As for heritability in the broad sense, it is of less practical importance and of more theoretical interest.

2.3.4.1 Estimation of heritability

Several methods have been employed to estimate heritability using data from observable variation of quantitative traits and its partitioning into genetically and environmentally controlled components (Poehlman, 1987). Genetic variance is comprised of additive, dominance and epistatis. The additive component of genetic variance is the variance contributed by genes having linear quantitative effects. The resemblance between parents and offspring is a result of additive genetic effects and largely determines the response of a population to selection. The dominance component represents the deviation of the heterozygote from the average of the homozygous parents. Contribution of dominance effects on quantitative characters is very small compared to additive effects. The

contribution of epistatis effects is smaller than that of the additive and dominance effect (Falconer, 1981; Poehlman, 1987).

The commonly used procedure for estimating heritability is progeny--parent regression. Regression of the progeny performance on the parent performance is based on resemblance between relatives and measures additive variance as a ratio of phenotypic variance. This is considered as heritability in the narrow sense and determines heritability more precisely than when heritability in the narrow sense is worked out (Poehlman, 1987). Analysis of variance is also used to estimate heritability (Singh & Chaudhary, 1977).

2.3.4.2 Heritability of wheat quality

Several researchers have conducted experiments to estimate heritability of wheat quality. Among these are Baker *et al.* (1971) who estimated heritability of wheat quality characteristics as the ratio G/(GxE), where G was the component of variance due to average genetic differences among cultivars and E was the component due to deviations from average performance. Results of heritability estimates of wheat quality were as follows; grain protein (80%), flour yield (66%), flour protein (88%), flour colour (62%), starch damage (82%), farinograph absorption (80%), farinograph development time (62%), extensograph length (47%), resistance (48%), area (71%) and baking volume (63%).

Similarly, Bhat & Derera (1975) reported heritability estimates of wheat quality characteristics to be as follows; hectolitre mass (66%), flour yield (75%), grain protein (72%), flour protein (78%) and colour (73%), which was not much different from that of Baker *et al.* (1971). In concurrence with the above researchers, O'Brien and Ronalds (1987) disclosed from their study that heritability estimates of protein quality, residue protein content and SDS sedimentation volume were all high.

2.4 Heterosis

The word 'Heterosis ' was coined by Shull (1908) when he was intensively crossing inbred lines of maize that resulted in a higher performance than either of the parents or average of the parents. Shull (1909) distinguished heterosis and hybrid vigour which were used interchangeably by many scientists. He defined heterosis as the developmental stimulation resulting from the union of different gametes, while hybrid vigour is the increase in the performance of F_1 over the mean of the parents or over the best parent. Hybrid vigour manifests itself in a variety of ways at different stages and plant parts. i.e. at that seedling or adult stage, vegetative or reproductive parts and in visible features like size or invisible like adaptive features (Grant,1975). It can appear in the F_1 generation and decline in subsequent generations or perpetuate for many seasons of growth in vegetatively propagated plants (Grant, 1975).

Many theories have been put forward to explain the phenomenon of hybrid vigour which include among others; dominance, over-dominance, complementary genes and cytoplasmic genes. Dominance theory states that where two different pure-lines are crossed, the offspring would be superior to both parents in that particular trait having at least one dominant gene in a pair. Over-dominance theory states that several different pairs may not be equal. Some have a greater effect than others. Complementary theory states that the superiority of F_1 performance is due to the favourable complementarity in enzyme action of two divergent alleles at a locus, while cytoplasmic theory suggests that hybrid vigour may arise from favourable interactions between nuclear genes and cytoplasmic genes or favourable combinations of different cytoplasmic genes (Hull, 1945; Mather & Jinks, 1971; Grant, 1975; Kearsey & Pooni, 1996; Chahal & Gosal, 2001).

According to Dobzhanski (1952), hybrid vigour is classified into hydrid luxuriance and euro-hybrid vigour, while Gustafusson (1951) classified hybrid vigour into somatic affecting vegetative system, reproductive capacity and adaptive features which have a greater capacity than average. Some traits expressed early in life, such as survival and growth rates are affected most. Some are moderately affected. Hybrid vigour may have little influence on some traits. Those traits showing the greatest degree of heterosis are the ones with adverse effects when inbreeding is practiced. The traits that are highly heritable are little affected by heterosis, whereas those that have low heritability are affected to

a greater degree. Hybrid vigour depends on the genetic diversity of the individual parents that are crossed. A physiological explanation states heterosis resulting from crossing of genetically unrelated plants may be due to the fact that such parents are less likely to possess the same detrimental or lethal recessive genes. The offspring from such parents is likely to carry at least one normal gene resulting in the normal functioning of biochemical reactions. Heterosis seems to be expressed in plants because of a more efficient metabolic system resulting from normal functioning of many pairs of genes in the heterozygous individual.

Heterosis has been explored in many crops for a variety of traits. In wheat yield, heterotic effects have been reported by Poehlman (1987) where the yield increased by 20 – 25%. Brears *et al.* (1988) as cited by Chahal and Gosal (2001) obtained heterosis of 13% of wheat yield. Some researchers obtained very high values of between 72 and 131% above mid-parent value (Mather & Jinks, 1971). Heterosis in yield components of wheat ranged from 11- 25% (Mayo, 1980).

2.5 High Performance Liquid Chromatography

It has become common knowledge that breadmaking quality of wheat flour is determined by its protein (Singh *et al.*, 1990), that is why studies have been conducted to explain the protein constituents that could account for quality differences. It was revealed that the varying ratio of glutenin to gliadin was responsible for flour quality. MacRitchie (1984) found that the ratio of glutenin to gliadin was directly related to mixograph development time and sedimentation volume in both weak and strong flours. Kim *et al.* (1988) showed that wheat quality could be changed by altering the glutenin/gliadin ratio leading to a 20 fold variation in dough resistance and 2 – 5 fold variation in extensibility. It was further stressed that a failure to correlate the glutenin to gliadin ratio with breadmaking quality could be attributed to inconsistency in solubility of proteins from different cultivars as well as the procedure followed in isolation of protein constituents (Bietz *et al.*, 1977). In order to effectively extract and fractionate protein constituents, several methods were tried which included among others; iso-electric focusing, SDS-PAGE, gel filtration and hydrophobic interaction chromatography. All these techniques proved to have insufficient selectivity and resolution for most cereal proteins (Bietz, 1983).

With continuous research for a method superior in separation, high performance liquid chromatography (HPLC) was developed, which was found to be highly reliable, provided improved speed, sensitivity, resolution reproducibility and ease of use (Popineau & Pineau, 1987). They further indicated that Reverse Phased (RP)-HPLC separates proteins on the basis of surface hydrophobicity and proved to be a new useful and complementary method for fractionation of cereal protein. Because RP-HPLC has stable columns giving high recoveries and no irreversible adsorption, it has succeeded where ordinary HPLC has failed (Marchylo & Kruger, 1984). Burnouf and Bietz (1984) discovered that RP-HPLC could be used to predict durum wheat quality when durum varieties were divided into two groups, corresponding exactly with varieties having PAGE bands 45 and 42, which are related to good and poor pasta quality, respectively. Damidaux *et al.* (1978) recommended RP-HPLC as a valuable alternative to PAGE in screening for durum quality in early generation of breeding. Payne and Lawrence (1983) noted that RP-HPLC revealed differences among HMW-GS of standard varieties typifying known alleles at *Glu*-A1, *Glu*-B1 and *Glu*-D1 proving that RP-HPLC can predict quality through glutenin subunit analysis. Huebner and Bietz (1985) confirmed that relative amounts of HMW and LMW glutenin subunits determined by quantitative RP-HPLC can also predict general scores, mixing time and bread quality.

Size-exclusion HPLC is a valuable tool widely used to quantify the relative proportions of the main endosperm proteins (glutenin, gliadin, albumin and globulins) of bread wheat. The proportions could be used to predict the relation between glutenin to gliadin ratio and molecular size distribution of polymeric protein affecting quality attributes such as mixing properties (Gupta *et al.*, 1995). Because SE-HPLC is reliable and needs a small amount of sample, it is an appropriate tool for the early prediction of promising material in a plant breeding programme. Marchylo and Kruger (1984) indicated that SE-HPLC is important in determining parameters such as polymeric protein percentages, glutenin to gliadin ratio, polymeric protein in the flour and percentage of extractable polymeric protein which could be useful markers for predicting breadmaking quality. Bietz (1988) indicated that SE-HPLC has a better reproducibility compared to RE-HPLC, is more accurate, is easy to quantify and permits rapid analysis, and also shows differences in the distribution of molecular weight of protein in wheat cultivars which may be correlated with breadmaking quality.

Identification of cultiars s becoming difficult with conventional methods because most of cultivars are utilized in breeding programmes narrowing down genetic pool such that distinction with some cultivars is impossible and this necessitates continouos search for methods that will evolve with new cultivars being developed. Molecular and biochemical markers are now gaining popularit to solve this problem, hence the study is conducted on storage protein profiles. Increasing knowledge and methods of testing cultivars for quality has conscientized Plant breders to embark on improvemnt of nutritional quality of wheart and meet nutritional requirementof people. This study attempts to contribute to the body of knowlegde on wheat quality on Lesotho genotypes.

2.6 References

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Chapter 3

Characterization of Lesotho wheat cultivars by storage proteins

Abstract

Thirty bread wheat cultivars grown in Lesotho were analysed using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). High and low molecular weight glutenin subunits (HMW-GS and LMW-GS) and gliadins were used to determine genetic variability and relationships between cultivars. The HMW-GS could not distinguish the cultivars, while both LMW-GS and gliadins could distinguish all cultivars. Cluster analysis performed using gliadin bands alone and in combination with LMW-GS, generated dendrograms which segregated cultivars according to genetic distance. Dendrograms were different from each other showing main groups and sub-groupings with different composition of cultivars. However, the genetic distance between cultivars were so close that it could be concluded that they are from the same gene-pool and have been used several times in breeding programmes.

Keywords: Bread wheat, gliadin, HMW-GS, LMW-GS, genetic diversity, SDS-PAGE

3.1 Introduction

Within a crop species, there are many cultivars that differ from each other in one or more characters that may be desirable or undesirable to the consumers, farmers and plant breeders. The accuracy of the method employed to detect differences amongs cultivars is of utmost importance. Morphological characterization used to be a popular method in the past when genetic distances amongst cultivars were far apart, but with selection and breeding the genetic distances became so close that it is difficult to distinguish cultivars using morphological characters (Smith & Smith, 1992). Recently, biochemical methods of establishing the differences between cultivars have superseded morphological characters. The method currently used is Polyacrylamide Gel Electrophoresis (PAGE) which has advantages such as handling of a large number of samples at a time, high resolving power and reproducibility, less time is required and lastly it is affordable (Bonnetti *et al.*, 1995). This method involves the separation of protein molecules according to their net charge and molecular

weight. Protein molecules move down the gel from cathode to anode separating according to their size to form banding patterns of different molecular weights (Cooke, 1991). The pattern of bands enables the cultivars to be distinguished.

Wheat, ranking third after maize and sorghum in Lesotho, has been used as an ideal model for the study because it (a) fetches a high price compared to other field crops, (b) can be grown in two seasons in a year, (c) utilises residue moisture in winter when all other field crops fail and (d) lastly, its water and nutrients requirement is low.

New cultivars are imported every year into Lesotho to be tested for adaptability, yield and pest resistance. The importation of these cultivars has necessitated cultivar fingerprinting to avoid duplication, fraudulence and violation of plant breeder's rights. To date, Lesotho does not have a cultivar release committee in existence (Ministry of Agriculture, 1997). The aim of this study was therefore to characterize wheat cultivars grown in Lesotho in terms of their glutenin and gliadin banding patterns, with the use of SDS-PAGE.

3.2 Materials and methods

Seeds of 30 wheat cultivars used in this study were obtained from the Agricultural Research Station of the Ministry of Agriculture and Food Security in Maseru, Lesotho (Table 1).

3.2.1 Methods

Methods adapted by Singh *et al.* (1991) were employed in the extraction of gliadin and glutenin. The method could be divided into the following according to the procedure; (i) extraction of seed storage proteins, (ii) casting of the gel, (iii) running the gel, (iv) fixing the gel, (v) staining and lastly, (vi) destaining. Appendix 1 presents the protocols followed in the preparation of stock solutions used, i.e. extraction buffer, separating and stacking gel, running buffer, fixing solution, staining and destaining solutions.

Table 1 Wheat cultivars grown in Lesotho

Cultivars	Classification	Growing season	Origin
Nata	HRS	Spring	Malawi
Wanda	HRI	Facultative	Malawi
Loerie 11	HRW	Winter	Zimbabwe
Sceptre	HRS	Spring	Zimbabwe
Chiriki	HRS	Spring	Malawi
Betana	HRI	Facultative	SGI-South Africa
Viri	HRS	Spring	Malawi
SST 65	HRW	Winter	Monsato-South Africa
SST 38	HRW	Winter	Monsato –South Africa
Boland	HRS	Spring	SGI-South Africa
Deka	HRS	Spring	Malawi
Manitoba	HRS	Spring	SGI-South Africa
SST 124	HRW	Winter	Monsanto –South Africa
Palmiet	HRS	Spring	SGI-South Africa
PNR 3342	HRI	Facultative	Pannar-South Africa
Tugela	HRW	Winter	SGI-South Africa
Kariega	HRS	Spring	SGI-South Africa
Caledon	HRW	Winter	SGI-South Africa
SST57	HRW	Winter	Monsato –South Africa
PNR3235	HRW	Winter	Pannar-South Africa
Scheepers 69	HRW	Winter	SGI-South Africa
Limpopo	HRS	Spring	SGI-South Africa
SST966	HRW	Winter	Monsato –South Africa
Bella	SRS	Spring	SGI-South Africa
Marico	HRS	Spring	SGI-South Africa
SST 367	HRW	Winter	Monsato –South Africa
SST 825	HRS	Spring	Monsato –South Africa
SST 363	HRW	Winter	Monsato –South Africa
Gariep	HRS	Spring	SGI-South Africa
Nkwale	HRS	Spring	Malawi

HRS=Hard Red Spring, HRW=Hard red winter,HRI=Hard Red Intermediate, SGI=Small Grain Institute

3.2.1 A.Gliadins

(i) Extraction of gliadins

(a) Two seeds of each cultivar were crushed to a fine powder using a pestle and mortar and were then transfered into 1.5ml Eppendorf tubes.

(b) 500µl of extraction buffer was added into each Eppendorf tube and was placed for 1hr in a waterbath set at 60^0C.

(c) After an hour, Eppendorf tubes were removed and vortexed for 20 minutes.

(d) Tubes were then centrifuged at 10 000 rpm for 2 minutes.

(e) 80µl supernatant was transferred into a new tube containing 80µl sample buffer. They were mixed well and centrifuged as before.

(ii) Running the gel

Supernatants from wheat cultivars including Chinese Spring (control) were shaken to mix it and loaded into the wells of the cast gel. Chinese Spring samples were loaded in the first two wells, two middle wells and one last well. The remaining wells were loaded with 100µl supernatants from wheat cultivars in triplicate for each cultivar.

The mould with a plastic spacer of 15mm containing the gel and supernatants were mounted on the stand of the electrophoretic apparatus (Hoefer Scientific Instrument, San Francisco, CA). Running buffers (cathode and anode) were added in the top and bottom tank, respectively. The power supply was switched on and set at 66mA for 3hrs. The cooler was set at 15^0C for circulation of water. The run was terminated when the blue marker dye reached the bottom of the gel. The mould was then dismantled.

The gel from the dismantled mould was immersed in the fixing solution over-night. The fixed gel was immersed in a staining solution and placed on the mechanical shaker for two to four hours. The gel was immersed in distilled water for four-six hours for the destaining of gels.

3.2.1 B. Glutenin

(i) Extraction of glutenin

(a) Two seeds of each wheat cultivar were crushed using a pestle and mortar to a fine powder and then transferred to a 1.5ml Eppendorf tube.

(b) 100µl of ethanol was added and placed in a waterbath set at 60^0C for 30 minutes after which ethanol was removed.

(c) 1 ml of 50% n-propanol was added, shaken and put in a waterbath at 60^0C for 30 minutes.

(d) Thereafter it was centrifuged for 2 minutes at 10 000 rpm, after which 50% n-propanol was removed. Step C and D were repeated three times.

(e) 85µl of extraction buffer containing 1.25% dithiothreitol (DDT) was added to the Eppendorf tube. Seed material and extraction buffer were loosened and left in the waterbath set at 60^0C for 1hour.

(f) 85µl of extraction buffer containing 16.8µl/ml vinyl pyridine was added to the tube and placed in the waterbath set at 60^0C for 1hour.

(g) 160µl sample buffer was added to the tubes and put in the waterbath for 15 minutes. It was then centrifuged for 5 minutes at 10 000 rpm, after which 100µl of supernatant was loaded.

Casting, running, fixing, staining and destaining of the gel were similar to those of gliadin in 2.2.2(ii), (iii), (iv), (v) and (vi), respectively.

Gel analysis

Gels were scanned, read and analyzed using Biorad Gel Doc 1000 Molecular Analyst Fingerprinting 1.1 version (1992 - 1994) software designed by Applied Maths Molecular Bioscience Group, California. Gels were scanned using an ultra-violet gel camera and VGA graphics in 256 colours. Gel analysis involved the following three steps; (a) conversion of the gel, (b) normalization of the tracks and (c) analysis of the tracks. During the last stage of analysis, a densitometric curve of every replication of every cultivar was drawn where both migration distance and band intensity were determined. Both were scored manually and average of the replications in each cultivar calculated. The intensity of the bands ranged from very light (1) to very dark (5). Where each replication had a

different intensity, a value of 6 was assigned to it.

The nomenclacture system developed by Konarev *et al.* (1979) was employed for gliadins to determine number of bands in each zone for each cultivar. Bands were further used to generate a matrix of 1 and 0 for presence and absence of bands in all the zones for each cultivar. The Gupta and Shepherd (1983) nomenclature system was used to analyze LMW-GS bands and that of Payne and Lawrence (1988) to analyze HMW-GS bands.

Statistical analysis

Data generated from the above were statistically analysed using NCSS 2000 computer software (Hintze, 1998). Cluster analysis was performed which generated a dendrogram. The index of genetic similarity was calculated as:

$$F=2n_{xy} /(nx + ny)$$

Where $2n_{xy}$ is the number of shared bands and nx and ny are the numbers of observed bands in cultivars x and y, respectively (Nei & Li, 1979).

3.3 Results and discussion

3.3.1 Gliadin bands

Gliadin band patterns of 30 wheat cultivars are presented in Table 2 showing the four major zones, their sub-division and relative mobility. The cultivars having the highest number of bands were Kariega, Manitoba, Nata, Nkwale, Scheepers 69 and SST 825 with 18 bands each, while the cultivars having the lowest number of bands were Chiruku, Karee and SST 57 with 11 bands each. Most of the cultivars had 14 bands. De Villiers and Bosman (1993) found the maximum and minimum number of bands in South African wheat cultivars to be 36 and 19, respectively. Menkovska and Knezevic (2002) obtained the maximum of 25 bands which enabled 10 Macedonian cultivars to be differentiated. Similarly, Tarekegne (2001) obtained 32 gliadin bands when examining 38 Ethiopian wheat cultivars and noted that each had a unique banding pattern. Labuschagne *et al.* (2002) examined 35 South African wheat cultivars for genetic diversity using gliadin bands and found gliadins to have high discriminatory power to distinguish all the cultivars, although some were closely related. The study revealed that four zones, γ and β could not distinguish the cultivars.

However, when all the zones were considered simultaneously each cultivar had an unique banding pattern which could distinguish the cultivars. This is consistent with De Villiers and Bosman (1993) who found bands in the A – region could not be used to distinguish the cultivars, only B-E were used.

Most of the cultivars possessed bands with the following relative mobility: ω - 9(58%), 8(77%), 7(71%), 5(65%), 4(58%) and 3(77%); γ - 4(61%) and 2(68%); β - 5(82%), 4(84%), 3(82%) and 2(77%) and α - 6(68%), 5(90%), 4(71%) and 2(77%). The figures in brackets show the percentage of cultivars having a particular band at a particular relative mobility. No one cultivar had a band at ω - 6 and β - 1. Few cultivars had bands at relative mobility of ω - 10, ω - 2, γ - 5, γ - 1 and α - 3.

Table 2. Electrophoretic formula for gliadin bands of 30 Lesotho wheat culivars

Cultivar	∝	β	γ	ω	Total bands
Bella	9,8,5,4,3	4,2	5,4,3,2	6,5,3	14
Betana	9,7,3	4,3,1	4,3,2	6,5,4,2,1	14
Boland	9,8,5,4,3,1	4,2	5,4,3	7,6,5,4,2	16
Caledon	9,8,4	4,2,	5,3,2	5,2	14
Chiriku	9,7,6,3	2	4,3,2	6,5,2	11
Deka	8,7,5,3,1	4,1	5,4,3	6,5,4	13
Gariep	10,8,7,5,3,1	4,2	5,4,2	5,2,1	14
Karee	5,1	3	5,4,2	7,6,5,4,2	11
Kariega	10,9,8,7,5,4,3	3,2	5,4,3,2	7,6,5,3,2	18
Limpopo	9,8,4,3,1	4,2	5,4,3,2	7,6,5,4,2	16
Loerie 11	10,9,7,5,3	5,4,2	5,4,2	7,6,4,2,1	16
Manitoba	10,8,7,5,4,3	4,2,1	5,4,3,2	7,5,4,2,1	18
Marico	9,8,5,3	5,4,3,2	5,3	5,3,1	13
Nata	9,5,3,1	4,3,2,1	5,4,2	7,6,5,4,2,1	18
Nkwale	9,8,7,5,4,1	5,4,2,1	5,4,3,2	7,5,4,2	18
PAN3235	8,2,	5,3,1	4,3,2	7,5,4,2	12
PAN3342	8,7,4,3	5,3,2	5,4,3,2	5,4,1	14
Palmiet	8,7,5,3	5,3,2	5,3,2	7,6,5,4	14
Sceptre	9,8,5,4,1	4	4,3,2	6,5,2,1	13
Scheepers 69	9,8,7,5,4,1	3,2	5,4,3,2	7,6,5,4,2,1	18
SST65	10,8,7,5,4,3,1	4,2	5,4,3,2	5,4,2,1	17
SST124	10,8,7,5,3	3,2	5,3,2	6,5,2,1	14
SST367	9,7,4,1	4	5,4,3,2	6,5,4,1	12
SST363	10,8,7,4,3,1	2	5,4,3,2	6,5,4,1	12
SST825	10,8,7,4,3,1	4,1	5,4,3,2	7,6,5,4,2,1	18
SST966	9,8,7,5,4,3	4,3	5,4,3	5,4,1	14
SST57	9,7,4,3	4	5,4,2	6,4,2	11
SST38	8,5,4,3	5,3,2	5,4,3	7,6,5,4,2,1	16
Tugela	9,8,7,5,3,1	4,2	5,4,3,2	6,4,2	15
Viri	10,9,7,4,3	4	5,4,3	7,6,5,2	13
Wanda	9,8,7,5,4,1	5,3,1	5,4,3,2	7,5,4,2	17

3.3.2 Gliadin band intensities

Band intensities were grouped into five categories: 1 – very light (1 – 50), 2 – light (51 – 100), 3- medium (101 – 150), 4 - dark (151 – 200), 5- very dark (above 200) and 6- impossible to give an average intensity class. The most common intensities were 1, 5 and 2. The intensities could be used as an indication of the concentration of proteins at a specific location on the gel (Tarekegne, 2001).

3.3.3 Low molecular weight glutenin subunits (LMW-GS)

It was easy to identify LMW-GS because of their fast electrophoretic mobility rate compared to HMW-GS and moreover, they are distinct. Among the 30 genotypes subjected to SDS-PAGE analysis, five to 14 distinct bands were observed having between slow and fast moving units. SST 367 possessed the least number of bands (5), while SST 38 and Deka had the maximum number of 14 bands. The frequency of bands observed between the least and maximum number of five and 14 were as follows, number of bands being in brackets; 3.3% (5), 6.6% (7), 13.3% (8), 13% (9), 6.6% (10), 23.3% (11), 16.6% (12), 10% (13) and 6.6% (14). A large number of genotypes (23.3%) had 11 bands, followed by 12 bands in 16.6% of the genotypes. Tarekegne (2001) obtained eight to 15 bands of LMW-GS bands when analysing 42 Ethiopian wheat genotypes. Maartens (1999) observed eight to 22 LMW-GS bands in 147 South African bread wheat genotypes. According to Gupta and Shepherd (1988) and Denery-Papini *et al.* (1995), two to 16 different bands of LMW-GS may be observed in hexaploid wheat.

The LMW-GS alone and frequency of LMW-GS combinations are presented in Table 3. In group 1 (*Glu*-A3 locus), unique band combinations were identified in 5 (16%) of 30 genotypes analysed. The remaining eight genotypes possessed two band combinations each. The band combination 'E' was not present. In group 11 (*Glu*-B3 locus), 28 (93%) out of 30 genotypes were identified, only two genotypes had two band combination each. Six out of nine combinations were represented. The missing ones were G, H and I. In group 111 (*Glu*-D3), out of 30 wheat genotypes, 28 (93%) were identified. The remaining two genotypes did not match with any of the five band combinations of group 111. In a similar study conducted by Tarekegne (2001), 32 of 42 Ethiopian bread wheat genotypes were identified in the group 1 band combination. In group 11, 28 genotypes out of 42 were identified. In group 111, 36 genotypes were also identified. Among these groups, there were some

genotypes not represented such as D, H and I in group 11.

It could be deduced from the results above that the LMW-GS had a high discriminating power in distinguishing genotypes on the basis of banding patterns and band combinations. Maartens and Labuschagne (1999) reported similar results for South African bread wheat cultivars.

Table 3: LMW-GS of Lesotho bread wheat genotypes

Cultivar	**A3**	**B3**	**D3**
Bella	A/C	B	A
Betana	A	C	C
Boland	B/C	D	E
Caledon	A/C	A	D
Chiriku	B	F	B
Deka	C	A	A
Gariep	B	C	B
Kariega	C	B	-
Limpopo	F	D	E
Lorie 11	A	C	B
Manitoba	B	D	B
Marico	C	A	A
Nata	A/B	A	C
Nkwale	C/F	A	C
PAN3235	F	C	E
PAN3342	B	A	B
Palmiet	D	E	C
Sceptre	A	B	C
Scheepers 69	F	C	C
SST65	B/F	D	E
SST124	C	C	A
SST367	A	C	C
SST363	A/F	B/E	B
SST825	D/F	C	A
SST966	C	A	A
SST57	B	A/B	C
SST38	B	D	-
Tugela	C	A	D
Viri	A	A	E

Glu-A3, *Glu*-B3, *Glu*-D3

Table 4: Frequency of LMW- GS band combinations in Lesotho bread wheat genotypes

Group	Band Combination	No. Genotypes	Frequency
1.	A	5	16.6
	B	6	20.0
	C	7	23.3
	D	1	3.3
	F	5	16.6
	A/B	1	3.3
	C/F	1	3.3
	B/F	1	3.3
	B/C	1	3.3
	A/C	2	3.3
	D/F	1	3.3
11	A	9	30.0
	B	3	10.0
	C	8	26.6
	D	5	16.6
	E	1	3.3
	F	2	6.6
	A/B	1	3.3
	B/E	1	3.3
111	A	8	26.6
	B	7	23.3
	C	7	23.3
	D	2	6.6
	E	4	13.3

Group1=*Glu*-A3, Group 11=*Glu*-B3 Group 111=*Glu*-D3

3.3.3 **High Molecular Weight Glutenin Subunits (HMW-GS)**

The HMW-GS are characterized by relatively slow electrophoretic mobility when compared with LMW-GS. They are resolved into four to five bands which are easily identified because of their different electrophoretic mobility. The results showing the frequency of HMW-GS among Lesotho bread wheat genotypes are presented in Table 5. This study revealed 11 different subunits which are as follows; three subunits at the *Glu*-A1 locus, five at *Glu*-B1 and three at the *Glu-D1 locus*. At the *Glu-A1* locus, the most frequent subunit was null accounting for 14 (6.6%) of 30 wheat genotypes. It was followed by subunit 1 with 11 (36.6%) of 30 genotypes which is slightly lower than subunit

'null'. The subunit 2* accounted for a very low number (5) of genotypes. The wheat genotypes grown in Lesotho could not be distinguished by these subunits. Nonetheless, all the subunits were represented in these genotypes. Similarly, reports on similar studies conducted in Great Britain, Afghanistan, Italy, Netherlands, Slovakia, Portugal, and Japan showed a high frequency of null form of *Glu*-A1 like that of Lesotho wheat genotypes (Lagudah *et al.*, 1987; Payne *et al.*, 1987; Pogna *et al.*, 1989; Kolster *et al.*, 1993; Gregova *et al.*, 1997; Igrejas *et al.*, 1999; Nakamura, 2000). Conversely, Tarekegne (2001) found that null form of *Glu*-A1 in a lower frequency in Ethiopian wheat.

At the *Glu*-B1 locus, the most frequent subunits were 7+9 accounting for 53.3% of the wheat genotypes. It was followed by 17+18 with a 23.3% frequency. The subunits in the lowest number of genotypes (3.3%) were 13+16. Payne and Lawrence (1983) reported subunits 7, 20, 13+19 and 14+15 to occur frequently in bread wheat cultivars worldwide. In Lesotho bread wheat genotypes, only subunit 7 occurred, the others were not present. Tarekegne (2001) found subunit 7+9 to be the most frequent in Ethiopian bread wheat genotypes, which is consistent with the results of this study. Furthermore, his study revealed subunits 13+16 to have occurred in very few genotypes (2.4%). This is also consistent with this study.

At the *Glu*-D1 locus, three pairs of subunits were represented, namely; 2+12, 3+10 and 5+10. The most frequent subunits were 5+10 accounting for 18 (60%) out of 30 genotypes. This was followed by 2+12 with 10 (33.3%) genotypes. The least was 3+10 in 2 (6.6%) out of 30 genotypes. Nakamura (2000) and Pogna *et al.* (1989) reported the subunits 3+12, 4+12 and 2+10 to be common in wheat cultivars grown in several countries. However, Lesotho wheat genotypes did not possess these subunits.

The variation in the HMW-GS has been associated with breadmaking quality in hexaploid wheat cultivars (Pogna *et al.*,1989). The Lesotho wheat genotypes formed 17 groups on the basis of HMW-GS. Table 6 showed HMW-GS composition of Lesotho Bread wheat gentypes. HMW-GS for Lesotho wheat genotypes formed 17 groups on the basis of their similarity when combining loci A1, B1 and D1, after which frequency was calculated. A large number of genotypes (10) had independent

combinations of HMW-GS. A combination that comprised a large number (5 %) of genotypes was 1, 7+9 and 5+ 10, followed by 0, 7+9 and 5+10, while the least were 0, 7+9, 2+12; 2*, 17+18, 5+10; 1, 7+8, 2+12, and 2*, 7+8 and 5+10.

Table 5: Frequency of HMW-GS among Lesotho bread wheat genotypes

Locus	**Subunit**	**Genotype No.**	**Frequency (%)**
A1	1	11	36.6
	2*	5	16.6
	Null	14	46.6
B1	7 + 9	16	53.3
	17 + 18	7	23.3
	7	2	6.6
	7 + 8	4	13.3
	13 + 16	1	3.3
D1	2 + 12	10	33.3
	3 + 10	2	6.6
	5 + 10	8	60.0
TOTAL	11	30	

Table 6: HMW- GS composition of Lesotho bread wheat genotypes

Group	HMW-GS			Frequency (%)	Genotypes
	A1	B1	D1		
1	1	7 + 9	2 + 12	3	Nata, Loerie 11, Viri
2	0	17 + 18	3 + 10	1	Nkwale
3	0	7 + 9	3 + 10	1	Wanda
4	0	17 + 18	5 + 10	1	Sceptre
5	0	7 + 9	2 + 12	2	Chiriku, SST 57
6	2	17 + 18	5 + 10	2	Betana, Kariega
7	0	7 + 9	5 + 10	4	SST 65, SST 966, Marico, Gariep
8	0	7	5 + 10	1	SST 38
9	0	17 + 18	2 + 12	1	Boland
10	1	7 + 8	2 + 12	2	Deka, SST 124
11	0	13 +16	5 + 10	1	Manitoba
12	1	7 + 9	5 + 10	5	Palmiet, Limpopo, SST 367, SST 363, SST 825
13	2	7 + 9	5+ 10	1	PNR 3342
14	2	7 + 8	5 + 10	2	Tugela, PNR 3235
15	1	17 + 18	5 + 10	1	Caledon
16	0	17 + 18	2 + 12	1	Scheepers 69
17	0	7	2 + 12	1	Bella

3.3.4 Cluster analysis

3.3.4.1 Gliadin

A dendrogram was constructed using gliadin bands to show genetic distances among Lesotho wheat cultivars. The dendogram is presented in Figure 1. Cultivars are divided into two main groups. Marico is the only one in one group and the rest in the second group. This second group again divide in to two subgroups with PAN 3235 and Karee in the first, and the rest in the second group. This second group contains most of the cultivars (27 of the 30), and is divided further. Thus Marico, PAN 3235 and Karee are the most distinct, dissimilar and genetically different from the other cultivars. The second big cluster of 27 cultivar is divided into three main groups with five subgroups. The three main groups are Viri, SST 57, Lorrie, SST 367, Chiruku and Betana, be followed by SST 38, Palmiet, PAN 3342 SST 124, Kariega SST 966, Deka, SST 825, Manitoba, SST 363, Gariep SST 65, Caledon Scheepers 69, Wanda, Nkwale, Nata, Tugela , Limpopo and Boland. The two most similar cultivars were Limpopo and Boland. The total genetic diversity for all cultivars was 0.70.

Stoyanova and Kolev (1996) examined genetic diversity in 32 wheat accessions using gliadin bands and generated a cluster analysis yielding eight groups. Similarly, Farshadfar *et al.* (1995) assessed the level of polymorphism in 54 wheat cultivars by gliadin bands and the cluster analysis produced 13 groups. Xiao *et al.* (2001) analyzed levels of polymophism in 20 wheat lines which produced 56 gliadin bands which produced cluster analysis with four groups. Several studies conducted observed high levels of genetic divergence in wheat (Tarekegne, 2001).

3.3.4.2 Combination of gliadin and LMW-GS

Combination of gliadin and LMW-GS bands were used to perform cluster analysis for 30 genotypes. Figure 2 shows the cluster analysis of 30 genotypes. The cluster analysis depicted two main groups, in which one group (A) had one cultivar standing on its own as an outlier. The other main group B comprising of 29 cultivars divided further into two sub-groups. Sub-group 1 comprised of two cultivars of different seed companies (Pannar and Sensako). Sub-group I I comprised of two further sub-groupings. One (A,2,i) had 19 cultivars which originated from different countries and seed companies. However, a large number of cultivars developed by Sensako appeared in this grouping. Cultivars originated from Malawi also fall under this grouping. The other sub-grouping (A,2,ii)

consisted of eight cultivars from different seed companies, although a large number of cultivars (SST 966, SST367 and SST 124) was developed by Monsanto.

Gliadin alone and in combination with LMW-GS were able to distinguish 30 wheat genotypes and were able to estimate genetic distance between them suggesting that they both have a high discriminatory power. Both showed a close relationship among the genotypes, which indicated that they originated from the same gene-pool or progenitors which have been used repeatedly in breeding programmes. Nonetheless, the dendrograms generated from the gliadin and a combination of gliadin and LMW-GS were completely different in terms of the number of clusters, main groups and sub-groupings. The genotypes in each cluster or main groups in gliadin and LMW-GS differed because they are produced independently by the genes that translated them.

It has been observed that PAN 3235 clustered separately in both dendrograms. Other cultivars that clustered together in both dendrogram are Wanda and Nkwale, Lorrie and SST57, and Chiruku and Betana. The Gliadin and LMW-GS data were combined to determine if they can increase the discriminatory power.

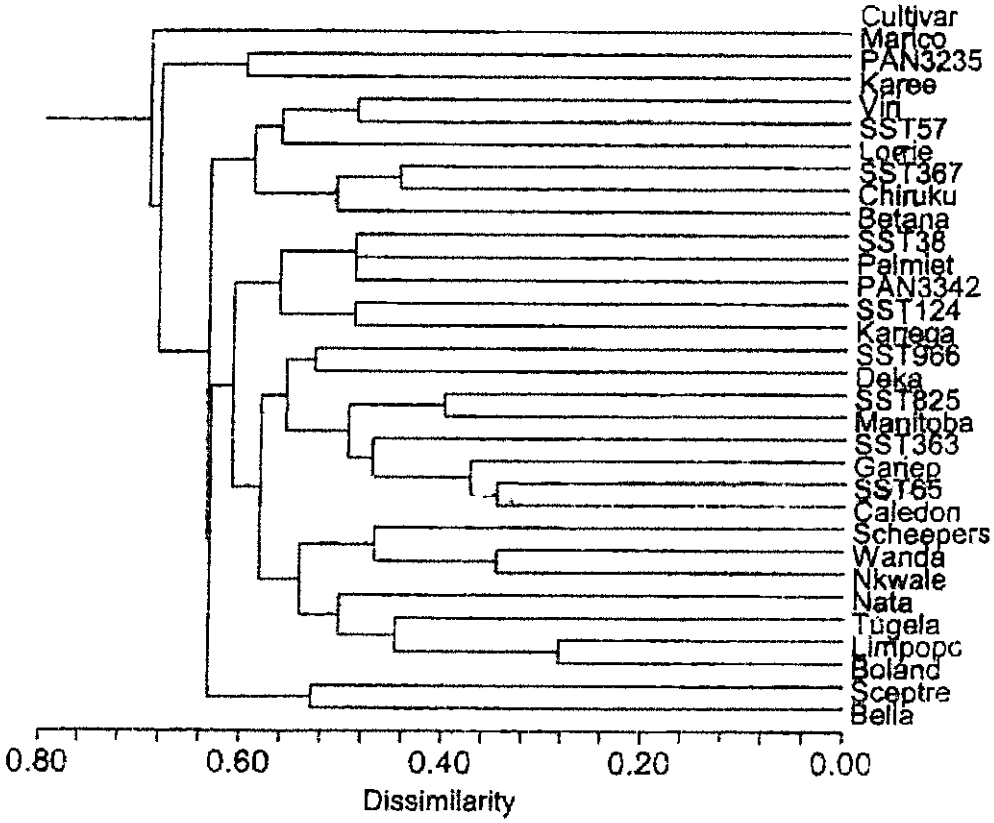

Fig. 1 Dendrogram generated from gliadin showing the distance among bread wheat genotypes grown in Lesotho

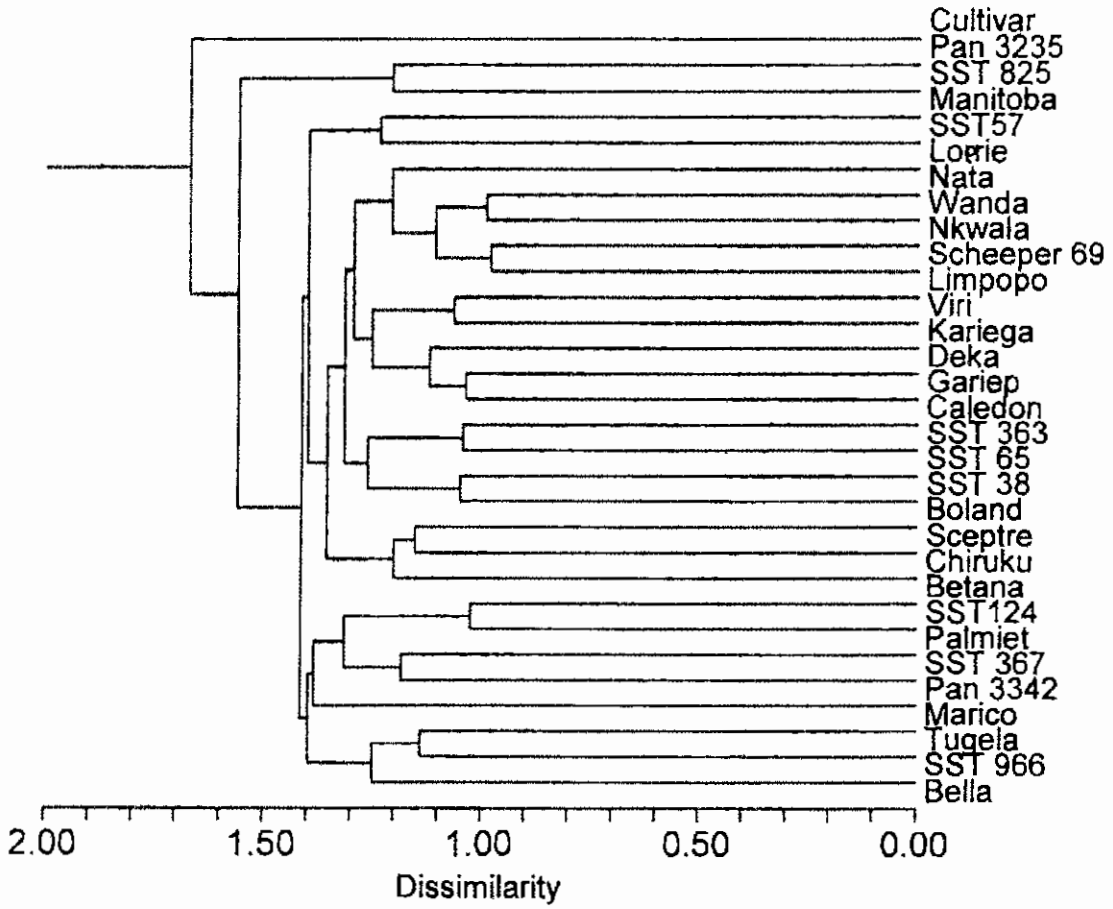

Fig.2 Dendrogram generated from a combination of gliadin and LMW-GS bands of bread wheat genotypes grown in Lesotho

3.4 Conclusions

The SDS-PAGE method employed was able to distinguish 30 wheat cultivars grown in Lesotho using three major protein storage groups, namely; gliadins, LMW-GS and HMW-GS. The variation in the number of bands and their different mobilities assisted in distinguishing the cultivars. However, some wheat cultivars had the same number of bands but at different locations, while others had a completely different number of bands. A large proportion of bands were at the same location and this could have attributed to the fact that they all belong to the family of Triticum and share most characteristics. The genetic distances between the cultivars were relatively large, implying that

genetic variability is available that could be explored in breeding programmes to improve some economic traits.

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Chapter 4

Analysis of quality characteristics in F_1 and F_2 diallels from cultivars varying in breadmaking quality

Abstract

The F_1 (grown in Maseru and Bloemfontein) and F_2 progeny (grown in Bloemfontein) of a 5 x 5 parental diallel cross of bread wheat (*Triticum aestivum* L.em Thell), were used to estimate general combining ability (GCA), specific combining ability (SCA), general combining ability to specific combining ability (GCA : SCA) ratios, components of genetic variance, heritability, heterosis and correlations of quality characteristics. Quality characteristics studied were flour yield (FLY), flour protein content (FPC), mixograph development time (MDT), Sodium Dodecyl Sulphate sedimentation volume (SDSS), seed weight (SKCSW), seed diameter (SKCSD) and seed hardness (SKCSH). Results revealed that significant differences existed between Maseru and Bloemfontein for SKCSW, SKCSD and SKCSH, while SDSS did not differ significantly. The analysis of variance (ANOVA) indicated significant differences between parents, F_1 and F_2 progeny for all characteristics studied. Significant differences were also seen between the means of F_1 and F_2 progeny for FLY and MDT, while insignificant differences were found in FPC, SDSS, SKCSW, SKCSD and SKCSH. The mean squares for GCA were significant for FLY, SDSS and SCKCH in the F_1 progeny, while SCA and reciprocals were significant for all wheat quality characteristics. Mean squares for GCA, SCA and reciprocals in the F_2 progeny were highly significant for all characteristics except MDT. GCA/SCA ratio in the F_1 progeny showed non-additive gene action in all characteristics except one. In the F_2 progeny, FLY, FPC, SKCSH and MDT were controlled by non-additive gene action, whereas SDSS, SKCSW and SKCSD were controlled by additive gene action. Variance of GCA and SCA in F_1 and F_2 progeny showed a large genotypic variability in most of the characteristics. Heritability in the broad sense (h_b^2) was high for all characteristics in F_1 and F_2 progeny, whereas heritability in the narrow sense (h_n^2) was high for F_2 and lower for F_1 progeny. Mid-parent heterosis and best parent heterosis were expressed in SDSS, SKCSH, FLY and SKCSW. Both significantly positive and negative correlations were observed between quality characteristics in the F_1 and F_2 generations.

Keywords: general combining ability, specific combining ability, wheat quality, heterosis, correlation, heritability.

4.1 Introduction

Wheat quality is of utmost importance to both millers and bakers in processing flour and for baking.wheat quality characteristics can be divided into milling and rheological characteristics. Milling characteristics include hectolitre mass, break flour yield and hardness or softness. Rheological characteristics include grain and flour protein, falling number, flour colour, mixograph, farinograph, loaf volume and SDS sedimentation (Finney *et al.*, 1987; Bechere *et al.* 2000). The most important protein is glutenin that makes the dough rise through formation of minute gas cells that retain the carbon dioxide produced during yeast formation or chemical leavening (Bushuk & Wrigley, 1974 ; Bonjean & Angus, 2001). Glutenin confers the elasticity and coherent properties of dough and allows the baking of leavened bread. Both leavened and unleavened bread remain the principal food product made from wheat. This could only be achieved by crossing the prospective candidates of wheat cultivars in all possible combinations and identify the progeny that have heterotic effects, after which the transgressive segregants would be produced and integrated into the breeding programme. According to Arunachalam (1976) and Baker (1978), combining ability is instrumental in facilitating improvement in plant breeding programmes. Diallel analysis provides an opportunity to evaluate a number of lines in all possible combinations (Singh, 2002). It is for this reason that this study has been undertaken to estimate the general combining ability (GCA), specific combining ability (SCA), heritability, heterosis and correlation from F_1 and F_2 diallel crosses between parents varying in breadmaking quality.

4.2 Materials and methods

4.2.1 Experimental design

Based on previous analysis, two cultivars with poor, one with medium and two with good breadmaking quality were selected (Table 7) and crossed with one another. This was performed in the greenhouse at the University of Free State from February 2002 to July 2002. Five parental lines were sown in 2 litre pots containing potting soil. Six seeds of each cultivar were sown in each pot. Parents were planted four times at an interval of two weeks to synchronise pollen for crossing.

F_1 progeny of those parental lines were grown in Maseru (Lesotho) and Bloemfontein (South Africa) in a randomized complete block design with three replications. Each plot measured 2m x 1.8m with the intra-row and inter-row spacing of 10cm and 45cm, respectively. Seeds obtained from the F_1 were analyzed for FLY, FPC, MDT, SDSS, SCKSW, SCKSD and SCKSH.

F_1 progeny harvested in Bloemfontein in November 2003 were planted in Bloemfontein at the same location, in June 2004 and harvested in November 2004 to compare results of breadmaking quality of the F_1 with the F_2 generations. Although the environment was the same, the season was different, and comparisons would therefore be influenced to some extent by this. Each F_2 progeny was planted in triplicate using a randomized complete block design. Harvested F_2 wheat samples were sent to Bethlehem at Small Grain Institute for quality analysis where approved methods of American Association of Cereal Chemists (200) were used.

4.2.2 Laboratory analysis for quality characteristics

Flour yield

All wheat samples were milled in a laboratory pneumatic mill using the Bühler model (MLU-202 manufactured by Bühler Bros., Inc., Uzwil, Switzeland). The AACC method 26-21A for milling hard wheat was followed (AACC, 2000). The amount of water added to a wheat sample for tempering was dependent on the vitreousness of the wheat kernels, the moisture of the wheat as well as the weight of the sample to be milled. A table that takes the above-mentioned into consideration is used to determine the desired moisture content. The quantity of water to be added to wheat was then determined by comparing the original moisture against the desired moisture. Wheat samples of 1.5kg were weighed into air-tight containers, conditioned and shaken to ensure equal distribution of water. Conditioning was performed 18hrs prior to milling. The Bühler Mill consists of break and reduction rollers which break the kernels and crush it into flour.

Flour Protein Content: AACC method 39-11.

This method was used where quantity of protein present in the flour was determined by a near infrared reflectance spectrophotometer which was calibrated against Kjeldahl data.

Mixograph: AACC method 54-40A was used.

Ten gram of flour sample was added to the mixing bowl, then water was added. The instrument was switched on, the agitator rotated to blend water and flour together to make a quasi-homogeneous mixture in order to develop a gluten matrix. As the mixture (dough) developed, the mixograph measured and recorded dough behavior and its resistance to mixing. The mixing curve indicates optimum development time, tolerance to over-mixing, descending graph width, estimates bake absorption and weak or strong dough characteristics.

SDS Sedimentation: AACC method 56-70 was used.

The sedimentation test reflects differences in quality and quantity of gluten in wheat, hence is a rough measure of baking strength. The SDSS test was performed in a 100ml measuring cylinder filled with 50ml water kept at 22^0 C in a warm bath. Four gram of flour was added to the water and shaken three times at regular intervals. A lactic acid mixture was added to the flour mixture and inverted at regular time interval. Six minutes after the third inversion, a reading was taken on the cylinder.

Single Kernel Characterization System

The Single Kernel Characterization System (SKCS model 4100 of Perten Instruments Co. Reno NV) was employed to determine kernel characteristics. Wheat seed was deposited ito the hopper, after which 300 individual wheat kernels were picked up by vacuum, weight and crushed. The computer software of the instrunnt then determined a hardness index for each sample based on the kernel crush profile, moisture, size and weight. Information obtained was then processed to furnish weight, diameter and hardness index on an individual basis. The hardness index was expressed as a percentage of the pressure of the two rollers to crush the kernels and is a function of moisture, size, weight and force-deformation curve derived from crushing individual kernels.

Table 7: Cultivars selected as parents for the diallel cross and their quality characteristics

Parents	Quality characteristics				
	SDSS(ml)	FLY(%)	FPC (%)	MDT(mins)	Rank
Kariega	92	57.3	14.9	4.5	Good
SST124	74	59.2	15.5	2.5	Good
Wanda	90	58.9	10.5	3.1	Medium
Nata	47	54.5	7.7	2.3	Poor
Sceptre	51	56.3	7.8	3.5	Poor

SDSS =SDS sedimentation volume, FLY= flour yield, FPC=flour protein content, MDT=mixograph development time

4.2.3 Statistical analysis

Analysis of variance

All data obtained from quality tests were subjected to analysis of variance using Agrobase (2000) to establish the level of significance among the genotypes for milling and rheological characteristics.

Combining ability

Combining ability analysis was carried out using Agrobase (2000) to determine significant differences among parents for quality characteristics. The full diallel crosses analysis of Method1 model 1 developed by Griffing (1956) was adopted for this study assuming fixed effects for genotypes. This method includes parents, F_1 and reciprocals. The analysis of variance provided the significance level for both general and specific combining ability and reciprocals as well. The general combining ability (GCA) effect, specific combining ability (SCA) effect and reciprocal effects were calculated separately for all quality characteristics. GCA to SCA means squares ratios were estimated to establish the presence or absence of additive gene action. Where GCA is larger than SCA variance, additive gene effect is predominant and vice versa.

Estimates of genetic variance

Analysis of variance for combining ability performed provided mean squares for general and specific combining abilities which were used to estimate GCA and SCA genetic variance. The formula for GCA and SCA as provided by Griffing (1956) were as follows;

$$\delta^2 gca = \frac{MSgsa - MSsca}{p-2} \qquad \text{while } \delta^2 sca = MSsca - MSe$$

According to Chaudhary and Singh (1977), Wricke and Weber (1986), and Falconer and Mackay (1996), genetic variance is partitioned into additive variance (δ_a) and dominance variance (δ_d). Where $\delta_a = 2\delta^2 gca$ and $\delta_d = \delta^2 sca$.

Phenotypic variance is obtained by applying this formula: $\delta_p = \delta_g + \delta_e$. δe = environment.

Estimation of degree of dominance was calculated using Chaudhary and Singh (1977) and Falconer and Mackay (1996) as follows;

$$\sqrt{H/D} = \sqrt{\frac{\delta^2 sca}{\delta^2 gca}}$$

The relative sizes of variances due to GCA and SCA were compared following Baker's predictability ratio:

$$PR = \frac{2\delta^2 gca}{2\delta^2 gca + \delta^2 sca.}$$

The closer the ratio is to unity, the greater its predictability of F_1 progeny performance.

Correlations

Agrobase (2000) was used to run a correlation matrix to determine relationships among the characteristics being studied. Genotypic correlations were determined from the general combining effects, and gave correlations with total exclusion of environmental effects.

Heritability

Heritability was calculated using the formula of Falconer and Mackay (1996), which is defined as regression coefficient of genotypic value on phenotypic value. Wricke and Weber (1986) defined it as the ratio of genotypic variance to phenotypic variance. However, they both divided heritability into narrow and broad sense. Heritability in the narrow sense was defined as the extent to which an individual is determined by its parents, while heritability in the broad sense was defined as the extent to which an individual is determined by its genetic make-up. The variances used in calculating heritability were obtained from combining ability performed by Agrobase (2000). The formula for heritability in the broad sense is as follows;

$$h_b^2 = \frac{\delta_g}{\delta_p}$$

where δ_g = genotypic variance ($2\delta gca + \delta^2 sca.$)

δ_p = phenotypic variance ($2\delta g = \delta^2 e$)

The formula for heritabilty in the narrow sense is as follows;

$$h_n^2 = \frac{\delta_a}{\delta_p}$$

where δa = additive genetic variance ($2\delta gca$)

δp = phenotypic variance($2\delta g + 2\delta^2 e$)

Estimates of heterosis

According to Mather and Jinks (1977) and Falconer and Mackay (1996), heterosis can be defined mathematically into two ways, namely; mid-parent heterosis (MPH) and high parent heterosis (HPH). Mid-parent heterosis is expressed as follows;

$$MPH = \frac{F_1 - MP}{MP} \times 100$$

High parent heterosis is expressed as follows:

$$HPH = \frac{F_1 - HP}{HP} \times 100$$

where F_1 = Offspring

MPH = Mid-parent value

HP = High parent value

4.3 Results and discussion

4.3.1 Analysis of variance for environments for the F_1 diallel trial

Experiments were conducted in two locations (Bloemfontein and Maseru) to compare the performance of the genotypes for quality characteristics. Because of problems encountered with the Lesotho trial, only 19 genotypes and four quality characteristics were used in the analysis. Table 8 and 9 show the analysis of variance of genotypes for quality characteristics and their means, respectively.

Significant differences were observed in these two environments for seed weight, seed diameter and seed hardness. No significant differences were obtained for SDSS for the two environments. Bloemfontein had the highest SDSS value of 78ml and the lowest value of 46ml, while the mean was 60ml. In Lesotho, the highest value was 73ml and the lowest was 46ml, while the mean was 58ml.

Lesotho had a highest seed weight value of 33g and lowest weight of 15g with the mean of 28g. Bloemfontein had a high seed weight value of 44g and low value of 26g, while the mean was 35.4g. Seed diameter of the same genotypes grown in Bloemfontein and Lesotho were dissimilar. Bloemfontein had a high value of 2.8mm and a low value of 2.0mm with the mean of 2.4mm. Lesotho had a high value of 2.1mm and low value of 1.4mm with the mean of 1.6mm. Bloemfontein had the highest seed hardness value of 74.12 and lowest value of 40.79 with the mean of 61.75. Lesotho had the highest hardness value of 64.24 and lowest value of 39.43 with the mean of 52.26. The difference that occurred in these locations showed clearly that seven characteristics are influenced by environment.

Table 8: Mean squares for quality characteristics in Bloemfontein and Maseru for the F_1 diallel

Source of variation	Df	SDSS (ml)	Seed weight	Seed diameter	Seed hardness
Locations	1	34.105	1271.370**	4.244**	857.375*
Genotypes	18	76.243	15.953	0.030	86.237
CV		14.45	16.25	12.06	18.75
LSD (0.05)		8.219	4.615	0.2408	7.486

*** $P \leq 0.01$

Table 9: Mean values for quality characteristics in Maseru and Bloemfontein

Location	SDSS (ml)	Seed weight (g)	Seed diameter (mm)	Seed hardness index
Bloemfontein	60	35.4	2.4	61.75
Lesotho	58	28.0	1.6	52.26
LSD (0.05)	8.219	4.615	0.2408	7.486

4.3.2 Analysis of variance for parents, and F_1 and F_2 progeny at Bloemfontein

Analysis of variance was carried out on parents and F_1 progeny and parents and F_2 progeny to establish the difference in milling and rheological characteristics. Results of analysis of variance performed revealed highly significant differences ($P<0.01$) among the parents, F_1 , F_2 and reciprocals for flour yield, flour protein content, mixograph development time, sodium dodecyl sulphate sedimentation, seed weight, seed diameter and hardness index (Table 10 and 11). Woldegiorgis (2003) found a significant difference between the parents, F_1 and F_2 analysed for SDS sedimentation, hardness index, mixing development time, seed weight, seed diameter, hardness index and break flour yield. Barnard (1999) studied above-mentioned quality parameters using parents from South Africa and F_1 and F_2 progeny and found significant differences for the said parameters. Van Lill and Purchase (1995) and Du Preez (2001) obtained similar results. The difference in parents and F1 and parents and F_2 revealed that in each generation there is a variability which could be exploited in breeng programmes to produce more improved cultivars.

Table 10: Analysis of variance for seven quality characteristics of parents and F_1 progeny

Source	df	FLY	FPC	MDT	SDSS	SKCSW	SKCSD	SKCSH
Total	74							
Block	2	5.588	0.244	0.011	7.453	17.940	0.038	7.452
Entry	24	24.088**	3.165**	0.825**	295.363**	53.840**	0.144**	397.096**
Residual	48	4.017	0.265	0.177	10.787	11.0740	0.041	20.655
Mean		47.282	12.259	3.096	60.147	34.927	2.383	61.053
R^2		0.7535	0.8572	0.6998	0.963	0.7142	0.6429	0.9059
CV(%)		4.24	4.20	13.60	5.46	9.53	8.49	7.44

FLY=break flour yield, FPC=flour protein content, MDT=mixograph development time, SDSS=SDS-sedimentation volume, SKCSW=seed weight, SCKCSD=seed diameter, SCKSH=seed hardness, **P≤0.05,

Table 11: Analysis of variance for seven quality characteristics of parents and F_2 progeny

		Mean squares for quality characteristics						
Source	**Df**	**FLY**	**FPC**	**MDT**	**SDSS**	**SKCSW**	**SKCSD**	**SKCSH**
Total	74							
Block	2	2.775	0.345	0.036	0.040	2.381	0.09	18.606
Entry	24	14.597***	1.239***	1.305***	147.330**	28.141**	0.083**	445.217**
Residual	48	2.638	0.126	0.266	17.040	3.416	0.012	39.849
Grand mean		58.9139	11.9593	3.2960	76.8800	33.9607	2.3032	67.6265
R^2		0.4559	0.3971	0.6100	0.6299		0.3971	0.4058
CV(%)		2.757	2.966	15.651	5.369	0.3640 5.443	4.782	9.335

FLY= flour yield, FPC=flour protein content, MDT=mixograph development time, SDSS=SDS-sedimentation volume, SKCSW=seed weight, SCKCSD=seed diameter, SCKSH=seed hardness, ***P≤0.01, **P≤0.05

4.3.3 Performance of parents and F_1 progeny at Bloemfontein

The results of mean values for break flour yield, flour protein content, mixograph development time, SDS sedimentation volume, seed weight, seed diameter and seed hardness clearly showed the differences among the parents and F_1 progeny (Table 12).

Flour yield: Nata x Wanda, Sceptre x Kariega, Sceptre x Wanda, Kariega x Sceptre and Nata x Sceptre had the highest values for FLY. These crosses performed significantly better than the lowest performing crosses Sceptre x Nata, Nata x Kariega, Kariega x Wanda, Wanda x Kariega, Kariega x Nata, Kariega x SST124 and Wanda x Nata. Of the parents Kariega had the highest value and Sceptre had the lowest value. This was interesting as Sceptre was a parent in four of the five highest FLY value crosses.

Flour protein content: Four of the crosses (Nata x Wanda, Sceptre x Kariega, SST124 x Nata and SST124 x Kariega) had FPC values higher than 13%. Three of the five parents (Wanda, Kariega and Sceptre) also had values above 13%. Of the crosses, nine had FPC values below 12% although only one parent (Nata) had a FPC value below 12%.

Mixograph development time: Two and a half minutes is the ideal mixing time in industry although up to 3 minutes is acceptable, therefore progeny of crosses should remain in this range. Of the parents, only SST124 was over stable, but of the crosses, 11 had mixing times of onger than 3 minutes and are therefore not suitable for bread making.

Sodium dodecyl sulphate -sedimentation: The highest possible SDSS values are required for good breadmaking quality. With the exception of Sceptre, all the parents had values of more than 60ml. In the progeny only six of the crosses had values of more than 60ml. It would seem as though the progeny performed very poorly compared to the parents.

Single kernel characterization seed weight: A significantly higher seed weight was obtained in five crosses compared to the rest and no significant difference was found between them. High seed weight is important as it is related to hectolitre mass used in the wheat grading system. SST 124 had a

significantly higher seed weight than other parents but significantly lower than the progeny. Four progeny had a low seed weight. Among the parents, Sceptre exhibited low seed weight which was lower than the lowest scoring progeny.

Single kernel characterization seed diameter: Wheat seed with diameter exceeding 2.00mm is desirable since it is correlated with seed weight. All the crosses and parents had a diameter above the desirable one except Sceptre. Sceptre x Wanda, Sceptre x Kariega, Kariega x Wanda, Nata x SST 124 and Wanda x Kariega had significantly higher diameters than the rest of the parents, while Nata x Kariega had the lowest diameter.

Single kernel characterization seed hardness : Kariega x Wanda, Sceptre x Nata, Nata x Kariega and Wanda x Sceptre showed a significantly higher hardness index than other progeny, which is unacceptable to the millers as more energy is required to break them during grinding. A large percentage (80%) of the crosses produced kernels whose hardness indices were within tolerable range.

4.3.4 Performance of parents and F_2 progeny at Bloemfontein

Results of mean values for break flour yield, flour protein content, mixograph development time, SDS sedimentation volume, seed weight, seed diameter and seed hardness clearly showed the differences among the parents and F_2 progeny (Table 13).

Flour yield: The breakflour yield considered to be ideal by millers is 60% and above because their interest lie on amount of flour in the kernels rather than the testa. Five of the crosses produced FLY of higher than 60%, while parents and remaining crosses obtained value close this.

Table 12: Means of quality characteristics of parents and F_1 progeny in the diallel analysis at Bloemfontein

Genotypes	Quality characteristics						
	FLY	FPC	MDT	SDSS	SKCSW	SKCSD	SKCSH
Nata x Wanda	49.95	13.03	2.53	51.00	36.25	2.46	66.2
Wanda xSceptre	47.36	12.63	4.17	58.33	29.01	2.15	73.0
Sceptre x Kariega	49.45	14.83	3.47	55.00	41.38	2.77	66.4
Sceptre x Nata	45.87	11.63	3.07	49.67	32.48	2.33	73.1
Nata x Kariega	43.57	11.47	3.90	55.00	26.29	2.03	72.8
Wanda x SST 124	47.24	10.90	3.90	56.67	31.79	2.26	69.3
Sceptre x Wanda	51.96	11.13	2.23	58.33	44.17	2.77	44.7
Sceptre x SST 124	48.89	10.63	3.10	54.33	35.85	2.42	60.0
Nata x SST 124	47.75	11.30	2.53	57.00	37.76	2.49	57.1
Kariega x Wanda	44.89	11.27	2.76	47.33	37.35	2.60	74.1
Wanda x Kariega	46.55	11.13	3.00	51.33	33.78	2.49	68.0
Kariega x Nata	41.29	12.27	3.40	56.33	34.80	2.48	56.5
Kariega x SST 124	42.25	11.47	3.77	67.00	34.52	2.29	37.4
Wanda x Sceptre	47.36	12.63	4.17	58.33	29.01	2.15	73.0
Kariega x Sceptre	51.63	12.60	2.77	76.67	38.88	2.47	48.9
Nata x Sceptre	49.58	12.60	2.73	78.00	33.90	2.21	40.7
Wanda x Nata	46.39	12.63	4.37	62.33	30.05	2.14	72.2
SST 124 x Nata	48.85	13.73	2.77	72.33	38.01	2.44	48.2
SST 124 x Kariega	48.25	13.27	3.10	53.33	33.65	2.31	72.2
SST 124 x Wanda	47.27	12.23	3.00	68.00	33.51	2.31	51.8
Nata	46.99	11.77	2.87	62.00	34.71	2.29	54.1
Wanda	49.29	13.27	2.70	76.33	34.33	2.31	58.5
SST 124	49.43	12.83	3.30	66.33	36.59	2.43	53.7
Kariega	49.38	13.20	2.73	76.33	34.07	2.28	57.3
Sceptre	41.77	13.13	2.93	48.67	27.91	1.95	75.1
Mean	47.00	12.00	3.10	60.40	34.93	2.37	60.82
Maximum	51.96	14.83	4.37	78.00	44.17	2.77	75.18
Minimum	41.29	10.63	2.23	47.33	26.29	1.95	37.40
Variance	2.80	1.02	0.52	9.46	4.15	0.20	11.06
LSD (0.05)	2.745	0.706	0.577	4.500	4.557	0.277	6.200

FLY=flour yield, FPC=flour protein content, MDT=mixogram development time, SDSS=SDS sedimentation volume, SKCSW=seed weight, SCKCSD=seed diameter, SCKSH=seed hardness,

Flour protein content : One half of the crosses produced a FPC above 12% , which is required by the bakers and they offer a higher price for it, below which there is a decline in price and grading class offered. Interestingly, in all the crosses where SST 124 was a paternal parent, FPC was above 12%. Wanda x Sceptre, Nata x Kariega and Kariega x SST 124 had a low FPC. FPC for all parents were above 12%.

Sodium dodecyl sulphate -sedimentation: SST 124 x Nata, Nata x Sceptre, Sceptre x SST 124 and Wanda x Sceptre had significantly higher SDSS than other progeny but no significant difference was realized among these progeny. Wanda, SST 124 and Kariega performed significantly better than other parents and these parents were insignificantly different from each other. Nata x Wanda, Kariega x Wanda and Sceptre x Nata performed significantly poorer than the other progeny and they were insignificantly different from each other. Sceptre was the only parent which did poorly, although its performance was better than the lowest of the parents.

Single kernel characterization seed weight: The yield of wheat is measured in terms of weight which is directly dependent on kernel weight. Anything above 30g is acceptable. All the crosses had seed weight of more than 30g except three, namely; Sceptre x SST 124, Wanda x Kariega and Kariega x SST 124. Parents gave a higher SKCSW than recommended with Nata obtaining the highest, followed by Sceptre.

Single kernel characterization seed diameter: Only one cross (Kariega x SST 124) obtained a value of 2.00mm for seed diameter while the rest were above it. Nata x Sceptre, Nata x Wanda and Sceptre x Wanda outperformed other crosses significantly and no significant difference was obtained among them. Seed diameter is directly related to the weight of the seed such that the larger the diameter the higher the seed weight.

Single kernel characterization seed hardness: High hardness index is undesirable to the millers as more energy is expended to break and grind the seed. Hardness index above 60 is unacceptable to the millers. A large number of crosses (15) had SKCSH values above 60, while one cross had dismally low hardness index which is also unacceptable. All parents fell within the acceptable range.

Table 13: Means of quality characteristics of parents and F_2 progeny in the diallel analysis

Genotypes	Quality characteristics						
	FLY	FPC	MDT	SDSS	SKCSW	SKCSD	SKCSH
Nata x Wanda	58.20	11.47	3.1	62	37.07	2.52	71.23
Wanda x Sceptre	58.93	10.97	3.9	76	30.86	2.19	71.82
Sceptre x Kariega	59.77	12.20	3.7	70	34.45	2.32	74.89
Sceptre x Nata	57.07	11.57	3.4	68	33.66	2.29	72.83
Nata x Kariega	56.82	10.10	4.2	70	30.17	2.21	75.29
Wanda x SST 124	63.75	11.75	3.8	84	31.11	2.20	80.45
Sceptre x Wanda	62.84	11.60	2.6	83	38.73	2.52	60.18
Sceptre x SST 124	59.53	12.20	4.0	85	29.65	2.06	67.68
Nata x SST 124	59.97	11.50	3.2	76	34.44	2.34	65.81
Kariega x Wanda	57.50	11.20	3.7	67	32.80	2.35	76.14
Wanda x Kariega	56.69	11.40	4.5	70	29.85	2.22	79.24
Kariega x Nata	58.19	12.30	3.2	77	33.71	2.19	73.20
Kariega x SST 124	53.99	10.60	2.5	76	28.81	1.78	21.11
Wanda x Kariega	58.59	12.30	3.8	76	33.06	2.29	77.09
SST124 x Sceptre	58.91	12.40	3.6	77	33.68	2.29	75.50
Nata x Sceptre	63.12	12.60	2.1	86	39.43	2.55	52.06
Wanda x Nata	60.54	12.50	4.3	83	32.29	2.17	76.65
SST124 x Nata	60.45	13.30	2.9	90	36.55	2.35	54.44
SST124 x Kariega	57.14	12.60	3.5	76	34.25	2.34	73.23
SST124 xWanda	58.61	12.50	2.7	76	33.63	2.34	71.20
Nata	59.72	12.00	2.6	76	38.82	2.51	61.86
Wanda	58.56	12.00	2.6	85	36.67	2.45	62.45
SST124	58.62	12.40	3.2	82	34.74	2.29	65.53
Kariega	59.48	12.70	2.7	81	36.00	2.43	67.65
Sceptre	56.00	12.00	2.6	67	37.04	2.39	62.51
Mean	58.92	11.92	3.3	77	34.02	2.30	67.60
Maximum	63.75	13.30	4.3	90	39.43	2.55	80.45
Minimum	53.99	10.10	2.1	62	28.81	1.78	21.11
Standard deviation	2.21	0.72	0.65	7.07	3.0	0.17	12.21
LSD (0.05)	9.114	2.041	0.908	12.172	0.444	6.845	19.765

FLY=flour yield, FPC=flour protein content, MDT=mixogram development time, SDSS=SDS sedimentation volume, SKCSW=seed weight, SCKCSD=seed diameter, SCKSH=seed hardness

Marais (1982) and Gaines (1991) found a wide variation in the F_2 progeny when studying protein content and kernel texture. Pooni (1993) obtained similar results when researching the yield performance of F_2 progeny.

4.3.5 Comparison of F_1 and F_2 progeny

Table 14 shows a comparison of F_1 and F_2 progeny. A reduction in the performance of some F_2 progeny were observed when compared to F_1 progeny which is caused by segregation of genes resulting from a decline in heterozygosity obtained in F_1 progeny. Homozygosity is starting to increase. Some F_1 progeny were similar to F_2 progeny which showed the impact of additive gene action which passed the genes combined in F_1 to F_2 in the same pattern. These progeny could be selected for further breeding. Those F_2 progeny with higher performance than F_1 progeny are due to additive or additive x additive gene action and these can be selected for further breeding purposes as well. Poshi *et al.* (1993) and Wricke and Weber (1986) indicated that segregation of genes in the F_2 results in varying performance of this generation from low to high. F_1 which exhibited higher performane than F_2 could be used in early generation testing, while F_2 that showed higher performance than F_1 could be used in other breeding methods where selection is done later.

4.3.6 Combining ability

Combining ability analysis for parents, F_1 and F_2 progeny

Analysis of variance for general and specific combining ability for parents and F_1 is presented in Table 15. The mean squares for general combining ability were significant ($P \leq 0.05$) for break flour yield, SDS sedimentation volume and kernel hardness. Specific combining ability and reciprocals were significantly different for break flour yield, flour protein content, mixograph development time, SDS sedimentation volume, seed diameter, seed weight and seed hardness index.

Analysis of variance for general and specific combining ability for parents and the F_2 generation as well as reciprocals is depicted in Table 16. General combining ability was highly significant for break flour yield, flour protein content, SDS sedimentation volume, seed weight, seed diameter and seed hardness index except for mixograph development time. Specific combining ability and reciprocals exhibited highly significant differences for all the seven wheat quality characteristics being studied. The magnitute of mean squares of the components for various characteristics varied gratly between F_1

and F_2 progeny indicating the presence of variability in the genetic expression of characteristics. The mean squares of GCA for FLY, SDSS and SKCSH in F_1 progeny were higher than F_2 progeny, while mean squares of SCA for FLY, FPC, SDSS, SKCSW AND SKCSD for F_1 were higher than in F_2 progeny, while MDT and SKCSHwere lower in F_1 than F_2 progeny. The difference in magnitude between F_1 and F_2 progeny revealed in this study is consistent with the findings of other researchers who found that magnitudinal differences between three generations comprising parents, F_1 and F_2 progeny (Paroda & Toshi, 1970; Mihaljev and Kovacev-Djolai, 1978; Sing *et al.*,1986; Singh, 1988; Mandal & Maity, 1992).

General combining ability effects in the F_1 progeny

Table 17 shows general combining ability effects for measured characteristics. GCA estimates revealed that among parents, Wanda and SST 124 were good general combiners for FLY, SDSS, SKCSD and SKCSD and poor for MDT, SKCSD and FPC. However, Wanda was also a good combiner for SKCSH, while SST 124 was a poor combiner for it. In general, Wanda ranked first, followed by SST 124 for GCA. The average combiners were Kariega and Sceptre for FPC, SKCSW and SKCSH, and FLY, FPC and SKCSH, respectively. They were poor combiners for MDT, SDSS and SKCSD. Nata showed poor combining ability for all wheat quality characteristics except SDSS and SKCSD. This necessitates further improvement of the combining ability for wheat quality characteristics, since none of the good combiners had good values for all the desirable characteristics. SST 124 and Wanda could be utilized extensively in the hybridisation programme to accelerate the pace of genetic improvement of wheat quality characteristics, while Nata could be discarded from the breeding programme.

Table 14: Comparison of F_1 and F_2 progeny for measured quality characteristics

Progeny	Quality characteristics													
	FLY		FPC		MDT		SDSS		SCKSW		SCKSD		SHI	
	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2
Sceptre x Wanda	51.96	62.84	11.13	11.60	2.33	2.60	58.33	83.67	44.17	38.73	2.77	2.52	44.7	60.1
SSt 124 x Sceptre	51.63	58.59	12.60	12.40	2.77	3.60	76.67	77.54	38.88	33.68	2.47	2.29	48.9	75.5
Nata x Wanda	49.95	58.20	13.30	11.47	2.53	3.10	51.00	62.85	36.25	37.07	2.46	2.52	66.2	71.2
Nata x Sceptre	49.58	63.12	12.60	12.60	2.73	2.10	78.00	86.64	33.90	39.43	2.21	2.55	40.7	52.0
Sceptrex Kariega	49.45	59.77	14.83	12.20	3.47	3.70	55.00	70.87	41.38	34.45	2.77	2.32	66.4	74.8
Sceptre x SST124	48.89	59.53	10.63	12.20	3.10	4.00	54.33	85.73	35.85	29.65	2.42	2.06	60.0	67.6
SST 124 x Nata	48.85	60.45	13.73	13.30	2.77	2.90	72.33	90.52	38.01	36.55	2.44	2.35	48.2	54.4
SST124x Kariega	48.25	57.14	13.27	12.60	3.10	3.50	53.33	76.41	33.65	34.25	2.31	2.34	72.2	73.2
Nata x SST 124	47.75	59.97	11.13	11.50	2.53	3.20	57.00	76.08	37.76	34.44	2.49	2.34	57.1	65.8
Wanda x Sceptre	47.36	58.93	12.63	10.97	4.17	3.90	58.33	76.54	29.01	30.86	2.15	2.19	73.0	71.8
SST124 x Wanda	47.27	58.61	12.23	12.50	3.00	2.70	68.00	76.52	33.51	33.63	2.31	2.34	51.8	71.2
Wanda x SST 124	47.24	63.75	10.90	11.75	3.90	3.80	56.67	84.73	31.79	31.11	2.26	2.20	69.3	80.4
Wanda x Kariega	46.55	56.69	11.13	11.40	3.00	4.50	51.33	70.72	33.70	29.85	2.49	2.22	68.0	79.2
Wanda x Nata	46.39	60.54	12.63	12.50	4.37	4.30	62.33	83.64	30.05	32.29	2.14	2.17	72.2	76.6
Wanda x Sceptre	46.19	58.59	11.13	12.30	2.93	3.80	51.33	76.54	42.22	30.86	2.49	2.19	68.0	77.0
Sceptre x Nata	45.89	57.07	11.63	11.57	3.07	3.40	49.67	68.96	32.48	33.66	2.33	2.29	73.1	72.8
Kariega x Wanda	44.89	57.50	11.27	11.20	2.76	3.70	47.33	67.54	37.35	32.80	2.60	2.35	74.1	76.1
Nata x Kariega	43.57	56.82	11.47	10.10	3.90	4.20	55.00	70.87	26.29	33.60	2.03	2.21	72.8	75.2
Kariega x SST124	42.25	53.99	11.47	10.60	3.17	2.50	67.00	76.53	34.52	28.81	2.29	1.78	37.4	21.1
Kariega x Nata	41.29	58.19	12.27	12.30	3.40	3.20	56.33	77.98	34.80	33.71	2.48	2.19	56.5	73.2

Table 15: Analysis of variance for general and specific combining ability in parents and F_1 progeny

		Mean squares for quality characteristics						
Source	**Df**	**FLY**	**FPC**	**MDT**	**SDSS**	**SKCSW**	**SKCSD**	**SKCSH**
Total	74							
GCA	4	7.242*	0.113*	0.011*	61.270*	5.467*	0.019*	137.185*
SCA	10	10.834*	0.991*	0.221*	107.827*	22.402*	0.071*	69.032*
Reciprocals	10	5.540*	1.496*	0.435*	103.956*	18.483*	0.037*	193.771*
Residual	48	1.339	0.088	0.059	3.596	3.611	0.014	6.885

FLY=break flour yield, FPC=flour protein content, MDT=mixogram development time, SDSS=SDS sedimentation
volume, SKCSW=seed weight, SCKCSD=seed diameter, SCKSH=seed hardness *P≤0.05

Table 16: Analysis of variance for general and specific combining ability in parents and F_2 progeny

		Mean squares for quality characteristics						
Source	**Df**	**FLY**	**FPC**	**MDT**	**SDSS**	**SKCSW**	**SKCSD**	**SKCSH**
Total	74							
GCA	4	4.503*	0.262*	0.113	55.942*	11.560*	0.034*	69.154**
SCA	10	4.907*	0.288*	0.536*	36.537*	7.272*	0.016*	151.826**
Reciprocals	10	4.970*	0.599*	0.436*	58.950*	10.617*	0.037*	176.686**
Residual	48	0.879	0.042	0.089	5.680	1.139	0.004	13.283

FLY=flour yield, FPC=flour protein content, MDT=mixogram development time, SDSS=SDS sedimentation volume,
SKCSW=seed weight, SCKCSD=seed diameter, SCKSH=seed hardness, ***P≤0.01, **P≤0.05, *P≤ 0.1

Table 17: General combining ability effects for parents and F_1 progeny

	FLY	FPC	MDT	SDSS	SKCSW	SKCSD	SKCSH
NATA	-0.5586	-0.0387	0.0073	0.4200	-1.0315	-0.0649	1.4990
WANDA	0.7387	-0.1087	0.0307	0.4533	-0.4778	-0.0019	3.2023
KARIEGA	-1.1603	0.1013	0.0207	-1.7467	0.2785	0.0571	2.5820
SST124	0.8161	-0.0787	-0.0027	3.6533	0.7879	0.0021	-5.7843
SCEPTRE	0.1641	0.1247	-0.0560	-2.7800	-0.4429	0.0075	1.4990

FLY=flour yield, FPC=flour protein content, MDT=mixogram development time, SDSS=SDS sedimentation volume, SKCSW=kernel weight, SCKCSD=kernel diameter, SCKSH=kernel hardness,

General combining ability effect in the F_2 progeny

Table 18 shows the GCA effects for F_2 progeny. Wanda showed a good combining ability for FLY, SDS and SKCSH and outperformed all other parents. It was followed by Nata which was a good combiner for FLY and SKCSW and poor for the rest. Sceptre was a good combiner for one characteristic (SKCSW), and average in two other characteristics (FPC and FLY), while poor for the rest. Kariega and SST 124 generally had poor combining ability. All parents revealed poor combining ability for SKCSD, followed by MDT and FPC.

Wanda was the only consistently good combiner in F_1 and F_2 progeny, while SST 124, which was a good combiner in the F_1 progeny had shown average combining ability in the F_2 progeny, indicating a decline which could be attributed to segregation of genes. On the other hand, Nata performed poorly in the F_1 but outperformed the rest of the F_2 progeny, except Wanda, for combining ability. Sceptre, which had an average combining ability in the F_1 generation, maintained status in the F_2 generation, showing consistency in its performance. Kariega, which was an average performer in the F_1 progeny, showed a poor combining ability in the F_2 progeny, indicating a decline in heterotic effect due to segregation of genes.

Only two parents showed consistency in their performance, which could be attributed to additive gene action, which is passed from generation to generation and new cultivars could be developed from them. The other three parents exhibited inconsistency in their performance which is caused by dominance gene action that appears in one generation and disappear in the next generation, which

could be utilized in hybrid production.

High GCA effects are mostly due to additive gene effects or additive x additive interactive effects (Griffing, 1956; Jensen, 1970). In view of this, breeders may utilize the good general combiners in specific breeding programmes for improvement of wheat quality. It is recommended that the breeder should breed for superior combining ability for wheat quality characteristics with the ultimate goal of improving overall GCA for quality in bread wheat.

Table 18: General combining ability effects for parents in the F_2 progeny

	FLY	FPC	MDT	SDSS	SKCSW	SKCSD	SKCSH
Nata	0.4665	0.0707	-0.1327	-0.2467	1.3697	0.0611	-0.9922
Wanda	0.4895	-0.2310	0.0840	0.3867	0.0287	0.0355	3.5598
Kariega	-1.1489	-0.0693	0.1307	-2.4467	-1.2177	-0.0482	0.9168
SST124	0.0448	0.2023	-0.0293	3.7533	-0.8803	-0.0739	-3.6255
Sceptre	0.1481	0.0273	-0.0527	-1.4467	0.6997	0.0255	0.1411

FLY=break flour yield, FPC=flour protein content, MDT=mixogram development time, SDSS=SDS sedimentation volume, SKCSW=kernel weight, SCKCSD=kernel diameter, SCKSH=kernel hardness,

Specific combining ability effects for F_1 progeny

Table 19 shows SCA effects for measured characteristics. The highest positive SCA effect was shown by the cross Sceptre x Kariega for SKCSW, SKCSH, SKCSD, MDT, FPC and FLY, followed by SST 124 Nata with five characteristics, then Wanda x Nata and Sceptre x Wanda with four. Other average combinations were Kariega x Wanda and Sceptre x SST 124. Poor combinations were SST 124 x Kariega, SST 124 x Wanda and Sceptre x Nata. In these combinations with high SCA effects, some of the parents (Sceptre, Nata, Kariega and SST 124) were of average GCA in one generation or the other suggesting that such combinations could give desirable transgressive segregants. It is recommended that new genotypes should be incorporated in future breeding programmes for recombining the desirable traits in the envisaged elite genotypes

Table 19: Specific combining ability effects for F_1 progeny

Progeny	FLY	FPC	MDT	SDSS	SKCSW	SKCSD	SKCSH
1	0.712	0.722	0.3160	-4.353	-0.267	-0.0148	6.455
2	-3.133	-0.455	0.5260	-3.153	-3.631	-0.1188	2.574
3	-1.137	-1.051	-0.3140	-9.520	0.797	0.1065	7.179
4	0.759	0.375	-0.4507	0.447	3.204	0.1462	-1.043
5	-1.583	-0.505	0.3260	-1.920	-2.586	-0.0968	2.112
6	-1.684	0.085	0.0193	-1.887	-1.907	-0.1425	-3.034
7	0.836	-0.228	-0.1473	6.047	-1.149	-0.1525	-4.103
8	1.474	-0.391	0.1293	0.513	1.697	0.0745	-6.855
9	1.535	0.682	0.1727	-5.120	6.153	0.3722	2.116
10	1.995	-0.688	-0.1040	4.480	1.205	0.0539	-2.286

1=Wanda x Nata, 2=Kariega x Nata, 3=Kariega x Wanda, 4=SST124 x Nata, 5=SST124 x Wanda, 6=SST124 x Kariega, 7=Sceptre x Nata, 8=Sceptre x Wanda, 9=Sceptre x Kariega, 10= Sceptre x SST124. FLY=break flour yield, FPC=flour protein content, MDT=mixogram development time, SDSS=SDS sedimentation volume, SKCSW=kernel weight, SCKCSD=kernel diameter, SCKSH=kernel hardness

Specific combining ability effects for F_2 progeny

Table 20 presents SCA effects for F_2 progeny. Kariega x Sceptre exhibited a high SCA effect for all wheat quality characteristics except one, while SST 124 x Sceptre and Nata x SST 124 followed with five different characteristics each. Wanda x SST 124 and Nata x Kariega showed high SCA effects for four characteristics. No combination of crosses had high SCA for FPC, MDT and SKCSD. SST 124, Nata and Sceptre appeared to be involved in a number of combinations with high SCA effects which were also observed in the F_1 generation. No combination with high SCA effects appeared in both F_1 and F_2 progeny, one combination would appear in the F_1 and disappear in the F_2 generation and another combination which did not appear in the F_1 would appear in the F_2 generation, which suggests that the genes conferring these characters are controlled by dominant gene action.

Normally, SCA effects do not significantly contribute to the improvement of self-fertilized crops, except where commercial exploitation of heterosis is feasible (Menon & Sharma, 1977; Singh, 2002). SCA effects represent dominance and epistasis interaction which can be related to heterosis. However, in self-pollinated crops like wheat, the additive x additive type of component is fixable in the later generations. The breeder's interest is vested in obtaining transgressive segregants through crosses and producing more potent homozygous lines. Jinks and Jones (1958) emphasized that the superiority of hybrids might not indicate their ability to produce transgressive segregants, rather SCA would provide satisfactory criteria.

Table 20: Specific combining ability effects for parents and F_2 progeny

	FLY	FPC	MDT	SDSS	SKCSW	SKCSD	SKCSH
Nata x Kariega	-0.501	0.1843	0.4360	-3.687	-0.576	-0.0531	3.80
Nata xWanda	-0.726	-0.2940	0.4227	-0.687	-2.603	-0.1178	6.90
Nata x SST124	0.783	0.1843	-0.0673	3.280	1.043	0.0562	-2.89
Nata x Sceptre	0.568	0.0260	-0.3273	1.813	0.015	0.0269	-4.03
Wanda x Kariega	-1.158	-0.3757	0.5893	-6.153	-1.445	-0.0038	5.59
Wanda x SST124	1.734	0.1777	-0.1173	-0.687	-0.739	0.0052	8.26
Wanda x Sceptre	1.262	-0.4890	-0.0773	-3.513	0.104	-0.0108	-5.33
Kariega x SST124	-2.246	-0.5090	-0.3640	-2.353	-0.734	-0.1228	17.98
Kariega x Sceptre	1.264	0.3327	0.4093	-0.153	0.311	0.0245	7.31
SST124 x Sceptre	0.113	0.1110	0.5860	1.813	-2.113	-0.0765	7.45

FLY=break flour yield, FPC=flour protein content, MDT=mixogram development time, SDSS=SDS sedimentation volume, SKCSW=kernel weight, SCKCSD=kernel diameter, SCKSH=kernel hardness

Reciprocal effects for F_1 progeny

Table 21 depicts the reciprocal effects for measured characeristics in F_1 progeny. Sceptre x SST 124 showed high reciprocal effects for FLY, FPC, SDS, SKCSW and SKCSD, followed by Wanda x Nata and Sceptre x Nata with four characteristics each. Four combinations had very low reciprocal effects for two characteristics which differed with each combination. Reciprocal effects exerted by the combinations mentioned earlier on, indicated that the marternal influence played a major role in expression of the said wheat quality characterstics, necessitating proper identification of male and female parents when planning the crossing block.

Table 21: Reciprocal effects for F_1 progeny

	FLY	FPC	MDT	SDSS	SKCSW	SKCSD	SKCSH
1	1.780	0.200	-0.9167	-5.667	3.102	0.1617	-2.99
2	1.137	-0.400	0.2500	-0.667	-4.257	-0.2267	8.16
3	0.830	-.0.067	0.1000	2.000	-1.828	-0.0517	-0.11
4	-0.548	-1.217	-0.1167	-7.667	-0.125	0.0233	4.44
5	-0.013	-0.667	0.4500	-5.667	-0.862	-0.0267	8.72
6	-3.000	-0.900	0.0333	6.833	0.433	-0.0100	-17.42
7	1.853	0.483	-0.1667	14.167	0.710	-0.0600	-16.16
8	-2.298	0.750	0.9667	0000	-7.577	-0.3100	14.18
9	-1.630	-1.667	-0.2333	-4.500	0.418	0.0500	0.80
10	1.370	0.983	-0.1667	11.167	1.517	0.0267	-5.59

1=Wanda x Nata, 2=Kariega x Nata, 3=Kariega x Wanda, 4=SST 124 x Nata, 5=SST 124 x Wanda, 6=SST 124 x Kariega, 7=Sceptre x Nata, 8=Sceptre x Wanda, 9=Scepter x Kariega, 10= Scepter x SST124. FLY=break flour yield, FPC=flour protein content, MDT=mixogram development time, SDSS=SDS sedimentation volume, SKCSW=kernel weight, SCKCSD=kernel diameter, SCKSH=kernel hardness.

Reciprocal effects in the F_2 progeny

Table 22 presents the reciprocal effects for the F_2 progeny. Four characteristics in the F_2 with high reciprocal effects were expressed in Nata x Sceptre, followed by Wanda x SST 124 with three. No reciprocal effect was expressed in Kariega x SST 124, Kariega x Sceptre and Nata x Kariega.

In this study, reciprocal effects differed largely within and between F_1 and F_2 progeny suggesting the presence of maternal effects. In some characteristics, F_1 progeny had larger maternal effects than F_2 progeny, while in some other characteristics, F_2 had larger maternal effects than F_1 progeny. Generally, the F_1 progeny showed larger maternal effects than F_2 progeny, which is brought about by the large cytoplasm acquired from both parents differing in a particular characteristic. In F_2 progeny, there is a regression due to a loss in some properties conferring the characteristics. Pooni (1993) indicated that the F_2 reciprocal effects are expected to differ in the presence of cytoplasmic or

maternal effects. Again, reciprocal differences in the F_2 diallel are not expected to be detected more often than in the F_1 diallel and the corresponding means squares of the two diallel may in fact differ greatly. This shows that a cultivar selected for its desirable traits may be determined by cytoplasm rather than nuclear genes which could be persistent or short lived.

Table 22: Reciprocal effects for parents in F_2 progeny

	FLY	FPC	MDT	SDSS	SKCSW	SKCSD	SKCSH
Nata x Kariega	-1.172	-0.517	-0.6167	-11.00	2.283	0.1700	-2.77
Nata xWanda	-0.685	-0.600	0.5167	-3.50	-1.337	0.0083	0.85
Nata x SST124	-0.242	-0.883	0.1333	-6.00	-1.053	-0.0067	5.69
Nata x Sceptre	3.027	0.517	-0.6500	8.67	3.388	0.1300	-10.08
Wanda x Kariega	-0.403	0.117	0.4000	1.33	-1.473	-0.0600	1.55
Wanda x SST124	2.572	-0.358	0.5667	4.00	-1.260	-0.0700	4.63
Wanda x Sceptre	-2.023	-0.367	0.6833	-3.33	-3.933	-0.1633	5.82
Kariega x SST124	-1.573	-1.017	-0.500	0.17	-3.118	-0.2983	-25.83
Kariega x Sceptre	-0.590	0.017	0.0500	2.83	-0.697	-0.0183	1.10
SST124 x Sceptre	-0.309	0.100	-0.1667	-3.67	2.013	0.1150	3.91

FLY=break flour yield, FPC=flour protein content, MDT=mixogram development time, SDSS=SDS sedimentation volume, SKCSW=kernel weight, SCKCSD=kernel diameter, SCKSH=kernel hardness.

GCA : SCA ratio

GCA: SCA ratio is used to determine the nature of genetic variability, where GCA to SCA mean squares ratio is below unity, there is dominance, which could be partial or complete. Ratio of unity is complete dominance indicating additive gene action, while low GCA to SCA ratio indicate over-dominance. Above unity, over-dominance exists (Falconer & Mackay, 1996).

Table 23 shows the GCA and SCA ratios. All the quality characteristics in the F_1 generation, except one, showed non-additive gene action with their GCA : SCA ratio being below one. This suggested that non-additive gene action was predominant, and that selection for these characteristics will not give a good response in following generations . GCA : SCA ratio for F_2 progeny is presented in

Table 24. Break flour yield, flour protein content, seed hardness and mixograph development time had values below unity, indicating that they are controlled by dominant gene action. SDS sedimentation volume, seed weight and seed diameter had values above unity, indicating that these characters are controlled by additive gene action. The characters with a ratio below unity could be improved through hybrid development, whereas the ones with the ratio above unity could be improved through normal breeding methods such as pedigree, single seed descent and others. Selection in this generation could give a good response.

Mihaljev and Kovacev-Djolai (1978) found additive gene action to be predominant for grain protein percentage, while Sayed (1978) reported yield to be the only character with non-additive gene action. Barnard (1999) observed that flour protein content, break flour yield and mixograph development time were controlled by additive gene action, while SDS sedimentation volume was controlled by non-additive gene action. Variation was obtained between the GCA : SCA ratios of F_1 and F_2 progeny, with the F_2 progeny showing that 50% of the characters were controlled by additive gene action, whereas in the F_1 progeny all characters were controlled by dominant gene action.

Table 23: Estimation of GCA:SCA ratio for the F_1 diallel

Quality characteristics	GCA	SCA	GCA:SCA
Beak flour yield	7.242	10.834	0.668
Flour protein content	0.113	0.991	0.114
Mixogram development time	0.011	0.221	0.050
SDS sedimentation volume	0.270	107.827	0.568
Kernel weight	5. 1467	22.402	0.244
Kernel diameter	0.038	0.144	0.264
Kernel hardness	137.185	69.032	1.987

GCA = general combining ability, SCA = specifc combining ability,

Table 24: Estimation of GCA:SCA ratio for the F_2 diallel

Quality characteristics	GCA	SCA	GCA:SCA
Beak flour yield	4.503	4.907	0.92
Flour protein content	0.262	0.288	0.91
Mixogram development time	0.113	0.536	0.21
SDS sedimentation volume	58.950	36.950	1.60
Kernel weight	11.560	7.272	1.59
Kernel diameter	0.034	0.016	2.125
Kernel hardness	69.154	151.826	0.456

GCA = general combining ability, SCA = specifc combining ability,

Variances of combining ability

The variance of combining ability and components of genetic variance are shown in Table 25. Results revealed that large genotypic variability existed in GCA amongst cultivars for characteristics in the F_1 progeny. SKCSH had the largest variability (137.185), followed by SDSS volume (61.270), FLY (7.242) and SKCSW (5.467) in GCA. There was a negligible variability in FPC (0.113), MDT (0.011) and SKCSD (0.019).

Genotypic variability in SCA for all characteristics except one was higher than that of GCA.

Characters with high genotypic variability were SDSS were (107.827), followed by SKCSH (69.032), SKCSW (22.402) and FLY (10.834). Least genotypic variability was obtained in SKCSD (0.071), MDT (0.221) and FPC (0.991).

In the F_2 progeny, results showed a large variance in GCA for wheat quality characteristics studied (Table 26).), SKCSH had the largest variability (69.154), followed by SDSS (55.942), seed weight (11.580) and FLY (4.503). Small variability was observed in FPC (0.262) and MDT (0.113), while SKCSD had a negligible value (0.034). This suggests that the parents that produce the characteristics with the lowest variance should be discarded from the breeding programme, while the ones with high GCA be retained for future breeding purpose.

Parents with low GCA variances suggested that directional selection was practised which resulted in narrowing down of genetic hetereogeneity. The variance for SCA was very large with SKCSH obtaining a high value of 151.826, followed by SDSS (36.537), SKCSW (7.272) and FLY (4.907). Low SCA was experienced in FPC (0.288) and MDT (0.536). The high SCA variance had resulted from an effective selection which had reduced the GCA. Plaisted *et al.*(1962) noted that previous selection methods may narrow down the genetic base of tested lines resulting in obtaining greater estimates of SCA. In agreement with this, Killick and Malcolmon (1973) suggested that characteristics subjected to directional selection, would be expected to show high SCA variance, while low SCA could be due to stabilizing selection practiced.

Consistency was obtained in the variance of F_1 and F_2 progeny for all characteristics. Similarly, the trend that was observed in general combining ability was found to be the same as with the specific combining ability. This indicated that the variances in general and specific combining ability were correlated, particularly where selfing is allowed. It could be concluded that variance in GCA and SCA is to the advantage of plant breeding because genetic material is broadened and can be utilized in different breeding programmes.

Components of genetic variance

It was observed that the relative proportion of V_a estimates to that of V_d varied greatly across the characteristics studied in the F_1 progeny. The characteristic with the highest V_a value was seed SKCSH, followed by SDSS, FLY and SKCSW. MDT, SKCSD and FLY had the lowest values. The values for V_d were high for SDSS, followed by SKCSH, SKCSH, FLY and FPC. The characteristics with lowest values of V_d were MDT and SKCSW. Three characteristics had high V_a estimates, while high V_d was observed in four characteristics. The characteristics with high V_a implied that they can be passed on to subsequent generations, whereas those indicating V_d can only be expressed in this particular generation and not be passed on to the subsequent generations. Wricke and Weber (1986) and Falconer and Mackay (1996) indicated that V_a is heritable, while V_d is non-heritable but can be expressed in a particular generation depending on the allelic interaction.

The relative proportion of V_a to V_d was relatively high for FLY, FPC, SDSS, SCKSW and SCKSD in F_1 progeny. SCKSH showed the highest V_a value, followed by SDSS, SCKSW and FLY. FPC and SKCSD had a very low V_a. However, MDT and SKCSH had relatively higher V_d than V_a. The former four characteristics are controlled by additive gene action which could be passed on from generation to generation, while the latter two are controlled by non-additive gene action suggesting that a change may occur in subsequent generation since dominance is not heritable (Falconer & Mackay, 1996).

Heritability

Heritability of seven characteristics under study were partitioned into h_n^2 and h_b^2. All characteristics showed very high h_b^2 ranging from 0.81 to 0.99 in the F_1 progeny. This showed that the combinations of genes in this particular generation were favourable for characteristics obtained under study. The major problem with the h_b^2 is that the good characteristics obtained in this generation cannot be passed on to the subsequent generations as with h_n^2. The major problem with h_b^2 is that dominance is also involved which changes from generation to generation and cannot be selected for. SDSS had the highest h_b^2, followed by SKCSH, FLY, FPC, SKCSW, SKCSD, then MDT (Table 25).

A wide range from 0.07 to 0.78 was obtained in h_n^2 across the characteristics in F_1 progeny. SCKSH had the highest value, followed by FLY, SDSS, SKCSD, SKCSW, FPC and lastly, MDT. The h_n^2 is

of significance because characteristics could be passed on to the subsequent generations. Since the h_n^2 estimates were low in some characteristics, it implied that to obtain high estimates, a number of breeding cycles have to be performed.

In F_2 progeny, h_b^2 ranged from 0.76 to 0.96 with characteristics having high values being SCKSH, SKCSD, SDSS and FLY. FLY followed, then MDT and SKCSW. The h_n^2 ranged from 0.27 to 0.77 with the highest value obtained from SKCSD, followed by SKCSW and SDSS, FLY, FPC, SCKSH and MDT.

The h_b^2 in F_1 and F_2 were higher than h_n^2 in both progeny. The range between these two progenies were not much different. However, h_n^2 in F_1 progeny in some characteristics were very low compared to h_n^2 in F_2 progeny. Both of them obtained the same highest value. Fowler and De la Roche (1975) showed that heritability of most quality characteristics is higher than those for yield. Similarly, Jalaluddin and Harrison (1989) found that the heritability of hectolitre mass was higher than that of grain yield. Baker *et al.* (1971) presented estimates of heritability of 11 quality characteristics which ranged between 47% and 88%. As mentioned earlier characteristics showing high h_b^2 can not be advised to select for since environment is included, while those with high h_n^2 selection can be conducted as early as possible.

Prediction ratio

In F_1 progeny (Table 25), SKCSH was the only characteristic of wheat which demonstrated a high prediction ratio, close to unity. Two of the seven characteristics showed moderate prediction ratioS, while others showed low to very low prediction ratios. The prediction ratio was found highly correlated to h_n^2, while h_b^2 was found to be not correlated. The prediction ratio was variable across wheat quality characteristics studied. In F_2 progeny, SKCSD, SKCSW, SDSS and FLY exhibited a high prediction ratio, while for FPC it was moderate. Low prediction ratios were observed in MDT and SKCSH. There was a positive trend between h_n^2 and h_b^2. The characteristics with high h_b^2 and h_n^2 showed a high prediction ratio as well, while those with low h_b^2 and h_{n2} revealed a low prediction ratio. The high prediction ratio which was closer to unity showed the relative importance of GCA and SCA in determining progeny performance. Tarekegne (2001) showed that h_n^2 and prediction ratio

are highly correlated when working on 18 wheat characteristicts related to yield and water-logging in Ethiopian wheat cultivars.

Degree of dominance

In the F_1 progeny (Table 25), all characteristics except one, indicated over-dominance. MDT had the highest value above unity, followed by FPC, SKSCW, SKCSD, SDSS and FLY. SKCSH exhibited a partial dominance with a value below unity. F_2 progeny (Table 26) demonstrated a varying degree of dominance with FLY and FPC exhibiting complete dominance. MDT and SKCSH expressed over-dominance, while SDSS, SKCSW and SKCSD showed partial dominance. The characteristics cut across all degrees of dominance. Tarekegne (2001) found a degree of dominance varying from partial to over-dominance for characteristics related to water-logging in wheat.

Estimates of heterosis

Means of the mid-parent and best parent heterosis for seven characteristics in F_1 and F_2 progeny are shown in Table 27 and 28. Both negative and positive heterosis were observed in all characteristics being studied. There was a great variation in the expression of heterosis. In the F_1 progeny, the maximum positive mid-parent heterosis was observed in SDSS, followed by SKCSH, FLY and seed SKCSW. The maximum positive best parent heterosis was obtained in SDSS, SKCSH, FLY and SKCSW.

A large number of hybrids showed a high mid-parent heterosis for SKCSW, followed by SKCSH and FLY, while in high best parent heterosis, it was SKCSH, followed by SKCSW, FLY and MDT. With MDT and SKCSD, hybrids showed very low mid-parent and best parent heterosis. Briggle (1963) noted that all parental combinations did not result in expression of hybrid vigour in wheat quality as some did not perform as well as either parent involved in hybridization. Johnson and Schmidt (1968) indicated that some F_1 hybrids out-performed their parents, while others were out-performed by their parents in wheat quality studies. Sayed (1978) observed a heterotic effect of 7.5% above high parent and about 44 – 48% hybrids showed heterosis with 40.7% being the maximum. He further indicated a maximum high parent heterosis ranging from 14.2% to 74% depending on the wheat quality characteristic being studied.

In the F_2 progeny, the characteristics that showed the highest positive mid-parent heterosis were MDT, followed by SKCSH and SDSS, while the highest positive best parent heterosis was observed in MDT, SKCSH, , SKCSD and SKCSW. A large number of hybrids exhibited positive mid-parent heterosis in MDT and SKCSH, followed by FLY. Positive best parent heterotic effects were expressed in SKCSH, MDT and FLY. Mid-parent heterosis and best parent hetrosis revealed that some characteristics being studied have improved in their performance over two generations .

The characteristics that showed low heterotic effects were SDSS and SKCSD in best parent heterosis, while seed weight and diameter had the lowest values in mid-parent heterosis.

Means squares of seven characteristics for hybrids are given in Table 29 and 30. The analysis showed that there were significant ($P \leq 0.05$) differences among hybrids for all seven characteristics being studied.

Table 25: F_1 estimates of combining ability and genetic parameters for the measured quality characteristics

Character	δgsa	δsca	$\delta a=2\delta^2 gca$	$\delta_d=\delta^2 sca$	$\delta_g=\delta_a+\delta_d$	δ_e	δp=δg+δe	h^2_b	h^2_n	PR	√H/D
FLY	7.242	10.834	14.484	10.834	25.318	1.339	26.657	0.95	0.54	0.57	1.22
FPC	0.113	0.991	0.226	0.991	1.217	0.088	1.305	0.93	0.17	0.19	2.96
MDT	0.011	0.221	0.022	0.221	0.243	0.059	0.302	0.81	0.07	0.09	4.48
SDSS	61.270	107.827	122.54	107.827	230.367	3.596	233.963	0.99	0.52	0.53	1.33
SKCSW	5.467	22.402	10.934	22.402	33.36	3.691	37.051	0.90	0.30	0.33	2.02
SKCSD	0.019	0.071	0.038	0.071	0.109	0.014	0.123	0.89	0.31	0.35	1.93
SKCSH	137.185	69.032	274.408	69.032	343.44	6.885	350.325	0.98	0.78	0.80	0.71

FLY=break flour yield, FPC=flour protein content, MDT=mixogram development time, SDSS=SDS sedimentation volume, SKCSW=kernel weight, SCKCSD=kernel diameter, SCKSH=kernel hardness, √H/D = degree of dominance , PR = predictability ratio.

Table 26: F_2 estimates of combining ability and genetic parameters for the measured quality characteristics

Character	δgsa	δsca	$\delta a=2\delta^2 gca$	$\delta_d=\delta^2 sca$	$\delta_g=\delta_a+\delta_d$	δ_e	$\delta p=\delta g+\delta e$	h^2_b	h^2_n	PR	√H/D
FLY	4.503	4.907	9.006	4.907	13.913	0.879	14.792	0.94	0.61	0.65	1.04
FPC	0.262	0.288	0.524	0.288	1.048	0.042	1.090	0.96	0.48	0.50	1.05
MDT	0.112	0.536	0.226	0.536	0.762	0.089	0.851	0.90	0.27	0.30	2.18
SDSS	55.942	36.537	111.884	36.537	148.421	5.680	154.101	0.96	0.73	0.75	0.81
SKCSW	11.580	7.272	23.16	7.272	30.432	1.139	31.571	0.76	0.73	0.76	0.79
SKCSD	0.034	0.016	0.068	0.016	0.084	0.004	0.088	0.96	0.77	0.81	0.69
SKCSH	69.154	151.826	138.308	151.826	290.134	13.283	303.417	0.96	0.46	0.48	1.48

FLY=break flour yield, FPC=flour protein content, MDT=mixogram development time, SDSS=SDS sedimentation volume, SKCSW=kernel weight, FSCKCSD=kernel diameter, SCKSH=kernel hardness, √H/D = degree of dominance , PR = predictability ratio.

Table 27: Estimates of mid-parent and best parent heterosis for seven characteristics of wheat quality in F_1 progeny

	FLY		FPC		MDT		SDSS		SKCSW		SKCSD		SKCSH	
Progeny	Mph	Bph	Mph	Bph	Mph	Bph	Mph	Bph	Mph	Bph	Mph	Bph	Mph	Bph
Sceptre x Wanda	6.43	2.67	-2.07	-2.14	-0.59	-0.70	-9.17	-23.00	13.05	9.84	0.64	0.46	-22.16	-30.46
Kariega x Nata	13.10	11.91	-0.38	-0.53	-0.35	-0.53	18.50	10.34	6.63	2.29	0.28	0.16	-15.56	-26.28
SST124 x Sceptre	6.03	2.2	0.51	-0.24	-0.26	-0.34	-18.17	-18.17	1.73	1.54	0.16	0.15	9.85	7.65
Nata x Wanda	1.81	0.66	0.15	-0.53	-0.17	-0.20	22.66	16.00	2.59	-0.81	0.09	-0.08	-23.89	-34.39
Nata x Sceptre	5.20	2.59	1.66	1.63	0.64	0.54	-7.50	-21.33	10.39	7.31	0.65	0.49	0.17	-8.73
Sceptre x Kariega	3.87	0.07	-2.35	-2.50	-0.02	-0.20	-3.17	-12.00	3.60	-0.74	0.23	-0.01	-4.39	-15.11
Sceptre x SST124	3.29	-0.54	1.43	0.90	-0.32	-0.32	8.16	6.00	2.36	1.42	0.08	0.01	-5.67	-5.88
SST124 x Nata	0.64	-0.58	0.25	0.07	0.08	-0.20	-18.00	-23.00	-1.68	-2.94	-0.05	0.12	16.68	14.86
SST 124 x Kariega	-1.12	-1.18	-1.50	-1.53	-0.56	-0.75	-7.17	-9.33	2.11	1.17	0.13	0.06	3.21	3.00
Nata x SST 124	0.46	-1.68	-0.57	-0.64	1.35	1.24	-4.17	-18.00	-2.11	-5.32	0.02	-0.16	6.20	-2.10
Wanda x Sceptre	1.83	-1.93	-0.82	-0.04	0.00	-0.33	-3.33	-3.53	1.95	-3.08	-0.06	-0.30	-4.30	-6.71
SST124 x Wanda	-2.09	-2.16	-2.18	-2.37	0.90	0.60	-14.66	-19.66	-3.67	-4.80	-0.11	-0.17	13.14	10.73
Wanda x SST124	-1.92	-2.06	-2.11	-2.14	0.28	0.27	-25.00	-26.20	-0.05	0.37	0.19	0.18	10.07	9.48
Wanda x Kariega	-2.79	-2.83	0.11	-0.64	1.58	1.50	-6.87	-14.00	4.02	-4.66	0.19	0.18	15.83	13.63
Wanda x Nata	-1.75	-2.90	-0.82	-1.05	0.17	0.14	-4.01	-4.21	11.01	7.99	0.21	0.04	3.37	-7.13
Scepter x Nata	-1.49	-1.12	-1.97	-2.00	0.01	0.00	-29.00	-29.00	-1.72	-1.59	0.10	0.09	16.14	15.55
Kariega x Wanda	-4.45	-4.49	-1.02	-1.73	1.10	1.03	-14.17	-21.33	-7.46	-7.78	-0.26	-0.26	17.09	15.49
Nata x Kariega	-4.62	-5.81	-1.55	-1.73	0.15	-0.13	-4.33	-9.33	-0.81	-2.07	-0.07	0.01	-18.16	-19.98
Kariega x SST124	-7.16	-7.18	-0.22	-0.93	0.60	0.53	-12.84	20.00	0.41	0.09	0.19	0.20	0.77	-0.83
Mean	0.80	-0.76	-0.71	-0.98	0.24	0.21	-6.96	-12.62	2.23	-0.13	0.14	0.07	2.02	-3.50
LSD ($P \leq 0.05$)	0.973	0.025	1.243	0.198	0.017	0.276	0.634	3.659	0.523	0.024	0.012	0.019	2.571	1.232

FLY=break flour yield, FPC=flour protein content, MDT=mixogram development time, SDSS=SDS sedimentation volume, SKCSW=kernel weight, FSCKCSD=kernel diameter, SCKSH=kernel hardness,

Table 28: Estimates of mid-parent and best parent heterosis for seven characteristics of wheat quality of F_2 progeny

	FLY		FPC		MDT		SDSS		SKCSW		SKCSD		SKCSH	
Progeny	Mph	Bph	Mph	Bph	Mph	Bph	Mph	Bph	Mph	Bph	Mph	Bph	Mph	Bph
Sceptre x Wanda	9.71	0.07	-3.33	-3.33	0.00	0.00	9.21	-2.35	5.13	4.62	4.13	2.86	-3.68	-3.73
Kariega x Nata	-0.02	-2.56	-0.41	-3.15	20.76	18.52	-1.91	-4.94	3.77	2.84	-11.34	-12.75	13.03	8.20
SST124 x Sceptre	10.20	0.50	0.82	1.64	24.14	12.50	3.36	-6.09	-6.16	-9.07	-2.14	-4.18	17.93	15.14
Nata x Wanda	-0.02	-0.03	-0.04	-0.04	19.2	19.20	-22.98	-27.06	20.37	4.51	1.61	0.40	14.59	14.06
Nata x Sceptre	9.09	5.69	5.00	5.00	-19.23	-19.23	20.28	13.16	6.30	1.57	10.00	1.59	-16.29	16.72
Sceptre x Kariega	0.04	0.01	1.21	3.93	39.62	37.04	-5.41	-13.58	-5.67	-6.99	-3.73	-4.53	17.11	16.51
Sceptre x SST124	0.04	0.02	0.00	-1.61	37.93	25.00	14.09	3.66	-17.39	-19.95	-11.97	-13.81	5.52	3.28
SST124 x Nata	2.16	1.22	9.02	1.21	11.54	-9.38	13.92	9.76	-0.63	-5.85	-2.08	-6.38	-14.54	-16.92
SST 124 x Kariega	-3.19	3.93	0.40	-0.79	18.6	9.38	-6.75	-7.32	-3.17	-3.5	-0.85	-3.70	9.97	8.25
Nata x SST 124	1.35	0.42	-5.74	-11.81	10.35	9.38	-3.80	-7.59	-6.36	-11.28	-2.50	-6.77	3.31	0.43
Wanda x Sceptre	2.88	0.63	-8.92	-8.81	50.00	50.00	0.00	-0.11	-16.28	-16.69	-9.50	-10.61	14.95	14.89
SST124 x Wanda	0.03	0.02	2.46	0.81	-6.70	-15.63	-8.98	-10.59	-5.82	-8.29	-1.27	-4.49	11.27	8.65
Wanda x SST124	0.09	0.09	-3.69	-5.24	31.03	18.75	0.60	-1.18	-12.51	-14.46	-7.17	-10.20	25.72	22.77
Wanda x Kariega	-0.04	0.469	-0.08	-10.24	43.40	40.74	-15.66	-17.68	-17.86	18.60	-9.02	-9.39	21.81	17.13
Wanda x Nata	2.37	1.37	4.17	4.17	11.54	-9.38	13.92	9.76	-0.63	-5.85	-2.08	-6.38	-14.54	-16.92
Scepter x Nata	0.01	-4.44	-3.58	-3.58	30.77	30.77	-5.29	-10.53	-13.89	-15.87	-6.53	-8.77	17.11	16.51
Kariega x Wanda	-2.58	-3.33	-9.31	-11.81	39.62	40.71	-19.28	-21.18	-9.74	-10.55	-3.69	-4.01	17.05	12.55
Nata x Kariega	-0.03	-0.05	-18.22	-20.47	58.49	55.56	-10.83	-13.58	-19.35	-22.28	-10.53	-11.95	16.26	11.29
Kariega x SST124	-8.57	-9.23	-15.54	-16.54	-15.25	-21.88	-6.75	-7.32	-18.55	-19.97	-21.24	-26.75	-68.30	-68.80
Mean	0.80	-0.76	-0.71	-0.98	0.24	0.21	-6.96	-12.62	2.23	-0.13	0.14	0.07	2.07	-3.50
LSD (P≤0.05)	0.532	1.034	0.416	2.767	4.828	3.659	2.767	1.745	4.476	2.321	0.982	1.099	3.231	4.768

FLY=break flour yield, FPC=flour protein content, MDT=mixogram development time, SDSS=SDS sedimentation volume, SKCSW=kernel weight, FSCKCSD=kernel diameter, SCKSH=kernel hardness,

Table 29: Mean squares for mid-parents for heterosis

Source	Df	FLY	FPC	MDT	SDSS	SW	SH	SD
Genotypes	18	66.608**	3.438**	1.534**	187.401**	52.532**	162.393**	8.887**
Error	38	0.335	0.217	0.207	0.344	3.904	1.944	3.361
Total	**56**							

FLY=break flour yield, FPC=flour protein content, MDT=mixogram development time, SDSS=SDS sedimentation volume, SKCSW=kernel weight, SCKCSD=kernel diameter, SCKSH=kernel hardness, **P≤0.05

Table 30: Mean squares for best parent heterosis

Source	Df	FLY	FPC	MDT	SDSS	SD	SW	SH
Genotypes	18	25.108**	1.836**	0.235**	165.643**	5.576**	26.180**	261.468**
Error	38	1.014	3.779	0.158	2.581	8.246	3.734	2.933
Total	**56**							

FLY=break flour yield, FPC=flour protein content, MDT=mixogram development time, SDSS=SDS sedimentation volume, SKCSW=kernel weight, SCKCSD=kernel diameter, SCKSH=kernel hardness, **P≤0.05

Comparison of F_1 and F_2 progeny

Significant differences between F1 and F2 progeny were found for all wheat quality characteristics, showing the presence of heterosis and inbreeding depression. A significant increase in mid-parent heterosis (MPH) was observed for five characteristics (FLY, SKCSD, SKCSW, SKCSH and SDSS) in two crosses (Sceptre x Wanda and SST 124 x Nata). Another significant increase in MPH occurred for four characteristics in seven crosses, namely; Nata x Wanda, Nata x Sceptre, Sceptre x Kariega, Sceptre x SST 124, Wanda x SST 124 and Sceptre x Nata. Two crosses revealed a significant increase in best parent heterosis (BPH) for all the characteristics studied, namely; SST 124 x Sceptre and Nata x Wanda, followed by SST 124 x Kariega and Kariega x Nata in six characteristics, respectively.

A large number of the crosses (6) exhibited an increase in four and three characteristics. According to Mackey (1976) expression of heterosis may result from one or two of the following circumstances; a) accumulation of favourable dominant genes dispersed among two parents and b) favourable allelic and non-allelic interaction (over-dominance) or complementary interaction of additive dominance on recessive genes at different loci (epistatis). Conversely, some crosses expressed a significant reduction in both MPH and BPH. The cross that showed decline for all characteristics in MPH was Kariega x SST 124, followed by Kariega x Nata exhibiting decline in five characteristics. Likewise, a large number of crosses showed a decline in four to three wheat quality characteristics. Kariega x SST 124 expressed inbreeding depression in both MPH and BPH. Decline in both MPH and BPH are accounted for by inbreeding depression which is expressed when dominance interaction effect disappeared in F_2 generation due to reduced heterozygosity and increase in homozygosity.

Crosses indicating either heterosis in the F_1 generation and decline in the F_2 generation should be used to produce hybrid cultivars, while the ones which showed consistent heterosis in the F_1 and F_2 generation should be utilized in cultivar development programmes. Crosses such as Sceptre x Wanda, SST 124 x Sceptre, SST 124 x Kariega, Kariega x Nata, Sceptre x Wanda and SST 124 x Nata could be used in cultivar development, while Nata x Wanda, Nata x Sceptre, Sceptre x Kariega, Sceptre x SST 124, SST 124 x Nata, SST 124 x Kariega, Nata x SST 124, Wanda x Sceptre, SST 124 x Wanda, Nata x SST 124, Wanda x Kariega, Wanda x Nata and Kariega x Wanda could be utilized in

hybridization programmes.

4.3.7 Phenotypic correlation

4.3.7.1 Correlation among wheat quality characteristics in F_1 progeny

Correlation was calculated between seven quality characteristics to determine the effect of one characteristic on the other. There was a negative and significant correlation (r =-0.2992) (Table 31) between MDT and FLY which means that as FLY increase, MDT decrease. A negative correlation existed between MDT and FPC but was not significant. Woldegiorgis (2003) and Graybosch *et al.* (1996) reported a negative, highly significant correlation between FLY and MDT.

Correlation between MDT and SKCSW was significant (r = -0.3525) and negative implying that as SKCSW increased, MDT decreased. Consistently, Woldegiorgis (2003) and Peterson *et al.* (1992) obtained results showing negative significant correlations between MDT and SKCSW. A significant correlation (r=0.4273) existed between MDT and SKCSD. Woldergiorgis (2003) reported a positive correlation between MDT and SKCSD.

No significant correlation existed between the SDSS and MDT. Peterson *et al.* (1992) found similar results. Contrarily, Woldegiorgis (2003) found significant and negative correlation between the SDSS and MDT. Gaines (1991) reported a negative correlation between FLY and FPC of red wheat. Basset *et al.* (1989) supported the argument.

A positive and significant correlation (r = 0.334) existed between SDSS and FLY indicating that as FLY increased SDSS increased. Du Preez (2001) found a negative correlation between FLY and MDT. FLY and SKCSW had a highly significant correlation (r=0.5006) meaning that SKCSW has a perceptible influence on FLY and an improvement or selection in SKCSW would increase FLY. Woldegiorgis (2003) found no correlations between FLY and SKCSW.

SKCSD and FLY had highly significant correlation (r = 0.394). This showed that if SKCSD is increased, FLY would also increase. Kosmolak and Dyck (1981) obtained similar results. SKCSH had a significant negative correlation (r = -0.2379) with FLY. These results are consistent with

Woldegiorgis (2003) who found SKCSD to be negatively and highly significantly correlated with FLY. Basset *et al.* (1989) found similar results.

No significant correlation existed between FLY and MDT. Finney *et al.* (1987) indicated they as FPC increased to about 12%, MDT decreased. Van Lill and Purchase (1995) indicated that low FPC appeared to increase requirement and more time and energy would be spend by a baker.

A significant positive correlation (r = 0.3450) existed between SDSS and FPC. However, Du Preez (2001) and Groger *et al.* (1997) found a negative correlation between SDSS and FPC. No significant correlation existed between kernel weight and flour protein content. Woldegiorgis (2003) reported no correlation between SKCSW and FPC which is consistent with the findings of this study.

FPC and SKCSH had no significant correlation. Woldegiorgis (2003) reported a significant positive correlation between FPC and SKCSH. A negative highly significant correlation (r =-0.6740) existed between SKCSH and SDSS. Woldegiorgis (2003) reported no correlation between SKCSH and SDSS .

SKCSD and SKCSH were positive and highly correlated (r =0.9444). An improvement in one characteristic would affect the other perceptibly. Similarly, Woldegiorgis (2003) found a highly significant positive correlation between SKCSD and SKCSH. SKCSH and SKCSW had significant negative correlation (r = -0.345). Similar results were obtained by Woldegiorgis (2003) who found a negative significant correlation between SKCSH and SKCSW.

Table 31: Phenotypic correlations for seven quality characteristics in parents and F_1 progeny

	FLY	FPC	MDT	SDSS	SKCSW	SKCSD
FPC	**0.1560**					
MDT	-0.2992*	-0.0311				
SDSS	0.3344**	0.3450**	-0.1501			
SKCSW	0.5006**	-0.0475	-0.3526**	-0.0178		
SKCSD	0.3935**	-0.1173	-0.2144	-0.2204	0.9444**	
SKCSH	-0.2379*	-0.0140	0.4273**	-0.6740**	-0.3457**	-0.1176

FLY=break flour yield, FPC=flour protein content, MDT=mixogram development time, SDSS=SDS sedimentation volume, SKCSW=kernel weight, SCKCSD=kernel diameter, SCKSH=kernel hardness,**P≤0.05,***P≤0.01

4.3.7.2 Correlations among wheat quality characteristics in F_2 progeny

FLY was positive significantly correlated with FPC (0.3240), SDSS (0.4954), SKCSW (0.4320), SKCSD (0.3963) and SKCSH (0.0805), and negatively correlated with MDT (-0.2042) (Table 32).

FPC was positively correlated with SDSS (0.4652), SKCSW (0.3190), SKCSD (0.2380) and SKCSW (0.5365). An increase in FPC would increase all the characteristics correlated with it. Conversely, FLY was negatively correlated with MDT. A negative correlation existed between MDT and SDSS (-0.1504), SKCSW (0.5614) and SKCSD (-0.3191), while a positive correlation was obtained for SKCSH (0.5365).

Similarly, a negative correlation was observed between SDSS and SKCSD (-0.0442) and SKCSH (-0.2373), while a positive correlation was found between SDSS and SKCSW. SKCSW was positively correlated with SKCSD (0.8920) and negatively correlated with SKCSH (-0.1590). As the SKCSW increased the SKCSD increased. SKCSD had a positive correlation with SKCSH (0.1905) meaning that a increase in SKCSD caused an increase in SKCSH.

Correlation of the wheat quality characteristics in F_1 and F_2 progeny were found consistent indicating that the degree of relationship among the characteristics did not change with generations. This results were similar to findings of other researchers (Baker *et al.*, 1971; Bhatt & Derera, 1975; Fowler & De la Roche, 1975; Levy & Feldman, 1989; Gaines, 1991; De Villiers & Laubscher, 1995)

Table 32. Phenotypic correlations for seven quality characteristics in the parents and F_2 progeny

	FLY	FPC	MDT	SDSS	SKCSW
FPC	**0.3240**				
MDT	-0.2042*	-0.1850			
SDSS	0.4954**	0.4652**	-0.1504		
SKCSW	0.4320**	0.3190	-0.5614**	0.1253	
SKCSD	0.3963**	0.2380	-0.3191	-0.0442	0.8920**
SKCSH	0.2028	-0.1963	0.3277	0.2832	0.1945

FLY=break flour yield, FPC=flour protein content, MDT=mixogram development time, SDSS=SDS sedimentation volume, SKCSW=kernel weight, SCKCSD=kernel diameter, SCKSH=kernel hardness, **P≤0.05, ***P≤0.01

4.3.8 Genotypic correlations

Correlations between seven wheat quality characteristics were calculated to determine the relationship among them with total exclusion of environmental factors. In F_1 progeny, FLY was negatively correlated with FPC (-0.1377), MDT (-0.5970), SDSS (-0.0918) and SKCSW (-0.2440) (Table 33).

A significantly high positive correlation was obtained between FPC and MDT (-0.5877) as well as SDSS (0.3058) indicating that when one of these characteristics increased, the other ones increased but not at the same rate. Conversely, FPC was negatively correlated with SKCSW (-0.8868), SKCKD (-0.5877) and SKCSH (-0.1059) suggesting an inverse relationship resulting in a decrease as one increased.

An insignificant positive correlation existed between SKCSW, SKCSD and SKCSH suggesting that as one of them change, the rest would change as well but with small amount. Negative correlation was observed between SKCSD and SKCSH showing an inverse relationship resulting in decrease as the other increased.

In the F_2 progeny (Table 34), FLY was negatively and insignificantly correlated to all characters except two which seemed to be positive and significant. MDT was highly correlated with FLY such that an increase in FLY would increase MDT. However, this increase is undesirable as the acceptable range is between 2.5 and 3.0min and if an effort is made to increase the FLY, then MDT will go above the upper limit. Fowler & De la Roche (1975) found the correlation between FLY and MDT to be positive and significant.

A positive correlation was observed between FPC and SDSS (0.1513), SKCKD (0.312) and SKCSH (0.919), while MDT (-0.382) and SKCSW (-0.6267) were negatively correlated with FPC. SDSS and SKCKD are important in wheat quality as a result an increase in SKSCD would increase SDSS and that will result in an improvement for both. SKCSH which attained a high correlation value is undesirable as more energy is required to break and grind the kernel.

MDT was negatively correlated with SDSS (-0.5152), SKSCD (-0.0204) and SKCSH (-0.4294). Both weight and MDT are economically important characteristics that determine quality, hence a high price is offered. However, the range of 2.5 – 3.0min for MDT should not be exceeded since a high price is offered within this range.

Correlation between SCKSD and SCKSW was significant and positive meaning that as SCKSD is improved, SCKSW would improve as well. The characteristics which are positively correlated indicated that they are conferred by one gene (pleotropic) or linked genes such that when manipulating this gene or genes to improve one character all other characters determined by this gene are affected and it is important to determine the inheritance pattern of the character before any effort is made to change a character.

Table 33: Genotypic correlations for parents and the F_1 generation for wheat quality characteristics

	FLY	FPC	MDT	SDSS	SKCSW	SKCSD
FPC	-0.1377					
MDT	-0.5970*	0.8100**				
SDS	-0.0918	0.3058*	0.0504			
SKCSW	-0.2440	-0.8868**	-0.6226**	-0.1224		
SKCSD	0.3273*	-0.5877*	-0.3709*	-0.4753*	0.2173	
SKCSH	0.0680	-0.1059	-0.4234*	0.4810*	0.3488*	-0.6763*

FLY=break flour yield, FPC=flour protein content, MDT=mixograph development time, SDSS=SDS-sedimentation volume, SKCSW=seed weight, SCKCSD=seed diameter, SCKSH=seed hardness, ***P≤0.01, **P≤0.05

Table 34: Genotypic correlations for parents and the F_2 generation for wheat quality characteristics

	FLY	FPC	MDT	SDSS	SKCSW	SKCSD
FPC	-0.2385					
MDT	0.816**	-0.382				
SDS	-0.121	0.1513	-0.5152*			
SKCSW	0.2942*	-0.6267**	0.4711*	0.2251		
SKCSD	-0.116	0.3102*	-0.0204	0.4962*	0.5249*	
SKCSH	-0.2854*	0.9919**	-0.4294*	0.2485	-0.5506**	0.4094*

FLY=break flour yield, FPC=flour protein content, MDT=mixograph development time, SDSS=SDS-sedimentation volume, SKCSW=seed weight, SCKCSD=seed diameter, SCKSH=seed hardness, ***P≤0.01, **P≤0.05

4.4 Conclusions

In Lesotho, the wheat industry is still at its infancy stage, without a breeding programme in place for improving breadmaking quality. Hence, cultivars with good breadmaking quality are required. To improve or enhance quality, parents Wanda, SST 124 and Sceptre could be incorporated into the breeding programme. Crosses such as Sceptre x Kariega, SST 124 x Nata, Wanda x Nata, Sceptre x

Wanda, Kariega x Wanda, Kariega x Sceptre, SST 124 x Sceptre, Wanda x SST 124, Nata x Kariega and Nata x SST 124 should be incorporated into the programme, while exotic promising candidates have to be sourced elsewhere. For tangible enhancement of wheat quality, emphasis should be placed on these crosses to develop new cultivars having good breadmaking quality required by millers and bakers alike. The study clearly demonstrated the presence of both dominance and additive components of genetic variance in controlling the inheritance of wheat quality characteristics. It could be inferred from this study that ample scope for exploitation of heterosis for cultivar development and hydrid varieties exists for the wheat industry in Lesotho, as long as economic wheat quality characteristics which need to be improved are identified and breeding programmes to be established is strategized to maximize the genetic potential of parents, F_1 and F_2 generations to the improvement of wheat industry.

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Chapter 5

The use of Size Exclusion - High Performance Liquid Chromatography (SE-HPLC) to predict wheat quality

Abstract

The predictive value of SE-HPLC for breadmaking quality in the parents, F_1 and F_2 progeny was studied. A two step procedure was followed involving extraction by SDS (Sodium dodecyl sulphate) and followed by sonication to remove remaining proteins. In the F_1 generation, SDS sedimentation was significantly correlated with the SDS insoluble LPP (large polymeric protein) and the large unextractable proteins. In the F_2 generation, the SDS soluble SPP (small polymeric protein) were significantly correlated with SDS sedimentation. The correlations with especially flour protein content and SDS sedimentation were not consistent for the F_1 to the F_2 generation. There was large variation for all the protein fractions and large significant differences between entries in the progeny, which indicated segregation, and selection should be possible for protein fractions to improve quality characteristics.

Keywords: breadmaking, protein fractions, SE-HPLC, predictors

5.1 Introduction

Breadmaking quality of wheat flour is determined by its protein composition (Singh *et al.*, 1990; Cornish *et al.*, 2001). Hence, much research has been conducted to explain which protein constituents account for the differences in quality (Bietz, 1990). On the basis of solubility, proteins are classified into globulin which is soluble in alkaline, albumin which is soluble in water, gliadin which is soluble in alcohol and glutenin which is soluble in acid solution (Osborne, 1907; Gianibelli *et al.*,2002).

Nonetheless, some researchers classified protein constituents according to molecular size, larger than 100 kilodaltons (kda) were glutenin, between 100 and 25 kda were gliadin and smaller than 25 kda were classified into either albumin or globulin (Meredith & Wren, 1966; Bushuk & Wrigley, 1971). The origin, structure, properties and relationships of the properties of proteins are well

documented (Bietz,1990; Masci *et al.*, 2003). Many cereal proteins interact non-covalently with endosperm constituents such as lipids and carbohydrates and associate either non-covalently through hydrogen or hydrophobic bonds or covalently through disulphides with each other to form high molecular weight complexes (Gianibelli *et al.*,2002).

Variations in glutenin to gliadin ratio were found highly correlated with wheat quality (Fleurent, 1986; Guess, 1900; Zhu & Khan, 2001). By varying the proportions of these fractions while maintaining the total protein level, it was possible to get 20-fold variation in dough resistance and 2.5-fold in extensibility of dough (Kim *et al.*, 1988). This showed that the properties of dough are determined by the relative proportions of these fractions. Furthermore, it was observed that a failure to correlate the glutenin to gliadin ratio with breadmaking quality of cultivars is attributed to inconsistent solubility of proteins from different wheat cultivars (Lookhart *et al.*, 1986). Similarly, baking studies employing classical flour reconstitution techniques have ascertained that glutenin to gliadin ratios are major factors governing wheat quality (Finney, 1943). The method by which these proteins are fractionated and quantified is a matter of concern to cereal chemists. Recently, high performance liquid chromatography was found to be the most successful method for predicting wheat quality, because of its speed, automation, quantitative ability and small sample required. The aim of this study was to determine the predictive value of SE-HPLC for quality characteristics in the parents and F_1 and F_2 generations.

5.2 Materials and methods

Two sets of seed materials obtained from parents and F_1 and parents and F_2 progeny, planted in Bloemfontein, were used in this experiment. Each set consisted of 25 entries, which was planted in triplicate in a randomized complete block design. Protein in the wheat kernels were extracted following a two step extaction procedure developed by Gupta *et al.* (1993). The first step extracts the proteins soluble in dilute SDS, while the second step extracts the proteins soluble only with sonication. The procedure in the first step involved suspension of 1.07mg white flour in 1.5ml of 0.5% (w/v) SDS phosphate buffer (pH 6.9). Thereafter it was vortexed for 5min at 2000rpm and centrifuged for 30min at 10000xg to obtain the supernatant protein. The procedure in the second step involved resuspension of pellets from the first step in 1.5ml SDS phosphate buffer, shaken for 5min

with mechanical shaker and sonicated in an ultra-sonic desintegrator (Branson B12 sonifier) amplitude 5 and fitted with 3mm exponential microtip for 30sec. The samples were centrifuged as above to get the supernatant proteins. The supernatants were filtered through 0.45µl filters (Millipore, Durapore membrane filters) before running on HPLC. Size exclusion HPLC analyses were carried out on a Varian HPLC system using a BIOSEP SECC-4000 column (Phenomenex). Separation was done by loading 20µl of sample into an eluant of 50% (v/v) acetonitrile and water containing 0.1% (v/v) trifluroacetic acid (TFA) at a flow rate of 0.2ml/min. Proteins were detected by UV absorbance at 210nm which produced graphs from which data were collected and calculated. The graphs are depicted in Fig 3a and 3b illustrating areas of the different peaks were calculated. The percentage of total unextractable polymeric protein in the total polymeric protein [(SDS-insoluble large and smaller protein polymers)/SDS – soluble and insoluble large and smaller protein polymers)] and the percentage of large unextractable polymeric protein in the total large polymeric protein (SDS-insoluble large protein polymers)/ (SDS-soluble and SDS – insoluble large protein polymers) was calculated according to Gupta *et al.* (1993).

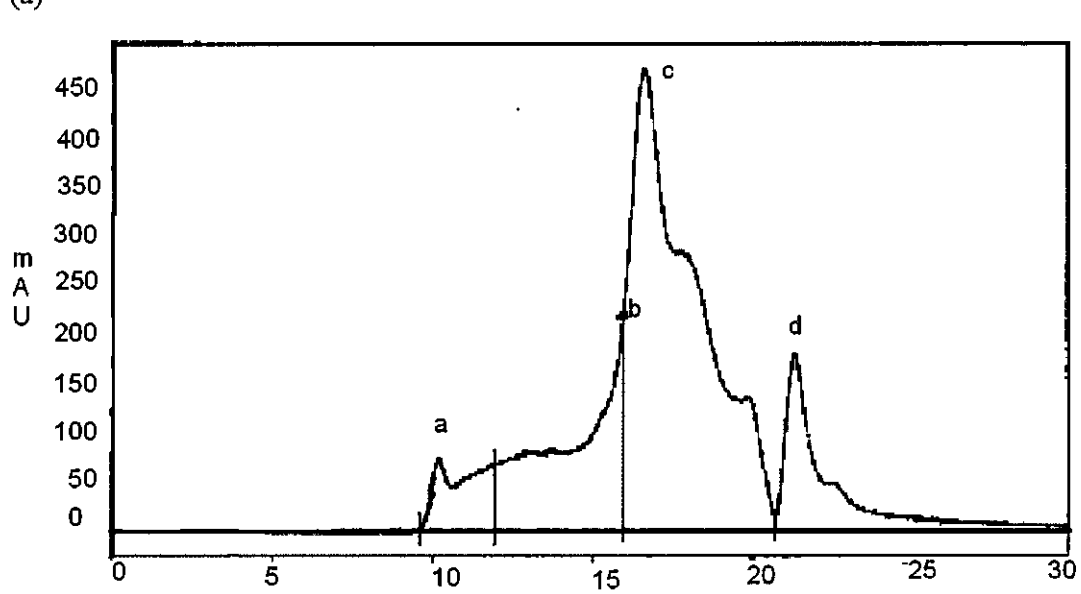
Data analysis

Means for protein fractions such as LPP, SPP, LMP and SMP in both SDS-soluble and insoluble proteins were calculated.

5.3 Results

The means of protein fractions for 20 F_1 progeny and five parents are presented in Table 35. The results of the ANOVA are presented in Table 36.

(a)



(b)

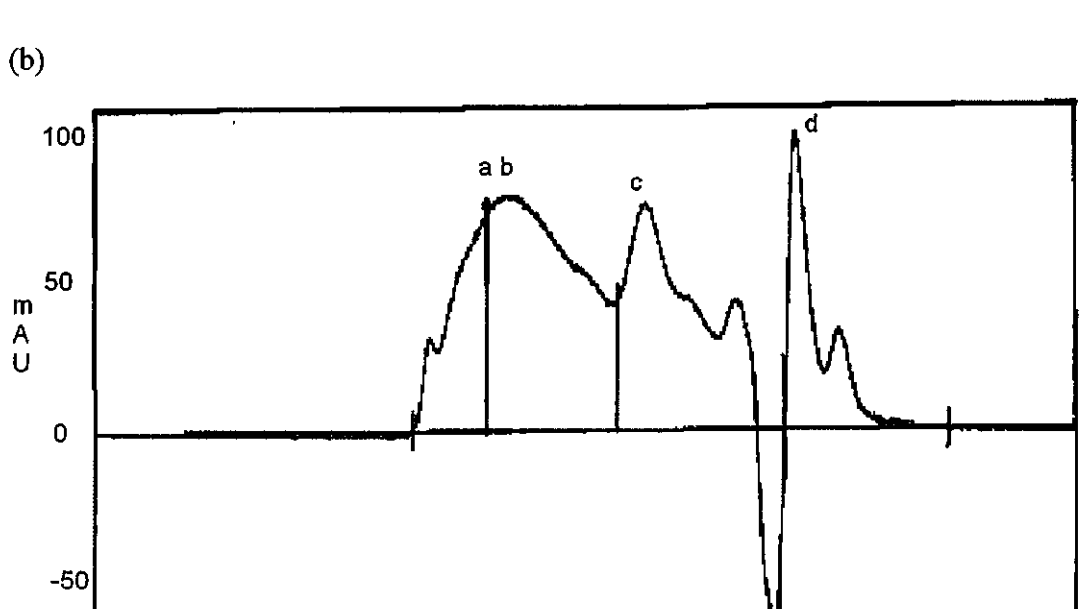


Figure 3: (a) SDS-soluble proteins and (b) SDS-insoluble proteins as separated with SE-HPLC

where: a = large polymeric proteins (LPP), b =small polymeric proteins (SPP), c = large monomeric proteins (LMP), d = small monomeric proteins (SMP)

5.3.1 Means of protein fractions for parents and their F_1 progeny

Large polymeric protein 1: Sceptre x Wanda, Kariega x SST 124, Kariega x Sceptre, Sceptre x Kariega, Kariega x Nata and Kariega x SST124 outperformed the other progeny significantly. Three parents (Kariega, SST 124 and Sceptre) performed significantly better than the progeny, but no significant differences existed among them. Nata performed poorly compared to the other parents.

Small polymeric protein 1: SST124 x Nata, Kariega x Sceptre, Kariega x SST 124, and Wanda x Nata performed significantly higher than the rest of the progeny. All parents performed like their best progeny, but not higher than the highest progeny.

Large monomeric protein1: Sceptre x Kariega had the highest value, followed by Wanda x Sceptre. No significant difference was obtained among the parents.

Small monomeric protein1: A wide range of SMP1 values were obtained among the progeny with the highest being 11.77 and the lowest 2.85. Four of the progeny (Sceptre x Nata, Kariega x Wanda, Sceptre x Kariega and Sceptre x Wanda) had the highest values. Parents formed two groups with a large difference between the groups.

Large polymeric protein 2: No significant differences were seen among the progeny and among the parents.

Small polymeric protein 2: Nata x Wanda, Kariega x Wanda, Nata x SST 124, Sceptre x Kariega and Nata x Sceptre had significantly higher values than the other progeny for SPP2. Among the parents, Wanda did significantly better than other parents but slightly lower than the best progeny. Kariega x SST 124 exhibited a lower SPP2 value than the other progeny and Sceptre showed a lower SPP2 value than the progeny.

Large monomeric protein 2: There was a wide variation among the progeny for LMP2 with the highest progeny obtaining a value of 15.70 and a lowest value of 7.95. Nata x SST 124 showed the highest performance, followed by Nata x Sceptre. Kariega x SST 124 had the lowest value. Wanda exhibited higher values than other parents, but it was lower than the best progeny. Nata showed a very low value compared to other parents.

Small monomeric protein 2: Wanda x Sceptre and Sceptre x Kariega performed significantly better than the other progeny, and their performance was similar to one of their parents. Nata x Sceptre, Kariega x Wanda and Nata x SST 124 performed significantly lower than the other

progeny, but higher than the lowest parent.

Total unextractable polymeric protein: Kariega x Wanda, Nata x SST 124 and Sceptre x Kariega obtained the highest TUPP values and no significant difference was observed between them. The best parent performed significantly lower than the best progeny.

Large unextractable polymeric protein: A significant difference was obtained among progeny with Wanda x Sceptre and Sceptre x Kariega showing the highest values and Sceptre x Wanda as well as SST 124 and Sceptre revealing the lowest LUPP values. Sceptre x Kariega and Wanda x Sceptre performed better than the other parents but lower than the best progeny. The progeny that showed the lowest values had a lower value than the lowest parent. Among the parents, no significant differences existed.

Large polymeric protein: Kariega x Sceptre, Kariega x SST 124 and Nata x Wanda showed higher values than the other progeny, even though no significant differences were seen. Sceptre x Kariega, Wanda x Sceptre and Nata X SST 124 performed poorly but better than the parent with the lowest value.

Large monomeric protein: Sceptre x Kariega had the highest value. The lowest value was expressed by Kariega x SST 124 and Kariega x Sceptre. Similarly, Wanda, among the parents outperformed the others but was lower than the best progeny.

Small polymeric protein: A large variation was observed among the progeny ranging from 61.38 to 108.54 where Nata x Sceptre and SST 124 x Kariega revealed highest values. However, the parents showed low variation among themselves with the highest being 103.85 and lowest 74.43.

Small monomeric protein: Sceptre x Kariega, Sceptre x Wanda, Nata x Kariega and Wanda x SST 124 showed significantly higher values than the other progeny for SMP. All parents except Wanda exhibited similar performance which was lower than the best progeny and better than the progeny with the lowest value.

Polymeric protein: Nata x Sceptre, Nata x Wanda and SST 124 x Kariega significantly outperformed the other progeny, while Sceptre x Wanda, Nata x Kariega and Sceptre x Nata performed significantly poorer for PP. No significant difference was obtained in the parents for PP. All parents except Sceptre, had values similar to their progeny.

Monomeric protein: The performance of all progeny was similar, except Sceptre x Kariega which

had a high value of 56.37. The rest ranged from 32.79 to 44.06, suggesting a narrow diversity among progeny. The performance of the parents was also similar.

Above results are confirmed from the mean squares (Table 36) for entries which were significant for all measured protein fractions. There were no significant differences between the blocks. Large variability was found among progeny for different protein fractions with some obtaining very low values while others obtained high values. Variabilty is important in plant breeding because it creats new genes that could be utilized to improve protein fractions. The parents showed value that fall within the extremes of progeny. Best parent could not perform better than best progeny in all fraction implying that there is a room for improvement of the desirable trait.

5.3.2 Means of protein fractions in F_2 progeny and parents

The means of protein fractions are presented in Table 37 and ANOVA is shown in Table 38.

Large polymeric protein 1: Highly significant difference were obtained among the progeny with SST 124 x Kariega ranking first, followed by SST 124 x Nata and lastly SST 124 x Wanda. Parents were insignificantly different from each other and their performance was similar to the best progeny.

Small polymeric protein 1: Kariega x Sceptre, Wanda x Nata, SST 124 x Wanda and Kariega x Wanda outperformed the others for SSP1. Most of the progeny obtained values above 50.00 and only Nata x Wanda, Wanda x Sceptre and Wanda x SST 124 got valùes below 49.00. The performance of the parents exceeded that of the progeny.

Large monomeric protein 1: Kariega x SST 124, Kariega x Nata, Kariega x Wanda and Sceptre x Nata showed a significantly higher performance than the other progeny. All parents had similar values close to 12.00 which were below most of their progeny.

Small monomeric protein 1: A large variation was observed among progeny and among parents. Wanda x Sceptre and Nata x Wanda had the highest values among the progeny. Sceptre showed the highest SMP1 value among the parents.

Large polymeric protein 2: Wanda x SST 124 and Kariega x Nata were significantly higher than the other progeny. All other progeny except one obtained values between 10.06 and 12.39 which was a very narrow range. The parents had a wider range between the lowest and highest

values from LLP2. However, progeny with highest values exceeded the best parents. The lowest progeny performed better than the lowest parent.

Small polymeric protein 2: Significant differences were obtained among progeny for SPP2 with Kariga x Sceptre and Kariega x Nata having the highest values. No significant differences were observed among parents.

Large monomeric protein 2: Sceptre x Nata and Kariega x Sceptre exhibited significantly higher LMP2 values than other progeny.

Small monomeric protein 2: Large variation was observed among progeny and among parents. The performance of the parents was higher than that of progeny and the lowest performing parent was far higher than the lowest performing progeny.

Total unextractable protein 2: difference was found among the progeny for TUPP, while significant differences were obtained among parents. Sceptre outperformed all parents.

Large unextractable polymeric protein: Kariega x Nata, Kariega x SST 124 and Sceptre x Wanda were found significantly higher than other progeny, but there were no significant differences between the parents.

Large polymeric protein: There were no significant differences for parents or progeny.

Large monomeric protein: Nata x SST 124 and Sceptre x Kariega showed a significantly higher value for LMP than Nata x SST 124 and Sceptre x Kariega. Wanda followed by Kariega had the highest LMP values.

Small polymeric protein: Nata x Sceptre and SST 124 x Wanda outperformed other progeny. Among the parents Kariega was the highest while SST 124 was the lowest. The performance of the progeny was higher than parents.

Small monomeric protein: The lowest parent was much higher than the lowest progeny for this fraction.

Polymeric protein: There was no significant difference among progeny and among parents.

Monomeric protein: Nata x SST 124 and Sceptre x Kariega significantly outperformed other progeny. Among the parents, Kariega had the highest value, followed by SST 124. There was no significant difference among parents.

Above results are confirmed from the mean squares (Table 38) for entries which were significant

for all measured protein fractions. There were no significant differences between the blocks. It is evident from results of F_1 and F_2 progeny that large variabilty existed for protein fractions in some progeny performing poorly while others showed high values. This could be attributed to segregation and reconmbination of genes which express protein fraction. Recombination of complementary genes make progeny to perform better than others and their parents, whereas combination of genes that work against protein fraction obtain low values of protein fractions. No significant difference was obtained among parents for most of protein fractions and their values were falling within thosose of progeny. This showed that progeny with high values for desirable fraction could selected for breeding programmes. Ratio of polymeric and monomeric protein fraction is used as a predictor of quality in bread wheat. High ratio of polymeric to monomeric showed that a cultivar producing such protein is good for breadmaking, while an inverse of that shows a poor breadmaking cultivar. Therefore, progeny obtained in these two generations can be selected basing on protein fraction.

5.3.3 Significant correlations between protein fractions and measured quality characteristics

Table 39 shows the significant correlations between protein fractions and measured quality characteristics.

F_1 progeny: SDS-soluble LPP showed a positive and significant correlation with SKCSD and FLY and was negatively correlated with FPC and SKCSW. SKCSH and FLY were positively and significantly correlated with SDS-soluble LPP, while SKSCW was negatively significantly correlated with it.

SDS-soluble LPP showed a high positive significant correlation with FPC, while SDS-soluble SMP was found negatively correlated with SKCSW and SKCSD. SDS-insoluble LPP was positively and significantly correlated with SDS sedimentation volume, SKCSW, SKCSD and FLY. A positively and significant correlation existed between SDS-insoluble SPP and SKCSH, SKCSW, SKCSD and FLY, while SDS-insoluble LMP was highly and positively correlated with FP.

LUPP expressed positive and significant correlations with SDS sedimentation volume while TUPP was also positively and significantly correlated with FPC. Significant correlation obtained in protein fractions was found mostly in kernel characteristics, while FLY, SDS-sedimentation and FPC also showed some correlation with protein fractions.

F_2 progeny: SDS-soluble LPP was significantly and positively correlated with SKCSW and SKCSD, and negatively correlated with FPC and FLY. Similarly SDS-soluble SPP was significantly and positively correlated with SDS sedimentation and MDT, and negatively and significantly correlated with FPC and FLY. FLY was negatively and significantly correlated with SDS-soluble SMP while a significant positive correlation was observed between SDS-soluble SMP and FPC. SDS-insoluble LPP was positively and significantly correlated with SKCSW, SKCSH and FP. LUPP was negatively and significantly correlated with FPC.

Table 35: Means of protein fractions from parents and F_1 progeny

	LPP1	SPP1	LMP1	SMP1	LPP2	SPP2	LMP2	SMP 2	TUPP	LUPP	LPP	LMP	SPP	SMP	PP	MP
1	12.16	47.34	11.56	5.60	12.97	51.20	12.88	11.19	51.89	51.61	25.13	24.44	98.54	16.79	123.67	41.23
2	9.44	50.40	15.68	5.50	11.29	28.21	9.85	13.03	39.76	54.46	20.73	25.53	78.61	18.53	99.34	44.06
3	9.33	44.83	13.30	6.21	11.20	38.50	9.64	8.58	47.85	54.55	20.53	22.94	83.33	14.79	103.86	37.73
4	11.77	34.34	10.82	11.22	11.19	33.27	9.54	7.60	49.09	48.74	22.96	20.36	67.61	18.82	90.57	39.18
5	10.73	32.87	10.82	10.67	12.02	33.72	10.80	10.11	51.20	52.84	22.75	21.62	66.59	20.78	89.34	42.40
6	11.56	43.01	12.35	10.00	10.72	33.81	11.43	10.02	44.93	48.11	22.28	23.78	75.82	20.62	98.10	43.80
7	13.80	27.96	8.68	10.65	11.05	33.42	11.92	10.32	51.57	44.47	24.85	20.60	61.38	20.97	86.23	41.57
8	13.06	36.86	17.18	10.99	11.54	36.19	14.33	13.87	54.46	46.91	24.60	31.51	73.05	24.86	97.65	56.37
9	11.34	37.29	7.29	9.49	9.63	49.71	15.70	5.73	55.00	45.92	20.97	25.99	87.00	15.22	107.97	41.21
10	11.85	30.12	8.29	11.17	10.25	50.32	14.14	5.35	59.07	46.38	22.10	22.43	80.44	16.52	102.54	39.95
11	12.98	50.88	12.24	5.26	10.98	32.16	10.42	11.99	40.32	45.83	23.96	26.22	83.04	17.25	107.00	39.91
12	13.06	52.57	11.92	4.54	10.51	25.55	10.51	12.14	35.99	44.59	23.57	22.43	78.12	16.68	122.71	39.11
13	13.76	53.72	11.50	4.57	11.88	24.29	7.95	8.87	34.86	46.88	25.64	19.45	78.01	13.44	103.65	32.89
14	13.70	54.07	12.22	5.27	12.28	28.26	8.66	9.74	37.43	47.27	25.98	20.88	82.33	15.01	108.31	35.89
15	13.05	52.77	12.28	4.57	10.93	33.49	8.91	6.97	40.29	45.58	23.98	21.19	86.26	11.54	110.24	32.73
16	11.74	52.57	13.41	4.28	12.61	55.97	14.90	5.01	51.61	51.79	24.35	28.31	108.54	9.29	132.89	37.60
17	12.60	53.93	11.92	3.99	11.26	39.69	11.46	8.09	43.37	47.15	23.86	23.38	93.62	12.08	117.48	35.46
18	10.63	54.50	13.72	2.85	11.11	41.97	11.18	7.00	44.90	51.10	21.74	24.90	96.47	9.85	118.21	34.75
19	10.09	50.65	13.70	5.01	10.54	51.49	12.69	4.68	50.53	51.09	20.63	25.39	102.14	9.69	122.77	36.08
20	10.52	52.10	13.60	4.97	12.28	41.60	12.03	6.72	46.25	53.86	22.80	25.63	93.70	11.69	116.50	37.32
21	10.31	48.70	12.62	7.22	10.79	34.05	9.64	10.01	43.18	51.14	21.10	22.26	82.75	17.23	103.85	39.49
22	9.73	45.14	12.47	6.63	9.89	52.04	14.38	5.25	53.02	50.41	19.62	26.85	97.18	11.88	116.78	38.73
23	11.05	51.94	12.75	4.97	9.10	51.91	11.49	12.77	49.20	45.16	20.15	24.24	103.85	17.74	124.00	41.98
24	11.59	53.29	11.98	4.98	10.67	27.99	10.13	13.38	37.34	47.93	22.26	22.11	81.28	18.36	103.54	40.47
25	11.71	52.55	12.46	4.48	10.36	21.88	10.09	12.21	33.41	46.94	22.07	22.55	74.43	16.69	96.50	39.24
Mean	11.66	46.58	13.39	69.60	11.08	40.11	11.39	9.24	45.86	48.83	22.74	23.54	81.56	15.83	108.15	40.65
LSD(0.05)	2.25	11.98	2.63	6.60	2.41	4.85	1.43	2.07	7.66	9.77	7.01	5.88	3.98	12.77	5.879	2.39

1=Nata x Wanda, 2=Wanda x Sceptre, 3=Sceptre x Kariega, 4=Sceptre x Nata, 5=Nata x Kariega, 6=Wanda x SST 124, 7=Sceptre x Wanda, 8 = Sceptre x Kariega, 9 = Nata x SST 124, 10 = Kariega x Wanda, 11 = Wanda x Kariega, 12 = Kariega x Nata, 13 = Kariega x SST 124, 14 = Kariega x Sceptre, 15 = SST 124 x Sceptre, 16 = Nata x Sceptre, 17 = Wanda x Nata, 18 = SST 124 x Nata, 19 = SST 124 x Kariega, 20 = SST 124 x Wanda, 21 = Nata, 22 = Wanda, 23 = Kariega, 24 = SST 124, 25 = Sceptre. LPP= Large polymeric proteins, SPP= Small polymeric proteins, LMP=Large monomeric protein, SMP=Small monomeric protein. TUPP=Total Unextractable Polymeric Protein, LUPP=Large unextractable polymeric protein, PP=Polymeric Protein..

Table 36: Analysis of variance for protein fractions in parents and F_1 progeny

		Mean squares for protein fractions in F_1 progeny							
Source	DF	LPP1	SPP1	LMP1	SMP1	LPP2	SPP2	LMP2	SMP2
Genotypes	24	5.488*	213.277*	6.94*	21.662*	3.620*	2284.694*	13.426*	24.807*
Reps	2	1.527	39.838	1.823	14.439	2.793	181.004	2.992	35.785
CV		14.09	10.83	15.83	4.30	15.72	3.11	2.20	7.80

Table 37: Means of protein fractions from parents and F_2 progeny

	LPP1	SPP1	LMP1	SMP1	LPP2	SPP2	LMP2	SMP 2	TUPP	LUPP	LPP	LMP	SPP	SMP	PP	MP
1	11.00	45.04	12.84	8.12	11.12	26.06	13.24	12.47	39.87	50.18	22.16	24.44	98.54	16.79	120.70	41.23
2	10.17	45.81	9.01	9.34	10.06	27.38	14.67	8.35	40.08	49.73	20.23	28.87	78.61	18.53	98.84	47.40
3	10.03	51.94	13.61	5.87	10.14	26.06	13.68	8.41	36.88	50.27	20.17	22.94	83.33	14.79	103.45	37.73
4	10.43	51.60	16.07	3.98	10.22	28.52	15.25	10.11	38.44	49.49	20.65	20.36	67.61	18.80	88.26	39.16
5	10.17	49.69	15.99	4.81	11.00	28.65	10.59	13.77	39.85	51.96	21.17	21.62	66.59	20.78	87.76	42.40
6	11.09	42.52	11.70	5.95	9.60	26.99	10.66	13.44	40.57	46.40	20.69	23.78	76.82	20.02	97.51	43.80
7	10.55	50.31	15.42	4.38	12.37	30.21	11.40	9.86	41.16	53.97	22.92	20.60	61.38	20.97	84.65	41.57
8	11.13	52.43	13.90	3.70	12.14	26.62	12.25	12.32	37.88	52.17	23.27	31.51	73.05	24.86	96.32	56.37
9	11.27	51.53	13.06	5.13	10.96	24.88	11.12	7.26	36.33	49.30	22.23	52.99	87.00	15.22	109.23	68.21
10	11.00	53.44	16.03	4.31	11.01	28.69	11.63	4.62	38.12	50.02	22.01	22.43	80.44	16.52	102.45	38.95
11	10.05	51.94	14.71	4.61	10.94	26.08	9.01	5.69	37.39	52.22	20.99	22.66	83.04	17.25	104.03	39.91
12	10.12	50.47	16.15	3.32	14.17	32.68	13.33	13.12	43.61	58.34	24.29	22.43	78.12	16.68	102.40	39.11
13	9.95	47.12	19.20	5.47	12.39	26.87	13.62	7.96	40.76	55.46	22.34	19.45	78.01	13.44	100.35	32.89
14	11.05	57.13	15.47	5.94	11.70	35.01	14.92	10.92	40.66	51.43	22.75	20.88	82.33	15.01	105.08	35.89
15	11.60	53.40	13.36	4.00	10.91	30.16	10.77	12.32	38.72	48.47	22.51	21.19	86.26	11.54	108.77	32.73
16	11.89	53.22	13.23	3.00	12.19	26.38	10.83	9.97	37.20	50.62	24.08	28.31	108.54	9.29	132.62	37.60
17	11.23	56.41	11.78	4.61	10.96	33.61	9.48	5.79	39.72	49.39	22.19	23.38	93.62	12.08	115.81	35.46
18	12.30	52.98	12.71	4.75	10.88	30.60	10.63	10.33	38.85	46.94	23.18	24.90	96.47	9.85	119.65	34.75
19	12.41	52.94	12.80	4.85	11.94	29.13	9.45	11.69	38.59	49.03	24.35	26.39	102.14	9.69	126.49	36.08
20	12.26	57.88	12.08	5.29	11.16	26.24	9.86	8.71	34.78	47.65	23.42	25.63	93.70	11.70	117.12	37.33
21	12.26	58.88	12.08	5.29	11.37	26.24	9.86	8.71	34.58	48.12	23.63	22.26	82.75	17.23	106.38	39.49
22	11.96	61.87	12.35	5.42	13.21	27.67	9.48	10.85	35.64	52.48	24.17	26.85	97.18	11.88	121.35	38.73
23	11.99	56.93	12.08	6.12	10.31	25.82	8.79	13.78	34.39	46.23	22.30	24.24	103.85	17.74	126.15	41.98
24	11.11	54.78	12.24	4.86	9.14	28.82	10.07	14.32	36.55	45.14	20.25	22.11	81.28	18.36	101.53	40.47
25	11.94	46.76	12.12	7.09	9.89	28.21	9.47	14.67	39.36	45.31	21.83	22.55	74.43	16.69	96.26	39.24
Mean	11.16	52.28	13.60	5.2	11.19	28.31	11.36	10.38	37.20	50.01	22.31	24.91	84.60	15.83	106.93	40.74
SD(0.05)	12.52	10.05	3.55	1.25	2.20	2.50	4.36	4.44	7.99	11.78	5.56	9.44	7.84	3.23	9.00	3.79

1 = Nata x Wanda, 2 x Wanda x Sceptre, 3 = Sceptre x Kariega, 4 = Sceptre x Nata, 5 = Nata x Kariega, 6 = Wanda x SST124, 7 = scepter x Wanda, 8 = Sceptre x Kariega, 9 = Nata x SST 124, 10 = Kariega x Wanda, 11 = Wanda x Kariega, 12 = Kariega x Nata, 13 = Kariega x SST 124, 14 = Kariega x Sceptre, 15 = SST 124 x Sceptre, 16 = Nata x Sceptre, 17 = Wanda x Nata, 18 = SST 124 x Nata, 19 = SST 124 x Kariega, 20 = SST 124 x Wanda, 21 = Nata, 22 = Wanda, 23 = Kariega, 24 = SST 124, 25 = Sceptre. LPP= Large polymeric proteins, SPP= Small polymeric proteins, LMP=Large monomeric protein, SMP=Small monomeric protein. TUPP=Total Unextractable Polymeric Protein, LUPP=Large unextractable polymeric protein, PP=Polymeric Protein.. 1=SDS soluble protein fractions, 2= SDS insoluble protein fractions.

Table 38: Analysis of variance for protein fractions in F_2 progeny

		Mean squares for protein fractions in F_2 progeny							
Source	DF	LPP1	SPP1	LMP1	SMP1	LPP2	SPP2	LMP2	SMP2
Genotypes	24	141.70*	57.963*	14.309*	7.271*	4.100*	17.446*	12.131*	22.960*
Reps	2	136.420	98.025	6.728	4.531	1.120	10.424	2.412	3.269
CV		9.15	14.17	9.36	4.13	2.09	2.19	2.79	3.1

LPP= Large polymeric proteins, SPP= Small polymeric proteins, LMP=Large monomeric protein, SMP=Small monomeric protein. TUPP=Total Unextractable Polymeric Protein, LUPP=Large unextractable polymeric protein, PP=Polymeric Protein.. 1=SDS soluble protein fractions, 2= SDS insoluble protein fractions. $P \leq 0.05$. CV= Coeffient of variation.

Table 39: Signification correlations between protein fractions and measured quality characteristics

F_1 progeny			F_2 progeny		
Protein fraction	**Characteristics**	**Correlation**	**Protein fractions**	**Characteristic**	**Correlation**
SDS-soluble LPP	FPC	-0.49*	SDS-soluble LPP	FPC	-0.32*
	SKCSW	-0.53*		SKCSW	0.38*
	SKCSD	0.55*		SKCSD	0.52*
	FLY	0.58*		FLY	-0.72**
SDS-soluble SPP	SKCSW	-0.41*	SDS-soluble SPP	SDSS	0.52**
	SKCSH	0.39*		MDT	0.56**
	FLY	0.62**		FPC	-0.32*
SDS-soluble LMP	FPC	0.71**		FLY	-0.41*
SDS-soluble SMP	SKCSW	-0.43*	SDS-soluble SMP	FLY	-0.44*
	SKCSH	-0.54**		FPC	0.39*
SDS-insoluble LPP	SDSS	0.62**	SDS-insoluble LPP	SKCSW	-0.34*
	SKCSW	0.55**		SKCSH	0.31*
	SKCSD	0.53**		FPC	-0.49*
	FLY	-0.39*	LUPP	FPC	-0.51*
SDS-insoluble SPP	SKCSH	0.44*			
	SKCSW	0.48*			
	SKCSD	0.45*			
	FLY	0.35*			
SDS-insoluble LMP	FPC	0.62**			
LUPP	SDSS	0.55**			
TUPP	FPC	0.54**			

FLY=flour yield, FPC=flour protein content, MDT=mixogram development time, SDSS=SDS sedimentation , SKCSW=kernel weight, SCKCSD=kernel diameter, SCKSH=kernel hardness, LPP= Large polymeric proteins, SPP= Small polymeric proteins, LMP=Large monomeric protein, SMP=Small monomeric protein.TUPP=Total Unextractable Polymeric Protein, LUPP=Large unextractable polymeric protein, PP=Polymeric Protein.. 1=SDS soluble protein fractions, 2= SDS insoluble protein fractions.*$P \leq 0.05$, **$P \leq 0.01$.

5.4 Discussion and conclusions

In the F_1 and F_2 material with the parents, SDS soluble LPP was negatively correlated with FPC. In the F_2 generation the SDS insoluble LPP and the large unextractable poteins were negatively correlated with FPC, and in the F_1 generation the SDS insoluble LMP was postively correlated with FPC. SDS sedimentation is often used as a selection parameter for breadmaking quality. In the F_1 generation, SDSS was significantly correlated with the SDS insoluble LPP and the large unextractable proteins. In the F_2 generation, the SDS soluble SPP were significantly correlated with SDSS. The correlations with especially FPC and SDSS were therefore not consistent for the F_1 to the F_2 generation. Quality is often associated with the occurrence of large protein aggregates (Autran, 1994). This was the case in the F_1 but not the F_2 generation. There was large variation for all the protein fractions and large significant differences between entries in the progeny, which indicates segregation, and selection should be possible for protein fractions to improve quality characteristics. In many of the fractions some of the progeny expressed much higher values than the parents, and this indicates that there may be heterosis for the expression of the protein fractions. Some progeny had a higher expression of this phenomenon than others, and they could be selected for this, to improve quality in this way.

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Chapter 6

Summary

- The objectives of this study were to characterize wheat cultivars grown in Lesotho according to their glutenin and gliadin banding patterns with the use of SDS-PAGE, estimate GCA, SCA, h^2, heterosis and correlation of wheat quality traits by crossing poor, medium and good breadmaking qualities in a full diallel design to create F_1 and F_2 progeny, and determine the potential of SE-HPLC in predicting quality of wheat cultivars.
- Glutenin and gliadin proteins were separated using SDS-PAGE. Cultivars used for estimating combining ability were grown in pots and crossed in the greenhouse at Bloemfontein. The F_1 progeny were grown in Lesotho and Bloemfontein while the F_2 progeny were grown in Bloemfontein. Protein extracted from the wheat flour of all this material were analysed by SE-HPLC.
- Seven wheat quality characteristics were analysed at the Agricultural Research Council, Bethlehem for parents, F_1 and F_2 progeny.
- Analysis of variance and correlations were employed as statistical tools to analyse the data generated from storage protein, combining ability trials and SE-HPLC.
- Results revealed that gliadins and LMW-GS were able to distinguish 30 cultivars of wheat grown in Lesotho, while HMW-GS enabled cultivars to be grouped.
- The ANOVA indcated significant differences between parents and F_1 progeny and parents and F_2 progeny for all characteristics studied. The mean squares for GCA, SCA and reciprocals were significant for all measured characteristics in the F_1 generation, while in the F_2, the mean squares for GCA were significant for all but one characterisics. Mean squares for SCA and reciprocals were significant for all characteristics in the F_2 generation.
- To improve or enhance quality, Wanda, SST 124 and Sceptre could be incorporated into the breeding programme. Crosses such as Sceptre x Kariega, SST 124 x Nata, Wanda x Nata, Sceptre x Wanda, Kariega x Wanda, Kariega x Sceptre, SST 124 x Sceptre, Wanda x SST 124, Nata x Kariega and Nata x SST 124 should also be incorporated into the programme.
- F_1 progeny showed non-additive gene action in all characteristics while F_2 progeny showed four characteristics to be controlled by non-additive gene action while the others were controlled by additive gene action.

- Heritability in the broad sense was high for all characteristics in the F_1 and F_2 progeny, whereas heritability in the narrow sense was higher for F_2 than F_1 progeny.
- Midparent heterosis and best parent heterosis was expressed in four characteristics.
- Highly significantly positive and negative correlations were observed between quality characteristics in F_1 and F_2 progeny.
- Results of SE-HPLC showed that large polymeric and large monomeric proteins can be used as predictors of good and poor quality respectively, in the parents and F_1 and F_2 progeny.

Opsomming

- Die doel van hierdie studie was om koring cultivars wat in Lesotho verbou word volgens hulle glutenien en gliadien bandpatrone te karakteriseer met SDS-PAGE, en om GCA, SCA, h^2, heterose and korrelasie van kwaliteits eienskape te bepaal deur swak, medium en goeie kwaliteit koring cultivars in 'n vol dialleel te kruis om F_1 en F_2 nageslag te genereer, en om die potensiaal van SE-HPLC vir die voorspelling van kwaliteit te bepaal.
- Glutenien en gliadien proteïene is met die gebruik van SDS-PAGE geskei. Cultivars wat gebruik is om kombineervermoë te bepaal, is in potte in die glashuis by Bloemfontein geplant. Die F_1 nageslag is in Lesotho en Bloemfontein geplant, en die F_2 nageslag is in Bloemfontein geplant. Proteïene is van al hierdie material geëkstraheer en met SE-HPLC geanaliseer.
- Sewe kwaliteits eienskappe is by die Landbou Navorsings Raad, Bethlehem geanaliseer vir ouers, F_1 en F_2 nageslag.
- Variansie analise en korrelasies is as statistiese metodes gebruik om data te analiseer wat is vanaf storings proteïene, kombineer vermoë proewe en SE-HPLC gegenereer.
- Die resultate het aangedui dat gliadiene en LMW-GS tussen 30 koring cultivars wat in Lesotho verbou word kon onderskei.
- Die ANOVA het betekenisvolle verskille tussen ouers en F_1 nageslag, en ouers en F_2 nageslag vir gemeette eienskappe aangetoon. Die gemiddelde kwadrate vir GCA, SCA en resiproke was betekenisvol vir alle eienskappe in die F_1 generasie, terwyl die gemiddelde kwadrate in die F_2 generasie betekenisvol vir GCA was vir alle eienskappe behalwe een. Gemiddelde kwaldrate was in die F_2 betekenisvol vir die SCA en resiproke vir alle eienskappe.
- Om kwaliteit te verbeter kan Wanda, SST 124 en Sceptre in die teelprogram ingesluit word.

Kruisings soos Sceptre x Kariega, SST 124 x Nata, Wanda x Nata, Sceptre x Wanda, Kariega x Wanda, Kariega x Sceptre, SST 124 x Sceptre, Wanda x SST 124, Nata x Kariega and Nata x SST 124 kan ook in die teelprogram ingesluit word.

- F_1 nageslag het nie-additiewe geen aksie vir alle eienskappe getoon, terwyl vier eienskappe in die F_2 nageslag deur nie-additiewe geen aksie bepaal is. Die ander eienskappe is deur additiewe geen aksie bepaal.
- Oorerflikheid in die breë sin was hoog vir alle eienskappe in die F_1 en F_2 nageslag, terwyl nou sin oorerflikheid hoër in die F_2 nageslag as die F_1 nageslag was.
- Mid-ouer en hoogste ouer heterose is vir vier eienskappe uitgedruk.
- Hoogs betekenisvolle positiewe en negatiewe korelasies is tussen kwaliteits eienskappe in die F_1 en F_2 nageslag gesien.
- Die resultate van SE-HPLC het getoon dat groot polimeriese en monomeriese proteïene as voorspellers van goeie en swak kwaliteit gebruik kan word onderskeidelik in die ouers en die F_1 en F_2 nageslagte.

Appendix 1

Stock solution for extraction

Extraction of gliadin

1. **Extraction buffer**

Urea	1.8g
B- Mercaptoethanol	0.1ml
Distilled water	10ml

2. **Sample buffer**

Tris (hydroxymethyl aminomethane)	1.0g
n-propanol	90ml

Titrate to pH 8 with N HCL. Make to 100 with 50% n-prpanol.

Add 40g glycerol, 2g SDS and 0.02g bromophemol blue

Extraction of HMW-GS

1. Stock solutions for extraction:

n-propanol (50%)	50ml
Distilled water	50ml

2 Extraction buffer:

80mM Tris HCL pH 8.0 made in 50% n-propanol

Tris	1.0g
50%n-propanol	90ml

Titrate to pH 8 with N HCl. Make up to 100ml with 50% n-prpanol.

3 Sample buffer same as above.

4 Stock solution for discontinous gel system

a) Separating buffer (2X) pH 8.88

Dissolve 45.412g Tris in 460ml distilled water

Titrate to pH 8.88 than add 1.0g SDS

Makes total of 500ml. Store at 4^0

b) Stacking buffer (2x) pH 6.8

Dissolve 6.06g Tris in 190 ml distilled water

Titrate to pH 6.8 then add 0.4g SDS

Make up to 200ml. Store at 4^0C

c) Separating acrylamide (30 % AC: 1% Bis)

Dissolve 75g acrylamide and 0.75g Bisacrylamide in 181 ml distilled water

Make up to 250ml. Store in dark at 4^0C

d) Stacking acrylamide (35% AC : 1.5% Bis)

Disslove 87.5 acrylamide and 1.32g bisacylamide in 181 ml distilled water

Make up to 250ml. Store in dark at 4^0C

Preparation of separating gel (10%)

	For 2 gels
Separating buffer	38 ml
Separating acrylamide	28.1 ml
Distilled water	14 ml
Temed	165 ml
Ammonium persulphate	190 µl

Preparation for stacking buffer

Stacking buffer	10 ml
Stacking acrylamide	2.6 ml
Distilled water	7.4 ml
Temed	40µl
APS (10%)	100µl

Electrode buffer

1. Cathode buffer

   Mix 30.28g Tris, 144g glycine, 10g SDS and make up to 1 litre with distilled water. Dilute 10x before use.

2. Anode buffer

   Mix 30.28g Tris with 800ml distilled water. Then titrate to pH 8.4, and make up to 1litre with distilled water. Dilute 10x before use.